

IEB 2014 Kyoto

Inner Ear Biology Workshop

2014 in Kyoto



Program & Abstracts

November 1 (Sat) – 4 (Tue), 2014
Kyoto, Japan



Inner Ear Biology Workshop

2014 in Kyoto

Program & Abstracts

November 1 (Sat) – 4 (Tue) 2014

Kyoto International Conference Center (ICC Kyoto)

Takaragaike, Sakyo-ku, Kyoto 606-0001, Japan

Tel: 075-705-1234



Welcome to IEB Workshop 2014 in Kyoto

Dear Friends and Colleagues,

It gives me great pleasure to welcome all of you to the "Inner Ear Biology Workshop 2014 in Kyoto." I am very pleased and honored that Kyoto is hosting this Workshop.

Regarded as an extraordinary meeting, this Inner Ear Biology Workshop will be the first to be held outside of Europe. I anticipate that, with your participation and contribution, it will be a great success.

The Workshop will include a high-quality scientific program focused on basic research of the inner ear function and inner ear disorders in order to reflect the remarkable recent progress in the field of basic science such as molecular biology, genetics, and regenerative medicine together with translational research and the clinical application of basic research.

Kyoto, the capital of Japan for more than 1000 years from 794 A.D. to 1868, is one of the most historic and beautiful cities in Japan. It is the heart of traditional arts and religions. It is also a city of science and culture. Kyoto has given rise to seven Nobel Laureates, most recently in 2012, the Nobel Prize in Physiology or Medicine.

November is one of the most beautiful seasons in Kyoto, especially as the mountains are covered with a brilliant brocade of red, yellow and orange leaves. The temperature is also most comfortable.

Many attractive social programs have been planned for the enjoyment of all participants and accompanying persons

I hope that you will all enjoy everything that Kyoto offers.



Juichi Ito, MD, PhD
Kyoto University

Organizing Committee

President Juichi Ito, MD, PhD (Kyoto University)

International Advisory Board

Karen B. Avraham (Israel)	Jean-Luc Puel (France)
Barbara Canlon (Sweden)	Ilmari Pyykkö (Finland)
Albert Edge (USA)	Yehoash Raphael (USA)
Anthony Gummer (Germany)	Helge Rask-Andersen (Sweden)
Stefan Heller (USA)	Edwin W. Rubel (USA)
Matthew W. Kelley (USA)	Joseph Santos Sacchi (USA)
Marlies Knipper (Germany)	Jochen Schacht (USA)
Sang-Heun Lee (Korea)	Isabel Varela-Nieto (Spain)
David J. Lim (USA)	Hans-Peter Zenner (Germany)
Seung-Ha Oh (Korea)	

Local Advisory Board

Katsumi Doi	Takashi Nakagawa
Kiyofumi Gyo	Kaoru Ogawa
Akira Hara	Koichi Omori
Katsuhisa Ikeda	Masafumi Sakagami
Tetsuo Ikezono	Hiroaki Sato
Kazuo Ishikawa	Mamoru Suzuki
Seiji Kakehata	Haruo Takahashi
Yukio Katori	Sugata Takahashi
Ken Kitamura	Noriaki Takeda
Izumi Koizuka	Tetsuya Tono
Kozo Kumakawa	Shin-ichi Usami
Shingo Murakami	Hiroshi Yamashita
Yasushi Naito	Tatsuya Yamasoba

Local Program Committee

Nobuhiro Hakuba	Takeshi Matsunobu
Harukazu Hiraumi	Ryosei Minoda
Kotaro Ishikawa	Yoshihiro Noguchi
Akinobu Kakigi	Takeshi Oshima
Sho Kanzaki	Hirofumi Sakaguchi
Tadashi Kitahara	Tatsunori Sakamoto
Shin-ichiro Kitajiri	Yutaka Takumi
Takuji Koike	Akiko Taura
Atsushi Matsubara	Daisuke Yamashita
Nozomu Matsumoto	

Secretary General

Takayuki Nakagawa (Kyoto University)
Norio Yamamoto (Kyoto University)

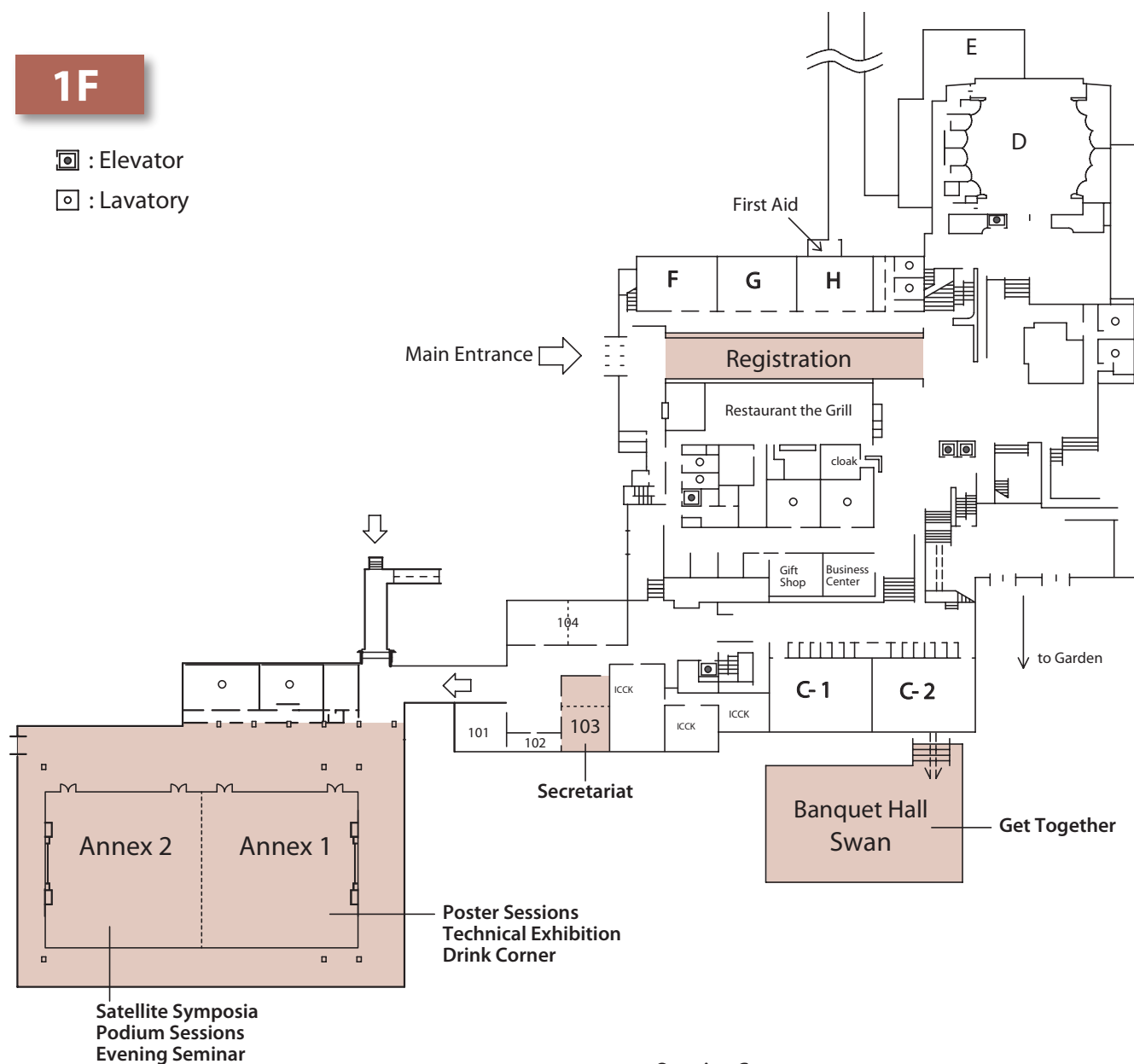


Kyoto International Conference Center

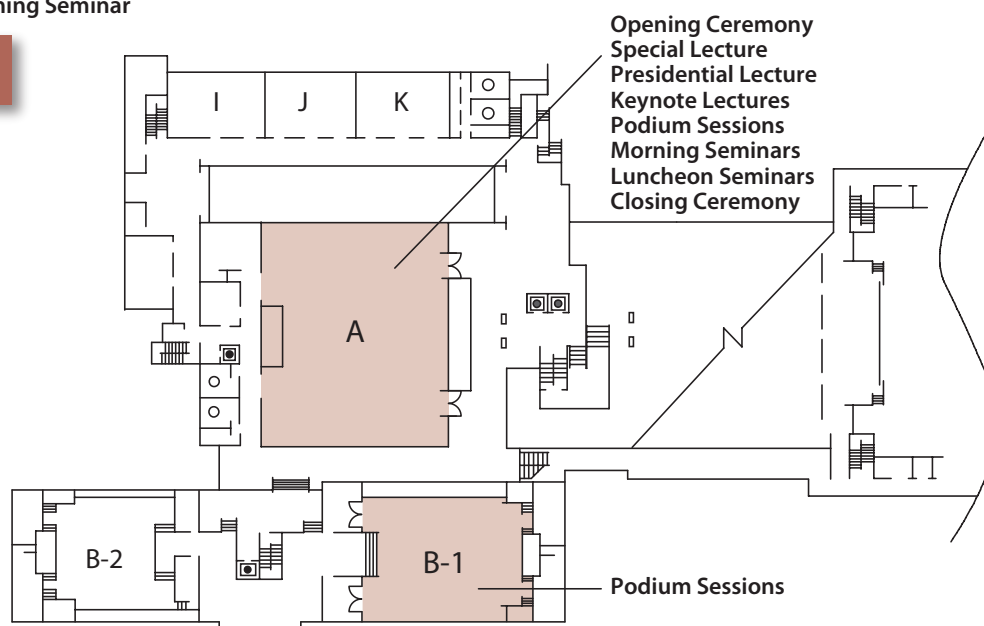
1F

◻ : Elevator

○ : Lavatory



2F



Inner Ear Biology Workshop 2014 in Kyoto

November 1, 2014

Annex 2	Lobby & Banquet Hall Swan
8:00	8:00
9:00	9:00
10:00	10:00
Symposium 1: Oto-stem cell Chairs: Löwenheim H (Germany) Nakagawa T (Japan) Speakers: Löwenheim H (Germany) Rivolta M (UK) Edge ASB (USA) Ealy M (USA) Nakagawa T (Japan)	11:00
12:00	12:00
13:00	13:00
Lunch	
14:00	14:00
Symposium 2: Genetics and Hearing Chair: Avraham KB (Israel) Speakers: Petit C (France) Choi BY (Korea) Neef J (Germany) Usami S (Japan)	15:00
16:00	16:00
17:00	17:00
	Registration
18:00	18:00
	Get Together Kyoto International Conference Center Banquet Hall, "Swan"
19:00	19:00
20:00	20:00



November 2, 2014

	Room A	Annex 2
8:00		
	Opening Ceremony Opening remarks Ito J History of IEB Gummer A	
9:00	Presidential Lecture Regeneration medicine for the inner ear disorders Speaker: Ito J Chair: Lim DJ	
	Keynote Lecture 1 Cell death and repair in hair cell epithelia Speaker: Gale JE Chair: Yamamoto N	
10:00	Coffee break	
	Podium 1 Ototoxicity 1 Chairs: Schacht J Hara A	Podium 2 Omics and genetic tools of inner ear Chairs: Ryan AF Kitamura K
11:00		
12:00	Luncheon Seminar 1 Speaker: van de Water TR Chair: Kumakawa K Co-sponsor: Med-El Medical Electronics Med-El Japan Co., Ltd.	
13:00		
	Poster session	
	Poster Presentation: Odd Number	
14:00		
	Coffee break	
	Keynote Lecture 2 The genomics of deafness: Exploring gene function and regulation Speaker: Avraham KB Chair: Usami S	
15:00		
	Podium 3 Inner ear damage Chairs: Canlon B Yamasoba T	Podium 4 Inner ear pathology Chairs: Neef J Ikeda K
16:00		
17:00		Evening Seminar Speaker: Takahashi H Chair: Murakami S Co-sponsor: J. Morita Mfg. Corp.
	17:50 Shuttle buses leave ICC Kyoto for "Welcome Reception"	
18:00		
19:00	Welcome Reception (The Sodoh Higashiyama & Kiyomizu Temple)	
20:00		

November 3, 2014

	Room A	Annex 2	Room B-1
8:00	Morning Seminar 1 Speakers: Treaba C, Patrick J, Verhoeven K Chair: Naito Y Co-sponsor: Nihon Cochlear Co., Ltd.		
9:00	Keynote Lecture 3 Application of human iPS cells for inner ear biology and human disease modeling Speaker: Okano H Chair: Takahashi S Coffee break		
10:00	Podium 5 Developmental biology 1 Chairs: Dabdoub A Kanzaki S	Podium 7 Cochlear implant Chairs: Pyykkö I Oh SH	
11:00	Podium 6 Developmental biology 2 Chairs: Ladher R Suzuki M		
12:00	Luncheon Seminar 2 Speakers: Shepherd RK Patrick J Chair: Yamasoba T Co-sponsor: Nihon Cochlear Co., Ltd.		
13:00	Poster session		
14:00	Poster Presentation: Even number		
15:00	Keynote Lecture 4 The Intricate, multifunctional roles of myosin motors in hair cell stereocilia Speaker: Kachar B Chair: Sakaguchi H		
16:00	Podium 8: Molecular structure of inner ear 1 Chairs: Kurima K Doi K	Podium 11 Tinnitus Chairs: Knipper M Ogawa K	Podium 14 Vestibular schwannoma -Clinical breakthrough- Chairs: Miyazaki H Kanemaru S Co-sponsor: Nihon Kohden Corp.
	Coffee break		
17:00	Podium 9: Molecular structure of inner ear 2 Chairs: Rask-Andersen H Katori Y	Podium 12 Mechanics and model of Cochlear and middle ear Chairs: Gummer A Koike T	Podium 15 Personalized medicine and genetics Chairs: Zenner HP Omori K
18:00	Podium 10: Auditory & vestibular function Chairs: Laurell G Sakamoto T	Podium 13 Developmental biology 3 Chairs: Varela-Nieto I Minoda R	
19:00	18:50 Shuttle buses leave ICC Kyoto for "Japanese Cuisine Experience"		
20:00	Japanese Cuisine Experience		



November 4, 2014

	Room A	Annex 2	Room B-1
8:00	Morning Seminar 2 Speakers: Takeda N Chair: Shojaku H Co-sponsor: Kowa Pharmaceutical Co. Ltd.		
9:00	Podium 16 Stem cells Chairs: Gale JE Taura A	Podium 17 Ototoxicity 2 Chairs: Rüttiger L Yamashita D	Podium 19 Vestibular Chairs: Magnusson M Ishikawa K
10:00		Podium 18 Regeneration Chairs: Edge ASB Okano T	
	Coffee break		
11:00	Special Lecture Recent progress in iPS cell research and application Speaker: Yamanaka S Chair: Ito J		
12:00			
	Luncheon Seminar 3 Speakers: Kulkarni A Chair: Sato H Co-sponsor: Advanced Bionics Japan		
13:00			
	Keynote Lecture 5 Synaptic diversity and the functional roles of Cochlear afferents Speakers: Fuchs PA Chair: Koizuka I		
14:00	Podium 20 Physiology Chairs: Santos-Sacchi J Nakagawa T	Podium 21 Gene therapy and drug delivery Chairs: Raphael Y Hakuba N	
15:00			
	Closing Ceremony		
16:00	15:50 Shuttle buses leave ICC Kyoto for "Private Noh Performance and Banquet"		
17:00			
18:00			
19:00	Private Noh Performance and Banquet		
20:00			

Social Programs

Open to all conference delegates and accompanying persons. Advance reservation required.

*You are requested to have your name tag and your reservation tickets for all events.

As for the Get Together, tickets are not required.

Saturday, November 1, 2014, 17:30 - 19:30

★Get Together

Place: ICC Kyoto, Banquet Hall, “Swan” (1F)

Dress code: Casual

* For the buffet style “Get Together” you will enjoy Western and Japanese cuisine in the “Swan” adjacent the beautiful Japanese garden.

Sunday, November 2, 2014, 18:30 – 22:00

★Welcome Reception

Place: The Sodoh Higashiyama and Kiyomizu Temple

17:50	Shuttle buses leave ICC Kyoto, Main entrance
18:30	Buffet style Welcome Reception at The Sodoh Higashiyama Walk to Kiyomizu Temple Special Concert and Private Visitation at Kiyomizu Temple
22:00	Shuttle buses depart Kiyomizu Temple for major hotels

Dress code: Smart Casual

* *Dress warmly and wear comfortable shoes*

The Sodoh Higashiyama

This restaurant was formerly the residence of the famous Japanese painter, Seihou Takeuchi.

Italian dishes are served with seasonal Kyoto ingredients.

The Illuminated Kiyomizu Temple, Private Visitation

Here you will witness the magnificent sight of the illuminated temple and the panoramic night view of Kyoto city, supremely beautiful, especially in autumn. A short, elegant concert will be offered in the ambience of the temple.

This is an exclusive opportunity only for the registered participants. It is a must-see.

~ Special Concert ~

A musical collaboration of the “Shakuhachi”, Japanese bamboo flute, and the harp accompanied by voice will be presented on the renowned veranda of the Kiyomizu stage.

Kiyomizu Temple: UNESCO World Heritage Site

This temple was established in 778. The Main Hall with its extended veranda is a National Treasure. The majority of the 13 halls and 2 pagodas in the precinct are designated as an Important Cultural Asset. The veranda on a 13 meter cliff is supported by wooden pillars without nails.

It commands a panoramic view of the city.



Tuesday, November 4, 2014, 17:00 – 21:00

★Private Noh Performance and Banquet

Place: Kyoto Kanze Kaikan Noh Theater and Westin Miyako Hotel Kyoto, “Mizuho”

15:50	Shuttle buses leave ICC Kyoto, Main entrance
17:00	Private Noh performance at the Kanze Kaikan Noh Theater
18:15	Depart for Westin Miyako Hotel Kyoto
19:00	Banquet at Westin Miyako Hotel Kyoto, “Mizuho”
21:00	Shuttle buses leave for major hotels

Dress code: Smart Casual

~ Special Private Noh Performance ~

Noh: “Aoi no Ue” (Lady Aoi from the “Tale of Genji”)
performed by Kurouemon Katayama, Kanze School

Noh

Noh is the oldest and highest level classical stage art dating back to the 14th century under the protection of the Ashikaga Shogunate. It was designated as an ‘Intangible Cultural Heritage’ by UNESCO in 2001. The Noh play is brought into perfect harmony by music, dance and narrative chorus. The beauty of Noh masks and colorful brocade costumes transfixed the eye of the viewer. Noh creates a deep beauty known as “yugen”, the subtle and profound.

Aoi no Ue

Lady Aoi, Prince Genji’s wife lies stricken with a mysterious illness due to the jealousy of Lady Rokujo, Prince Genji’s former mistress. A priestess is summoned to evoke the evil spirit of Lady Rokujo.

Lady Rokujo then becomes subdued by the incantations of a priest.

This should most definitely be an unforgettable experience.

Banquet entertainment by Maiko and Geiko (dancing girls in Kyoto)

The Maiko are young apprentice Geiko who are trained in dancing, singing, and playing traditional Japanese musical instruments for the ultimate entertainment of selected guests.

You will have an opportunity to talk with them and take photos.

■ Timetable of Social Programs and Tours

	9	10	11	12	13	14	15	16	17	18	19	20	21	22
November 1 (Sat)										Get Together				
										17:30	19:30			
November 2 (Sun)												Welcome Reception Private visitation & Concert at Kiyomizu Temple		
											18:30			
November 3 (Mon)		OP-1 Half-Day Tour (Kinkakuji, Ryoanji & Kyoto Museum of Traditional Crafts) and Lunch										Japanese Cuisine Experience for Dinner		
	9:00			13:00								19:30		
November 4 (Tue)		OP-2 Half-Day Tour (Zazen, Nishiki Market & Nijo Castle) and Lunch								Private Noh Performance and Banquet				
	9:00			13:30					17:00					

OP: optional tour

Monday, November 3, 2014 19:30~

★Japanese Cuisine Experience for Dinner

***Regretfully all reservations have been filled.**

Departure time: 18:50

Shuttle buses leave ICC Kyoto, Main entrance

**Shuttle bus will be provided from ICC Kyoto to the restaurants, however, not from the restaurants after dinner so that you can enjoy the city night life.*

Japanese cuisine may be described as traditional products, exquisitely prepared and presented, emphasizing delicate shades of color, seasonality, taste and texture. It was designated as an Intangible World Heritage by UNESCO in autumn 2013.

This will no doubt be a memorable experience for you.

Choices of Japanese Cuisine for Dinner

A. Sushi (Nigirizushi)

Place: Ganko Takasegawa Nijo En

Dress Code: Casual

Nigirizushi is one of the varieties of sushi. Vinegar seasoned rice is molded into small oval shapes. Then a very small amount of wasabi or horse radish, and a slice of raw fish or other delicacies top the rice. You can enjoy a hands-on experience.

B. Izakaya

Place: Iroha-karuta, Honten

Dress Code: Casual

Izakaya is a Japanese-style eating and drinking venue. It is especially popular among young adults as a casual and reasonably priced place for socializing. Many office workers and students enjoy food and drinks with friends at Izakaya.

C. Kaiseki Cuisine

Place: Shimogamo Saryo

Dress code: Smart Casual

Kaiseki cuisine began as an intricate part of chanoyu or the Way of Tea. Later it was blended with imperial court cuisine and shojin cuisine (vegetarian dishes for Buddhist priests) to become an elaborate, gorgeous multi-course meal. Each dish is presented beautifully in exquisite dinnerware with the sense of season. Things from nature, such as foliage and flowers, often adorn the dish to heighten seasonality. High-end Japanese restaurants and inns serve kaiseki dinners, the ultimate in Japanese cuisine.



Optional Half-Day Tours (OP-1, OP-2)

*An English speaking guide will be available

Fee: ¥ 3,000

OP-1 Monday, November 3, 2014

Time:	9:00-13:00
	9:00 Shuttle bus leaves ICC Kyoto, Main entrance
	Kinkakuji Temple (Golden Pavilion) Ryoanji Temple (Rock Garden) Kyoto Museum of Traditional Crafts
	13:00 ICC Kyoto (Lunch)

Kinkakuji Temple: UNESCO World Heritage Site

Kinkakuji Temple was originally the villa of Yoshimitsu Ashikaga, the Shogun of the 14th century and was later converted to a Zen temple. It is renowned for its three-story pavilion with gold-leaf gilding which reflects beautifully on the surface of the Mirror Pond.

Ryoanji Temple: UNESCO World Heritage Site

Ryoanji Temple was previously the villa of noble families and became a Zen temple in the 15th century. The rock garden is famous for the fifteen rocks arranged on the raked sand, a masterly example of the beauty and harmony of the dry landscape garden of Zen temples.

Kyoto Museum of Traditional Crafts

Beautiful examples of Kyoto's fine traditional arts and crafts are gathered and displayed here with detailed English explanation. You will have a chance to absorb the technique of how these are created. Master craftsmen will be demonstrating.

OP-2 Tuesday, November 4, 2014

Time:	9:00-13:30
	9:00 Shuttle bus leaves ICC Kyoto, Main entrance
	Zazen (Zen Meditation) at Kenninji Temple Nishiki Market Nijo Castle
	13:30 ICC Kyoto (Lunch)

Zazen (sitting Zen Meditation)

Zazen is a unique form of meditation of central importance in Zen Buddhism. By minimizing external thoughts during meditation, practitioners seek to attain the highest possible state of mental concentration, and the inner experience towards enlightenment. Zazen is the study of the self.

** Wear loose clothing. Chairs will be available.*

Kenninji Temple

Kenninji is the oldest Zen temple in Kyoto. It ranks among the top five Rinzai sect Zen temples in Kyoto.

Nishiki Market

In Nishiki Market, the Kitchen of Kyoto, food for Japanese cuisine is sold. You will experience the feeling of Kyoto's food culture!

Nijo Castle: UNESCO World Heritage Site

Nijo Castle was built in 1603 as the residence of the Tokugawa Shogunate who ruled in Edo, Tokyo. The gorgeous paintings on the sliding doors are by famous artists of the Kano school.

10:00-13:00 Satellite Symposium 1

Annex 2

Oto-stem Cell

Chairs: Löwenheim H (Germany)
Nakagawa T (Japan)

SS1-1 The OTOSTEM Project: Human Stem Cell Applications for the Treatment of Hearing Loss
Löwenheim H (Germany), the OTOSTEM Consortium (Switzerland)

SS1-2 Generation of Otic Progenitors from Human Pluripotent Stem Cells
Boddy SL, Romero-Guevara R, Abbas L, Eshtan SJ, Barrott D, Unger C, Corns L, Marcotti W, Andrews PW, Rivolta MN (UK)

SS1-3 Human ES Cell-derived Neurons and Innervation of Hair Cells
Edge ASB (USA)

SS1-4 Single Cell Analysis of Monolayer Otic Placode Generation from Human Stem Cells
Ealy M, Heller S (USA)

SS1-5 Transplantation of Human iPS Cell-derived Neurons Cultured on 3D Collagen Matrix
Nakagawa T (Japan)

13:00-14:00 Lunch time

14:00-17:00 Satellite Symposium 2

Annex 2

Genetics and Hearing

Chair: Avraham KB (Israel)

SS2-1 Genetic and Biochemical Approaches of the Mechano-Electrical Transduction (MET) Machinery
Pepermans E, Michel V, Bonnet C, Bahloul A, El-Amraoui A, Avan P, Petit C (France)

SS2-2 Disruption of Inner Ear Tricellular Tight Junctions by a Mutation of *ILDR1* in a Family Segregating Mild Hearing Loss
Kim NKD (Korea), Higashi T (Japan), Lee KY (Korea), Kim AR (Korea), Kitajiri S (Japan), Kim MY (Korea), Chang MY (Korea), Kim V (Canada), Oh SH (Korea), Kim D (Korea), Furuse M (Japan), Park WY (Korea), Choi BY (Korea)

SS2-3 Molecular Physiology and Pathology of Sound Encoding in the Inner Ear
Neef J, Picher MM, Reisinger E, Jung S, Moser T (Germany)

SS2-4 Screening Strategy for the Molecular Diagnosis of Deafness: From Social Health Insurance-Based Screening to Massively Parallel DNA Sequencing
Usami S, Miyagawa M, Naito T, Moteki H, Nishio S (Japan)

Presentation time of IEB Kyoto 2014

Podium session: Target Lectures have 20 minutes including discussion, while other presentations have 12 minutes including discussion.



8:30-9:00 Opening Ceremony

Room A

Opening Remarks by President

Ito J (Japan)

History of IEB

Gummer A (Germany)

9:00-9:30 Presidential Lecture

Room A

Chair: Lim DJ (USA)

PL

Regeneration Medicine for the Inner Ear DisordersIto J (Japan)

9:30-10:15 Keynote Lecture 1

Room A

Chair: Yamamoto N (Japan)

KL1

Cell Death and Repair in Hair Cell EpitheliaGale JE (UK)

10:30-11:50 Podium 1: Ototoxicity 1

Room A

Chairs: Schacht J (USA)
Hara A (Japan)10:30 1
Target Lecture**Development of Designer Aminoglycoside Antibiotics without Ototoxicity**Matt T (Switzerland), Shcherbakov D (Switzerland), Sha S-H (USA), Perez-Fernandez D (Switzerland), Akbergenov R (Switzerland), Meyer M (Switzerland), Duscha S (Switzerland), Freihofer P (Switzerland), Dubbaka SR (Switzerland), Xie J (USA), Vasella A (Switzerland), Böttger EC (Switzerland), Schacht J (USA)

10:50

2

How the Exposure to Styrene Affects the Different Sources of DPOAEsSisto R, Moleti A, Botti T, Sanjust F (Italy)

11:02

3

The Influence of Sphingosine Kinase Inhibitor and Sphingosin on Cisplatin-induced Hair Cell Loss of the Rat CochleaTani K, Tabuchi K, Nakayama M, Hara A (Japan)

11:14

4

Protective Role of Ecabet Sodium against Neomycin-induced Hair Cell Damage in ZebrafishChoi J, Chang J, Im GJ, Chae SW, Jung HH (Korea)

11:26

5

Purinergic P2 Receptor Signaling Enhances Neomycin OtotoxicityLin SCY, Vlajkovic SM, Thorne PR (New Zealand)

11:38

6

Netrin-1 Protects Hair Cells against AminoglycosideYamahara K, Yamamoto N, Nakagawa T, Ito J (Japan)

12:00-13:00 Luncheon Seminar 1

Room A

Chair: Kumakawa K (Japan)

LS1

EAS- DXMb-eluting Electrodes Protect against Electrode Insertion Trauma (EIT)-induced Increases in Hearing Thresholds, Impedance and Loss of Cochlear Sensory Receptor CellsVan De Water TR, Bas E, Bohorquez J, Eshraghi AA, He J, Perez E, Melchionna T, Garnham C, Jolly C (USA, Austria)

Co-sponsor: Med-El Medical Electronics, Med-El Japan Co., Ltd.

Chairs: Ryan AF (USA)
Kitamura K (Japan)

10:30 7 Proteomics of Noise-induced Hearing Loss

Target Lecture Savas J, Pak K, Ryan AF (USA)

10:50 8 Variation Analysis of RNA-seq Data for Identification of Candidate Genes Associated with Individual Variation in Cochlear Responses to Acoustic Trauma

Hu BH, Yang S, Cai Q, Vethanayagam RR, Dong YY, Bard J, Jamison J (USA)

11:02 9 Characterization of Transcriptomes of Cochlear Inner and Outer Hair Cells

Liu HZ, Pecka Jason L, Zhang Q, Soukup GA, Beisel KW, He DZZ (USA)

11:14 10 An Inducible Gene Expression System Using a Modified Hair Cell Promoter for Myosin 7a

Asai Y, Géléoc GSG, Holt JR (USA)

11:26 11 Establishment of a Novel Mouse Model for Hearing Impairment by Selective Ablation of Outer Hair Cells in the Inner Ear

Kikkawa Y, Miyasaka Y, Wada K, Tokano H, Kitamura K, Matsuoka K (Japan)



14:30-15:15 Keynote Lecture 2

Room A

Chair: Usami S (Japan)

- KL2 The Genomics of Deafness: Exploring Gene Function and Regulation**
Avraham KB (Israel)

15:30-17:38 Podium 3: Inner Ear Damage

Room A

Chairs: Canlon B (Sweden)
Yamasoba T (Japan)

- 15:30 12 TrkB Mediated Protection against Circadian Sensitivity to Noise Trauma**
 Target Lecture Meltser I, Cederroth CR, Basinou V, Savelyev S, Schmitz Lundkvist G, Canlon B (Sweden)
- 15:50 13 Mitophagy can be Observed in the Inner Ear but is not an Universal Response to Mitochondrial Damage**
Bodmer D, Setz C (Switzerland)
- 16:02 14 Physiological Significance of p62-mediated Autophagy in Auditory Cells**
Hayashi K, Goto F, Tsuchihashi N, Nomura Y, Masuda T, Fujioka M, Kanzaki S, Ogawa K (Japan)
- 16:14 15 The Role of Purinergic Signalling in Cochlear Response to Stress and Injury**
Vlajkovic SM (New Zealand), Housley GD (Australia), Thorne PR (New Zealand)
- 16:26 16 Assessing Ototoxicity of Terbinafine Eardrops in Guinea Pigs Using Caloric, OVEMP and CVEMP Tests**
Yang TH, Chung FL, Young YH (Taiwan)
- 16:38 17 Protective Role of Edaravone against Cisplatin Induced Apoptosis in HEI-OC1 Cell**
Chang J, Park S-K, Kim H-J, Im GJ, Lee S, Jung HJ, Choi J, Chae SW, Jung HH (Korea)
- 16:50 18 Hyaluronan Up-regulation is Linked to Renal Dysfunction and Hearing Loss Induced by Silver Nanoparticles**
 Feng H (Finland), Pyykkö I (Finland), Zou J (Finland, China)
- 17:02 19 Epithelial Healing in the Organ of Corti after a Deafening Insult**
Anttonen T, Belevich I, Kirjavainen A, Laos M, Brakebusch C, Jokitalo E, Pirvola E (Finland)
- 17:14 20 Accelerated Noise-induced Hearing Loss and Audiogenic Seizure in Mice Lacking Thrombospondins**
 Wangsawihardja FV, Sundaresan S, Mendus D, Leu R, Grillet N, Ghoddoussi F, Holt AG, Mustapha M (USA)
- 17:26 21 Routes, Dynamics, and Correlates of Bacterial Invasion and Inflammatory Cell Infiltration in the Inner Ear during Pneumococcal Meningitis**
Thomasen PC, Miyazaki H, Nue Møller M, Worsøe L, Brandt CT, Ostergaard C (Denmark)

15:30-16:54 Podium 4: Inner Ear Pathology

Annex 2

Chairs: Neef J (Germany)
Ikeda K (Japan)

- 15:30 22 Assembly and Disruption of Cochlear Gap Junction Macromolecular Complex are Regulated by Connexin 26**
Kamiya K, Minowa O, Ikeda K (Japan)
- 15:42 23 Different Contribution of NKCCs in Two Epithelial Layers of the Lateral Cochlear Wall to the Unidirectional K⁺-transport in the Inner Ear**
Yoshida T, Nin F, Ogata G, Komune S, Hibino H (Japan)
- 15:54 24 Water Homeostasis of the Inner Ear can be Influenced by the Anti-diuretic Hormone**
Gleiser C (Germany), Runggaldier D (Germany), Brockhues J (Germany), Taguchi D (Japan), Müller M (Germany), Löwenheim H (Germany), Hirt B (Germany)
- 16:06 25 Targeting Translation during Cellular Stress: Manipulating Stress Granule Formation and Identifying RNA Components**
Goncalves AC, Dawson SJ, Gale JE (UK)
- 16:18 26 The Quantitative Analysis of the Aquaporin Expression Levels in the Inner Ear of Slc26a4^{-/-} Mice**
Miyoshi T, Yamamoto N, Yamaguchi T, Tona Y, Ogita K, Nakagawa T, Ito J (Japan)
- 16 :30 27 Glucocorticoids Stimulate Endolymphatic Water Reabsorption in Inner Ear through Aquaporin 3 Regulation**
Nevoux J, Viengchareun S, Lema I, Lecoq AL, Lombès M, Ferrary E (France)
- 16:42 28 Natural History of Resident Macrophages in the Mouse Cochlea**
Okano T, Ito J (Japan)

17:00-17:40 Evening Seminar

Annex 2

Chair: Murakami S (Japan)

- ES Mystery of Inner Ear Anomaly - Incomplete Partition Type II with Large Vestibular Aqueduct**
Takahashi H, Hara M, Kanda Y, Hatachi K (Japan)

Co-sponsor: J.Morita Mfg. Corp.



8:00-8:50 Morning Seminar 1

Room A

Chair: Naito Y (Japan)

MS1-1 Perimodiolar Electrodes: Designed for Optimal Placement
Treaba C (USA), Patrick J (Australia)

MS1-2 Slim Electrodes: Designed to Preserve Inner Ear Structures
Patrick J (Australia)

MS1-3 Cochlear Implant Biology and Surgery Research
Verhoeven K (Belgium)

Co-sponsor: Nihon Cochlear Co., Ltd.

9:00-9:45 Keynote Lecture 3

Room A

Chair: Takahashi S (Japan)

KL3 Application of Human iPS Cells for Inner Ear Biology and Human Disease Modeling
Okano H, Hosoya M, Fujioka M, Ogawa K (Japan)

10:00-11:08 Podium 5: Developmental Biology 1

Room A

Chairs: Dabdoub A (Canada)
Kanzaki S (Japan)

10:00 29 Wnt4 Signaling Inhibits Hair Cell Formation in the Developing Mammalian Cochlea
 Target Lecture through the Non-Canonical Wnt/Calcium/PKC Pathway
Dabdoub A (Canada)

10:20 30 β -catenin is Required for Hair-cell Differentiation in the Cochlea
 Shi F (USA), Hu L (USA, China), Edge AS (USA)

10:32 31 In Vivo Overactivation of Notch Signaling Pathway in Cochlear Prosensory Epithelium
Tateya T, Sakamoto S, Imayoshi I, Kageyama R (Japan)

10:44 32 Replacing Atoh1 with Neurog1 can Differentiate and Maintain Hair Cells
Jahan I, Pan N, Kersigo J, Fritsch B (USA)

10:56 33 CASK Function in the Inner Ear
Kita T, Honda A, Matsumoto Y, Aruga J, Kudo M, Nagao S, Ladher RK (Japan)

11:10-12:06 Podium 6: Developmental Biology 2

Room A

Chairs: Ladher R (Japan)
Suzuki M (Japan)

11:10 34 Controlling Inner Ear Fate and Shape
 Target Lecture Ladher R, Honda A, Kita T, Freeman S, Sai XR (Japan)

11:30 35 Expression of bHLH Genes in Developing Cochlear Epithelium
Sakamoto S, Tateya T, Harima Y, Imayoshi I, Ito J, Kageyama R (Japan)

11:42 36 Molecular Mechanisms for Human Anterior/Posterior Cranial Placode Cell Lineage Specification
Ealy M, Ronaghi M, Bravo D, Nasr M, Waldhaus J, Heller S (USA)

11:54 37 Septin7 Regulates the Formation of Inner Ear during Early Developmental Stage
Torii H, Yamamoto N, Yoshida A, Nakagawa T, Ito J (Japan)

Chair: Yamasoba T (Japan)

LS2-1 Electro-Acoustic Stimulation of the Cochlear: Fundamental Studies

Shepherd RK, Fallon JB, Irving S, Wise AK (Australia)

LS2-2 Electroporation

Patrick J, Housley G (Australia)

Co-sponsor: Nihon Cochlear Co., Ltd.



10:00-12:14 Podium 7: Cochlear Implant

Annex 2

Chairs: Pyykkö I (Finland)
Oh SH (Korea)

- 10:00 38 Inner Ear Imaging of Cochlear Implant Using Optimization of Cone Beam CT**
Target Lecture Pyykkö I, Zou J, Koivisto J, Lähelmä J, Aarnisalo AC (Finland)
- 10:30 39 Inner Ear Health and Cochlear Implant Function**
Pfingst BE, Colesa DJ, Watts MM, Budenz CJ, Raphael Y (USA)
- 10:42 40 Speech Perception and Speech Production after Cochlear Implantation in Prelingually Deaf Children**
Sziklai I, Pongrácz I, Sziklainé Héjja M, Kunkli F, Szilvássy J, Batta JT (Hungary)
- 10:54 41 Recent and Future Trends in the Bioelectrical Interface of Auditory Implants**
Volkenstein S, Kwiatkowska M, Gahlen F, Dazert S (Germany)
- 11:06 42 The Effect of Systemic Steroid Pump in Preservation of Residual Hearing after Cochlear Implantation**
Rah YC (Korea), Lee MY (Korea), Lee HS (Korea), Choi JJ (Korea), Park MN (Korea), Suh MW (Korea), Oh SH (Korea), Chang SO (Korea), O'Leary S (Australia), Lee JH (Korea)
- 11:18 43 Detection of Cochlin-Tomoprotein in the Fluid Leakage during Cochlear Implantation**
Shirai K, Ogawa Y, Kawano A, Ikezono T, Suzuki M (Japan)
- 11:30 44 Effects of Congruent and Incongruent Visual Cues on Speech Perception and Brain Activity in Cochlear Implant Users**
Target Lecture Song J-J, Lee H-J, Kang H, Lee DS, Chang SO, Oh SH (Korea)
- 11:50 45 Simultaneous 2nd Cochlear Implantation and Translabrynthine Removal of Vestibular Schwannoma after 1st Cochlear Implantation on the Other Side**
Doi K, Sato M, Kobayashi T, Shiraishi K, Saito K, Kohama K, Kinoshita T (Japan)
- 12:02 46 Development of a Piezoelectric Electrode with a Modiolus Penetration Needle**
Tona Y, Nakagawa T, Kawano S, Ito J (Japan)

14:15-15:00 Keynote Lecture 4

Room A

Chair: Sakaguchi H (Japan)

- KL4 The Intricate, Multifunctional Roles of Myosin Motors in Hair Cell Stereocilia**
Ebrahim S, Grati M, Kachar B (USA)

15:10-16:18 Podium 8: Molecular Structure of Inner Ear 1

Room A

Chairs: Kurima K (USA)
Doi K (Japan)

- 15:10 47 Transmembrane Channel-like 1 and 2 are Localized at Stereociliary Tips of Mammalian Inner Ear Hair Cells**
Target Lecture Kurima K, Ebrahim S, Millis BA, Pan B, Nakanishi H, Fujikawa T, Kawashima Y, Holt JR, Griffith AJ, Kachar B (USA)
- 15:30 48 Role of Rho-GTPases in Inner Ear Hair Cells**
Sakaguchi H, Ueyama T, Nakamura T, Morioka S, Ninoyu Y, Saito N, Hisa Y (Japan)
- 15:42 49 Localization-delocalization Transition of Tricellulin by Occludin in the Inner Ear**
Kitajiri S, Katsuno T, Sasaki H, Nagao K, Ito J, Furuse M, Tsukita S (Japan)
- 15:54 50 Human Basilar Membrane - An Immunohistochemistry and Electron Microscopic Study**
Liu W (Sweden), Atturo F (Sweden, Italy), Santi P (USA), Glueckert R (Austria), Pfaller K (Austria), Schrott-Fischer A (Austria), Rask-Andersen H (Sweden)
- 16:06 51 Lipid Droplets from Guinea Pig Hensen Cells are Protein Storage Organelles**
Kalinec GM, Lomberg G, Thein P, Parsa A, Park C, Urrutia R, Kalinec F (USA)

16:35-17:35 Podium 9: Molecular Structure of Inner Ear 2

Room A

Chairs: Rask-Andersen H (Sweden)
Katori Y (Japan)

- 16:35 52 The Human Endolymphatic Sac is the Endocrine Organ of the Inner Ear and Produces Multiple Potent Natriuretic Hormones**
Nue Møller M, Kirkeby S, Vikeså J, Cilius Nielsen F, Thomasen PC (Denmark)
- 16:47 53 Further Characterization of the Striated Organelle and Apical Mitochondria in Rat Inner Ear Hair Cells**
Lysakowski A, Price SD, Chidavaenzi RL (USA)
- 16:59 54 Targeting of Prestin and Slo to the Basolateral Surface: Hair Cells are Epithelial and not Neuronal**
Navaratnam D, Moeini-Naghani I, Bai J-P, Zhang Y, Santos-Sacchi J (USA)
- 17:11 55 Constitutive Gai Coupling Activity of VLGR1 and its Regulation by PDZD7**
Hu Q-X, Dong J-H, Du H-B, Zhang D-L, Ren H-Z, Ma M-L, Cai Y, Zhao T-C, Yin X-L, Yu X, Xue T, Xu Z-G, Sun J-P (China)
- 17:23 56 The Role of TRIOBP in Stereocilia Rootlets Formation**
Katsuno T (Japan), Yamahara K (Japan), Kita T (Japan), Sakamoto T (USA), Ono K (USA), Segawa K (Japan), Ito J (Japan), Kitajiri S (Japan)



17:40-18:40 Podium 10: Auditory & Vestibular Function

Room A

Chairs: Laurell G (Sweden)
Sakamoto T (Japan)

- 17:40 57 Auditory Characteristics in Mouse Model of the Sclerosteosis**
Moon IJ, Kim KR, Cho YS, Chung WH, Jin DK, Hong SH (Korea)
- 17:52 58 Cochlear Pathology Contributes to Hearing Loss in Vestibulo-cochlear Schwannomas and Allows for New Diagnostic Evaluation and Treatment**
Nue Møller M, Hansen S, Miayzaki H, Thomasen PC (Denmark)
- 18:04 59 Evaluation of Hearing Aid's Success Probability Based on Aided Auditory Steady State Response Thresholds**
Sardari S, Sameni Seyed J, Jafari Z (Iran)
- 18:16 60 Phonological Loop Activation by Maintenance of Pseudo-words with Auditory and Visual Presentation in Functional Magnetic Resonance Imaging**
Kanazawa Y, Yamazaki H, Aso T, Ishii T, Nakamura K, Fukuyama H, Ito J (Japan)
- 18:28 61 Evaluation of Cerebral Hemodynamic Responses during Phonological Working Memory Tasks Using Functional Near-infrared Spectroscopy (fNIRS)**
Yamazaki H, Hiraumi H, Kanazawa Y, Ito J (Japan)

15:10-16:18 Podium 11: Tinnitus

Annex 2

Chairs: Knipper M (Germany)
Ogawa K (Japan)

- 15:10 62 Future of Biomedical Research in Inner Ear Biology**
Target Lecture Knipper M (Germany)
- 15:30 63 Neuroimaging of Brain Regions Responsible for Tinnitus Loudness and Distress**
Ueyama T, Donishi T, Ukai S, Shinosaki K, Hotomi M, Ikeda Y, Tamagawa S, Terada M, Yamanaka N, Kaneoke Y (Japan)
- 15:42 64 The Efficacy of Tinnitus Counselling Compared to Cognitive-Behavioral Therapy on Tinnitus**
Jeong H-J, Oh S-J (Korea)
- 15:54 65 Dysfunctional Noise Cancelling of the Rostral Anterior Cingulate Cortex in Tinnitus Patients**
Song J-J (Korea), Vanneste S (USA), Jang JH (Korea), De Ridder D (New Zealand)
- 16:06 66 Toward an Objectification of Tinnitus: Machine Learning Approach of Resting-State Cortical Oscillation Pattern Can Detect the Presence of Tinnitus**
Vanneste S (USA), De Ridder D (New Zealand), Song J-J (Korea)

16:35-17:43 Podium 12: Mechanics and Model of Cochlear and Middle Ear Annex 2

Chairs: Gummer A (Germany)
Koike T (Japan)

- 16:35 67 Modeling of Human Active Cochlea Using Finite-Element Method: Simulation of DPOAEs**
Target Lecture Koike T, Mochizuki H, Sakashita T (Japan)
- 16:55 68 The Use of a Fast Method of Recording Schroeder Phase Masking Function for Measuring Nonlinear Cochlear Function**
Rahmat S (New Zealand, Malaysia), O'Beirne GA (New Zealand)
- 17:07 69 Otoacoustic Emissions and Hearing Functionality in Patients Affected by Neurodegenerative Diseases**
Sisto R, Pisani V, Moleti A, Di Girolamo S, Mazzone S, Di Mauro R, Botti T, Sanjust F, Cerini L, Tirabasso A (Italy)
- 17:19 70 Frequency Tuning and Phase-locking Estimated from Cochlear Potentials in Normal Hearing Human Volunteers**
Verschooten E, Desloovere C, Joris PX (Belgium)
- 17:31 71 Cochlear Response in a Model of Middle Ear Surgery**
Bergin MJ, Bird PA, Vlajkovic SM, Thorne PR (New Zealand)

17:45-18:41 Podium 13: Developmental Biology 3

Annex 2

Chairs: Varela-Nieto I (Spain)
Minoda R (Japan)

- 17:45 72 Programmed Cell Senescence in Inner Ear Development and Ageing**
Target Lecture Varela-Nieto I, Gibaja A, Celaya A, de Iriarte R, Magariños M, Zubeldia JM, Murillo-Cuesta S, Contreras J (Spain)



- 18:05 73 Live Cochlear Explant Imaging Reveals Reorganization Processes Underlying Robust Development of the Organ of Corti**
Amir L (Israel), Hersch M (Switzerland), Bhonker Y (Israel), Chen P (USA), Matsuzaki F (Japan), Avraham KB (Israel), Sprinzak D (Israel)
- 18:17 74 Live Imaging to Explore Dynamics of Stereocilia Formation in the Developing Mammalian Cochlea**
Bhonker Y (Israel), Amir L (Israel), Kim SM (USA), Chen P (USA), Matsuzaki F (Japan), Sprinzak D (Israel), Avraham KB (Israel)
- 18:29 75 Defining Spontaneous Morphological Activity in the Kölliker's Organ of the Developing Cochlea**
Dayaratne N, Vlajkovic SM, Lipski J, Thorne PR (New Zealand)

15:10-16:20 Podium 14: Vestibular Schwannoma -Clinical Breakthrough- Room B-1

Chairs: Miyazaki H (Denmark)
Kanemaru S (Japan)

- 15:10 76 **Inner Ear Symptoms and Quality of Life in 1000 Observed Patients with Vestibular Schwannomas**
Thomasen PC, Hansen S, Yde J, Stangerup S, Møller M, Workman C (Denmark)
- 15:25 77 **The Cutting Edge of Hearing Preservation Surgery -Using Intraoperative Monitoring of ABR and Dorsal Cochlear Nucleus Action Potential with CE-chirp Stimuli-**
Miyazaki H (Denmark)
- 15:40 78 **The Prehabilitation Concept (PREHAB) to Enhance Postoperative Recovery in Vestibular Schwannoma Patients**
Magnusson M, Tjernström F (Sweden)
- 15:55 79 **Clinical Application for Regeneration of the Skull/Temporal Bone**
Kanemaru S, Kanai R, Tsuji T, Yamamoto M, Toda H, Yamashita M, Maetani T, Nishida A (Japan)
- 16:10 Discussion
Co-sponsor: Nihon Kohden Corporation

16:35-18:31 Podium 15: Personalized Medicine and Genetics Room B-1

Chairs: Zenner HP (Germany)
Omori K (Japan)

- 16:35 80 **Personalized Medicine**
Target Lecture Zenner HP (Germany), Pfister M (Switzerland), Friese N (Germany), Zrenner E (Germany), Röcken M (Germany)
- 16:55 81 **Development of an *in vitro* Bioassay to Analyze Interface-dependent Response Profiles of Auditory Neurons on Multi-electrode Arrays**
Hahnewald S (Switzerland), Roccio M (Switzerland), Marconi E (Switzerland), Garnham C (Austria), Melchionna T (Austria), Tschertter A (Switzerland), Streit J (Switzerland), Brossard J (Switzerland), Homsy A (Switzerland), Keppner H (Switzerland), Widmer H-R (Switzerland), Senn P (Switzerland)
- 17:07 82 **Genetic Background of Single Nuclear Polymorphism Causing Hearing Loss Caused by Tobacco Smoking**
Pyykkö I, Iltanen K, Zou J, Juhola M (Finland)
- 17:19 83 **Genetic Background of Single Nuclear Polymorphism in Causing Noise Susceptibility**
Pyykkö I, Iltanen K, Zou J, Juhola M (Finland)
- 17:31 84 **Genetic Etiology of Presbycusis in Portugal**
Chora JR, Pereira L, Simões-Teixeira H, Matos TD, Fialho MG, Caria MH (Portugal)
- 17:43 85 **ILDR1 Deficiency Causes Degeneration of Cochlear Outer Hair Cells and Disrupts the Structure of the Organ of Corti: A Mouse Model for Human Deafness DFNB42**
Sang Q, Li W, Feng Y, Xu Y, Feng R, Xing Q, Zhao X, Jin L, He L, Li H, Wang L (China)
- 17:55 86 **Novel *MITF* Mutation as a Molecular Etiology of Hereditary Unilateral or Asymmetric Sensorineural Hearing Loss**
Kim SH, Kim AR, Choi HS, Kim MY, Chun EH, Oh SH, Choi BY (Korea)
- 18:07 87 **Characterization of Expression and Transcriptional Regulation in Short Isoform of the Deafness Gene *Whirlin* in Mice**
Yasuda SP, Kikkawa Y (Japan)
- 18:19 88 **The Inner Ear Pharmacokinetics Depends on Systemic Injection Dose**
Kanzaki S, Fujioka M, Inagaki Y, Oishi N, Ogawa K (Japan)



8:00-8:50 Morning Seminar 2

Room A

Chair: Shojaku H (Japan)

MS2 Neural Mechanisms of Motion Sickness and Spatial Disorientation
Takeda N (Japan)

Co-sponsor: Kowa Pharmaceutical Co. Ltd.

9:00-10:24 Podium 16: Stem Cells

Room A

Chairs: Gale JE (UK)
Taura A (Japan)

- 9:00 89 The Regenerative Therapy for Vestibular Disorders with Human Induced Pluripotent Stem (iPS) Cells**
Taura A, Nakashima N, Onishi H, Nakagawa T, Ito J (Japan)
- 9:12 90 The Paracrine Effect of Mesenchymal Stem Cells Restored Autoimmune Sensorineural Hearing Loss (hASC)**
 Zhou B (USA), Du X (USA), Di Girolamo S (Italy), Barbieri M (Italy), Yoo TJ (USA, Korea)
- 9:24 91 Auditory Brainstem Whole Mounts Promote the Differentiation of Neuronal Stem Cells of the Cochlear Nucleus in Co-culture Experiments**
Rak K, Voelker J, Voelker C, Schendzielorz P, Radeloff A, Hagen R (Germany)
- 9:36 92 Toxicity Evaluation of Hydroxyapatite Powder Prepared for Tissue Engineering Using Adipo Derived Stem Cell (ADSC)**
Pham TK, Do QM, Le MD, Pham VP (Vietnam)
- 9:48 93 In vitro Transition of Mouse Embryonic Stem-Cell Differentiation into Inner Ear Progenitors**
 Abboud N, Fontbonne A, Brézun JM, Feron F, Zine A (France)
- 10:12 94 Differentiation of Human Induced Pluripotent Stem Cells into Glutamatergic Neurons on 3D Scaffolds**
Ohnishi H, Skerleva D, Sakamoto T, Yamamoto N, Ito J, Nakagawa T (Japan)
- 10:00 95 Transplantation of Neurons Derived from Human Induced Pluripotent Stem Cells into Guinea Pig Cochleae**
Ishikawa M, Onishi H, Skerleva D, Sakamoto T, Yamamoto N, Ito J, Nakagawa T (Japan)

11:00-12:00 Special Lecture

Room A

Chair: Ito J (Japan)

SL Recent Progress in iPS Cell Research and Application
Yamanaka S (Japan)

12:15-13:15 Luncheon Seminar 3

Room A

Chair: Sato H (Japan)

LS3 Cochlear Implant Technologies – An Evolution
Kulkarni A (USA)

Additional Speaker: Gulock R

Co-sponsor: Advanced Bionics Japan

8:45-9:29 Podium 17: Ototoxicity 2

Annex 2

Chairs: Rüttiger L (Germany)
Yamashita D (Japan)

- 8:45 96 Environmental Demands and Pharmacological Activation of Soluble Guanylyl Cyclase (sGC) Interact with the Progression of Age Related and Noise Induced Hearing Loss**
Target Lecture Rüttiger L, Varakina K, Möhrle D, Bing D, Knipper M (Germany)
- 9:05 97 Noise-induced Cochlear F-actin Depolymerization is Mediated by ROCK2/p-ERM Signaling Pathway**
Sha S-H, Han Y (USA)
- 9:17 98 Characterisation of Noise-Induced Cochlear Inflammation in a Mouse Model**
Tan WJT, Telang RS, Thorne PR, Vlajkovic SM (New Zealand)

9:30-10:38 Podium 18: Regeneration

Annex 2

Chairs: Edge ASB (USA)
Okano T (Japan)

- 9:30 99 Cochlear Hair Cell Generation from Lgr5-Positive Supporting Cells**
Target Lecture Bramhall NF, Shi F, Edge ASB (USA)
- 9:50 100 DAPT Enhances Atoh1 Activity to Generate New Hair Cells in situ Following Neomycin Ototoxicity in Rat Cochleae in vitro**
Yang J-M, Luo W-W, Han Z, Chi F-L (China)
- 10:02 101 DNA Damage Signaling Regulates Age-Dependent Proliferative Capacity of the Inner Ear Supporting Cells**
Laos M, Pirvola U (Finland)
- 10:14 102 Induction of Auditory Neurons in Cochlear Endogenous Cells in the Mammalian Cochlea**
Nishimura K, Dabdoub A (Canada)
- 10:26 103 Hearing Regeneration for Severe Sudden Deafness**
Ishizaki H (Japan)



8:45-10:33 Podium 19: Vestibular

Room B-1

Chairs: Magnusson M (Sweden)
Ishikawa K (Japan)

- 8:45 104 Conservatively Managed Sporadic Vestibular Schwannoma: Audiovestibular Factors Influencing Quality of Life**
Hansen S, Yde J, Stangerup S, Nue Møller M, Workman C, Thomasen PC (Denmark)
- 8:57 105 Otolith Dysfunction Caused by Acoustic Neuroma Affects Head Stability during Gait**
Ishikawa K, Itasaka Y, Omi E, Koizumi K (Japan)
- 9:09 106 Long-term Administration of Vasopressin Can Cause Meniere's Disease in Mice**
Takumida M (Japan), Katagiri Y (Japan), Hirakawa K (Japan), Anniko M (Sweden)
- 9:21 107 Psychiatric Comorbidity in Patients with Dizziness and the Therapy of Psychotropic Drugs**
Kiyomizu K, Matsuda K, Torihara K, Yoshida K, Tono T (Japan)
- 9:33 108 Hearing Preservation on Intra Tympanic Gentamicin Treatment for Meniere's Disease**
Ishizaki H (Japan)
- 9:45 109 Balance Deficit Enhances Anxiety and Balance Training Decreases Anxiety in Vestibular Mutant Mice**
Mintz M, Shefer S, Gordon C, Avraham KB (Israel)
- 9:57 110 Utilizing the Oval Window as a Route for Gentamicin in Ablation of the Vestibular Apparatus, When Transtympanic Installments Fail in Patients with Meniere's Disease**
Magnusson M, Karlberg M, Tjernström F, Degerman E (Sweden)
- 10:09 111 Dysfunction of the Peripheral Vestibular Organs may Contribute to Vertigo in Vestibulo-cochlear Schwannomas. A Human Temporal Bone Histopathology Study**
Nue Møller M, Hansen S, Miyazaki H, Thomasen PC (Denmark)
- 10:21 112 Nondestructive Observation of the Vestibular Systems of *Slc26a4* K.O. Mice Using Optical Coherence Tomography**
Sakamoto T, Tona Y, Taura A, Nakagawa T, Ito J (Japan)

13:30-14:15 Keynote Lecture 5

Room A

Chair: Koizuka I (Japan)

KL5 **Synaptic Diversity and the Functional Roles of Cochlear Afferents**
Fuchs PA (USA)

14:20-15:40 Podium 20: Physiology

Room A

Chairs: Santos-Sacchi J (USA)
Nakagawa T (Japan)

14:20 113 **On the Area Motor Model of Prestin Activity**
Target Lecture Santos-Sacchi J, Song L (USA)

14:40 114 **Biophysical Properties of Mouse Ca^{2+} Channels Alter Before and After Onset of Hearing**
Inagaki A, Murakami S (Japan)

14:52 115 **Rab Interacting Molecule 2a (RIM2a) Regulates the Number and Function of $\text{Ca}_v1.3$ Ca^{2+} Channels at the Mouse Inner Hair Cell Afferent Synapse**
Oshima-Takago T, Jung S, Chakrabarti R, Picher MM, Wong AB, Jing Z, Michel K, Yamanbaeva G, Göttfert F, Predoehl F, Hell S, Schoch S, Strenzke N, Wichmann C, Moser T (Germany)

15:04 116 **Evaluation of Synaptic Function of Otoferlin at the Mouse Inner Hair Cell Ribbon Synapse by Postsynaptic Recording**
Takago H (Germany, Japan), Moser T (Germany)

117 **Assessment of Efferent Control of the Cochlea Using Wide Band Reflectance Measurement**
Harada T (Japan) \Rightarrow moved to Poster presentation

15:16 118 **Cochlear Adaptation Underpins Some Aspects of Temporary Threshold Shift**
Thorne PR (New Zealand), Vljakovic SM (New Zealand), Telang RS (New Zealand), Ryan AF (USA), Housley GD (Australia)

15:40- Closing Ceremony

Room A

Closing Remarks

Ito J (Japan)



14:20-15:28 Podium 21: Gene Therapy and Drug Delivery

Annex 2

Chairs: Raphael Y (USA)
Hakuba N (Japan)

14:20 119 Neurotrophin Gene Therapy Enhances the Neural Substrate of the Deaf Cochlea

Target Lecture

Takada Y (USA), Fukui H (USA), Shibata SB (USA), Budenz CJ (USA), Colesa DJ (USA), Swiderski DL (USA), Shivatzki S (Israel), Avraham KB (Israel), Pfingst BE (USA), Raphael Y (USA)

14:40 120 Protein Transduction Using Arginine-rich Cell Penetrating Peptides into the Inner Ear through the Round Window

Takeda H, Minoda R, Yamada T, Ise M, Yumoto E (Japan)

14:52 121 How Long should Patients Remain in the Supine Treatment Position after Intratympanic Dexamethasone Injection?

Park SH, Park C, Seo JY, Moon IS (Korea)

15:04 122 Round Window Membrane Vibration may Increase the Effect of Intratympanic Dexamethasone Injection

Park SH, Moon IS (Korea)

15:16 123 Virally-mediated Gene Therapy to Restore Hearing for the Most Common Types of Human Congenital Deafness Caused by Null Mutations in *Gjb6*, *Gjb2*, *Slc26a4* and *Kcnq1* Show Promising Therapeutic Effects in Mouse Models

Wang JJ, Chang Q, Li Q, Zhou BF, Wall S, Lin X (USA)

Poster Session

Schedule

Posters should be mounted, presented and removed based on the following schedules. The presenters for poster sessions are requested to be at the site of the posters at the designated time to answer questions from participants. The poster sessions will not be chaired. Posters which are not removed during the designated time will be disposed of at the discretion of the secretariat.

Mounting: November 1, 2014, 16:00-17:30 or November 2, 2014, 9:00-12:00

Presentation:

Odd Number: November 2nd, 13:15-14:15

Even Number: November 3rd, 13:15-14:15

Removal: November 4, 2014, 14:00-15:30

Room: Annex 1

Posters

- 124 Behavior of Tricellular Junctions in Temperature-sensitive Mouse Cochlear Precursor Hair Cells**
Kakuki T, Kojima T, Takano K, Miyata R, Kohno T, Kaneko Y, Himi T (Japan)
- 125 Primary Cilia in Temperature-sensitive Mouse Cochlear Precursor Hair Cells**
Takano K, Kojima T, Kakuki T, Miyata R, Ninomiya T, Konno T, Kaneko Y, Himi T (Japan)
- 126 The Expression of PTEN in the Developmental Spiral Limbus**
Dong YY (USA, Japan), Sui L (Japan), Tokuda M (Japan), Hu BH (USA)
- 127 Dominant Negative Connexin26 Mutation R75W Causing Severe Hearing Loss Influences Normal Programmed Cell Death In Postnatal Organ Of Corti**
Inoshita A, Karasawa K, Funakubo M, Miwa A, Ikeda K, Kamiya K (Japan)
- 128 Glutamatergic Signaling is Required for Maintenance of Inner Hair Cell, but Not Outer Hair Cell Innervation during Early Postnatal Development**
Barclay M, Robertson IP, Montgomery JM, Thorne PR (New Zealand)
- 129 FGFR1 Regulates the Kinocilia-specific Transport during Hair Cell Development**
Honda A, Kita T, Misaki K, Nakagawa R, Yonemura S, Ladher RK (Japan)
- 130 Expression of TRPM4 in the Mouse Cochlea and its Implicated Functions in the Depolarization of Inner Hair Cells and the Endolymph Formation**
Sakuraba M (Japan), Murata J (Japan), Teruyama R (USA), Kamiya K (Japan), Yamaguchi J (Japan), Okano H (Japan), Uchiyama Y (Japan), Ikeda K (Japan)
- 131 Distribution of TBC Proteins in the Sensory Epithelium of the Cochlea**
Juergens L, Rak K, Mlynski R (Germany)
- 132 Shaping the Mammalian Inner Ear Sensory Organs by the Vertebrate Planar Cell Polarity Pathway**
Kim SM, Padmashree R, Tower-Gilchrist C, Knapp L, Jones C, Qian D, Li SD, Chen P (USA)
- 255 The Rho GTPase Cdc42 Regulates Patterning and Polarization of Hair Cells in the Embryonic Organ of Corti**
Kirjavainen A, Laos M, Anttonen A, Pirvola U (Finland)



- 133 **Regulation of Inner Ear Hair Cell Fate: Transcription Factor Combination and Epigenetics**
Ikeda R (USA, Japan), Pak K (USA), Eduardo C (USA), Hashimoto S (Japan), Katori Y (Japan), Ryan AF (USA)
- 134 **The Role of Planar Cell Polarity Pathway in the Ectopic Regenerated Hair Cells Regulated by the Testosterone Treatment**
Ren D-D, Yang X-Y, Jin K, Ma R, Luo W-W, Yang J-M, Liu X, Chi F-L (China)
- 135 **The Effects of Surface Patterning in Cell Expansion of Auricular Chondrocytes**
Oh JH, Hwang SH, Park SH, Kim DS, Kim SW (Korea)
- 136 **Gap Junctional Coupling is Essential for Epithelial Repair in the Chicken Cochlea**
Jagger D, Nickel R, Forge A (UK)
- 137 **Inner Ear Neuron Culture in 3-D Gradient**
Edin F, Li H, Liu W, Rask-Andersen H (Sweden)
- 138 **Defining the Role of Integrins in the Repair and Regeneration of Hair Cells in the Human Vestibular System**
Hussain K, Forge A, Saeed SRS, Dawson S, Taylor R (UK)
- 139 **Effect of Netrin-1 for Spiral Ganglion Afferent Dendrite and Synapse Regeneration**
Asaka N, Shiina K, Yamahara K, Yamamoto N, Nakagawa T, Ito J, Ishikawa K (Japan)
- 140 **The Activation of Stem Cell Homing Factors Highly Induces the Cochlear Invasion of Bone Marrow Mesenchymal Stem Cells**
Fukunaga I, Karasawa K, Hatakeyama K, Minowa O, Ikeda K, Kamiya K (Japan)
- 141 **From Hair Follicles in the Human Ear Canal to Neuron-like Cells**
Li H (Sweden), Edin F (Sweden), Hayashi H (Sweden, Japan), Rask-Andersen H (Sweden)
- 142 **Differentiation of Human Embryonic Stem Cells towards Sensory Neuronal Phenotype**
Ali RQ, Cheng YF, Kostyszyn B, Ährlund-Richter L, Ulfendahl M (Sweden)
- 143 **Natural Pathway of Stem Cells within the Cochlear Nucleus of Rats**
Voelker J, Rak K, Schendzielorz P, Radeloff A, Hagen R (Germany)
- 144 **Characterization of Differentiating Neural Stem Cells from the Rat Cochlear Nucleus by Spontaneous Calcium Activity Analysis**
Voelker C, Rak K, Voelker J, Schendzielorz P, Radeloff A, Hagen R (Germany)
- 145 **Hypoxia Induces a Metabolic Shift and Enhances the Stemness of Cochlear Spiral Ganglion Stem/Progenitor Cells**
Wang CH, Chen HC, Li SL, Chen HK, Shih CP, Lin YC (Taiwan)
- 146 **H₂O₂ Treatment Accelerates Autophagy and Cellular Senescence in Auditory Cell Line**
Tsuchihashi NA, Hayashi K, Goto F, Nomura Y, Masuda T, Fujioka M, Kanzaki S, Ogawa K (Japan)
- 147 **Inhibition of Histone Deacetylases Attenuates Noise-induced Hearing Loss and Outer Hair Cell Death**
Hill K, Chen J, Sha S-H (USA)
- 148 **Expression of Insulin and cAMP Signaling Components in Hair Cells and Supporting Cells of the Human Inner Ear-implications for Diabetes and Menieres Disease**
Degerman E, Rauch U, Hultgårdh A, Lindberg S, Caye-Thomasen P, Magnusson M (Sweden)

- 149 **Arc Gene Expression Difference in the Adult Rat Brain between Unilateral and Bilateral Deafness**
Park MH, Lee HS (Korea)
- 150 **Two Novel Causative Proteins for Hereditary Hearing Loss, ATP6B1 and PENDRIN, co-expressed in the Inner Ear Lateral Wall of Common Marmoset**
Hosoya M, Fujioka M, Okano H, Ogawa K (Japan)
- 151 **Assembly and Disruption of Cochlear Gap Junction Macromolecular Complex are Regulated by Connexin 26**
Hatakeyama K, Fukunaga I, Ikeda K, Kamiya K (Japan)
- 152 **Tricellulin Difficiency Causes Deafness due to Cochlear Rapid Hair Cell Degeneration**
Kamitani T, Sakaguchi H, Miyashita T, Inamoto R, Tokumasu R, Tamura A, Mori N, Tsukita S, Hisa Y (Japan)
- 153 **Three Dimensional Organization of Inner Hair Cell Membrane Systems and Their Relationship to Synaptic Structures**
Bullen A, West T, Fleck R, Ashmore J, MacLellan-Gibson K, Moores C, Forge A (UK)
- 154 **Acoustic Overstimulation Activates 5'-AMP-activated Protein Kinase through a Temporary Decrease in ATP Level in the Cochlear Spiral Ligament Prior to Noise-induced Hearing Loss**
Yoneyama M, Yamaguchi T, Kuramoto N, Ogita K (Japan)
- 155 **The Effect of Mutation of the ATP-gated P2Y₂ Receptor on Hearing and Susceptibility to Noise**
Park M, Moon JH, Suh MW, Lee JH (Korea)
- 156 **Notch Signaling Negatively Regulates Formation and Growth of the Stereocilia Bundle**
Li Y (China, USA), Jia S (USA), Gong S (China), He DZZ (USA)
- 157 **Regulation of KCNQ1/KCNE1 Channels by Sphingomyelin Synthase 1**
Wu MK, Takemoto M, Okazaki T, Song W-J (Japan)
- 158 **The Oxidative Stress Role in the Pathophysiology of Otosclerosis**
Rudic M (Croatia), Wagner R (USA), Wilkinsson E (USA), Kiros N (Ethiopia), Ferrary E (France), Sterkers O (France), Bozorg Grayeli A (France), Zarkovic K (Croatia), Zarkovic N (Croatia)
- 159 **The $\alpha_2\delta_3$ Ca²⁺ Channel Subunit is Essential for Normal Function of Auditory Nerve Synapses and is a Novel Candidate for Auditory Processing Disorders**
Kurt S (Germany), Pirone A (Germany, USA), Zuccotti A (Germany), Rüttiger L (Germany), Pilz P (Germany), Brown DH (Germany, USA), Franz C (Germany), Schweizer M (Germany), Rust MB (Germany), Rübsamen R (Germany), Friauf E (Germany), Knipper M (Germany), Engel J (Germany)
- 160 **Effect of Prestin on Cell Volume Regulation**
Nabekura T, Matsuda K, Nakamura T, Tono T (Japan)
- 161 **Evaluation of the Mouse Vestibulo-ocular Reflex with Video-oculography**
Takimoto Y, Imai T, Uno A, Ishida Y, Kitahara T, Kamakura T, Okumura T, Horii A, Inohara H, Shimada S (Japan)
- 162 **Whole-Mount Fluorescent Imaging of the Endolymphatic Sac using Prox1-GFP Transgenic Mice**
Miyashita T (Japan), Burford JL (USA), Hong Y-K (USA), Gevorgyan H (USA), Lam L (USA), Hoshickawa H (Japan), Mori N (Japan), Peti-Peterdi J (USA)
- 163 **A Cochlear Origin for Binaural Time and Phase Delay?**
Karino S (Belgium, Japan), Joris PX (Belgium)



- 164 **Evidence of the K⁺-circulation Current that Controls the Electrochemical Properties of the Mammalian Cochlea**
Sato M, Yoshida T, Nin F, Ogata G, Doi K, Hibino H (Japan)
- 165 **The Susceptibility to Noise Injury is Increased in Streptozotocin-induced Diabetic Mice**
Fujita T, Yamashita D, Katsunuma S, Hasegawa S, Tanimoto H, Nibu K (Japan)
- 166 **Adrenergic and Cholinergic Stimulation-Mediated Changes of Transepithelial Current from Human Endolymphatic Sac Epithelium**
Kim SH, Kim BG, Kim JY, Jeong J, Choi JY (Korea)
- 167 **Developmental Increase in Hyperpolarization-activated Current Regulates Intrinsic Firing Properties in Rat Vestibular Ganglion Cells**
Yoshimoto R, Iwasaki S, Takago H, Nakajima T, Sahara Y, Kitamura K (Japan)
- 168 **Measuring Glutathione Content in the Organ of Corti Using Live Imaging**
Majumder P, Duchen MR, Gale JE (UK)
- 169 **Quantal Release at the Auditory Hair Cell Synapse in the Turtle**
Schnee ME, Ricci AJ (USA)
- 170 **Identification of Factors Underlying Spiral Ganglion Neurons' Dynamic Range**
Niwa M, Ricci AJ (USA)
- 171 **Metabolome Analysis of Inner Ear Fluid in Guinea Pigs Cochlea after Intense Noise**
Yamashita D, Fujita T, Shinomiya H, Inokuchi G, Hasegawa S, Nibu K (Japan)
- 172 **Type I IFN is Produced in Supporting Cells against Virus Infection of the Cochlear Sensory Epithelium via RIG-I Like Receptor Signaling Pathway**
Hayashi Y, Onomoto K, Narita R, Yoneyama M, Kato H, Taura A, Nakagawa T, Ito J, Kaga K, Fujita T (Japan)
- 173 **Interferon Gamma Activity Research in Case of Acute Sensorineural Hearing Loss**
Petrova NN, Perevozchikova VN, Maslova YA, Petrova EV (Russia)
- 174 **ALH-L1005 Attenuates Endotoxin Induced Inner Ear Damage**
Jang CH, Jo SY, Cho YB, Park BY, Kim MY (Korea)
- 175 **Activation of MiR-34a/SIRT1/p53 Signaling Contributes to Cochlear Hair Cell Apoptosis: Implications for Age-related Hearing Loss**
Xiong H, Pang J, Lai L (China)
- 176 **Absence of Serpinb6 Causes Sensorineural Hearing Loss with Multiple Histopathologies in the Mouse Inner Ear: Implications for Non-syndromic Hearing Loss in Humans**
Tan J, Kaiserman D, Prakash M, Bird PI (Australia)
- 177 **Vacuolar Formation in Intermediate Cells of Stria Vascularis from Rat with Vasopressin-induced Hearing Loss**
Naganuma H, Kawahara K, Sato R, Ochiai A, Tokumasu K, Okamoto M (Japan)
- 178 **Indicators Acoustic Reflex in Persons Employed in the Furniture Industry**
Petrova NN, Panshina VS (Russia)
- 179 **Accumulated Caveolins in the Models of Connexin26 Associated Deafness**
Anzai T, Kamiya K, Ikeda K (Japan)
- 180 **Disruption of Ion-trafficking System in the Cochlear Spiral Ligament Prior to Noise-induced Hearing Loss**
Yamaguchi T, Yoneyama M, Ogita K (Japan)

- 181 **Analysis of Sirt3 Gene and Mitochondrial Genome in Japanese Patients with Presbycusis**
Ohtsuka H, Shimamoto K, Fukuda K, Sato H (Japan)
- 182 **Novel *TECTA* Mutations Identified in Stable Sensorineural Hearing Loss and Their Clinical Implication**
Chang MY, Kim AR, Koo JW, Oh SH, Choi BY (Korea)
- 183 **Characterization of Pathogenic Mechanism of a Nonstop Extension Mutation of *EYAI* Detected in BOR Syndrome**
Kim AR, Choi HS, Koo JW, Jeon EH, Kim MY, Choi BY (Korea)
- 184 **Down Regulated Connexin26 at Different Postnatal Stage Displayed Different Types of Cellular Degeneration and Formation of Organ of Corti**
Chen S (China), Sun Y (China), Lin X (USA), Kong W (China)
- 185 **Targeted Exome Sequencing Identified a Novel Truncation Mutation of *EYA4* in Moderate Degree Hearing Loss**
Choi HS, Kim AR, Kim SH, Koo JW, Choi BY (Korea)
- 186 ***MARVELD2* (DFNB49) Mutations in the Hearing Impaired Central European Roma Population: Prevalence and Founder Effect**
Varga L (Slovakia), Mašindová I (Slovakia), Šoltýsová A (Slovakia), Matyás P (Hungary), Ficek A (Slovakia), Hučková M (Slovakia), Sůrová M (Slovakia), Anwar S (USA), Bene J (Hungary), Janicsek I (Hungary), Seeman P (Czech Republic), Riazuddin S (USA), Melegh B (Hungary), Klimeš I (Slovakia), Kádasi L (Slovakia), Profant M (Slovakia), Gašperíková D (Slovakia)
- 187 **Identification of Deafness Genes in Middle Eastern and Brazilian Families Using Exome Sequencing**
Martins FTA (Israel, Brazil), Brownstein Z (Israel), Abu-Rayyan A (Palestinian Authority), Birkan M (Israel), Sokolov M (Israel), Farran ND (Israel), Isakov O (Israel), Shomron N (Israel), Shalev S (Israel), Kanaan M (Palestinian Authority), Sartorato EL (Brazil), Avraham KB (Israel)
- 188 **Analysis of a Conserved *GJB2* Intronic Region in Hearing Impaired Monoallelic Portuguese Patients**
Matos TD, Simões-Teixeira H, Gonçalves AC, Chora JR, Rosa H, Monteiro L, O'Neill A, Dias ÓP, Andrea M, Caria H, Fialho G (Portugal)
- 189 **Vascular Endothelial Growth Factor Polymorphisms and Hearing Impairment in Japanese Aged Population**
Uchida Y, Sugiura S, Nakashima T, Ueda H, Otsuka R, Ando F, Shimokata H (Japan)
- 190 **Massively Parallel DNA Sequencing Successfully Identifies New Causative *TMPRSS3* Mutations in Patients with EAS**
Miyagawa M, Nishio S, Tsukada K, Moteki H, Usami S (Japan)
- 191 **Major Susceptibility Gene (s) on Chromosome 10 for Congenital Hearing Loss in NOD/Shi Mice**
Suzuki S, Obara Y, Yasuda SP, Takada T, Shiroishi T, Kikkawa Y (Japan)
- 192 **Comprehensive Genetic Testing in a Japanese Hearing-Loss Population using Targeted Genomic Enrichment and Massively Parallel Sequencing**
Moteki H (USA, Japan), Azaiez H (USA), Booth KT (USA), Shearer AE (USA), Sloan CM (USA), Kolbe DL (USA), Usami SI (Japan), Smith RJH (USA)
- 193 **Microarray Analysis of Tonotopic Gene Expression Patterns in the Mouse Cochlea**
Yoshimura H, Nishio S, Takumi Y, Iwasa Y, Usami S (Japan)
- 194 **Clinical Genetic Testing Based on Massively Parallel DNA Sequencing**
Nishio S, Miyagawa M, Naito T, Iwasa Y, Ichinose A, Usami S (Japan)



- 196 **Difference in Ototoxicity of Acetic Acid and Maturity of the Guinea Pigs**
Yamano T, Higuchi H, Ueno T, Nakagawa T, Morizono T (Japan)
- 197 **Pyrroloquinoline Quinone Protects Vestibular Hair Cells against the Aminoglycoside Ototoxicity**
Sugahara K, Hirose Y, Hashimoto M, Shimogori H, Yamashita H (Japan)
- 198 **Ototoxic Effect of Ultrastop Anti-fog Solution Applied to the Guinea Pig Middle Ear**
Nomura K, Oshima H, Yamauch D, Hidaka H, Kawase T, Katori Y (Japan)
- 199 **Cochlear Lesion of the Animal Model of Eosinophilic Otitis Media**
Kudo N, Matsubara A, Nishizawa H, Takahata J, Sasaki A (Japan)
- 200 **p53 Plays a Key Role in Cisplatin-induced Cochlear Cell Death: Toward the New Therapeutic Strategy**
Ogita H, Puel J-L, Wang J (France)
- 201 **The Effect of Sphingosine-1-phosphate Receptor Antagonists on Gentamicin-ototoxicity of the Rat Cochlea**
Nakayama M, Tabuchi K, Nishimura B, Nakamagoe M, Hara A (Japan)
- 202 **Redox Imbalance in Styrene Ototoxicity and Acoustic Trauma: in vivo Protective Effect of Q-ter**
Fetoni AR, Rolesi R, Paciello F, Eramo SML, Paludetti G, Troiani D (Italy)
- 203 **Protective Effects of Curcumin against Cisplatin Induced Hearing Loss in vitro and in vivo Studies**
Fetoni AR, Mezzogori D, Eramo SML, Paciello F, Rolesi R, Paludetti G, Troiani D (Italy)
- 204 **In vivo Overexpression of X-linked Inhibitor of Apoptosis Protein Protects against Neomycin-induced Hair Cell Loss in the Apical Turn of the Cochlea during the Ototoxic-sensitive Period**
Sun S, Zhang YP, Sun MZ, Cheng C, Waqas M, Yu HQ, He YZ, Xu B, Wang L, Yin SK, Cha RJ, Li HW (China)
- 205 **CTP Detection Test and Idiopathic Sudden Sensorineural Hearing Loss**
Shibasaki O, Ikezono T, Matsuda H, Matsumura T, Saito S, Ito A (Japan)
- 206 **Protective Effect of Resveratrol against Cisplatin-induced Ototoxicity in HEI-OC1 Auditory Cells**
Lee SH, Kim HS, An YS, Chang J, Choi J, Im GJ (Korea)
- 207 **Clinical Characteristics and Course of Recurrent Vestibulopathy Following Diuretics**
Ahn S-K, Hur DG, Ko J-S, Won S-J (Korea)
- 208 **Changes in Eye Movements Elicited by a Vestibular Prosthesis Following Ototoxic Lesioning**
Phillips C, Shepherd SJ, Nowack A, Nie K, Rubinstein JT, Ling L, Phillips JO (USA)
- 209 **Control of Vestibular Symptoms in Patients with Meniere's Disease Using Middle Ear Pressure Applications**
Futaki T, Fukaya T (Japan)
- 210 **The Endolymphatic Sac is the Primary Immunological Organ of the Human Inner Ear**
Nue Møller M, Kirkeby S, Vikeså J, Cilius Nielsen F, Cayé-Thomasen P (Denmark)
- 211 **Influence of Tinnitus on Auditory Spectral and Temporal Resolution, and Speech Perception Ability in Tinnitus Patients**
Kim DH (Korea), Kang HW (Korea), An YH (Korea), Won JH (USA), Shim HJ (Korea)

- 212 **A Population-based Cohort Study of Tinnitus in Japan**
Sugiura S, Nakashima T, Yasue M, Uchida Y, Otsuka R, Ando F, Shimokata H (Japan)
- 213 **Difference of Autoantibody between Meniere's Disease and Bilateral Sudden Hearing Loss : Is There a Different Autoimmunity Mechanism?**
Kim HJ, Roh KJ, Kim BG, Choi JY , Kim SH (Korea)
- 214 **Results on Video Head Impulse Test Differ from Those on Caloric Testing in the Patients with Meniere's Disease**
Seo T , Kobayashi T, Miyashita M, Saito K, Doi K (Japan)
- 215 **Increased Risk of Stroke and Post-stroke Mortality in Patients with Meniere's Disease: Two Nationwide Studies**
Liao C-C, Chen T-L (Taiwan)
- 216 **Triple Combined Intravenous and Intratympanic Steroid, Hyperbaric Oxygen Therapy for Severe Idiopathic Sudden Sensorineural Hearing Loss**
Kawano T, Ishitoya J, Sakuma Y, Matsuura T, Ikoma R, Takahashi M, Oridate N (Japan)
- 217 **Controlled Release of Dexamethasone Protects TNF alpha Apoptosis of Auditory Hair Cells**
Horie RT, Saito T, Jo J, Tabata Y (Japan)
- 218 **Translational Research of Meniere's Disease; Morphological Changes of the Stria Vascularis in Animal Model**
Nishimura M, Kakigi A, Takeda T, Takeda S, Okada T, Sato H (Japan)
- 219 **Otoprotective Treatments: Pharmacokinetic Properties of Adenosine Amine Congener (ADAC) in Rat Plasma and Cochlea**
Chang H, Telang RS, Sreebhavan S, Tingle M, Thorne PR, Vlajkovic SM (New Zealand)
- 220 **Liposome-Encapsulated Hemoglobin Alleviates Hearing Loss after Transient Cochlear Ischemia**
Okada M, Kawaguchi AT, Hakuba N, Hyodo J, Hato N, Gyo K (Japan)
- 221 **Screening for Protective Effect in Kampo (Herbal Medicine) Using the Zebrafish Lateral Line Hair Cell**
Hirose Y, Sugahara K, Yamashita H (Japan)
- 222 **Enhancing the Cochlear Protection of X-linked Inhibitor of Apoptosis Protein by Using Arginine-rich Cell-penetrating Peptide**
Kurioka T, Matsunobu T, Minoda R, Miwa T, Yamada T, Mizutari K, Niwa K, Tamura A, Shiotani A (Japan)
- 223 **Intra-tympanic Injection of Isosorbide for Endolymphatic Hydrops in Guinea Pig**
Kim M, Do KH, Kim KS (Korea)
- 224 **The Change of Vocal Performance according to Cochlear Implantation: for 1 year**
Oh S-J, Kong S-K, Lee I-W, Goh E-K (Korea)
- 225 **Cochlear Implantation in Patients with Eosinophilic Otitis Media**
Takahashi M, Sudo N, Isono Y, Arai Y, Sakuma N, Oridate N, Iwasaki S (Japan)
- 226 **Evaluation of Intraoperative Electrically Evoked Auditory Brainstem Response Findings in Patients with Bilateral Cochlear Implants**
Kishimoto I, Yamazaki H, Fujiwara K, Shinohara S, Kikuchi M, Suehiro A, Tona R, Harada H, Kuwata F, Moroto S, Yamamoto R, Naito Y (Japan)
- 227 **Reversed Internal Magnet of Cochlear Implant after Magnetic Resonance Imaging**
Oh S-J, Jeong H-J (Korea)



- 228 Outcome of Bilateral Cochlear Implantation for Children with SNHL**
Matsui T, Ogawa H, Yamada N, Baba Y, Suzuki Y, Nomoto M, Omori K (Japan)
- 229 The Basal Turn: Variability of an Ideal Insertion Vector for Cochlear Implantation**
Breinbauer HA (Germany, Chile), Plinkert PK (Germany), Praetorius MJ (Germany)
- 230 Real-time Impedance Measurement for Intracochlear Electrode Insertion**
Tan C-T (USA), Svirsky M (USA), Anwar A (USA), Kumar S (Australia), Caessens B (Australia), Treaba C (Australia), Carter P (Australia), Roland JT Jr (USA)
- 231 Observation of Cortical Activity During Speech Stimulation in Prelingually-Deaf Adolescent and Adult Patients with Cochlear Implantation by PET-CT**
Yoshida H, Kanda Y, Usui T, Miyamoto I, Tiba K, Takahashi H (Japan)
- 232 The Effect of the Cochlear Implantation in Teenagers with Progressive Hearing Loss**
Kitaoka K, Takahashi H, Kanda Y (Japan)
- 233 The Strategy of Cochlear Implantation in Patients with Chronic Otitis Media**
Kanai R, Kanemaru S, Nishida A, Yamamoto M, Maetani T, Yamashita M, Furuta I, Kitada Y (Japan)
- 234 Localization of Kainate Receptors in Inner and Outer Hair Cell Synapses**
Fujikawa T (Japan, USA), Petralia RS (USA), Fitzgerald TS (USA), Wang YX (USA), Millis B (USA), Kitamura K (Japan), Kachar B (USA)
- 235 A Rare Case of Meningeal Carcinomatosis with Multiple Nerve Palsy, Deaf and Facial Palsy**
Ida Y, Yasuda M, Sasaki Y, Sugo N, Edamatsu H (Japan)
- 236 Audiologic Tests Results of Patients Referred from Newborn Hearing Screening Program**
Song CI, Kang HS, Ahn JH (Korea)
- 237 Effect of Edaravone on Acute Brainstem-cerebellar Infarction with Vertigo and Sudden Hearing Loss**
Inoue Y, Yabe T, Okada K (Japan)
- 238 Analysis of the Input/Output Functions of Different Components of Otoacoustic Emissions**
Moleti A, Sisto R (Italy)
- 239 New Otolith and Semicircular Functional Test Using Eccentric Center Rotation**
Okumura T, Imai T, Takimoto Y, Inohara H (Japan)
- 240 Ex vivo MR Histology of Nerve Fibers in a Human Temporal Bone by High-resolution Diffusion Tensor Imaging Using 9.4 T MRI**
Fujioka M, Hikishima K, Oishi N, Ohta H, Kawai Y, Kawaura M, Ogawa K, Okano H (Japan)
- 241 Three-dimensional Analysis of the Eye Movement of a Patient Whose Lateral Semicircular Canal Has Morphological Malformation**
Higashi-Shingai K, Imai T, Kitahara T, Ohta Y, Morihana T, Inohara H (Japan).
- 242 Temporal Changes of c-Fos Expression in Medial Vestibular Nuclei after Unilateral Chemical Labyrinthectomy in Rats**
Lee H, Kim H (Korea)
- 243 Clearing of the Mouse Temporal Bone Using a Modified SeeDB Protocol**
Cook R, Shimizu N, Tamura A, Perachio A, Makishima T (USA)
- 244 Evaluation of the Endolymphatic Hydrops of Guinea Pigs Using Optical Coherence Tomography**
Kakigi A, Takubo Y, Egami N, Matsumoto Y, Sakamoto T, Yamashita S, Yamasoba T (Japan)

- 245 Highlighting Small Contrast Agent Variations in Cochlear DCE-MRI using VIBE Sequences**
Plumat JM (New Zealand), Rooney WB (USA), Nuttall AL (USA), Vljakovic SM (New Zealand), Thorne PR (New Zealand)
- 246 Preliminary Results of TEN(HL) Test for Patients with Sensorineural Hearing Loss in South Korea**
Lee HY, Park SK, Cho CS (Korea)
- 247 3D-imaging of Optical Coherence Tomography for Endolymphatic Hydrops Model**
Jang JH, Cho NH, Jung WG, Kim JH, Lee SH (Korea)
- 248 Morphofunctional Alterations in the GASH:Sal Induced by Audiogenic Kindling**
Hernández-Noriega S (Spain), Sánchez-Benito D (Spain), Côrtes de Oliveira JA (Brazil), Garcia Cairasco N (Brazil), López DE (Spain), Sánchez C (Spain), Castellano C (Spain)
- 249 Espin-like is a Novel Myosin III Cargo, which Participates in the Regulation of Stereocilia Length**
Ebrahim S, Grati M, Windsor A, Dong L, Millis BA, Cui R, de Sousa A, Salles FT, Raval M, Yengo CM, Kachar B (USA)
- 250 Alterations in the Efferent System in the Epileptic Hamster GASH:Sal**
Sánchez-Benito D (Spain), Hernández-Noriega S (Spain), Hyppolito MA (Brazil), Gómez-Nieto R (Spain), Castellano O (Spain), Muñoz LJ (Spain), Batista Murashima A (Brazil), Garcia-Cairasco N (Brazil), Morujo A (Spain), López DE (Spain)
- 251 Labyrinthine Fistulae Secondary to Middle Ear Cholesteatoma**
Gyo K, Miura M, Ikeda H, Takebayashi S, Ohno S, Yasumoto A, Hayashi Y (Japan)
- 252 Hearing Loss in Xeroderma Pigmentosum (XP) and Mechanism of Inner Ear Disorder**
Yamashita D, Fujita T, Shinomiya H, Inokuchi G, Hasegawa S, Nibu K (Japan)
- 253 Effects of Caffeic Acid on Cisplatin-induced Hair Cell Damage in HEI-OC1 Auditory Cells**
 Choi J, Chae SW, Lee JD, Lee BD, Park MK (Korea)
- 254 The Effect of Microgravity on mRNA Expression in the Vestibular Endorgans: Comparison of the 90- day and 15-day Space Flight**
Takumi Y (Japan), Oguchi T (Japan), Suzuki N (Japan), Nishio S (Japan), Boyle R (USA), Usami S (Japan)

Abstracts

Satellite Symposium.....	41
Special Lecture	46
Presidential Lecture.....	48
Keynote Lectures	49
Morning/Luncheon/Evening Seminars..	54

Note:

Abstracts in PDF file format are available in the following URL.

<http://www.acplan.jp/ieb-kyoto2014/abstracts.html>

SS1-1

The OTOSTEM Project: Human Stem Cell Applications For The Treatment Of Hearing Loss

Löwenheim H^{1,2} and the OTOSTEM Consortium³

¹Carl von Ossietzky University of Oldenburg, Oldenburg, Germany; ²Eberhard Karls University of Tübingen, Tübingen, Germany; ³www.otostem.org; by SCIPROM Dr. Peter Ulrich, Sàrl St-Sulpice, Switzerland

The lack of human otic cell models represents a significant roadblock that has hampered the development of drug-based and cell-based therapies for the treatment of hearing loss. In a collaborative effort OTOSTEM wishes to further develop approaches to utilize for such therapies human otic progenitors and differentiated otic cells from different human stem cell sources. OTOSTEM will devise guidance protocols for human pluripotent stem cells toward inner ear cell types that make use of principles of early germ layer formation and otic induction. Addressing this systematically, collaboratively, and with multiple sources for human otic progenitors from ESCs/iPSCs and native human otic tissues from fetal and adult stages will serve for one to enable the development of novel bioassays for drug screening efforts, as well as generating cells with decreased tumorigenicity for cell transplantation studies. Screening in human stem cell derived assays will identify new hit compounds that will be validated in tissue and organ culture models as well as in in vivo models of noise and ototoxic drug induced injury. Cell transplantation studies will be undertaken in in vivo animal models of auditory neuropathy and meningitis. The scope of OTOSTEM involves a collaborative team effort, with nine groups from Europe and the USA that combine their experience in human ESC/iPSC work, inner ear stem cell biology, high-throughput assay development, and in translating research findings into the clinic as well as into the biotechnology realm. The OTOSTEM translational goal is to bring human otic stem cell derived technology towards inner ear medical applications aiming at the restoration of hearing function.

SS1-2

Generation of Otic Progenitors from Human Pluripotent Stem Cells

Sarah L. Boddy^{1,2}, Ricardo Romero-Guevara^{1,2}, Leila Abbas^{1,2}, Sarah Jacob Eshtan^{1,2}, Darrell Barrott^{1,2}, Christian Unger^{1,2}, Laura Corns², Walter Marcotti², Peter W. Andrews^{1,2} and Marcelo N. Rivolta^{1,2}

¹Centre for Stem Cell Biology and ²Department of Biomedical Sciences, University of Sheffield, Sheffield S10 2TN, United Kingdom.

Previously, otic progenitors have been derived from human embryonic stem cells (hESCs) using FGF signalling. The reprogramming of adult somatic cells into a pluripotent state has expanded the potential application of stem cells by conceptually allowing the generation of patient-specific, induced pluripotent stem cells (hiPSCs). We sought to verify the applicability of hESC otic differentiation protocols to hiPSCs. Human iPSC lines were subjected to the same two-stage method developed with hESCs, first to generate otic progenitor cells and then to further differentiate along either a sensory hair cell or neuronal lineage. Expression of proteins associated with the early otic and later neural/hair cell phenotypes were assessed via antibody probing and immunofluorescence detection. RNA transcript levels were compared between samples by qPCR. Recording of electrophysiological properties was undertaken on differentiated cells, to explore the presence of currents typical of the lineages under study. Human iPSCs were induced to differentiate into cells expressing otic progenitor markers such as PAX8, PAX2, SOX2 and FOXG1. As for hESCs, two types of otic progenitors were obtained, otic neuroprogenitors (ONPs) and otic epithelial progenitors (OEPs). ONPs were further differentiated towards sensory neuronal fates as evidenced both by the expression of neuronal markers and the detection of sodium and potassium currents. Human iPSC-derived OEPs were used to generate cells co-expressing POU4F3 with MYO7A and ATOH1, and which presented electrophysiological profiles similar to immature hair cells. In contrast to hESCs, hiPSCs appear less dependent on exogenous FGF3 and FGF10 for induction during the first phase of differentiation, and the terminal phenotypes are less mature and more variable.

SS1-3

Human ES Cell-Derived Neurons and Innervation of Hair Cells

Edge, A.S.B.^{1,2}

¹Harvard Medical School, Boston, MA, USA, ²Massachusetts Eye and Ear Infirmary, Boston, MA. USA.

We have used an *in vitro* organ of Corti explant to study genes that enhance regeneration of the synapse between hair cells and auditory neurons and have used *in vivo* approaches to assess the functional replacement of the auditory nerve. After adding newborn mouse spiral ganglion neurons to an organ of Corti explant that lacked afferent connections, contact of neural processes with hair cells and elaboration of postsynaptic densities at sites of the ribbon synapse were increased by treatment with a blocking antibody to an axonal repulsive guidance molecule, RGMA. Human ES cells and neural progenitors made by direct reprogramming were treated with a combination of growth factor and morphogens to direct the cells to a sensory neural fate. Using a mouse model, in which type 1 spiral ganglion neurons were removed by ouabain leading to a loss of synaptic ribbons at hair cells and elevation of ABR thresholds, sensory neurons were transplanted into the cochlear modiolus. A decrease in ABR thresholds was observed in transplanted animals at 3 months, and cells positive for neural markers had sent out extensive processes into the cochlear nucleus and the cochlea. The engrafted cells were positive for postsynaptic glutamatergic markers at the hair cell synapse and for presynaptic glutamatergic markers at the endings in the cochlear nucleus. Thus, neuronal replacement and synaptogenesis can be achieved with human neurons, but the extent of reinnervation is low. Inhibition of RGMA is being tested for its enhancement of regeneration of peripheral processes *in vivo*.

SS1-4

Single Cell Analysis of Monolayer Otic Placode Generation from Human Stem Cells

Ealy M¹, Heller S¹

¹Department of Otolaryngology – Head and Neck Surgery, Stanford University, Stanford, California, USA,

In vitro stem cell guidance protocols have traditionally relied on immunostaining and bulk cell population qPCR data to assess the production of cell types of interest. To get a better understanding of the cell types generated during otic induction, we used multiplex single cell qPCR to analyze 96 marker genes in parallel within a single cell. We utilize a monolayer human stem cell guidance protocol to develop first non-neural ectoderm followed by placodal ectoderm and finally otic placode cells. Each step of the protocol is analyzed at the single cell level to determine cellular transcriptional profiles. We found that a subset of the cells adopt a transcript expression pattern signifying non-neural ectoderm. This is dependent on time in culture as well as type of cell signaling pathway manipulation. It also appears that cells develop along the non-neural ectoderm differentiation pathway at different rates. Additionally we find that a portion of non-neural ectoderm cells also express markers of other cell lineages. In an effort to enrich for the non-neural ectoderm population we are undertaking a cell surface marker screen to enrich for the target cell population. We hypothesize that enriching for the correct non-neural ectoderm cell population will increase the efficiency of downstream cell lineage production.

SS1-5

Transplantation of Human iPS cell-derived neurons cultured on 3D collagen matrix

Takayuki Nakagawa

Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

The present study examined the efficacy of the neural induction method for human induced pluripotent stem (iPS) cells to eliminate undifferentiated cells and to determine the feasibility of transplanting neurally induced human iPS cells into guinea pig cochleae for replacement of spiral ganglion neurons (SGNs). A stepwise method for differentiation of human iPS cells to neurons was used. In the primary step, the combination of a glycogen synthase kinase 3 β inhibitor and a transforming growth factor- β -receptor antagonist was used for neuroectodermal induction. In the second step, induced cells were further cultured on a collagen matrix in N2B27 medium. The characteristics of cell populations at each differentiation step were assessed by reverse transcription-polymerase chain reaction and immunocytochemistry. Human iPS cell-derived neurons cultured on a collagen matrix were transplanted into intact or damaged guinea pig cochleae, followed by histological analysis. *In vitro* analyses revealed successful induction of neural stem cells from human iPS cells with no retention of undifferentiated cells expressing OCT3/4. After the second step, approximately 70% of cultured cells expressed a neuronal marker, 90% of which were positive for vesicular glutamate transporter 1 (VGLUT1). In intact cochleae, the survival of transplant-derived neurons was achieved when inflammatory responses were appropriately controlled. Our preparation method for human iPS cell-derived neurons efficiently eliminated undifferentiated cells and contributed to the settlement of transplant-derived neurons expressing VGLUT1 in guinea pig cochleae.

SS2-1

Genetic And Biochemical Approaches Of The Mechano-Electrical Transduction (MET) Machinery

PEPERMANS Elise^{1,2,3}, MICHEL Vincent^{1,2,3}, BONNET Crystel^{2,3,4}, BAHLOUL Amel^{1,2,3}, EL-AMRAOUI Aziz^{1,2,3,4}, AVAN Paul⁵ and PETIT Christine^{1,2,3,4,6}

¹Institut Pasteur, Unité de Génétique et Physiologie de l'Audition, Paris, France, ²INSERM UMRS 1120, Paris, France

³Sorbonne Universités, UPMC Université Paris06, Paris, France, ⁴Institut de la vision, Syndrome de Usher et autres Atteintes Rétino-Cochléaires, Paris, France, ⁵Laboratoire de Biophysique Sensorielle, INSERM UMR 1107, Faculté de Médecine, Université d'Auvergne; 63000 Clermont-Ferrand, France, ⁶Collège de France, Paris, France

The study's objective: This study aims at elucidating the molecular composition of the MET machinery in order to understand how these components interplay to determine the physiological characteristics of the auditory transduction. We addressed the roles of the protocadherin-15 splice-subclasses (CD1, CD2 and CD3) and their isoforms, that only differ by their cytoplasmic regions, and of harmonin, a PDZ-domain containing protein.

Methods: We generated full knockout mice and postnatal hair cell-specific conditional knockout mice for several alternatively spliced exons that encode cytoplasmic sequences of protocadherin-15 (exons 35 to 39 were deleted individually), and of exon 5 to obtain a full protocadherin-15 knockout. Biochemical studies were based on 3D structures analysed by small-angle-X-ray-scattering (SAXS) and crystallography.

Results: Mutant mice with conditional deletion of exon 38, encoding the sequence specific to CD2, have correctly oriented hair bundles (in contrast to full CD2 knockouts). At P21, loss of CD2 results in the appearance of deafness with the loss of tip-links. Mutant mice with a conditional deletion of exon 5, additionally display anomalies of the tectorial membrane as well as of stereocilia length. During hair bundle development, mutant mice with a complete knockout of exon 37 (encoding the sequence common to CD2 and CD3), display residual MET currents. Mutant mice with a deletion of exon 36, display no hair bundle anomalies except at the very base of the cochlea. Harmonin has a dynamic scaffolding behavior.

Conclusions: At mature stage, only the CD2 protocadherin-15 isoform is required to form the tip-link whilst during hair bundle morphogenesis the two other subclasses are together compensating the loss of CD2. A more detailed definition of the particular isoforms involved in the tip-link could be obtained. We uncovered an unsuspected role of protocadherin-15 at the interface between the tectorial membrane and the stereocilia.

Acknowledgments: This work was supported by European Union Seventh Framework Programme, under grant agreement HEALTH-F2-2010-242013 (TREATRUSH), ERC-Hair bundle (ERC-2011-ADG_20110310), BNP-Paribas, Fondation Raymonde & Guy Strittmatter, FAUN Stiftung (Suchert Foundation), the French State program "Investissements d'Avenir" managed by the Agence Nationale de la Recherche [ANR-10-LABX-65] and the Foundation "Voir et Entendre".

SS2-2

Disruption of Inner Ear Tricellular Tight Junctions by a Mutation of *ILDR1* in a Family Segregating Mild Hearing Loss

Kim NKD^{1*}, Higashi T^{2*}, Lee KY³, Kim AR⁴, Kitajiri S⁵, Kim MY⁶, Chang MY⁴, Kim V⁷, Oh SH⁴, Kim D³, Furuse M², Park WY^{1,8#}, Choi BY^{6#}

¹Samsung Medical Center, Seoul, Korea; ²Kobe University Graduate School of Medicine, Kobe, Japan;

³Korea Advanced Institute of Science and Technology, Daejeon, Korea; ⁴Seoul National University Hospital, Seoul, Korea; ⁵Kyoto University Graduate School of Medicine, Kyoto, Japan; ⁶Seoul National University Bundang Hospital, Seongnam, Korea; ⁷University of Toronto St. George, Toronto, Canada; ⁸Sungkyunkwan University School of Medicine, Suwon, Korea

Compartments within the inner ear are protected by tight junctions (TJ) barriers between, epithelial cells, which are necessary for normal hearing. The immunoglobulin-like domain containing receptor 1 (*ILDR1*) gene encodes angulin-2/*ILDR1*, a recently discovered tight junction protein, which forms tricellular tight junction (tTJ) structures with tricellulin and angulin-1/lipolysis-stimulated lipoprotein receptor (LSR) at tricellular contacts (TCs) in the inner ear. Autosomal recessive mutations in *ILDR1* cause non-progressive, severe to profound nonsyndromic hearing loss (NSHL) DFNB42. Whole exome sequencing of a multiplex NSHL family with a milder auditory phenotype (ski slope type hearing loss) identified a novel variant in *ILDR1* (p.P69H) in an immunoglobulin-like (Ig-like) domain. This variant was not detected among 276 chromosomes from ethnically matched normal hearing controls. We observed that the Angulin-2/*ILDR1* p.P69H variant protein was not well-localized at TCs in angulin-1/LSR knockdown epithelial cell lines. Protein modeling showed that angulin-2/*ILDR1* contributed to tTJ by forming a homo-trimer structure through the Ig-like domain, and the p.P69H variant was predicted to disturb the homo-trimer formation. In this study, we propose a wider audiologic phenotypic spectrum of DFNB42 caused by some mutations of *ILDR1* and a possible role of angulin-2/*ILDR1* in tTJ formation in the inner ear.

SS2-3

Molecular Physiology and Pathology of Sound Encoding in the Inner Ear

Neef J¹, Picher M M¹, Reisinger E², Jung S¹, Moser T¹

¹InnerEarLab, University Medical Center Göttingen, Göttingen, Germany, ²Molecular Biology of Cochlear Neurotransmission group, InnerEarLab, University Medical Center Göttingen, Göttingen, Germany

The ribbon-type synapses between inner hair cells (IHCs) and spiral ganglion neurons encode acoustic information with remarkable precision, reliability, and dynamics, demonstrating very high rates of vesicle release and resupply. This delicate balance relies on the function of a specialized presynaptic machinery, whose disruption leads to hearing impairment. We examined the roles of the proteins CaBP2, otoferlin, and AP-2 in this process using electrophysiological recordings from IHCs and spiral ganglion neurons, immunohistochemistry, and electron microscopy.

The release of synaptic vesicles is triggered by the opening of voltage-dependent Ca²⁺ channels, which are regulated by Ca²⁺ binding proteins (CaBP). We have analyzed the role of CaBP2 in IHCs of *CaBP2*^{-/-} mice and found an increase in Ca²⁺-dependent inactivation of the Ca²⁺ current in CaBP2-deficient hair cells, which leads to a marked hearing impairment and likely also contributes to deafness in human DFNB93.

Synaptic transmission following Ca²⁺ influx in IHCs requires the multi-C₂ domain protein otoferlin, whose disruption leads to profound prelingual deafness DFNB9. The study of a mutant variant of otoferlin, which in humans leads to a temperature-sensitive deafness (O515T), in mice demonstrated a decrease in otoferlin levels, which caused a hearing impairment due to decreased resupply of vesicles to the active zone.

Otoferlin interacts with the endocytic clathrin adaptor protein AP-2. We show that disruption of the μ -subunit of AP-2 in IHCs impairs the clearance of the active zone of previously exocytosed material as well as vesicle regeneration from internalized endosome-like vacuoles leading to severe deafness.

SS2-4

Screening Strategy for the Molecular Diagnosis of Deafness: From Social Health Insurance-Based Screening to Massively Parallel DNA Sequencing

Shin-ichi Usami¹, Maiko Miyagawa¹, Takehiko Naito¹, Hideaki Moteki¹, Shin-ya Nishio¹

¹Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621 Japan

Approximately one hundred genes are estimated to cause hereditary hearing loss, but a number of these may result in similar phenotypes that entail no abnormalities other than hearing loss. The cost and time required for screening genes one-by-one is prohibitive, but it is now thought that certain mutations are recurrent. We have developed an Invader assay-based screening tool that focuses on frequently recurring mutations that are most likely to be encountered in a clinical setting and that can identify approximately 40% of deafness patients. This indicates that the deafness observed in 30-40% of patients is due to commonly occurring mutations, such as in *GJB2* or *SLC26A4*. In Japan, genetic testing for deafness using an Invader assay has been covered by social health insurance since 2012. Currently, we first apply the Invader assay to screen for 46 known mutations in 13 known deafness genes, followed by direct sequencing as necessary (Usami et al., 2012).

For the remainder of the patients with deafness of unknown etiology, we are now applying Massively Parallel DNA Sequencing (MPS) for the identification of rare causative genes. Exome sequencing using MPS is a powerful new strategy for rare Mendelian disorders such as deafness. The analysis of randomly selected Japanese deafness patients previously evaluated for common genes/mutations by Invader assay has allowed us to efficiently identify rare causative mutations and/or mutation candidates. Our data suggests that targeted exon sequencing of selected genes using MPS technology will enable the identification of rare genes, including new candidate genes, responsible for deafness in individual patients and improve molecular diagnosis in the clinical setting (Miyagawa et al., 2013).

Shinya Yamanaka

Center for iPS Cell Research and Application, Kyoto University, Sakyo-ku, Kyoto, Japan

Induced pluripotent stem cells (iPSCs) were originally generated from mouse and human fibroblasts by the retroviral transduction of four genes, *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*. The iPSCs have the ability to proliferate almost indefinitely, and to differentiate into multiple lineages. iPSCs can be made using various somatic cells, which may come from genetically characterized individuals, providing better opportunities for versatile medical applications. As a result, cell-based therapies, disease mechanisms and new drug development are being studied worldwide using iPSCs, and the iPSC technology has evolved at an accelerating pace.

We are currently trying to establish optimally safe and efficient technologies for iPSC generation, which could be made the world standard to realize medical applications in accordance with GMP. In terms of the safety of iPSC derivation, we have reported an integration-free method that does not result in any chromosomal damage using episomal vectors. In extended studies of iPSC-inducing factors, we proposed the use of L-Myc as an alternative to the oncogenic c-Myc in order to reduce the risk of tumorigenicity, while keeping the high efficiency. In order to avoid the need for conventional feeder cells or culture materials from different species, and to make them more suitable for the GMP setting, feeder cells were replaced with a recombinant laminin-based matrix and a culture medium free of animal-derived constituents (xeno-free) was developed. Regarding the quality control, some marker genes for neural differentiation-defective clones were identified, indicating that there may be a possibility of screening out the low-quality iPSCs before use, such as prior to their application for regenerative medicine. Thus, many improvements have been achieved in iPSC production in terms of both safety and efficiency.

This year, the world's first clinical trial using iPSCs was initiated to study the transplantation of iPSC-derived RPE (retinal pigment epithelium) sheets for age-related macular degeneration. In addition, iPSC studies have recently shown major progress for other conditions, such as corneal diseases, blood diseases and Parkinson's disease, suggesting that these human conditions may also be treated using iPSC-based regenerative medicine in the near future. From a broad perspective, we are proceeding with an iPSC stock project in which iPSC clones are being established from donors with a homologous HLA haplotype, which is associated with a decreased immune response, in order to provide quality-assured iPSCs for future cell transplantation.

Another application of iPSCs is to provide more effective systems for drug screening, toxicity studies and the elucidation of disease mechanisms using disease-specific iPSCs from patients with intractable diseases. In addition, using individual iPSCs may make it possible to predict the patient condition and provide a preemptive therapeutic approach to protect against the onset of the disease or personalized medicine. Moreover, it is expected that the repositioning of drug candidates which used to be categorized as false-positive or false-negative with conventional testing may be feasible.

Shinya Yamanaka

Titles: *Director/Professor, Center for iPS Cell Research and Application*
Kyoto University

Senior Investigator, Gladstone Institute of Cardiovascular Disease,
and L.K. Whittier Foundation Investigator in Stem Cell Biology
The J. David Gladstone Institutes

Educational History:

1981–1987 Kobe University, Kobe, Japan
School of Medicine (M.D. awarded in March, 1987)

1989–1993 Osaka City University Graduate School, Osaka, Japan
Division of Medicine (Ph.D. awarded in March, 1993)

Professional History:

1987–1989 *Resident*, National Osaka Hospital, Osaka, Japan

1993–1995 *Postdoctoral Fellow*, Gladstone Institute of Cardiovascular Disease
The J. David Gladstone Institutes, San Francisco, CA, USA

1995–1996 *Staff Research Investigator*, Gladstone Institute of Cardiovascular Disease
The J. David Gladstone Institutes, San Francisco, CA, USA

1996–1999 *Assistant Professor*, Osaka City University, Medical School, Osaka, Japan

1999–2003 *Associate Professor*, Nara Institute of Science and Technology, Nara, Japan

2003–2005 *Professor*, Nara Institute of Science and Technology, Nara, Japan

2004–2007 *Professor*, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

2007– *Professor of Anatomy*, University of California, San Francisco, CA, USA

2007– *Senior Investigator*, Gladstone Institute of Cardiovascular Disease
The J. David Gladstone Institutes, San Francisco, CA USA

2007–2012 *Professor*, Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan

2008–2010 *Director*, Center for iPS Cell Research and Application (CiRA), iCeMS, Kyoto University, Kyoto, Japan

2010– *Professor and Director*, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

2012– *Principal Investigator*, Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, Japan

2013– *Scientific Advisor*, Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, Japan

Honors and Awards:

2008 The Special Prize for Science and Technology, the Minister of Education, Culture, Sports, Science and Technology, Japan

2008 The Shaw Prize in Life Science and Medicine, Hong Kong

2008 Robert-Koch Preis 2008, Germany

2009 Albert Lasker Basic Medical Research Award, USA

2009 Canada Gairdner International Award, The Gairdner Foundation, Canada

2010 100th Imperial Prize and Japan Academy Prize, The Japan Academy, Japan

2010 Selected as Person of Cultural Merit (“Bunka Korosha”), Japan

2010 26th annual Kyoto Prize in Advanced Technology, Japan

2010 Balzan Prize for Stem Cells: Biology and Potential Applications, Italy

2011 King Faisal International Prize, Saudi Arabia

2011 Elected a foreign associate of the National Academy of Sciences, USA

2011 Wolf Prize in Medicine, Israel

2012 Millennium Technology Grand Prize, Finland

2012 The Nobel Prize in Physiology or Medicine 2012, Sweden

2012 Order of Cultural Merit, Japan

2013 Selected as a member of the Pontifical Academy of Sciences, Vatican

2013 Elected to the Japan Academy as a member, Japan etc.

Regeneration Medicine for the Inner Ear Disorders

Juichi Ito

Department of Otolaryngology Head and Neck Surgery
Graduate School of Medicine, Kyoto University, Kyoto, Japan

Latest findings on application of regenerative medicine for the inner ear disorders will be presented. In the early phase of inner ear damage, we try to rescue inner ear cells from cell death and promote self-repair activity using new drug delivery system (DDS) to the inner ear. Together with the experimental results, result of clinical trial of local drug application into the inner ear using cell growth factors will be presented. Induction of transdifferentiation is a next possible strategy and induction of cell proliferation is an alternative approach. As for the cell transplantation therapy to the inner ear we started using embryonic stem cells (ES cells) and autologous cell sources such as bone marrow stromal cells (BMSCs) and then induced pluripotent stem cells (iPS cells) as donor cells. Transplantation of those stem cells recovered cells (spiral ganglion neurons and hair cells) from degeneration. Cell transplantation therapy is a useful method for treatment of inner ear diseases. Then as a novel therapeutic method for sensorineural hearing loss we developed newly invented auditory device called artificial auditory epithelium (AAE) or HIBIKI device. HIBIKI device utilizes oscillation of the basilar membrane and is a totally implantable device without use of battery. The detailed HIBIKI device will be explained.

KL1

Cell death and repair in hair cell epithelia

Gale JE

UCL Ear Institute, London, UK.

Sensory hair cell death is a major cause of acquired sensorineural hearing loss. It is well established that, in mammals, cochlear hair cells are not regenerated after loss and thus hearing loss resulting from hair cell death is permanent. The loss of vestibular hair cells is also thought to underlie many forms of vestibular dysfunction including those associated with aminoglycoside ototoxicity and ageing. When hair cells are damaged or die, in either the cochlea or the vestibular system, the sensory epithelium undergoes a repair process that can be compared to a wound healing response. We know surprisingly little about the molecular mechanisms that regulate epithelial repair yet understanding these, along with the mechanisms that regulate hair cell death, is essential if we are to provide effective therapies for protecting the inner ear.

I will present an overview of the current understanding of how hair cell epithelia respond to ototoxic drugs at the cellular and molecular level, focusing on the potential roles for intracellular calcium signaling and mitochondria. I will also present recent work that reveals the importance of supporting cells in the response to damage, including new data on the regulation of phagocytic-like behavior of supporting cells by the ERK signaling pathway.

The Genomics of Deafness: Exploring Gene Function and RegulationAvraham KB

Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

The field of deafness genetics has undergone a remarkable transition in recent years. Genomic tools enable deep sequencing of all DNA and RNA molecules in a cell and are being applied to studying the auditory and vestibular systems. In the context of DNA, deep sequencing, or next-generation sequencing (NGS), is being used to discover new genes for deafness, as well as rapidly identify variants associated with this sensory disorder. In fact, approaches combining targeted gene capture of known deafness genes and deep sequencing, as well as whole exome sequencing, have doubled the number of genes associated with deafness. The speed and low cost have made this approach feasible for incorporating into clinical diagnosis protocols. In the context of RNA, deep sequencing (RNA-seq) is being used to discover and characterize noncoding RNAs, including microRNAs and lincRNAs, key regulators in multiple cellular processes. Additional layers of function and regulation are available by dissecting the full spectrum of the epigenome, including differential chromatin conformation, histone post-translational modifications and DNA methylation of the highly heterogeneous tissue of the inner ear. Together, these genomic tools facilitate functional studies towards a comprehensive understanding of auditory and vestibular function and the pathology associated with deafness.

KL3

Application of human iPS cells for inner ear biology and human disease modeling.

Hideyuki Okano¹, Makoto Hosoya^{1,2}, Masato Fujioka^{1,2}, Kaoru Ogawa²

¹Department of Physiology and ²Department of Otolaryngology, Keio University School of Medicine, 35-Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.

There is an increasing interest in the application of iPS cells technology in medical science in terms of regenerative medicine and human disease modeling. Particularly, the iPSCs technology is attracting strong attentions for their potential to broaden our understanding of the pathogenesis of psychiatric disorders (Bundo et al., Neuron, 2014; Horiuchi et al., Neurosci Res, 2013) and many neurological diseases, including those of pediatric (Higurashi et al., Mol Brain, 2013; Kuroiwa-Numasawa et al., Stem Cell Reports, 2014) and late onset (Yagi et al., Human Mol Genet, 2011; Ito et al., Annals of Neurol, 2012; Imaizumi et al., Mol Brain, 2012; Nihei et al., J. Biol. Chem, 2013; Imaizumi and Okano, J Neurochem, 2013; Yamanaka and Okano, Mol Brain, 2014) and functional and degenerative diseases of sensory organs (Yoshida et al., Mol Brain, 2014). However, in fact, there have been fewer applications of iPS cells in the disease modeling in the field of otolaryngology.

Nevertheless, I believe that application of iPS cells-based disease modeling would achieve a major breakthrough in inner ear biology and human disease modeling in otolaryngology on the following reasons. Due to the anatomical and physiological limitations, it is nearly impossible to obtain inner ear cells without destroying hearing function. Thus, generating cell types of interest through human iPS (hiPS) cells is believed as a potential approach to overcome those issues. Here we propose a novel efficient method of the otic induction from human ES/iPS cells. In the talk I will present data showing how powerful the method is by showing not only phenotypes of the cells from hiPS cells but the clinical case using patient derived- hiPS cells.

The Intricate, Multifunctional Roles Of Myosin Motors In Hair Cell Stereocilia

Seham Ebrahim, M'hamed Grati, and Bechara Kachar

¹National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD, USA

Stereocilia are actin protrusions on the surface of sensory hair cells that hold the mechano-electrical transduction (MET) molecular complex. Despite their exquisite sensitivity to mechanical vibration, orderly structure, and potential for damage by overstimulation, stereocilia are largely maintained in proper working order for a lifetime. When it does occur, however, stereocilia malformation or disruption causes many forms of inherited, acquired, or age-related hearing loss and vestibular dysfunction. Several myosins, multifunctional motor proteins that use energy derived from ATP hydrolysis to move and transport cargo along polarized actin filament tracks, have been identified as essential for hearing and vestibular function. Some of these myosins and their cargos have been shown to play vital and intricate roles in stereocilia formation, regulation, and function. The plus end directed motors, myosin IIIa, myosin IIIb, and myosin XVa localize to stereocilia tips, where they regulate the precisely graded lengths of stereocilia essential for accurate and robust mechanotransduction, via their actin-regulatory cargos. Preliminary data now suggest that the differential regulation of stereocilia length is in fact the result of more sophisticated coordinated/competing interactions between different myosin isoforms and their multiple associated cargo proteins. These myosins are also likely to transport molecular components of the mechanotransduction complex located at the stereocilia tips. Myosin VIIa was recently shown to localize to the stereocilia mechanotransduction apparatus where it forms, together with the scaffolding proteins sans and harmonin, a molecular complex that connects cadherin 23 to the actin core and exerts tension on the tip link. Myosin Ic has been implicated in the mechanism of adaptation but its localization remains controversial. The multifunctional, minus end directed motor, myosin VI concentrates at the tapered stereocilia base, where it is thought to play a role in shaping, trafficking and/or stabilizing at this region of stereocilium. A comprehensive understanding of the molecular mechanisms of stereocilia regulation and function, and changes that occur during development, aging and deafness, thus requires the continued elucidation of the distinct and integrated functions of myosins. In this presentation we will review the current state of knowledge regarding stereocilia myosins and highlight important future directions to identify ways that their regulation and compensatory potential may be exploited for repair and recovery of stereocilia from malformation or disruption.

Keywords: Myosins; hair cells; stereocilia; deafness, actin regulation

Acknowledgements: This work has been sponsored by NIDCD, IRP, NIH.

KL5

Synaptic Diversity and the Functional Roles of Cochlear Afferents

Fuchs P.A. The Center for Hearing and Balance, Otolaryngology- Head and Neck Surgery and the Center for Sensory Biology, Institute for Basic Biomedical Sciences, the Johns Hopkins University School of Medicine, Baltimore, Maryland USA

Type I and type II cochlear afferents differ markedly in number, morphology and innervation pattern. The predominant type I afferents transmit the elemental features of acoustic information to the central nervous system. Excitation of these large diameter myelinated neurons occurs at a single ribbon synapse of a single inner hair cell. This solitary transmission point depends on efficient multi-vesicular release that produces very large, rapid, suprathreshold excitatory postsynaptic potentials. In contrast, the many fewer, thinner, unmyelinated type II afferents cross the tunnel of Corti, turning basally for hundreds of microns to form contacts with dozens of outer hair cells. Although each type II afferent is postsynaptic to many outer hair cells, transmission from each occurs by the infrequent release of single vesicles, producing receptor potentials of only a few millivolts. Analysis of membrane properties and the site of spike initiation suggest that the type II afferent could be activated only if all its presynaptic outer hair cells were maximally stimulated. Thus, the details of synaptic transfer inform the functional distinctions between type I and type II afferents. High efficiency, multi-vesicular release from the inner hair cell's ribbon synapse provides the type I afferent's window onto the acoustic world. The much sparser transfer from outer hair cells to type II afferents imply that these could only respond to the loudest, sustained sounds, consistent with previous reports from limited *in vivo* recordings. However, type II afferents could be excited additionally by ATP released during acoustic stress of the cochlea.

MS1-1

Perimodiolar Electrodes: Designed for Optimal Placement

Claudiu Treaba¹, Jim Patrick²

¹Cochlear Denver Research & Technology Labs, Denver, Colorado, USA, ²Cochlear Limited, Macquarie University, New South Wales, Australia

Computer modeling and animal psychophysics studies predicted better stimulation with electrodes placed close to the cochlear modiolus. Two decades on, clinical studies show that expected performance of cochlear implant (CI) users is significantly improved with perimodiolar electrodes.

A review of the stimulation paradigms affected by a specific location of electrode contacts include, lower stimulation thresholds, smaller spread of excitation, and a pre-defined, optimal stimulation range. The precise perimodiolar location of the electrode is also a pre-requisite for advanced sound coding strategies, like “phased array”. The charge and electrical currents delivered can be manipulated and optimized only if there is sufficient insulation between the individual electrode channels.

From a different perspective, perimodiolar electrode designs can create significant challenges as well as opportunities relative to the surgical technique employed for insertion. Current technology still relies on the mechanical interaction with the cochlear inner or outer wall, which influences final electrode placement and in particular any trauma to the inner ear.

Finally, due to their location under the osseous spiral lamina, perimodiolar electrodes (in particular, slim curved electrodes) have the potential to preserve the mechanical function of the cochlea (basilar membrane).

Considering the potential clinical and surgical advantages, perimodiolar electrode designs remain an important focus of research and development work from university labs as well as design groups.

MS1-2

Slim Electrodes: Designed to Preserve Inner Ear Structures

Jim Patrick

Cochlear Limited, Macquarie University, New South Wales, Australia

Growing interest in the area of Electro-Acoustic Stimulation (EAS) with Hybrid cochlear implants (CI) and the development of advanced technologies for perimodiolar electrodes have led to a new generation of slim, thin and flexible CI electrodes.

One design approach focuses on the mechanics of insertion along the cochlear lateral wall, as well as the different insertion depths that suit clinical cases from profoundly deaf patients to patients with moderate to severe hearing loss. Cochlear has a portfolio of designs optimized for different clinical categories. These electrodes are easiest to insert via a cochleostomy, the Round Window or enlarged Round Window.

A second approach aims to place a thin pre-curved electrode in close proximity to the cochlear modiolus, for improved stimulation and location under the osseous spiral lamina. To achieve this, advanced designs are used, based on insertion stylets or sheaths, which deploy the electrode in a safe location in the scala tympani, with minimal risk of tip foldover. Such electrode designs emerged from the combination of slim electrode technologies with perimodiolar designs.

Slim electrodes have the best potential to preserve inner ear structures while requiring easy insertion.

MS1-3

Cochlear Implant Biology and Surgery Research

Kristien Verhoeven

Cochlear Technology Centre Europe, Mechelen, Belgium

After peacemakers, cochlear implants (CI) are the most successful medical devices. In order to improve CI even further, it is necessary to study the interaction of the CI with the human body and improve the surgical procedure and associated techniques and technologies. To investigate these topics, research tools (electrodes-stimulators) and animal models have been developed, in collaboration with selected research partners. These research tools are essential to investigating three major implant research topics: preservation of residual hearing (RH), biofilm and infection control, and electrode insertion guidance tools.

Electro-Acoustic or Hybrid stimulation has proven benefits for sound perception, including improved speech discrimination, and hearing in noise and tonal languages, compared to CI alone. However, its expansion is hampered by RH loss. The approach taken is a systematic investigation and study of the hypotheses formulated for the fundamental causes of RH loss.

A new technique has been developed to analyse explanted human devices for causes of infection. This technique is used to understand explanted devices better and make CI infection proof. Implantable devices also interact and integrate with the human body, and this integration can reduce surgical complications.

Finally, physical and neural responses may be used to improve clinicians' confidence in implant placement, such as advancement of the CI electrode during insertion into the cochlea. These methods offer possibilities to assess electrode location and any affect on cochlear structures, beyond regular imaging techniques. They may also be used to predict and monitor CI user performance.

MS2

Neural Mechanisms of Motion Sickness and Spatial Disorientation

Takeda N

Department of Otolaryngology, University of Tokushima School of Medicine, Japan

My presentation will concentrate on histaminergic mechanisms of motion sickness and the roles of hippocampus and amygdala in spatial disorientation. The neural mismatch hypothesis in the development of motion sickness is widely accepted. Essential to the hypothesis is the neural mismatch signal encoding spatial disorientation. Provocative motion disturbs the spatial orientation and generates neural mismatch signal, which activates the histaminergic neurons in the hypothalamus. Histaminergic descending impulse stimulates H1 receptors on the emetic center of the brainstem to induce vomiting. H1 antagonist or antihistamine inhibits symptoms of motion sickness. Thus, the hypothalamus plays an important role in the vestibulo-autonomic reflex. Although hippocampus has a spatial map, it does not generate neural mismatch signal, but counteracts the development of motion sickness. The amygdala is essential in the development of motion sickness to evaluate the emotional significance of neural mismatch signal. The vestibular cerebellum is not the region generating neural mismatch signal.

LS1

EAS- DXMb-eluting Electrodes Protect Against Electrode Insertion Trauma (EIT)-Induced Increases in Hearing Thresholds, Impedance and Loss of Cochlear Sensory Receptor Cells

Van De Water, T. R., PhD, Bas, E., PharmD, PhD, Bohorquez, J., PhD, Eshraghi, A.A., MD, He, J., MD, Perez, E. MD, Melchionna, T., PhD, Garnham, C., PhD, Jolly, C., PhD

¹ Cochlear Implant Research Program, University of Miami Ear Institute, Miami, FL, USA, ² MED-EL, Innsbruck, Austria.

Dexamethasone (DXM) infusion into the scala tympani of electrode insertion trauma (EIT) damaged guinea pig cochleae protects hearing thresholds and against loss of hair cells.

In vivo: Guinea pigs were implanted. Implants were: 1) silicone; 2) silicone+10% DXM; silicone+1% DXM; or silicone+0.1% DXM electrodes. Hearing thresholds determined by ABR, impedance was measured, hair cell counts and evaluation of neural elements determined by confocal microscopy. *In vitro*: P-3 rat organ of Corti with lateral wall tissues (OC+LW) explants were studied following EIT and compared to controls with or without DXM treatment. Time-lapse movies analyzed explant/monocyte interactions. Signaling pathways studied by qRT-PCR.

In vivo: Cochleae implanted with silicone electrodes experienced >30 dB SPL increase in ABR thresholds and significant hair cell loss at 3 months post-EIT. Threshold increase at 3 months post-EIT in 10% DXM ears was <5 dB SPL with no significant loss of hair cells. One% and 0.1% DXM electrodes hearing protection was dose dependent. Guinea pigs implanted with control electrodes showed increased impedance. Impedances of DXM electrode animals remained low. *In vitro*: EIT-OC+LW explants demonstrated increases in inflammatory cytokines, inducible enzymes, cell adhesion molecules and chemokines. The EIT-OC+LW explants enter a proliferative-fibrosis phase with increased growth factors. DXM inhibited the inflammatory and fibrosis responses initiated by EIT and increase the anti-inflammatory cytokine IL-10.

The guinea pig model of EIT-induced hearing loss demonstrated that DXM released from a drug loaded electrode protects against trauma-induced increases in hearing thresholds, loss of hair cells, damage to neural elements and increases in impedance.

LS2-1

Electro-Acoustic Stimulation of the Cochlear: Fundamental Studies

Robert K. Shepherd^{1,2}, James B. Fallon^{1,2}, Sam Irving¹, Andrew K. Wise^{1,2}

¹The Bionics Institute, Melbourne, Victoria, Australia

²Medical Bionics Department, University of Melbourne, Melbourne, Victoria, Australia

Objectives: Electro-Acoustic Stimulation (EAS) has proven benefits for sound perception, including improved speech discrimination, and hearing in noise and tonal languages, compared to users of cochlear implants (CI) alone. However, EAS is hampered by the pathophysiological changes in the cochlear associated with loss of residual hearing (RH). **Methods:** We developed a feline model of EAS that exhibits a symmetrical high frequency hearing loss with normal thresholds below ~4 kHz [1]. Animals were bilaterally implanted with a Hybrid L electrode array and received unilateral electrical stimulation for 5 months via a Nucleus cochlear implant system. Hearing thresholds were measured throughout the implantation period and cochleae were examined histologically to measure spiral ganglion neuron and hair cell survival, and tissue response. **Results:** CI resulted in a deterioration of residual hearing over the implantation period for both stimulated and unstimulated ears, although hearing loss was not observed in all animals. Electrical stimulation resulted in small but significant increases in threshold at frequencies corresponding to the site of the electrode array but not in more apical (low frequency) regions of the cochlea. Despite increased hearing loss, there was no evidence that electrical stimulation affected hair cell survival. There was significantly more tissue response in the basal turn electrically stimulated cochleae however there was no correlation between the extent of the tissue response and the loss of hearing. **Conclusions:** We have developed a robust animal model of EAS and have shown a reduction in RH that matches the clinical observation. Importantly, chronic electrical stimulation did not adversely affect RH or hair cell survival at sites distal to the electrode array. The model can be used with pharmacological agents to improve RH outcomes with CIs. [1] Irving et al, J. Neural Eng. **11** (2014) 046008.

LS2-2

Electroporation

Jim Patrick¹, Gary Housley²

¹Cochlear Limited, Macquarie University, New South Wales, Australia, ²University of New South Wales, Kensington, New South Wales, Australia

A derivative of electroporation gene delivery has been developed, which provides a direct translational application to enhance cochlear implant (CI) performance. A CI microarray is used as the means to electroporate mesenchymal cells which form a contiguous lining of the perilymphatic chambers of the guinea pig cochlea. This new mode of electroporation delivered transfection efficiencies approaching 50% using a circularized plasmid including a bicystronic expression cassette comprising Flag-tagged brain-derived neurotrophic factor (BDNF) and green fluorescence protein (GFP) reporter elements.

Cell transformation is site-specific, with GFP nuclear labeling occurring within both scala tympani and scala vestibuli mesenchymal cells in the basal turn region. Control experiments where DNA was perfused into the cochlea, but electroporation was omitted, failed to exhibit significant cell transfection. Electroporation-driven BDNF expression in the deafened cochlea drove regeneration of the peripheral neurites of the primary afferent spiral ganglion neurons, as well as increase in the somata size.

The restoration of neural structure to the deafened cochlea resulted in functional improvement in CI performance. This was established using electrically-evoked auditory brainstem response (eABR) analysis. The average current stimulus levels required to evoke eABR threshold responses in cochleae treated by BDNF electroporation gene therapy were half that required for cochleae treated with a gutted control plasmid (GFP expression only). Complementing this, the dynamic range (growth function) of the eABR was significantly increased in the BDNF gene therapy treatment group. This study paves the way for safe therapeutic delivery of naked DNA gene constructs to the implanted cochlea.

LS3

Cochlear Implant Technologies – An Evolution

Kulkarni, A¹

¹ Vice President, Research and Technology, Advanced Bionics, LLC, Valencia, CA, USA

The story of modern day cochlear implants is one of an evolution where, over time, fundamental learnings from auditory neurophysiology have been translated into strategies that electrically stimulate the cochlea in an attempt to mimic the neural code of the normal ear. During the course of this evolution seemingly fundamental limitations to delivering natural stimulation to the auditory nerve have been overcome through the use of ingenious techniques paving the way for users of modern day cochlear implants (CIs) to experience the world of sound in increasingly normal ways.

This talk will cover the evolution CI technology from Advanced Bionics (AB) over the last decade. The significance of AB's flexible implant electronic platform with independent current sources will be discussed in the context of stimulation techniques capable of rendering, high-rate, biomimetic stimulation patterns to the inner ear. The state-of-the-art in electrode technologies embodied in AB's Hifocus Mid-Scala electrode design will be covered in the context of providing the optimal electrode-nerve interface for the delivery of electrical stimulation while also preserving the delicate structures of the cochlea. The integration sound processing technologies from the hearing-aid domain will be covered in the context of improving CI performance outcomes in, every day, noisy listening conditions. The integration of wireless technologies in CI processor hardware will be covered in the context of improving ease-of use and providing CI users with unprecedented opportunities for binaural hearing. Finally, current challenges with electrical hearing will be discussed along with the solutions being considered to address them.

ES

Mystery of inner ear anomaly - incomplete partition type II with large vestibular aqueduct

Haruo Takahashi¹⁾, Minoru Hara¹⁾, Yukihiro Kanda²⁾, Kensuke Hatachi³⁾

- 1) Department of ORL-HNS, Nagasaki University Graduate School of Biomedical Sciences
- 2) Kanda ent Clinic, Nagasaki bell Hearing Center
- 3) Department of ORL-HNS, Nagasaki University Hospital

Incomplete partition type II (IP II), which is characterized by hypoplastic cochlea, often accompanies large vestibular aqueduct (LVA). Since both anomalies are often combined and have similar characteristics in the audiological aspect, we retrospectively analyzed patients with IP II and/or LVA. We have experienced 5 patients (10 ears) having only LVA, 7 patients (14 ears) having both LVA and IP II, and 5 patients (10 ears) having only IP II. Most of them had severe or profound hearing loss with apparent air-bone gap with comparatively well-preserved bone conduction thresholds in the low tones. Family history of hearing loss was found totally in 7 patients with LVA or with LVA & IP II, and genetic analysis revealed abnormality in SLC26A4 in one of the two patients with only LVA, in all the five patients with LVA and IP II, but in none of those with only IP II. A patient with LVA and IP II, who have had some benefit from a hearing aid and whose exploratory tympanotomy revealed hypoplasia of the oval window, showed no benefit from stapedotomy or vibrant sound bridge with round window application, and the round window reflex was not observed during the surgery. Those results suggest that hearing loss seen in patients with LVA and LVA with IP II may be caused by similar congenital structural abnormalities within the cochlea, which result in an impairment of intracochlear sound conduction, and that the abnormalities may be derived from genetic abnormality.

Abstracts

Podium Sessions	63
Poster Sessions	132

Note:

Abstracts in PDF file format are available in the following URL.

<http://www.acplan.jp/ieb-kyoto2014/abstracts.html>

Development of Designer Aminoglycoside Antibiotics Without Ototoxicity

Matt, T¹, Shcherbakov, D¹, Sha, S-H², Perez-Fernandez, D³, Akbergenov, R¹, Meyer, M¹, Duscha, S¹, Freihofer, P¹, Dubbaka, SR³, Xie, J², Vasella, A³, Böttger, EC¹, Schacht, J²

¹Institut für Medizinische Mikrobiologie, Universität Zürich, Switzerland; ³Kresge Hearing Research Institute, University of Michigan, Ann Arbor, USA; ⁴Laboratorium für Organische Chemie, ETH Zürich, Switzerland.

Objective: Based on our research into basic mechanisms of ototoxicity, we have shown clinically successful pharmacological mitigation of aminoglycoside-induced hearing loss. However, chemotherapy of the future will require aminoglycosides effective against multi-drug resistant bacteria without ototoxic potential. Our mechanistic concept postulates a key role for the mitochondrial ribosome (mitoribosome) in aminoglycoside ototoxicity. We have previously reported that apramycin, a structurally unique aminoglycoside in veterinary oral use, shows low activity towards eukaryotic ribosomes and also low ototoxicity.

Methods: Chemical synthesis; in-vitro translation; antibacterial activity; ototoxicity in vitro and in vivo.

Results: Based on the proof-of-concept that antibacterial activity can be dissected from aminoglycoside ototoxicity, we have developed new lead compounds that promise a safety margin (antibacterial efficacy vs. ototoxicity) more than an order of magnitude better than gentamicin.

Conclusion: We are able to develop less toxic aminoglycosides by hypothesis-driven chemical synthesis.

Support: Grants from the University of Zurich and the European Community (PAR, FP-7 HEALTH-2009-241476) to ECB and grant DC-003685 from the National Institutes of Health, NIDCD, to JS.

How the exposure to styrene affects the different sources of DPOAEs

Sisto R.¹, Moleti A.², Botti T.¹, Sanjust F.¹

¹INAIL Research, Monteporzio Catone, Rome, Italy

²University Tor Vergata, Rome, Italy

Objective

Objective of this study is the investigation of the cochlear functionality in workers exposed to styrene during their daily working activity. Otoacoustic emissions (OAEs), recordable sounds produced by active movements of cochlear OHC, are a simple, objective, quantitative and non-invasive biomarkers of healthy cochlear function.

Methods

DPOAEs have been recorded alternatively with or in absence of a contralateral suppression stimulus of 80 dB. The contralateral stimulation (CAS) permits to test the efficacy of the efferent system (MOH). High-resolution DPOAE spectra were measured using an advanced chirp technique and separated with a time-frequency technique. The DPOAEs measured in the groups of exposed workers were compared to those of a control group. A careful matching of age and sex was performed

Results

Statistically significant differences were found between the exposed and control group both in the short and long latency DP components. The expected suppression effect due to the CAS was found both in the control as in the exposed group. No dysfunction in the MOH efferent system was evidenced in workers exposed to styrene.

Conclusions

The OAEs can be an important objective tool for the understanding of the effect of ambiental ototoxic agents on the auditory function. The styrene seems to induce a specific damage at the cochlear level.

The Influence Of Sphingosine Kinase Inhibitor And Sphingosin On Cisplatin-Induced Hair Cell Loss Of The Rat Cochlea

Tani K¹, MD, Tabuchi K¹, MD, Nakayama M¹, MD, and Hara A¹, MD

¹Department of Otolaryngology, Faculty of Medicine, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8576, Japan

Sphingolipid metabolites including ceramide, sphingosine (Sph), and sphingosine-1-phosphate (S1P) play important roles in the regulation of cell survival and death. Sphingosine kinase (Sk) phosphorylates Sph to S1P. Sk is reported to be over expressed in various cancer cells, and Sk inhibitors are focused for anti-tumor therapy. However, the effects of Sk inhibitor on cochlea hair cells have not been known. In the present study, expression of Sk isotypes in the cochlea was examined. In addition, the changes of Sk activity by CDDP and the effects of an Sk inhibitor and Sph on CDDP ototoxicity were investigated using tissue culture techniques. The cochlea was dissected from Sprague-Dawley rats on postnatal days 3–5. Basal turn organ of Corti explants were exposed to 5 μ M CDDP for 48 h with or without an Sk inhibitor or Sph. Both Sk1 and Sk2 were expressed in the cochlea. An Sk inhibitor alone caused hair cell loss at a high concentration. Moreover, CDDP activated Sk, and an Sk inhibitor enhanced CDDP-induced hair cell loss. Sph with or without CDDP also caused hair cell loss. Consideration for a possibility of ototoxicity is required for usage of an Sk inhibitor.

Protective role of ecabet sodium against neomycin-induced hair cell damage in zebrafish

June Choi¹, Jiwon Chang², Gi jung Im¹, Sung Won Chae¹, Hak Hyun Jung¹

¹ Department of otolaryngology-Head & Neck Surgery, Korea University Medical Center

² Department of otolaryngology-Head & Neck Surgery, Hallym University Medical Center

OBJECTIVES: Ecabet sodium, developed as a gastric mucosal defensive agent for the treatment of peptic ulcer, has an anti-pepsin effect and induces prostaglandins in gastric mucosa. The objective of the present study was to evaluate the effects of ecabet sodium on neomycin-induced ototoxicity in transgenic zebrafish (Brn3C: EGFP).

METHODS: Five-day, post-fertilization zebrafish larvae were exposed to 125 μ M neomycin and one of the following ecabet sodium concentrations for 1 hr: 5 μ g, 10 μ g, 20 μ g, 40 μ g and 80 μ g. Hair cells within the neuromasts of the supraorbital (SO1 and SO2), otic (O1), and occipital (OC1) lateral lines were analyzed using fluorescence microscopy and confocal microscopy (n = 10). Hair cell survival was calculated as a percentage of hair cells in the control group that were not exposed to neomycin. Ultrastructural changes were evaluated using scanning electron microscopy.

RESULTS: Ecabet sodium protected against neomycin-induced hair cell loss in the neuromasts (ecabet sodium 40 μ g: 13.0 ± 0.4 cells, 125 μ M neomycin only: 7.5 ± 0.5 cells; n = 10, P < 0.05) and decreased the Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) reaction. In the ultrastructural analysis, structures of mitochondria and hair cells within the neuromasts were preserved in zebrafish exposed to 125 μ M neomycin and 40 μ g ecabet sodium.

CONCLUSION: Ecabet sodium attenuated neomycin-induced hair cell loss in zebrafish. The results of this study suggest that neomycin induces apoptosis, and that apoptotic cell death can be prevented by treatment with ecabet sodium.

Purinergic P2 Receptor Signaling Enhances Neomycin Ototoxicity

Lin SCY¹, Vlakovic SM¹, Thorne PR^{1,2}

¹Department of Physiology and Centre for Brain Research and ²Section of Audiology, The University of Auckland, Auckland, New Zealand

Extracellular purine and pyrimidine nucleotides acting via P2X and P2Y receptors regulate many aspects of normal cochlear function and its response to stress. The aim of this study was to determine the role of extracellular nucleotides in the maintenance of sensory hair cells under stress using a model of neomycin ototoxicity. **Methods:** The explants of the organ of Corti were obtained from C57BL/6 mice at postnatal day 3. The explants were pre-incubated in normal culture medium (2:1 mixture of Eagle's medium and Earle's balanced salt solution with 5% fetal bovine serum) or in culture medium enriched with P2 receptors agonists (ATP or UTP; 100 μ M) or their slowly hydrolysable analogues (ATP γ S or UTP γ S; 1 μ M) for 20 hours, followed by exposure to aminoglycoside antibiotic neomycin (1 mM) for 3 hours. The explants were then incubated (37°C, 5% CO₂) in normal or enriched culture medium for a further 20 hours, fixed with 4% paraformaldehyde and stained with Alexa 488-Phalloidin for hair cell counting. **Results:** Neomycin caused a substantial loss of the outer hair cells, mostly in the middle turn of the cochlea (52.2 ± 1.91 , $n=5$). Culture media enriched with ATP and UTP did not enhance neomycin ototoxicity, whilst the slowly hydrolysable analogues ATP γ S and UTP γ S significantly ($p < 0.05$) potentiated the hair cell loss (73.6 ± 2.01 and 83.1 ± 2.88 , respectively) in the organ of Corti exposed to neomycin. **Conclusion:** This study demonstrates that P2 receptor signaling aggravates hair cell loss after exposure to neomycin. Enzymatic hydrolysis of ATP and UTP by endogenous ectonucleotidases, however, reduces P2 receptor-mediated cytotoxicity.

Netrin-1 protects hair cells against aminoglycoside

Yamahara Kohei, Yamamoto Norio, Nakagawa Takayuki, Ito Juichi

Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, JAPAN

Background and objectives: We have demonstrated that insulin-like growth factor - 1 (IGF-1) protects cochlear hair cells of neonatal mice against aminoglycoside (Hayashi et al. Molecular and Cellular Neuroscience 2013). We identified two genes whose expression were increased by IGF-1 treatment using microarray and quantitative RT-PCR (qRT-PCR) (Hayashi et al. Neuroscience Letters 2014). One of such genes was netrin-1 (*Ntn1*). In rodents, netrin-1 is expressed in many tissues including the inner ear as well as the central nervous system. Recent work suggested tissue morphogenesis or an anti-apoptotic survival effect as functions of NTN1 other than their conventional roles, axon guidance and cell migration. The aim of this study is to test if NTN1 is an effector of IGF-1 signaling by evaluating the hair-cell protection effect of NTN1 against aminoglycoside.

Methods: We counted numbers of surviving hair cells in the basal turn of mice cochlear explant cultures when treated with neomycin only or both neomycin and NTN1. After 24 hours of treatment, we counted the numbers of surviving hair cells in the basal turn of each group. The survived hair cells are defined as those with remaining stereocilia labeled with phalloidin.

Results: NTN1 significantly attenuated the loss of hair cells in neomycin-damaged cochlear sensory epithelia

Proteomics of Noise-Induced Hearing Loss

Jeffrey Savas¹, Kwang Pak^{2,3}, Allen F Ryan^{2,3}

¹The Scripps Research Institute, ²The University of California, San Diego and ³San Diego VA Medical Center, La Jolla, CA, USA

OBJECTIVES: Noise-induced hearing loss can occur too rapidly for gene transcription to occur, suggesting that protein changes mediate initial noise effects. We performed discovery-based, quantitative cochlear proteomics after brief exposures to noise that produce temporary (TTS) or permanent (PTS) threshold shift.

METHODS: Mice were metabolically labeled over two generations with an N¹⁵ diet, to label all proteins, sacrificed, their cochleas rapidly isolated, and the protein extracted and pooled. N¹⁴ mice were exposed to brief noise (4-16 kHz; 0, 70, 100 or 105 dB SPL; 30 minutes) to minimize gene transcription. Proteins from N¹⁵ and N¹⁴ mice were mixed 1:1 so a heavy nitrogen standard provided relative quantitation. Samples were peptide digested and analyzed by multidimensional protein identification technology (MudPIT) with high resolution Orbitrap Velos mass spectrometers. Threshold shifts were measured separately.

RESULTS: 70 dB SPL produced no hearing loss; 100 dB SPL produced ~55 dB of TTS but no PTS; 105 dB SPL produced >60 dB TTS and 25 dB of PTS. Protein changes were minimal at 70 dB, but increased dramatically after TTS- or PTS-inducing exposures. Comparison of TTS to PTS revealed 142 significantly altered proteins out of 3,280. Of 91 decreased proteins, most fell into five categories: protein synthesis/transport; actin/stereociliary; damage/stress, neural/synaptic and cell division/differentiation.

CONCLUSIONS: Proteins decreased after brief noise are likely damaged or heavily used/degraded. Comparison of TTS versus PTS proteins may identify those involved in irreversible cochlear damage, while TTS changes re 0 dB reveal proteins that may contribute to temporary hearing loss.

Supported by grants from the US NIH/NIDCD (R01DC012595) and the Veterans Administration (RX000977 and BX001205).

Variation Analysis of RNA-seq Data for Identification of Candidate Genes Associated with Individual Variation in Cochlear Responses to Acoustic Trauma

Hu, BH¹, Yang, S¹, Cai, Q¹, Vethanayagam, RR¹, Dong, YY¹, Bard J², Jamison J²

¹Center for Hearing and Deafness, University at Buffalo, Buffalo, NY, USA,

²Next-Generation Sequencing and Expression Analysis Core, New York State Center of Excellence in Bioinformatics and Life Sciences, University at Buffalo, Buffalo, NY, USA

Objective: Individual variation is a feature change of acoustic trauma. To define the molecular mechanisms of the variation, efforts have been placed on defining the contributing genes. Given the abundance of cochlear genes, exploring candidate genes for further investigation is a valuable strategy for a targeted search. In the current investigation, we examined the biological variation in RNA-sequencing data to explore potential parameters for screening of candidate genes.

Methods: C57BL/6J mice and Sprague–Dawley rats were exposed to a broadband noise at 120 dB SPL for 1 hour and the cochlear sensory epithelia were collected at 1 day post-noise exposure for RNA-seq analyses. To identify the genes that are associated with the magnitude of noise damage, the expression of a panel of 84 mitochondrial genes were examined in the ears displayed different levels of hearing loss. The data were analyzed using multiple statistical and bioinformatic methods.

Results: Normal RNA-seq data exhibited diverse values of coefficient of variation (CV) of the expression levels of detectable cochlear genes. This variation was significantly increased after the noise trauma. Bioinformatics analyses revealed that the genes with increased variation were related to the molecular pathways of apoptosis, cell damage and defense. Further screening of the genes with mitochondrial function revealed three genes displaying a damage-level dependent expression change. All these genes displayed increased CV values in the RNA-seq data after the noise injury, suggesting that the genes with the increased expression variation are likely to contribute to the individual variation in cochlear responses to acoustic trauma.

Conclusion: Variation analysis of RNA-seq data is a valuable strategy for identification of candidate genes for further investigation of the molecular basis of individual variation in acoustic trauma.

Characterization of Transcriptomes of Cochlear Inner and Outer Hair Cells

Liu HZ, Pecka Jason L, Zhang Q, Soukup GA, Beisel KW, He DZZ

Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178, USA

Inner hair cells (IHCs) and outer hair cells (OHCs) are the two types of sensory receptor cells that are critical for hearing in the mammalian cochlea. IHCs and OHCs have different morphology and function. The genetic mechanisms that define their morphological and functional specializations are largely unknown. The transcriptome reflects the genes that are being actively expressed in a cell and holds the key to understanding the molecular mechanisms of the biological properties of the cell. Using DNA microarray, we examined the transcriptome of 2,000 individually collected IHCs and OHCs. We show that 16,647 and 17,711 transcripts are expressed in IHCs and OHCs, respectively. Of those genes, approximately 73% are known genes, 22% are uncharacterized sequences, and 5.0% are non-coding RNAs in both populations. 16,117 transcripts are expressed in both populations. Uniquely and differentially expressed genes account for less than 15% of all genes in either cell type. The top ten differentially expressed genes include *Slc17a8*, *Dnajc5b*, *Slc1a3*, *Atp2a3*, *Osbpl6*, *Slc7a14*, *Bcl2*, *Bin1*, *Prkd1*, *Map4k4* in IHCs, and *Slc26a5*, *C1ql1*, *Strc*, *Dnm3*, *Plbd1*, *Lbh*, *Olfm1*, *Plce1*, *Tectb*, *Ankrd22* in OHCs. We analyzed commonly and differentially expressed genes with focus on genes that are related with hair cell specializations in the apical, basolateral and synaptic membranes. Eighty-three percent of the known deafness-related genes are expressed in hair cells. We also analyzed genes involved in cell cycle regulation. Our dataset holds an extraordinary trove of information about the molecular mechanisms underlying hair cell morphology, function, pathology, and cell-cycle control.

An Inducible Gene Expression System Using a Modified Hair Cell Promoter for Myosin 7a

Asai Y, Géléoc GSG, Holt JR

Department of Otolaryngology, F. M. Kirby Neurobiology Center, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA

Cell type-selective, tightly-regulated, reversible gene expression systems are valuable for interpretation of gene/cell function in basic research and for development of gene therapy. We designed a system that allows precise spatiotemporal control of gene expression in hair cells. In the *lac* regulatory system, gene expression is regulated by binding of LacI protein to *lac* operator sequences in promoter regions that flank the transcriptional start site. The regulation is reversible, as addition or removal of the inducer, IPTG, turns on or off gene expression at any point during the animal's life. We engineered this system for use with a modified Myosin7a (*Myo7a**) promoter, whose activity in the inner ear is restricted to hair cells. We tested *lac* operators placed at various positions within the *Myo7a** promoter for regulatability of luciferase gene expression in HeLa cells. After the initial screen, we prepared adenoviral vectors with the optimized *Myo7a** promoter and the coding sequence for GFP and transfected inner ear tissue that expressed LacI. GFP expression was restricted to hair cells and was tightly regulated by IPTG induction. Next, we generated a transgenic mouse that expressed TREK1 K⁺ channels regulated by the *Myo7a** promoter. Induced expression of TREK1 clamped the hair cell resting potential near E_{K+}, which effectively silenced hair cell electrical activity. This novel mouse model will be used to investigate the role of hair cell activity during inner ear development. Conclusions: 1) The *Myo7a** promoter was regulatable using *lac* operators and IPTG induction. 2) Hair cell expression was confirmed *in vitro* via adenoviral transfection. 3) The *Myo7a** promoter drives TREK1 expression in hair cells of transgenic mice and successfully silenced the cells. We suspect our inducible hair cell-specific promoter and the TREK1 mouse developed for this study will be valuable genetic tools to regulate gene expression and hair cell function *in vivo*.

Establishment of a Novel Mouse Model for Hearing Impairment by Selective Ablation of Outer Hair Cells in the Inner Ear

Kikkawa Y¹, Miyasaka Y¹, Wada K^{1,2}, Tokano H³, Kitamura K³, Matsuoka K¹

¹Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, ²Tokyo University of Agriculture, Abashiri, Hokkaido, Japan, ³Tokyo Medical and Dental University, Tokyo, Japan.

Mechanosensory outer hair cells (OHC) play an essential role in the amplification of sound-induced vibrations in the cochlea because of their ability to contract or elongate following changes in the intracellular potential. To study the critical roles of OHC, we generated a novel mouse model, OHC-TRECK, for hearing impairment by introducing the human diphtheria toxin (DT) receptor gene under the control of the OHC-specific promoter of the mouse prestin gene. DT administration to OHC-TRECK mice resulted in severe hearing impairment with a decrease in the amplitude of distortion product otoacoustic emissions and elevated auditory brainstem responses. Next, we assessed the phenotype for inner ear hair cells by immunohistochemistry using markers for hair cells, and observed that DT-administered OHC-TRECK mice exhibited specific depletion of OHC without any effects on inner hair cells and vestibular hair cells. Moreover, we performed differential expression analysis between DT-administered wild-type and OHC-TRECK mice using microarrays. In this analysis, we found that the expression of the *Oncomodulin* (*Ocm*) gene, which codes the EF-hand Ca^{2+} -binding protein, was significantly decreased in DT-administered OHC-TRECK mice. Interestingly, OCM expression was specifically detected in OHC of adult wild-type mice. These results suggest that OCM performs an important function associated with Ca^{2+} -binding ability to facilitate mechanotransduction in OHC.

TrkB mediated protection against circadian sensitivity to noise trauma

Inna Meltser¹, Christopher R. Cederroth¹, Vasiliki Basinou¹, Sergey Savelyev², Gabriella Schmitz Lundkvist^{1,2} and Barbara Canlon¹

¹ Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, 17177, Sweden;

² Department of Neuroscience, Karolinska Institutet, Stockholm 17177, Sweden

Noise-induced hearing loss is a permanent impairment affecting 10-15% of the population and there is no cure for this debilitating disorder. New innovative strategies are therefore needed. The identification of a robust circadian expression of *Period 2* mRNA transcripts in the mouse cochlea prompted us to evaluate if there is a functional response to this audio clock. Different recovery patterns were found in response to noise trauma delivered during day or night. Complete recovery was found 2 weeks after day trauma, whereas a permanent loss persisted after night trauma. Recovery from day noise trauma coincided with a greater induction of brain-derived neurotrophic factor (BDNF) mRNA transcripts in the cochlea compared to night trauma. *In vivo* administration of the selective TrkB receptor agonist 7,8-dihydroxyflavone (DHF) in the night, but not in the day, lead to a complete auditory recovery after noise and maintenance of inner hair cell synaptic integrity. *In vitro* application of DHF phase shifted and boosted the amplitude of cochlear PER2::LUC rhythms, which effects were blocked by the specific TrkB receptor antagonist, ANA12. We report for the first time that noise trauma during the night contributes to more severe consequences compared to noise exposure given during the day and that this daily variance in noise sensitivity is adjusted by a self-sustained circadian cochlear clock gating the protective functions of TrkB on synaptic integrity. These findings highlight the coupling of circadian rhythmicity and TrkB receptor for the successful prevention and treatment of noise-induced hearing loss. Moreover, drug treatments for hearing disorders will be refined when the time point of application is taken into consideration.

Mitophagy Can Be Observed In The Inner Ear But Is Not An Universal Response To Mitochondrial Damage

Daniel Bodmer, Cristian Setz

Otolaryngology, Inner Ear Research Laboratory, University Hospital Basel, Switzerland

Objectives: Mitophagy is a selective mechanism within cells in which malfunctioning mitochondria are degraded. The involvement of mitochondria in neurodegenerative diseases has become general acceptance. The central role of mitochondria in the cell makes them suspects in many disorders, including their involvement in hearing loss. In this study, we hypothesized that mitophagy can be observed in the cochlea.

Methods: we first analyzed whether PINK1/parkin mRNA are expressed in the cochlea of 5-day-old C57BL/6 mice by RT-PCR. We focused thereafter on whether mitophagy can be observed within the organ of Corti (OC) of 5-day-old Wistar rats and the House Ear Institute-Organ of Corti 1 (HEI-OC1) cell line *in vitro*. For this purposes, we analyzed the 24 h-effect of a well described mitophagy-inducing agent, the mitochondrial uncoupler carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and compared it with the ototoxic aminoglycoside agent Gentamicin and untreated control organs/cells. We used different system to detect mitophagy: COXIV protein level, Atg12 and LC3 protein levels, oxygen consumption rate and finally mitochondrial morphology.

Results: PINK1 and parkin mRNA could be detected in all cochlear compartments. Mitophagy could be observed in both the OC and the HEI-OC1 cell line after CCCP treatment but not after Gentamicin treatment.

Conclusion: Mitophagy can be observed in the inner ear, however it is not an universal response after cellular and mitochondrial damage to the inner ear.

Physiological significance of p62-mediated autophagy in auditory cells

Ken Hayashi^{1,2}, Fumiyuki Goto², Nana Tsuchihashi, Yasuyuki Nomura³, Takeshi Masuda³, Masato Fujioka², Sho Kanzaki², Kaoru Ogawa²

1. Dept. of Otorhinolaryngology, Kamio Memorial Hospital, Tokyo, Japan, 2. Dept. of Otorhinolaryngology, Keio University, Tokyo, Japan, 3. Dept. of Otorhinolaryngology, Nihon University, Tokyo, Japan

Objectives: The objectives of this study were to elucidate how Keap1, Nrf2 and p62 provide molecular foundation for a possible crosstalk between autophagy and necrosis pathways in auditory cells.

Methods: We used auditory cell line (HEI-OC1) in this study. The viability of HEI-OC1 was determined by cell viability assays. The samples after treatment of HEI-OC1 were analyzed by a flow cytometer. Immunofluorescent confocal laser microscopy was used. Western blot was performed. Morphological observation was performed by Transmission electron microscope (TEM). Transient GFP-LC3 and p62 siRNA were transfected into HEI-OC1 with electroporation.

Results: HEI-OC1 treated with H₂O₂ exhibited dose- and time-dependent cell death. After treatment of H₂O₂, not apoptotic cell but necrotic cell was detected by a flow cytometer. H₂O₂ treatment resulted in time-dependent accumulation of LC3-II and the expression of GFP-LC3 in HEI-OC1 cells. Autophagosomes were confirmed under TEM in H₂O₂ treated-HEI-OC1. Immunostaining analysis demonstrated that the majority proportion of Nrf2 translocated into the nucleus in p62 KD HEI-OC1 cells, while the majority proportion of Nrf2 disappeared from nucleus after treatment of H₂O₂ in p62 KD HEI-OC1. The suppression of autophagy by p62 knockdown accelerated cell death in H₂O₂-treated HEI-OC1.

Conclusion: Our results demonstrated that the intracellular clearance is decreased under oxidative stress due to the impairment of crosstalk of p62, Nrf2 and Keap1. This leads to the progression of auditory cell death. Taken together, a crosstalk between Keap1/Nrf2 pathway and the p62-mediated autophagy play an important role in cell survival of auditory cells under oxidative stress.

The Role of Purinergic Signalling in Cochlear Response to Stress and Injury

Vlajkovic SM¹, Housley GD², Thorne PR¹

¹Department of Physiology and Centre for Brain Research, The University of Auckland, Auckland, New Zealand, ²Translational Neuroscience Facility, University of New South Wales, Sydney, Australia

In the past two decades, we and others have demonstrated a major role of extracellular purines in cochlear physiology, and purinergic signaling pathways are now regarded as significant regulatory elements in cochlear homeostasis and sensory transduction. Our current research incorporates a series of projects that directly investigate the protective role of extracellular purines (adenosine and ATP) on the development of noise-induced cochlear injury and other forms of oxidative stress in the inner ear. Understanding how purines are involved in cochlear response to loud sound has revealed novel mechanisms of adaptation to noise and identified novel therapeutic targets for the treatment of hearing loss. Our recent work demonstrates that ATP-mediated mechanisms underlying temporary hearing loss from noise exposure actually represent cochlear adaptation to the noisy environment, rather than the injury mechanism. If the pathway through ATP receptors is removed by genetic manipulation, this adaptive mechanism does not occur and the ear becomes vulnerable to longer term noise exposure and the effects of age, eventually resulting in permanent hearing loss. Here, we also present an overview of studies looking at the role of adenosine receptors in cochlear response to noise, ototoxic drugs and aging. We have provided evidence that adenosine receptor agonists mitigate oxidative stress, inflammation and apoptosis in the cochlea, and thus protect it from injury. Together, these studies present novel concepts in understanding how our ears respond to stress and injury, and lay an evidence-based foundation for the development of clinically relevant compounds for therapeutic management of sensorineural hearing loss.

Assessing Ototoxicity of Terbinafine Eardrops in Guinea Pigs Using Caloric, OVEMP and CVEMP Tests

Yang TH¹, Chung FL¹, Young YH¹

¹Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan

Objectives: Commercially available terbinafine solution (10 mg/ml) is effective against athlete's foot and has the potential of treating fungal infections of the ear (otomycosis). Its toxicity on the inner ear was not clear and must be verified before clinical use. For this purpose, we developed an inner ear test battery including ABR, caloric, ocular VEMP (oVEMP) and cervical VEMP (cVEMP) tests on the guinea pigs. This test battery is able to monitor global inner ear function and also serves as a tool for the evaluation of ototoxicity of any new medicine, for example, the topical terbinafine eardrops.

Methods: Guinea pigs were treated with either 100 µl saline, terbinafine (10 to 100 mg/ml) or gentamicin (50 mg/ml) daily over the round window membrane for one week by intratympanic administration. Each animal underwent ABR, caloric, oVEMP and cVEMP tests 2 weeks later. The same tests were repeated another 2 weeks later. The inner ear endorgans of the animals were then processed for morphological study.

Results: Terbinafine (10-25 mg/ml) and saline-treated ears showed normal results in ABR, caloric, oVEMP and cVEMP tests. Their inner ear endorgans demonstrated normal morphology. On the contrary, gentamicin-treated ears showed significantly higher rate of abnormal test results and damaged inner ear endorgans. Guinea pigs treated with terbinafine 50-100 mg/ml had solvent-induced dermatitis in the ear and were excluded from this study.

Conclusion: The four-combined inner ear test battery discloses convincingly the cochleo- and vestibulo-toxicity of medical agents. In this study, it verifies that the topical terbinafine eardrops are non-ototoxic for the guinea pigs at appropriated doses which are effective against otomycosis.

Protective Role of Edaravone against Cisplatin Induced Apoptosis in HEI-OC1 cell

Jiwon Chang¹, Su-Kyoung Park¹, Hyung-Jong Kim¹, Gi Jung, Im², Sehee Lee², Hyung Jin Jung², June Choi², Sung Won Chae², Hak Hyun Jung²

¹Department of Otorhinolaryngology-Head and Neck Surgery, Hallym University College of Medicine, Seoul, Republic of Korea; ²Department of Otorhinolaryngology Head and Neck Center, Korea University College of Medicine, Seoul, Republic of Korea

Objectives: Edaravone is a neuroprotective agent with a potent free radical scavenging and antioxidant actions. Edaravone is known to reduce the amount of ROS by inhibiting lipoxxygenase metabolism, preventing lipid peroxidation, and enhancing prostacyclin production. In the present study we investigated the influence of edaravone on cisplatin ototoxicity in auditory cells.

Materials and Methods: Cell viability was determined by MTT cell proliferation assay, while oxidative stress and apoptosis were assessed by ROS measurement, flow cytometry, Hoechst 33258 staining, caspase-3 activity assay and western blot for PARP. Intracellular calcium concentration changes were detected with calcium imaging.

Results: Pretreatment with 100µM of edaravone prior to application of 15 µM of cisplatin significantly increased cell viability after 48 h of incubation in HEI-OC1 cells (from 51.9% to 64.6% viability) and also, significantly attenuated the cisplatin-induced increase in reactive oxygen species (ROS)(from 2.3 fold to 1.9 fold). Edaravone also inhibited the expression of caspase-3 and poly-ADP-ribose polymerase related to cisplatin-induced apoptosis because a major mechanism of cisplatin-induced toxicity involves ROS production.

Conclusion: We propose that edaravone protects against cisplatin- induced ototoxicity by preventing apoptosis, and limiting ROS production in HEI-OC1 cells.

Hyaluronan up-regulation is linked to renal dysfunction and hearing loss induced by silver nanoparticles

Hao Feng¹, Ilmari Pyykkö¹, Jing Zou^{1,2}

¹Hearing and Balance Research Unit, Field of Oto-laryngology, School of Medicine, University of Tampere, Tampere, Finland

²Department of Otolaryngology-Head and Neck Surgery, Center for Otolaryngology-Head & Neck Surgery of Chinese PLA, Changhai Hospital, Second Military Medical University, Shanghai, China

Increased application of silver nanoparticles (AgNPs) has raised concerns on their potential adverse effects on human health. However, the precise toxicological mechanisms are unknown in detail. The current study hypothesized that AgNPs induced glycosaminoglycan accumulation in the basement membrane that associated with the up-regulation of its component hyaluronic acid, known as a hydrophilic molecule of binding and retaining water, and caused toxicities in the kidney and cochlea. Rats administered AgNPs through either intravenous or intratympanic injection were observed at different time points after exposure. The concentrations of creatinine and urea in the serum were elevated remarkably, and proteins leaked into the urine were increased. A significant hearing loss over a broad range of frequencies was indicated. AgNP exposure induced glycosaminoglycan accumulation and hyaluronic acid up-regulation in the basement membrane. Abundant apoptotic cell death was demonstrated in the AgNP-exposed organs. Our results suggested that glycosaminoglycan accumulation associated with the up-regulation of hyaluronic acid was involved in the toxicities of kidney and cochlea caused by AgNPs.

Keywords silver nanoparticles, basement membrane, hyaluronic acid, glycosaminoglycan, kidney, cochlea

Epithelial Healing in the Organ of Corti after a Deafening Insult

Anttonen T, Belevich I, Kirjavainen A, Laos M, Brakebusch C, Jokitalo E, Pirvola E
University of Helsinki, Helsinki, Finland

We have studied the acute pathophysiological changes occurring in the ototoxically traumatized murine organ of Corti *in vivo* with serial block-face scanning electron microscopy (SBEM). This knowledge is essential for the development of repair and regenerative therapies for sensorineural hearing loss. SBEM allows automated imaging of thousands of serial EM sections, from which objects of interest can be modeled in 3D.

We show that Deiters' cells are tissue-resident phagocytes that digest apoptotic outer hair cell debris and thereby maintain tissue homeostasis. Phagocytosis is performed by phalangeal processes of DCs that swell during acute trauma. Clearance of an individual degenerated OHC and actin remodeling based surface closure i.e. scar formation are mainly performed by different supporting cells, because of the complex cytoarchitecture of this epithelium.

While limiting scar formation ability of supporting cells might rescue OHCs from the decapitation activity of supporting cells, absence of epithelial healing predisposes the whole organ to additional damage. Cdc42 inactivated supporting cells fail to form phalangeal scars upon outer hair cell loss and leave open wounds on the epithelial surface that permit the leakage of endolymph into the epithelium. This causes massive damage to the residual nerve terminals of innervating neurons and shifts the death mode of outer hair cells from apoptosis to necrosis. Our results highlight the importance of the intact epithelial barrier during wound healing. Future repair and regenerative interventions should therefore maintain this barrier.

Accelerated noise-induced hearing loss and audiogenic seizure in mice lacking thrombospondins

Felix V. Wangsawihardja¹, Srividya Sundaresan¹, Diana Mendus¹, Rose Leu¹, Nicolas Grillet¹, Farhad Ghoddoussi², Avril Genene Holt², Mirna Mustapha¹

¹ Department of Otolaryngology – Head and Neck Surgery, Stanford University, Stanford, CA, USA

² Department of Anatomy and Cell Biology, Wayne State University, Detroit, MI, USA

Background. Recent studies revealed important roles for astrocyte-secreted cell adhesion molecules such as thrombospondins (TSPs) in promoting brain synapse formation and in repairing synaptic connection after stroke. We have recently shown these genes to be important in cochlear synapse formation and in normal hearing. Here we further elucidate the role of TSPs in the maintenance of cochlear synapses and hearing after noise injury. We investigated 1) whether TSPs play a protective role in high noise level environments; and 2) the behavioral consequences of TSP disruption in peripheral and central auditory systems following noise-induced stress.

Methods. We used quantitative RT-PCR to measure the expression of TSPs at different developmental ages, and before and after noise exposure. We exposed mice to 30 minutes 100 dB SPL broad-band noise. Manganese-enhanced magnetic resonance imaging (MEMRI) was used to examine the auditory pathways in TSP mice. Auditory functions were assessed by electrophysiological tests.

Results. Analysis of TSP1 and TSP2 expression levels revealed up-regulation of these genes after noise injury. Noise exposure experiments revealed that TSP mutants have a higher sensitivity to noise-induced trauma and have higher rates of hearing loss upon noise exposure. Interestingly, the external noise stimulus triggered seizure behavior, in TSP2 and TSP1/2, but not in TSP1 mutants. The seizure was specifically triggered by mid and high frequency noise stimulus. Furthermore, our MEMRI studies showed a trend toward increased neuronal activity in TSP2 mutants and significantly decreased neuronal activity in the brain of TSP1 mutants when compared to control mice.

Conclusion. Based on our study, TSP1 and TSP2 genes are likely to be protective in high noise level environments. Exposure to noise in individuals carrying TSP mutations may predispose them to rapid hearing loss and audiogenic epilepsy.

21

Routes, dynamics, and correlates of bacterial invasion and inflammatory cell infiltration in the inner ear during pneumococcal meningitis.

Cayé-Thomasen P, Miyazaki H, Møller MN, Worsøe L, Brandt CT, Ostergaard C.

OBJECTIVES/HYPOTHESIS: To examine the routes, dynamics and correlates of cochlear bacterial spread and inflammatory cell infiltration in meningitis in order to provide information on the pathogenesis of the associated hearing loss and indications for rational pharmacotherapeutical intervention.

STUDY DESIGN: A well-established rat model of *Streptococcus pneumoniae* meningitis was employed.

METHODS: Rats were inoculated intrathecally with *Streptococcus pneumoniae* and sacrificed when reaching terminal illness or after 7 days, followed by light microscopy. Routes of cochlear bacterial invasion and inflammatory infiltration were examined. The volume fraction of inflammatory infiltration was estimated and correlated to bacterial and leukocyte counts in cerebrospinal fluid (CSF) and blood.

RESULTS: During the first few days after inoculation, bacteria and inflammatory cells invade the inner ear through the cochlear aqueduct, into the scala tympani of the cochlea (perilymphatic space). From here, spread occurs apically toward the helicotrema and subsequently basally through the scala vestibuli, toward the vestibule and the vestibular system. After 5 to 6 days, hematogenous bacterial spreading occurred to the spiral ligament and into the cochlear endolymph, subsequently to the vestibular endolymph. Rosenthal's canal was infiltrated through osseous spiral lamina canaliculi.

Perilymphatic inflammation correlated significantly with the CSF leukocyte count, whereas endolymphatic inflammation correlated with spiral ligament inflammation. The degree of inflammation correlates positively with time of death in untreated meningitis, whereas antibiotic treatment leads to subsiding infiltration during recovery. Bacterial elimination was evidenced by engulfment by macrophages within the inner ear.

CONCLUSIONS: Meningogenic bacterial invasion and inflammation of the rat cochlea occur via the cochlear aqueduct and the spiral ligament capillary bed. The spiral ganglion is infiltrated through the osseous spiral lamina. The degree of inflammation correlates positively with time of death in untreated meningitis, whereas antibiotic treatment leads to subsiding infiltration during recovery.

Although internal barriers exist within the inner ear, the spreading of bacteria occurs via the natural pathways of the fluid compartments. Bacterial elimination occurs by local macrophage engulfment.

Assembly and Disruption of Cochlear Gap Junction Macromolecular Complex are Regulated by Connexin 26

Kamiya, K¹, Minowa, O², Ikeda, K¹

¹Department of Otorhinolaryngology, Juntendo University School of Medicine, Tokyo, Japan,

²BioResource Center, RIKEN, Tsukuba, Japan

Objectives: Hereditary deafness affects about 1 in 2000 children and GJB2 gene mutation is most frequent cause for this disease. GJB2 encodes connexin26 (Cx26), a component in cochlear gap junction. To elucidate the molecular pathway for the gap junction dysfunction, biochemical analysis of gap junction plaques (GJPs) with multiple Cx26 mutant mouse models are needed.

Methods: In this study, we analyzed macromolecular change of gap junction plaques with two different types of Cx26 mutation as major classification of clinical case, one is a model of dominant negative type, Cx26R75W+ and the other is conditional gene deficient mouse, Cx26f/fP0Cre as a model for insufficiency of gap junction protein.

Results: Gap junction composed mainly of Cx26 and Cx30 in wild type mice formed large planar GJPs. In contrast, Cx26R75W+ and Cx26f/fP0Cre showed fragmented small round GJPs around the cell border. In Cx26f/fP0Cre, some of the cells with Cx26 expression due to their cellular mosaicism showed normal large GJP with Cx26 and Cx30 only at the cell junction site between two Cx26 positive cells. These indicate that bilateral Cx26 expressions from both adjacent cells are essential for the formation of the cochlear linear GJP, and it is not compensated by other cochlear Connexins such as Connexin30.

Conclusions: In the present study, we demonstrated a new molecular pathology in most common hereditary deafness with different types of Connexin26 mutations, and this machinery can be a new target for drug design of hereditary deafness (Kamiya et al., J. Clin. Invest., 2014, ;124(4):1598–1607).

Different Contribution of NKCCs in Two Epithelial Layers of the Lateral Cochlear Wall to the Unidirectional K⁺-transport in the Inner Ear

Yoshida T^{1,2}, Nin F¹, Ogata G¹, Komune S², Hibino H¹

¹Molecular Physiology, Niigata Univ, Niigata, Japan, ²Otolaryngology, Kyushu Univ, Fukuoka, Japan

Backgrounds: The lateral cochlear wall, which contains the stria vascularis and the spiral ligament, are thought to be functionally composed of two epithelial layers; the inner layer and the outer layer. The unidirectional K⁺-transport is driven by K⁺-uptake molecules expressed in each layer and involved in generation of the EP by controlling [K⁺] in extracellular and intracellular spaces of the lateral wall (Nin et al., PNAS 109:9191-6, 2012). This arrangement contributes to the generation of the endocochlear potential (EP) essential for hearing. The fibrocytes of the ligament, which form the basolateral surface of the outer layer, express Na⁺,K⁺-ATPases and Na⁺,K⁺,2Cl⁻-cotransporters (NKCCs), like the basolateral surface of the inner layer. Inhibition of the outer-layer's NKCCs by perilymphatic perfusion of bumetanide reduces the EP. It suggests that the NKCCs are crucial for the unidirectional K⁺-transport and contributes to the EP. **Objective and Methods:** To test this idea, electrochemical properties inside the lateral cochlear wall of guinea pigs were recorded with microelectrodes sensitive to [K⁺] and potential. **Results:** We first confirmed that pharmacological blockage of Na⁺,K⁺-ATPases in the outer layer reduced the intracellular [K⁺] of the outer layer and thus the molecules play pivotal roles in the K⁺-transport (Adachi et al., J Physiol 591:4459-72, 2013). Unexpectedly, the perilymphatic perfusion of bumetanide barely affected [K⁺] of the outer layer. Furthermore, this perturbation elevated [K⁺] in the extracellular space between the inner and the outer layers and declined [K⁺] of the inner layer. These observations resemble those when the inner-layer's NKCCs, which have been shown to be essential for the K⁺-transport, were inhibited by vascular injection of the blocker. **Conclusions:** It is plausible that the NKCCs in the inner layer but not those in the outer layer would be required for the unidirectional K⁺-transport and contribute to the EP.

Water Homeostasis Of The Inner Ear Can Be Influenced By The Anti-diuretic Hormone

Gleiser C¹, Runggaldier D¹, Brockhues J¹, Taguchi D³, Müller M², Löwenheim H², Hirt B¹

¹University of Tübingen, Department of Clinical Anatomy and Cell Analytics, ²University of Tübingen Medical Center, Hearing Research Center, Department of Otorhinolaryngology – Head & Neck Surgery, Tübingen, Germany, ³Kochi Medical School, Department of Otolaryngology, Kochi, Japan.

Water homeostasis of the endolymphatic fluid is essential to maintain the function of hearing and balance. Disturbance of this homeostasis might lead to pathologic conditions like in the endolymphatic hydrops (EH). However, knowledge about the molecular determinants of endolymphatic water homeostasis and its volume regulation is rather limited. A positive correlation between the presence of EH and blood concentration of the anti-diuretic hormone (ADH) was reported from clinical observations and animal experiments. The endolymphatic sac (ES) has been suggested to be a direct target of ADH.

Using confocal laser scanning technique in acute cultures of ES of postnatal rats, the transepithelial and transcellular water flow in the ES epithelium was measured in response to osmotic challenge under different pharmacological treatment conditions.

Our data show that the ES is a direct target of ADH and that the cellular water homeostasis of the ES epithelium may be influenced by pharmacological substances which are involved in an ADH-mediated signal transduction cascade. Besides Aquaporins, Na⁺-K⁺-Cl⁻ cotransporters (NKCC) respond to ADH and facilitate water transport across cell membranes along osmotic gradients. As knowledge of the expression pattern of NKCC subtypes in the ES is restricted to the cellular level, we analyzed their subcellular localization in the cells of the ES.

These results may support the understanding of the mechanism of water related volume regulation of the inner ear and direct implications for potential treatment options in Menière's Disease.

25

Targeting Translation During Cellular Stress: Manipulating Stress Granule Formation and Identifying RNA components

Goncalves AC, Dawson SJ* and Gale JE*

UCL Ear Institute, London, UK (*equal contribution).

Stress granules (SGs) are cytoplasmic aggregates of mRNA and protein that form in response to cellular stress. SG-sequestered mRNAs are not translated, whilst the production of essential proteins continues. The dysregulation of SG-formation has recently been linked to age-related disease.

We used inner ear-derived OC-2 cells to investigate SG formation and regulation. We evaluated two compounds: pp242, an inhibitor of mTOR that prevents SG formation in HeLa cells (Fournier et al 2013) and 9-hydroxamate, which disrupts the eIF4F translation-complex resulting in SG formation (Rodrigo et al 2012). OC-2 cells were labeled, immunocytochemically, with two SG-markers, TIA-1 and Caprin-1 and SG properties were quantified using ImageJ (NIH). Exposing OC-2 cells to heat shock (43°C, 1-hour) caused a significant increase in TIA-1/Caprin-1-positive SGs. Pre-incubation with pp242 (2µM, 24-hours) significantly reduced the number of SGs activated by heat shock. Incubating OC2 cells with 9-hydroxamate for 2, 4 or 8 hours resulted in SG formation; at 8 hours, both SG-size and numbers were equivalent to that observed with heat shock. We have also developed an immuno-RNA-FISH protocol allowing us to determine the specific nature of the RNAs sequestered to SGs. We confirmed co-localization of polyA-mRNA (using a Cy3-labelled-probe) with TIA-1 and are currently assessing sequence specific probes to localise specific RNAs to SGs. Having demonstrated that we can manipulate, quantify and characterise the SGs that form in OC-2 cells we now aim to evaluate the effect of SG manipulation on hair cell survival and to clarify the role of SG in the inner ear.

The Quantitative Analysis of the Aquaporin Expression Levels in the Inner Ear of Slc26a4^{-/-} Mice

Takushi Miyoshi¹, Norio Yamamoto¹, Taro Yamaguchi², Yosuke Tona¹, Kiyokazu Ogita², Takayuki Nakagawa¹ and Juichi Ito¹

¹ Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, JAPAN

² Department of Pharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, JAPAN

Objectives: Aquaporins (AQPs) are water channel proteins, which are major candidates for water regulation in the inner ear. The exact function of AQP in the inner ear remains unclear although localizations of AQPs are being reported. To elucidate functions of AQPs in the inner ear, we measured expression levels of AQP subtypes in abnormal water homeostasis. We used Slc26a4 knockout mice (model for Pendred syndrome) as a genetic model, which present sensorineural hearing loss complicating endolymphatic hydrops.

Methods: Whole inner ears were dissected from Slc26a4 (Pendrin) mice at postnatal day (P) 21. Total RNA was extracted and the expression level of each AQP subtype was determined by qRT-PCR. The expression levels of Slc26a4^{-/-} mice inner ears were compared to those of Slc26a4^{+/+} mice. For AQP4, 6 and 11, the expression levels at P3 and P10 were also compared between Slc26a4^{-/-} and Slc26a4^{+/+} mice.

Results: The expression levels of AQP4, 6 and 11 at P21 were significantly increased in Slc26a4^{-/-} mice compared to those of Slc26a4^{+/+} mice ($p < 0.01$ for AQP4, $p < 0.05$ for AQP6 and 11 by Student's t-test). The expression level of AQP4 was also increased at P10 ($p < 0.05$). The expression levels of AQP4, 6 and 11 at P3 showed no significant difference between Slc26a4^{-/-} and Slc26a4^{+/+} mice.

Conclusions: The increase of AQP4, 6 and 11 in Slc26a4^{-/-} mice occurs at the later stage of inner ear maturation, P10 – 21. The time course suggests that AQP4, 6 and 11 are up-regulated to compensate endolymphatic hydrops and keep the water homeostasis stable. These results will contribute to the elucidation of the mechanisms how AQPs are involved in the pathogenesis of sensorineural hearing loss.

Glucocorticoids Stimulate Endolymphatic Water Reabsorption in Inner Ear Through Aquaporin 3 Regulation

Nevoux J^{1,2,3,4}, Viengchareun S^{1,2}, Lema I^{1,2}, Lecoq AL^{1,2}, Lombès M^{1,2}, Ferrary E⁴.

¹Inserm, U693, Le Kremlin-Bicêtre, F-94276, France; ²Université Paris-Sud, Faculté de Médecine Paris-Sud, UMR-S693, Le Kremlin-Bicêtre, France; ³Service d'ORL, Assistance Publique-Hôpitaux de Paris, CHU de Bicêtre, Le Kremlin-Bicêtre, France; ⁴Inserm, U867, Université Paris Diderot, France;

Menière's disease, clinically characterized by fluctuating, recurrent, and invalidating vertigo, hearing loss, and tinnitus, is linked to an increase in endolymph volume, the so-called endolymphatic hydrops. Since dysregulation of water transport could account for the generation of this hydrops, we investigated the role of aquaporin 3 (AQP3) in water transport into endolymph, the K-rich, hyperosmotic fluid that bathes the apical ciliated membrane of sensory cells, and we studied the regulatory effect of dexamethasone upon AQP3 expression and water fluxes.

The different AQP subtypes were identified in inner ear by RT-PCR. AQP3 was localized in human utricle and mouse inner ear by immunohistochemistry, and confocal microscopy. Unidirectional transepithelial water fluxes were studied by means of ³H₂O transport in murine EC5v vestibular cells cultured on filters, treated or not with dexamethasone (10⁻⁷ M). The stimulatory effect of dexamethasone upon AQP3 expression was assessed in EC5v cells and *in vivo* in mice.

AQP3 was unambiguously detected in human utricle, and was highly expressed in both endolymph secretory structures of the mouse inner ear, and EC5v cells. We demonstrated that water reabsorption, from the apical (endolymphatic) to the basolateral (perilymphatic) compartments, was stimulated by dexamethasone in EC5v cells. This was accompanied by a glucocorticoid-dependent increase in AQP3 expression at both mRNA and protein level, presumably through glucocorticoid receptor-mediated AQP3 transcriptional activation.

We show for the first time that glucocorticoids enhance AQP3 expression in human inner ear, and stimulate endolymphatic water reabsorption. These findings should encourage further clinical trials evaluating glucocorticoids efficacy in Menière's disease.

Natural history of resident macrophages in the mouse cochlea

Takayuki Okano and Juichi Ito

Department of Otolaryngology, Head and Neck Surgery

Kyoto University Graduate School of Medicine

The clinical entity of immune-mediated inner ear disorder which responds to treatment with corticosteroid and cyclophosphamide was introduced by McCabe in 1979, whereas immune-mediating cells have been thought to be excluded from the inner ear for a long time. Recent studies suggested that various kinds of immune response are involved in pathophysiology in the inner ear, including Meniere disease, ANCA-associated vasculitis with sensorineural hearing loss, and virus-mediated inner ear disorder. However, the precise mechanism underlying the pathology of immune-mediated inner ear disorder is yet to be elucidated, and our knowledge on the innate immune system in the inner ear still remains limited.

Previously, we reported that bone marrow-derived cells reside in the cochlea as tissue-resident macrophages, and cochlear macrophages gradually turn over for several months during steady state condition via replacement by bone marrow-derived cells. Furthermore, cochlear macrophages demonstrate phenotypes morphologically and immunohistologically similar to microglia which are thought to be resident macrophages in the central nervous system. These findings suggest that cochlear macrophages play important roles in maintenance of inner ear homeostasis and pathophysiology in various types of hearing loss. To begin to identify the roles of cochlear macrophages on hearing loss, we will discuss distribution of macrophages in the developing mouse cochlea in the present study. In addition, we will present age-related change in the number of cochlear macrophages associating with age-related hearing loss.

29

Wnt4 Signaling Inhibits Hair Cell Formation in the Developing Mammalian Cochlea through the Non-Canonical Wnt/Calcium/PKC Pathway

Dabdoub, Alain^{1,2,3}

¹Sunnybrook Research Institute, ²Department of Otolaryngology – HNS, ³Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada

Determination of cell fate and establishment of boundaries between cell types is an essential step in the development of every organ. Precise specification of phenotype is particularly important within structures that are comprised of highly ordered cellular patterns such as the mammalian cochlea. Using a combination of genetic, molecular and biochemical approaches, we demonstrate for the first time a role for Wnt/Calcium/PKC signaling in hair cell development in the mammalian cochlea. We identified Wnt4 as a ligand expressed in the medial region of the cochlear duct that inhibits hair cell formation. Utilizing gain- and loss-of-function experiments we demonstrate a key role for Wnt4 signaling in the establishment of the sensory/non-sensory boundary in the cochlea. Furthermore, Wnt4 activity is likely inhibited by a gradient of sFRP2, a Wnt antagonist, originating from the lateral edge of the duct. Using biochemical assays we show that the activity of Wnt4 is mediated through the non-canonical Wnt/Calcium/PKC pathway, and that inhibition of PKC results in the formation of extra inner hair cells. These results demonstrate a key role for Wnt4 in the establishment of hair cell fate and the sensory/non-sensory boundary within the cochlea. Moreover, our data suggest that phosphorylation by PKC plays an important role in regulating hair cell formation and we are currently investigating its targets.

30

β -catenin is Required for Hair-cell Differentiation in the Cochlea

Shi F^{1,2}, Hu L^{1,2,3}, Edge AS^{1,2}

¹Department of Otology and Laryngology, Harvard Medical School, Boston, Massachusetts USA,

²Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts USA,

³Department of Otolaryngology-Head and Neck Surgery, Xinhua Hospital, Ear Institute, Shanghai Jiaotong University School of Medicine, Shanghai, China.

Objectives: To know the role of canonical Wnt pathway mediator, β -catenin in the hair cell development in the mouse cochlea.

Methods: We assessed the role of Wnt/ β -catenin in both steps in gain and loss-of-function models in mice. In different embryonic stages, after down-regulating or up-regulating the expression of β -catenin in sensory progenitors, supporting cells or hair cells, we analyzed the pattern of Organ of Corti including the amount of hair cells and supporting cells. The hair cell amount in utricle was also analyzed. We knocked out the β -catenin in hair cells when it formed and tested the ABR and DPOAE after acquisition of hearing.

Results: β -catenin can be knocked out or overexpressed within 24 hours after activation of Cre. KO of β -catenin before or in phase of hair cell differentiation can inhibit the hair cell differentiation and maturation. KO of β -catenin in phase of differentiation led to lack of pillar cells. KO of β -catenin also resulted in a shortening of the Organ of Corti. The development of the utricle was also inhibited by KO of β -catenin. When β -catenin was knocked out after hair cell differentiation, the pattern of Organ of Corti did not change and the ABR and DPOAE kept normal. Overexpression of β -catenin can induce supporting cell reenter in the cell cycle, but the hair cell differentiation interrupted, resulted in expansion of Organ of Corti and length shortening.

Conclusions: Our data demonstrate that β -catenin plays a role in cell division and differentiation in the cochlear sensory epithelium.

In Vivo Overactivation of Notch Signaling Pathway in Cochlear Prosensory Epithelium

Tomoko Tateya^{1,2}, Susumu Sakamoto^{1,3}, Itaru Imayoshi^{1,2,4}, Ryoichiro Kageyama^{1,5,6}

¹Institute for Virus Research, Kyoto University, Kyoto, Japan, ²The Hakubi Center, Kyoto University, Kyoto, Japan, ³Department of Otolaryngology - Head and Neck Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan, ⁴Japan Science and Technology Agency, PRESTO, Kyoto, Japan, ⁵World Premier International Research Initiative–Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan, ⁶Japan Science and Technology Agency, CREST, Shogoin-Kawahara, Sakyo-ku, Kyoto, Japan.

Objectives: To examine the effects of Notch activation on the cochlear prosensory epithelium and to know the precise roles of Notch signaling on cochlear prosensory epithelium.

Methods: We used the conditional NICD expressing transgenic mice, and analyzed cochlear epithelium histologically.

Results: The conditional NICD cochleae were short. Hair cell number reduced especially in apical turn though hair cells developed where NICD was overexpressed. Ectopic expression of early prosensory markers such as Sox2 and Jag1, and Hes/Hey genes except for Hes5 was seen where NICD was overexpressed in cochlear epithelium. However, ectopic expression of p27Kip1, Hes5 and Prox1 was not observed. Hes5- and Prox1-positive supporting cells were reduced and observed around hair cells. Hair cells were reduced but Atoh1 and MyosinVI were occasionally expressed where NICD was overexpressed.

Conclusions: Our data shows that NICD overexpression in vivo decreased both hair cells and supporting cells in the cochlear epithelium. Our results indicate that Notch signaling may be involved in the determination of total numbers of hair cells and supporting cells.

Replacing Atoh1 With Neurog1 Can Differentiate And Maintain Hair Cells

Jahan I, Pan N, Kersigo J, Fritzsche B

Department of Biology, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA, USA

Atoh1, a basic helix loop helix transcription factor (bHLH TF), is essential for the patterned development of the organ of Corti (OC) hair cells (HCs). Generating the topologically correct OC cell type is indispensable to transform movement of the basilar membrane into hearing. Without *Atoh1* a featureless, flat epithelium forms; overexpression of *Atoh1* causes formation of ectopic HCs and replacing *Atoh1* with the fly ortholog, *atonal*, results in normal HCs. We replaced *Atoh1* with another bHLH TF, *Neurog1*. This leads to quantitatively near normal HCs formation, if *Atoh1* is transiently expressed. Misexpression of *Neurog1* maintains the inner hair cells (IHCs) of the OC, but rescue is qualitatively incomplete. *Fgf8*, an essential factor for pillar cells development, is reduced in this mutant, resulting in topologically inappropriate HCs: IHCs develop stereocilia bundles of OHCs and *vice versa*, indicating a correlation of *Fgf8* level with 'IHC' specification, known for *Neurod1* mutant mice. *Fgf10* or *Bmp4* expression is also downregulated, resulting in disruption of the medial and lateral boundaries of the OC in this mutant. This study shows for the first time that replacement of *Atoh1* with the pro-neuronal gene *Neurog1* can rescue HCs, indicating that HC gene networks can be driven by other bHLH TFs'. It provides a novel approach to molecularly dissect the development of the OC through modulating the intracellular gene network. The outcome of this approach will provide an understanding for the complex autocrine and paracrine gene regulation to reconstruct the functionally essential cellular mosaic of the OC.

CASK Function in The Inner Ear

Kita T^{1,4}, Honda A¹, Matsumoto Y², Aruga J², Kudo M³, Nagao S³ and Ladher RK¹

¹Laboratory for Sensory Development, RIKEN Center for Developmental Biology, Kobe, Japan,

²Laboratory for Behavioral and Developmental Disorders, ³Laboratory for Motor Learning Control, RIKEN Brain Science Institute (BSI), Wako, Japan, ⁴Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Calcium/calmodulin-dependent serine protein kinase (CASK) is an evolutionarily conserved multidomain protein that plays an important role in brain development. Sensorineural hearing loss is also observed in patients with *CASK* mutations. Moreover, CASK is found in inner ear hair cells, suggesting an important role of this molecule in inner ear development and/or function. However, there has been no comprehensive study of CASK in the inner ear. To better understand the physiological importance of CASK, we first examined the expression pattern of CASK in the inner ear. In situ hybridization of CASK in mouse cochlea revealed that in immature cochlea CASK expresses in the spiral ganglion neurons (SGNs) and Organ of Corti. The cochlea from CASK conditional KO mice shows a clear defect with a decrease in the number of SGNs and outer hair cells. ABR and VOR data suggest that CASK is required for the function of the inner ear, where CASK may play as a multifunctional molecule both in SGNs and hair cells. We are now beginning to dissect the function of CASK in each organelle.

34

CONTROLLING INNER EAR FATE AND SHAPE

Raj Ladher, Akira Honda, Tomoko Kita, Stephen Freeman, XaioRei Sai
RIKEN Center for Developmental Biology, Kobe, Japan

The signals involved in specifying the inner ear, change the developmental trajectory of non-neural ectoderm from epidermis into inner ear. Understanding the signals involved in this change, allows us to control the development of other cells to cajole them into adopting an inner ear fate. Members of the fibroblast growth factor (FGF) family play a significant role in specifying the fate of the inner ear precursor, the otic placode. The balance of signals ensures that the otic placode is appropriately positioned, appropriately proportioned and appropriately patterned. We have found that FGF signalling converts the fate of non-neural ectoderm such that it forms the precursor of the inner ear. Inner ear fate is then confirmed by the action of yet another signalling system, the Wnt signal. Once induced the superficially located inner ear placode must be transformed into an internal cystic structure. We have found that here, external signals control cytoskeletal reorganization that drive the changes in cell and tissue shape. Our results show that the precise control of the signals the inner ear precursors encounter is important in the development, morphogenesis and maturation of the inner ear.

35

Expression of bHLH Genes in Developing Cochlear Epithelium

Susumu Sakamoto^{1,2}, Tomoko Tateya^{1,3}, Yukiko Harima¹, Itaru Imayoshi^{1,3,4}, Juichi Ito², Ryoichiro Kageyama^{1,5,6}

¹Institute for Virus Research, Kyoto University, Kyoto, Japan, ²Department of Otolaryngology - Head and Neck Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan, ³The Hakubi Center, Kyoto University, Kyoto, Japan, ⁴Japan Science and Technology Agency, PRESTO, Kyoto, Japan, ⁵World Premier International Research Initiative-Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan, ⁶Japan Science and Technology Agency, CREST, Shogoin-Kawahara, Sakyo-ku, Kyoto, Japan.

Objectives: To clarify the spatiotemporal expression pattern of bHLH genes in developing auditory epithelium.

Methods: We used Hes1-Venus fusion mice and Atoh1-GFP fusion mice, and analyzed cochlear epithelium histologically.

Results: Atoh1 was expressed at various levels in the prosensory domain of E14.5 cochlear epithelium. Atoh1-positive cells localized both near the apical surface of the epithelium and near the basilar membrane in prosensory domain. At E15.5 Atoh1-positive cells began to be arranged in rows near the apical surface of the epithelium from basal region of the cochlear duct. Hes1 expression was delayed compared with the first Atoh1 expression, observed at various levels in prosensory domain forming mosaic with Atoh1-positive cells at E15.5, and then gradually limited to the lateral periphery of prosensory domain.

Conclusions: Atoh1 expression pattern was consistent with Atoh1 function as a proneural gene. Hes1 expression was simultaneous with ongoing hair cell differentiation and thought to be involved in hair cell differentiation.

Molecular Mechanisms for Human Anterior/Posterior Cranial Placode Cell Lineage Specification

Ealy M¹, Ronaghi M¹, Bravo D¹, Nasr M¹, Waldhaus J¹, Heller S¹

¹Department of Otolaryngology – Head and Neck Surgery, Stanford University, Stanford, California, USA,

The ability to produce human cell types of interest from stem cells requires efficient stem cell guidance protocols that reliably produce the desired cell types. To produce sensory epithelia of the human inner ear, we need a better understanding of the molecular mechanisms that lead to human cranial placode development and subsequent otic lineage development. In-depth understanding of the molecular events that give rise to specific lineages is a prerequisite for downstream applications such as phenotype studies, disease models, screens for ameliorating drugs or treatments, or transplantation. To accomplish this, we are utilizing both three-dimensional and monolayer cell culture of hESCs to generate pre-placodal cells that can be further manipulated using signaling environments that steer the cells either towards anterior (olfactory and upper respiratory) or posterior (otic) placodal lineages. We show that 3D culture can become self-guiding once directed to the placode stage. To get a better understanding of the lineage bifurcations required for generating the different cranial placodes, we are utilizing a monolayer human stem cell culture system. By controlling each step from induction of non-neural ectoderm, pre-placodal cells, and the different placodes we are able to determine both the inductive and restrictive molecular mechanisms involved in development of various placodal lineages. Preliminary evidence suggests that in monolayer cultures, retinoic acid signaling plays a role in determining anterior/posterior patterning of the cranial placodes, with higher levels of retinoic acid inducing posterior placode markers. Together the two different model systems (monolayer and 3D) are providing more insight into human otic development, which will ultimately lead to better understanding of hearing loss and balance disorders, for example via analysis of otic lineages derived from patient-derived iPSCs.

Septin7 Regulates the Formation of Inner Ear During Early Developmental Stage

Hiroko Torii, Norio Yamamoto, Atsuhiko Yoshida, Takayuki Nakagawa, Juichi Ito

Dep. Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Kyoto, Japan

Septin proteins are GTP-binding proteins that are evolutionally conserved through eukaryotes except plants. Septin complex can interact with membrane, myosin, and microtubules. Their functions contribute to the formation of the cellular polarity that is important characteristics of epithelial cells. Our previous reports showed that Septin7, a core protein of multimeric Septin complex, was expressed in the embryonic cochlea.

To identify the roles of Septin7 in inner ear development, we analyzed the phenotypes of Septin7 cKO mice embryos more precisely. We used Foxg1Cre which works as Cre from E8.5 and Emx2Cre which works from E11.5 in inner ear. We performed immunohistochemistry to characterize the cell proliferation, apoptosis, neural development and hair cell differentiation in the developing mice inner ear from -E8.5 to E18.5. We also investigated the macroscopic morphology of the inner ear by using paintfilling.

In Foxg1Cre⁺-Septin7^{floxed/floxed}, macroscopic morphology of the inner ear was normal until E10.5. But the inner ear hypoplasia was found from E11.5 and severe cystic hypoplasia was seen at E13.5. This suggests that the development of the inner ear gross morphology was impaired between E10.5 and E13.5 in Septin7 cKO mice. The promotion of apoptosis was detected by immunohistochemistry at E12.5. Sox2 positive and Myo6 positive cells were detected within the remnant inner ears even at E18.5, suggesting that the differentiation of sensory epithelia happened in Septin7 cKO inner ears. These cell populations had innervation from the β 3-tubulin positive auditory nerves. But Emx2Cre⁺-Septin7^{floxed/floxed} doesn't show any gross morphological deformity.

Our results indicate that Septin7 involved in the formation of inner ear gross morphology in early embryonic stage but not in the differentiation of the sensory epithelia.

Inner Ear Imaging Of Cochlear Implant Using Optimization Of Cone Beam CT.

Pyykkö I¹, Zou J¹, Koivisto J², Lähelmä J², Aarnisalo AC³. Hearing and Balance ¹Research Unit, Field of Oto-laryngology, School of Medicine, University of Tampere, Tampere, Finland, ²PlanmecaOy, Helsinki, Finland, ³Department of Otolaryngology, University Hospital of Helsinki, Helsinki, Finland.

The aim was to search for methods to improve image quality rendered by Cone-Beam Computed Tomography (CBCT) in the temporal bone with control of the radiation emission.

In experimental set up the effect of different tube voltage (79-120 kV), tube current (8-11 mA), pulse number (number of frames of 300, 450, 900) and magnification factor on image quality were studied. The effect of radiation was evaluated MOSFET dosimeter on RANDO head phantom. In hardware filtering we used copper and aluminium filter combination.

Changes in the tube current from 8 mA to 12.5 mA resulted in a minimal change of the temporal bone image quality. The tube voltage of 80 kV provided best images using 900 frames. In 2D imaging all inner ear structures were imaged satisfactory such as osseous spiral lamina, Rosenthal's canal and bony wall of the cochlea. In 3D images contrasts adjustment allowed sophisticated evaluation of stapes superstructure and oval window. Depending on the number of frames, tube voltage and tube current the total radiation dose of the inner ear varied from 35.2 µSv to 105.6 µSv (900 frames, 88 kV, 495 mAs). For 1.7 magnification the effective doses increased about 25% (max. 138 µSv). The most dominant contributor to the effective dose was bone marrow (36-37 %).

Selection of filter, number of frames and magnification affect the image quality in CBCT temporal bone imaging. The radiation dose is about 1/10 of respective dose in medical CT. We recommend CBCT as a primary tool for inner ear imaging.

39

Inner Ear Health and Cochlear Implant Function

Pfingst, B.E., Colesa, D.J., Watts, M.M., Budenz, C.J., Raphael, Y.
Department of Otolaryngology, University of Michigan, Ann Arbor, MI, USA

The objective of this study was to determine the relationships between the health of the implanted cochlea, assessed histologically and measures of cochlear implant function.

Subjects were mature male SPF guinea pigs. Several treatments were used to achieve a broad range of inner-ear health including hearing preservation, deafening with neomycin and deafening followed by neurotrophin gene therapy. Functional measures including psychophysical detection thresholds, ECAP and EABR amplitude-growth functions and ensemble spontaneous activity (ESA) were obtained over a period of 5 to 14 months and then the animals were euthanized and the cochleae were processed histologically.

Spiral ganglion neuron (SGN) survival was generally best in ears with preserved hearing, worst in animals with neomycin deafening and moderate following deafening plus neurotrophin therapy. Steepness of ECAP and EABR growth functions correlated with the degree of nerve survival over a wide range of SGN densities. In contrast, good psychophysical multipulse integration and temporal integration were seen only in cases with surviving inner hair cells, ESA, and very high SGN densities.

The functional measures affected by these preservation procedures are correlated with speech recognition in humans reinforcing the importance of inner-ear health for cochlear implant function. The psychophysical and electrophysiological measures could be useful for monitoring tissue-engineering procedures and for identifying the strongest and weakest stimulation sites when programming an individual's processor map.

Supported by R01 DC010412, P30 DC05188, MED-EL and the UM Center for Organogenesis.

Speech perception and speech production after cochlear implantation in prelingually deaf children

Istvan Sziklai, Ildikó Pongrácz, Marianna Sziklainé Héjja, Ferenc Kunkli, Judit Szilvássy, Jozsef Tamas Batta

Dept. ORL HNS, Debrecen University, Debrecen, Hungary

Cochlear implantation is an advent in speech and language acquisition for prelingually deaf children. Intensive investigations are performed worldwide to establish those dependent variables in the function of independent variables which may decide the quality and speed of the language acquisition process. Independent variables are e.g. age at implantation, duration of implant use, additional handicap, bilinguality, etiology, familiar background etc. All these should be accounted in calculating the potential expectation reality in speech development and should be classified in term of predictive value for developmental characteristics. Dependent variables are a.g. number of functioning electrodes of the implant, segregated or integrated education, density of training etc.

A retrospective study was performed in prelingually deaf children (n=32) who received cochlear implant in the age range of 1-7 years. The patients received Med-El and Nucleus 24 implants. The effect of the following dependent variables were examined on speech perception and speech production: 1. Number of functioning electrodes, 2. Integrated or segregated education, 3. Intensity of training on speech development in the functions of age at implantation and duration of implant use. The control group was consisted of age matching normal hearing listeners (n=50, 10 in all age groups between 2 yrs old to 6 yrs old). Speech and language skills were measured by the same speech therapist. Closed and open set word and sentence recognition as perceptive language skill measurements were performed. Expressive language skill was measured by picture naming and word and sentence intelligibility established by naive listeners.

Prelingually deaf children acquire language faster under the age of 3 yrs old than after this age. Integrated education is preferable for better speech production skill acquisition. Speech production lags behind speech perception. Accordingly, new methods of audiovisual support should be introduced in the habilitation to support expressive speech development. Aiming this, a new cross-modal (vision for audition) training system is demonstrated.

Recent And Future Trends In The Bioelectrical Interface Of Auditory Implants

Volkenstein S, Kwiatkowska M, Gahlen F, Dazert S

Department of Otorhinolaryngology, Head & Neck Surgery, Ruhr-University of Bochum, St. Elisabeth-Hospital, Bochum, Germany

In patients with severe to profound hearing loss, cochlear implants (CIs) are currently the only therapeutic option when the amplification with conventional hearing aids does no longer lead to a useful hearing experience. Despite its great success, there are patients in which benefit from these devices is rather limited. One reason may be a poor neuron-device interaction, where the electric fields generated by the electrode array excite a wide range of tonotopically organized spiral ganglion neurons at the cost of spatial resolution. Coating of CI electrodes to provide a welcoming environment combined with suitable surface chemistry (e.g. with neurotrophic factors) has been suggested to create a closer bioelectrical interface between the electrode array and the target tissue, which might lead to better spatial resolution, better frequency discrimination, and ultimately may improve speech perception in patients. In addition, the use of progenitor/stem cells may help to improve this interaction as well in the future.

Materials and Methods: We will present a general concept for future cochlear implant electrode design and show first results of experiments including coating strategies, the use of growth factors and progenitor cells to achieve a better neuron:device interaction.

Conclusion: There have been different approaches suggested to improve the bioelectrical interface of cochlear implants and therefore the hearing performance of these patients. Coating strategies of the CI electrode array to achieve neuroprotection and a directed neurite outgrowth in combination with growth factors or small molecules as well as the use of mini-pumps integrated in the CI device seem to be the most promising approaches right now, whereas the use of stem or progenitor cells may play a role in the future after discovering more insights of neuronal differentiation and neurite outgrowth pathways.

The Effect of Systemic Steroid Pump in Preservation of Residual Hearing after Cochlear Implantation

Rah YC¹, Lee MY¹, Lee HS¹, Choi JJ¹, Park MN¹, Suh MW¹, Oh SH¹, Chang SO¹, O'Leary S², Lee JH¹

¹Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University College of Medicine, Seoul, Korea, ²Department of Otolaryngology, University of Melbourne, Melbourne, Australia

This study aimed to evaluate minimal administration time of systemic steroid for hearing preservation after cochlear implantation by analyzing correlation between the duration of steroid administration and long term serial change of hearing level.

Continuous mini-osmotic infusion pump (10µl/hr, Alzet, USA) filled with dexamethasone (4mg/ml) was inserted a day prior to implantation and removed 3 days or 7 days after implantation. Electrode insertion was carried out through cochleostomy approach for Guinea pigs at 7 weeks. Hearing thresholds (tone burst at 2, 8, 16, 24, 32 kHz) were measured prior to implantation and at 7 days, 30 days, 90 days after implantation. Histologic evaluation of cochlea was carried out.

Initially no definite difference in hearing threshold was identified among groups. Significant hearing preservation than control group especially in high frequency were observed in both 3-day (24, 32 kHz) and 7-day (16, 24, 32 kHz) infusion groups after 7 days from implantation. After 30 days, only 7-day-infusion group showed significant hearing preservation than control group (2, 8, 16 kHz). Significant hearing preservation of 7-day infusion group continued at 90 days after implantation. (16, 24 kHz) Histologic review showed more significant fibrosis and inflammatory cell infiltration along the electrode insertion site of scala tympani in control group than in steroid-infusion groups.

In conclusion, systemic steroid administration was effective in long term hearing preservation only in 7-day-infusion group whereas the hearing of 3-day-infusion group was worsened after 1 week. Suspected mechanism was the suppression of inflammation and subsequent tissue reaction by steroid.

Detection of Cochlin-Tomoprotein in the Fluid Leakage during Cochlear Implantation

K.Shirai¹, Y.Ogawa¹, A.Kawano,¹ T.Ikezono², and M.Suzuki¹

¹Tokyo Medical University: Department of Otolaryngology, Tokyo, Japan, ²Saitama Medical University: Department of Otolaryngology, Saitama, Japan

Study's objectives

The purpose of this study was to investigate the specificity of the Cochlin-Tomoprotein (CTP).

Methods

We studied 20 cases aged between 1 and 79 years, who had been implanted in Tokyo Medical University Hospital between 2011 and 2012. Perilymph was collected during cochlear implantation. Samples were collected by lavaging the middle ear cavity four times, before, during, and after the fenestration of the cochlea and after the insertion of an electrode. CTP detection test was performed on the samples.

Results

In all of the samples taken before and during the fenestration, CTP was negative. Fourteen samples (70%) taken after the fenestration and 13 samples (65%) taken after the electrode insertion were positive for CTP. Only in 3 cases, CTP was negative in both of the samples taken after the fenestration and insertion.

Conclusion

In all cases, CTP was negative before the fenestration of the cochlea. After fenestration, CTP was positive in 17 cases (85%) at least one time. These results indicate that CTP detection test can be used to make a definite, objective diagnosis of perilymph fistula.

Effects of congruent and incongruent visual cues on speech perception and brain activity in cochlear implant users

Jae-Jin Song¹, Hyo-Jeong Lee^{2,3*}, Hyejin Kang⁴, Dong Soo Lee⁴, Sun O Chang^{1,3}, **Seung Ha Oh^{3,5*} (presenter)**

¹Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, Korea, ²Department of Otorhinolaryngology-Head and Neck Surgery, Hallym University College of Medicine, Chun-Cheon, Korea, ³Sensory Organ Research Institute, Seoul National University Medical Research Center, Seoul, Korea, ⁴Department of Nuclear Medicine, Seoul National University Hospital, Seoul, Korea. ⁵Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Hospital, Seoul, Korea

Objectives: While deafness-induced plasticity has been investigated in the visual and auditory domains, not much is known about language processing in audiovisual multimodal environments for patients with restored hearing via cochlear implant (CI) devices. Here we examined the effect of agreeing or conflicting visual inputs on auditory processing in deaf patients equipped with degraded artificial hearing.

Methods: Ten post-lingually deafened CI users and matched controls underwent H₂¹⁵O-PET scans while carrying out a behavioral task requiring the extraction of speech information from unimodal auditory stimuli, bimodal audiovisual congruent (conAV) stimuli, and incongruent (incAV) stimuli.

Results: Regardless of congruency, the controls demonstrated activation of the auditory and visual sensory cortices, as well as the superior temporal sulcus, the classical multisensory integration area, indicating a bottom-up multisensory processing strategy. While the controls exhibited activation of the right ventral premotor-supramarginal pathway, CI users activated primarily the visual cortices more in the conAV condition. Also, compared to controls, CI users displayed an activation focus in the right amygdala for conAV stimuli. Most notably, an activation focus was displayed in the left inferior frontal gyrus in CI users in the conAV condition, suggesting top-down cognitive modulation for audiovisual conflict.

Conclusions: Taken together these results suggest that for multimodal inputs, cochlear implant users are more vision-reliant when processing congruent stimuli and are disturbed more by visual distractors when confronted with incAV stimuli. To cope with this multimodal conflict, CI users activate the left inferior frontal gyrus to adopt a top-down cognitive modulation pathway, whereas normal hearing individuals primarily adopt a bottom-up strategy.

Simultaneous 2nd cochlear implantation and translabyrinthine removal of vestibular schwannoma after 1st cochlear implantation on the other side.

Doi K, Sato M, Kobayashi T, Shiraishi K, Saito K, Kohama K, Kinoshita T.

Department of Otolaryngology, Kinki University Graduate School of Medicine

Decision making in patients with a vestibular schwannoma (VS) in the only hearing ear is challenging. Restoration of hearing when they become deaf will depend on the status of the remaining cochlear and the integrity and function of the cochlear nerve. In terms of hearing restoration, cochlear implantation (CI) is the most effective option if the cochlear nerve could be preserved intact and the cochlea could be remain responsive to electrical stimulation. Auditory Brainstem Implants (ABI) would be another option for the patients in which the cochlear nerve could not be spared during VS removal, while the hearing results with an ABI are still far poor than those with a CI. If CI is being considered, it should be performed near the time of the surgery for VS removal, because cochlear fibrosis and/or ossification might occur in a short time after the surgery. When the translabyrinthine approach for VS removal is selected, CI surgery should be done as close as possible to the procedure and optimally.

Simultaneous CI and translabyrinthine removal of VS was considered for the patient who suffered from severe sensorineural hearing loss on the same side with VS and profound deafness on the contralateral side due to the past sudden deafness. The first CI was preceded on the contralateral side, then the second CI was performed one year after. Simultaneous CI and translabyrinthine VS removal is a good option that should be considered when discussing and planning the most appropriate strategy.

Development of a Piezoelectric Electrode with a Modiolus Penetration Needle

Tona Yosuke¹, Nakagawa Takayuki¹, Kawano Satoyuki², Ito Juichi¹

¹Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto City, Kyoto Prefecture, Japan, ²Second affiliation information: Organization, City, State, Country
Department of Mechanical Science and Bioengineering, Graduate School of Engineering Science, Osaka University, Toyonaka City, Osaka Prefecture, Japan

Objectives: We previously reported that a piezoelectric membrane generated electrical potentials in response to sound stimuli that were able to induce auditory brainstem responses in deafened guinea pigs. However, the electrical output from our device was insufficient in comparison to that from conventional cochlear implants. The device should be fixed in the same position without rotation or migration. We developed a new electrode with penetrating needles into the modiolus, in order to reduce the electrical demand.

Methods: Hartley guinea pigs were used. The device was implanted into the scala tympani of the cochlear basal turn with the needle inserted into the bony wall of the modiolus. Histological analysis was performed at 1 month after implantation surgery. For functional analysis, the eABR thresholds were compared before and after insertion of the electrode needle into the modiolus.

Results: At the cochlear harvest, the device remained fixed in the scala tympani with the needle electrode inserted into the modiolus. The histological findings of the cochleae showed limited cell infiltration around the rim of the scala tympani in all the samples. The eABR thresholds were improved with penetration of the needle.

Conclusion: These findings indicated that our new device with needle electrode is efficient and safe for implantation, although some problems need to be overcome before its clinical application.

47

Transmembrane Channel-like 1 and 2 are Localized at Stereociliary Tips of Mammalian Inner Ear Hair Cells

Kurima K¹, Ebrahim S², Millis BA², Pan B³, Nakanishi H¹, Fujikawa T², Kawashima Y¹, Holt JR³, Griffith AJ¹, Kachar B².

¹Molecular Biology and Genetic Section, ²Section on Structural Cell Biology, NIDCD, NIH, Bethesda/MD, USA. ³Department of Otolaryngology, Harvard Medical School, Boston/MA, USA

Transmembrane channel-like 1 and 2 (TMC1 and TMC2) are essential for mechanoelectrical transduction (MET) in sensory hair cells of the inner ear. We generated transgenic mice expressing TMC1-mCherry and TMC2-AcGFP to determine the spatial and temporal expression pattern of these proteins in stereocilia. Both TMC proteins were distributed along the length of stereocilia at early postnatal days, but this distribution was increasingly restricted to stereociliary tips as the hair cells matured. Both TMC1-mCherry and TMC2-AcGFP were excluded from the tips of the tallest row of stereocilia in the bundle where MET activity and tip links are thought to be absent. This distribution was confirmed for native TMC1 and TMC2 by immunofluorescence using specific antibodies. Interestingly, TMC1 and TMC2 fluorescence signals rarely colocalize outside the stereocilia tips, suggesting that they do not traffic and targeting to the tips as a heteromeric complex. Consistent with the hypothesis that TMC2 can compensate for TMC1 only during early bundle formation in cochlear hair cells, TMC2 signals at stereociliary tips rapidly disappear, first from outer hair cells and then from inner hair cells, in the early postnatal period. Conversely, TMC1 signal at the stereociliary tips remains in both OHCs and IHCs to adulthood. TMC1-mCherry and TMC2-AcGFP transgenic mice restored hair cell MET currents as well as normal hearing and vestibular function in homozygous null mutants lacking TMC1 and TMC2. These data collectively establish the localization of TMC1 and TMC2 at stereocilia tips, consistent with the hypothesis that they play a local role in the MET channel complex.

48

Role of Rho-GTPases in Inner Ear Hair Cells

Sakaguchi H¹, Ueyama T², Nakamura T¹, Morioka S^{1,2}, Ninoyu Y^{1,2}, Saito N², Hisa Y¹

¹Otolaryngology-Head and Neck Surgery : Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan, ²Laboratory of Molecular Pharmacology, Biosignal Research Center: Kobe University, Kobe, Hyoto, Japan

Several members of Rho GTPases are known to be expressed in the inner ear sensory epithelia. Each member of Rho GTPases is known to regulate specific actin structures in fibrocytes and neurons, suggesting that they could also contribute to organization of the unique actin structures in inner ear hair cells (HC), such as stereocilia and apical cell junctions. To get insights into the function of Rho GTPases in inner ear hair cells, we utilized gene-targeting strategy to generate several strains of HC-specific conditional knockout mice, and analyzed their hearing function and HC morphology. Although some of the strains did not show any phenotype in the inner ears, a strain carrying Cdc42-deletion showed progressive hearing loss associated with HC degeneration. HCs of Cdc42-KO mice developed normally but progressively degenerated after maturation, resulting in progressive hearing loss particularly for high frequencies. HC degeneration began as stereocilia fusion and depletion, accompanied with thinning and waving circumferential actin belt at apical junctional complexes. Cdc42-knockdown MDCK cells displayed phenotypes similar to Cdc42-deleted HC and down-regulated actin turnover that was represented by the enhanced levels of phospho-Cofilin. Our data demonstrated that Cdc42 plays important roles in maintenance but not development of stereocilia and apical junctional complex in HC by elaborate tuning of actin-turnover.

Localization-delocalization transition of tricellulin by occludin in the inner ear

Shin-ichiro Kitajiri^{1,2}, Tatsuya Katsuno¹, Hiroyuki Sasaki³, Kunihiro Nagao³, Juichi Ito¹, Mikio Furuse^{4,5} and Shoichiro Tsukita²

¹ Department of Otolaryngology Head and Neck Surgery, Kyoto University Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan.

² Department of Cell Biology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.

³ Department of Physical Therapy, Faculty of Community Health Care, Teikyo Heisei University, Uruido Minami, Ichihara, Chiba 290-0193, Japan

⁴ Department of Cell Biology, Division of Cell Biology, Kobe University Graduate School of Medicine, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan.

⁵ Division of Cerebral Structure, National Institute for Physiological Sciences, Okazaki 444-8787, Japan

Occludin is the first identified protein in tight junction (TJ), but its function has remained for the most part obscure. TJs have been demonstrated to play important roles in the inner ear function, and occludin is expressed in all the epithelial TJs in the inner ear. Thus, we examined the inner ears of occludin-deficient (*Occ*^{-/-}) mice. Although inner ears initially developed normally in *Occ*^{-/-} mice, apoptosis occurs in hair cells in the organ of Corti around day 12 after birth, and deafness developed. Since hair cell degeneration was not observed in cochlear explant cultures of *Occ*^{-/-} mice, environmental changes were considered to be the trigger of cell death. As for the vestibular system, both the morphologies and functions are normal in *Occ*^{-/-} mice. These phenotypes of *Occ*^{-/-} mice are very similar with those of claudin-14 or claudin-9 deficient mice, leading us to speculate the existence of imbalance induced by TJ abnormalities, such as localized ionic components. Moreover, the occludin deficiency led to dislocalization of tricellulin, a gene responsible for human deafness DFNB49. The deafness in *Occ*^{-/-} mice may be due to this dislocalization of tricellulin.

Human Basilar Membrane - An immunohistochemistry and electron microscopic study

Liu W¹, Atturo F^{1,2}, Santi P³, Glueckert R⁴, Pfaller K⁵, Schrott-Fischer A⁴ and Rask-Andersen H¹

¹Dept. of Surgical Sciences, Head and Neck Surgery, section of Otolaryngology, Uppsala University Hospital, Depts. of Otolaryngology, Uppsala University Hospital, Sweden, ²Dept. of Neurology, Mental Health and Sensory Organs, Otorhinolaryngologic Unit, Medicine and Psychology, Rome, Italy, ³Dept. of Otolaryngology, University of Minnesota, USA, ⁴Dept. of Otolaryngology, Medical University of Innsbruck, Austria, ⁵Dept. of Histology and Molecular Cell Biology, Institute of Anatomy and Histology, Medical University of Innsbruck, Austria.

Objectives: The molecular composition of the human basilar membrane (BM) is still unclear. Understanding cochlear micromechanics and frequency tuning depends on knowledge about macromolecular organization. **Materials and Methods:** We studied freshly obtained cochleae from patients undergoing meningioma surgery. Immunohistochemistry was performed on the cryo-sections, using various anti-human-antibodies. High resolution scanning (SEM) and transmission electron microscopy (TEM) were carried out in addition to laser-confocal microscopy. **Results:** The human BM consisted of four separate layers. 1. Epithelial basement membrane positive for laminin-β2 and collagen IV; 2. BM “proper” composed of radial fibers expressing collagen II; 3. Layer of collagen IV; 4. Tympanic covering layer expressing collagen IV, fibronectin and integrin. BM width (outer pillar region) ranged from 126 μm at high frequencies to 418 μm in the apex. Thickness varied both radially and along the spiral. BM was thinnest at the OHC region and laterally (mean values respectively 0.55 – 1.16 μm). Tympanic covering layer (TCL) thickness increased apically, but disappeared in the apex. **Conclusions:** Human BM consists of acellular layers of highly organized ECM molecules and cellular TCL with fibers expressing collagen IV and blood vessels. The BM width increased and thickness decreased from base to apex partly explaining reduction in stiffness. Findings suggest that The TCL layer may play a fundamental role for the assembly and maintenance of the ECM strata. It is speculated that a fibronectin/trans-membrane β-integrin receptor pathway in TCL can monitor ECM homeostasis and may be triggered in cochlear implantation leading to foreign body reaction, inflammation and fibrosis.

Lipid Droplets from Guinea Pig Hensen Cells are Protein Storage Organelles

Kalinec, GM¹, Lomberg, G², Thein, P¹, Parsa, A¹, Park, C¹, Urrutia, R² and Kalinec, F¹

¹Laboratory of Cell Structure and Function, Department of Head & Neck Surgery, David Geffen School of Medicine at UCLA, Los Angeles, California 90095, USA.

²Epigenetics and Chromatin Dynamics Laboratory, Translational Epigenomic Program, Center for Individualized Medicine (CIM) Mayo Clinic, Rochester, Minnesota 55905, USA.

The cytoplasm of Hensen cells (HCs), one of the most prominent supporting cells in the inner ear, is filled with numerous lipid droplets (LDs). We investigated the proteome of LDs isolated from guinea pig HCs with nano-LC-ESI-MS/MS, and the data was analyzed by searching in a guinea pig protein database with ProteinPilot, spectral counting-based quantification with QSpec, gene ontology classification, and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis. After eliminating resident proteins from other organelles, we identify a total of 309 LDs-associated proteins. We recognized 23 of them (7.4%) as associated with LD metabolism, 23 (7.4%) related to protein folding and processing, 71 (23.0%) to vesicular formation and maintenance, 111 (36.0%) to vesicular transport, and 81 (26.2%) to the cytoskeleton. Further studies with laser confocal and immune electron microscopy confirmed this association in selected proteins, and led us to conclude that: i) LDs have resident proteins and others that are cargo, with some of them probably being recycling products of other membranous compartments (e.g.: ER, endosomes, mitochondria); ii) some of these cargo proteins are in the surface but others are actually stored inside the lipid core of the droplets; iii) LDs are tightly associated with cytoskeletal as well as vesicular maintenance and transport proteins; iv) binding of the glucocorticoid dexamethasone to mineralocorticoid receptors in the surface of HCs induce signals that change the biophysical properties of the LDs surface, facilitating both their fusion and/or fragmentation and the release of cargo proteins. These responses could be crucial, among other functions, for immunological responses in the organ of Corti.

52

The human endolymphatic sac is the endocrine organ of the inner ear and produces multiple potent natriuretic hormones.

Martin Nue Møller¹, Svend Kirkeby², Jonas Vikeså³, Finn Cilius Nielsen³, Per Cayé-Thomasen^{1,4}

¹ Dept. Of Oto-rhino-laryngology, head and neck surgery, Rigshospitalet DK – 2100 Copenhagen Denmark

² Department of Oral Medicine, Dental School, Panum Institute, University of Copenhagen, Copenhagen, Denmark

³ Center for Genomic Medicine, University of Copenhagen, Rigshospitalet, DK - 2100 Copenhagen, Denmark

⁴ The Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

OBJECTIVES/HYPOTHESIS: The purpose of the present study is to explore, demonstrate and describe the expression of genes related to natriuretic peptide activity in the human endolymphatic sac. It is hypothesized, that the endolymphatic sac is the endocrine organ of the human inner ear, and is responsible for regulation of endolymphatic fluid homeostasis.

STUDY DESIGN: DNA micro-arrays and immuno-histochemistry were used for analyses of fresh human endolymphatic sac tissue samples.

METHODS: Twelve tissue samples from the human endolymphatic sac were obtained during translabyrinthine surgery for vestibular schwannoma. Microarray technology was used to investigate tissue sample gene expression, using adjacent dura mater as control. The expression of genes specific for natriuretic hormonal activity was explored and results for selected key molecules verified by immuno-histochemistry.

RESULTS: A comprehensive overview of expressed genes related to hormonal natriuretic activity was obtained. Multiple key natriuretic peptides were expressed in the arrays. Immuno-histochemical verification included peptides: uroguanylin, brain natriuretic peptide, c type natriuretic peptide and intermedin.

CONCLUSIONS: The present data provides the first direct evidence of a substantial endocrine capacity of the human endolymphatic sac. The ES expresses and secretes potent natriuretic molecules, likely in a paracrine manner. The evidence strongly supports the role of the endolymphatic sac in the regulation of endolymphatic fluid homeostasis, which closely resembles that of the water regulatory function of the kidney. Additionally, it is possible that the human ES plays a role in intracranial pressure regulation.

53

Further Characterization of the Striated Organelle and Apical Mitochondria in Rat Inner Ear Hair Cells

Anna Lysakowski, Steven D. Price and Robstein L. Chidavaenzi

Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL, USA.
a.lysakow@uic.edu

We have been characterizing the striated organelle, a cytoskeletal specialization found in the apical region of hair cells [Vranceanu et al., PNAS 109 (12), 4473-4478, 2012]. This organelle interacts with stereociliar rootlets and mitochondria and may thus affect mechanotransduction. We used confocal, EM immunogold and EM tomography methods, coupled with Western blots, co-immunoprecipitation and mass spectrometry. We have previously described the localization of α 2- and β 2-spectrin in the striated organelle (SO). Recently, we found that nebulin, the largest known actin-bundling protein (600-900kDa), is closely associated with the SO. Each nebulin molecule can bind 200 actin monomers. In vertebrate skeletal muscle, nebulin has been investigated for its role in determining the length of actin filaments and in regulating actin-myosin interactions in a Ca²⁺-calmodulin dependent manner. Hence an integral role in various cytoskeletal assemblages is implied. We sought to determine its presence and distribution in inner ear auditory and vestibular hair cells. Nebulin is co-extensive with, but extends beyond, the two spectrin isoforms in both the cuticular plate and SO. We have also been examining the relationship of subcuticular mitochondria, which are especially large in central type I hair cells, to the striated organelle. Some are polarized toward the SO and stereociliar rootlets, which is intriguing and we are attempting to further characterize mitochondria in hair cells. In conclusion, the striated organelle and associated elements continue to be fascinating structures.

Acknowledgements: This work was sponsored by a Knowles Center for Hearing Research Collaborative Grant and the Amer. Hearing Research Fndn. We thank Drs. Jose Antonio López Escámez and Maria Teresa Requena Navarro for steering us toward nebulin and Mr. Steven D. Price for excellent technical assistance.

Targeting of prestin and Slo to the basolateral surface: hair cells are epithelial and not neuronal

Dhasakumar Navaratnam^{*,^}, Iman Moeini-Naghani⁺, Jun-Ping Bai⁺, Yifan Zhang⁺, and Joseph Santos-Sacchi^{^*#}.

Depts of Neurology⁺, Neurobiology^{*}, Surgery[^] and Cell and Molecular Physiology[#], Yale School of Medicine, New Haven, CT.

In polarized epithelial cells proteins are segregated to the basolateral and apical ends of the cell that results in a segregation of function. This segregation of proteins occurs by sorting of proteins after exit from the Golgi. Since neurons demonstrate a similar segregation of function, it has been proposed that dendritic and axonal compartments are analogous to the basolateral and apical surface respectively of polarized epithelial cells. Hair cells however, pose a dilemma since they have features of both epithelial cells and neurons. Critically, the dendritic and axonal ends of a hair cell are at opposite ends to the expected basolateral and apical ends of the cell. Thus mechanosensitive channels that serve as its receptors are present in stereocillia and not at the basolateral surface as would be expected by its dendritic extrapolation. Similarly, the synaptic apparatus of inner hair cells is located at the basal pole and not at the stereocilliary apical end as would be expected by its axonal extrapolation.

Prestin, the outer hair cell motor, and BK channels, which are important for determining inner and outer hair cell membrane potentials, are located along the basolateral surface of hair cells. Using MDCK cells as a model system we show that these proteins are targeted to the basolateral surface of MDCK cells. Additionally, these proteins use different motifs to target the basolateral surface of these cells. Thus, prestin uses a tyrosine residue and BK (Slo) uses a dileucine motif to achieve basolateral localization. Moreover, prestin is dependent on the clathrin adapter protein AP1mu1B to localize to the basolateral surface while Slo is not. Together with recent data showing disordered hair cell function in AP1mu1B knockouts in Zebrafish, these data suggest that hair cells are more analogous to polarized epithelial cells than neurons.

Constitutive G_{ai} coupling activity of VLGR1 and its regulation by PDZD7

Qiao-Xia Hu^{1#}, Jun-Hong Dong^{1#}, Hai-Bo Du^{2#}, Dao-Lai Zhang¹, Hong-Ze Ren², Ming-Liang Ma¹, Yuan Cai³, Tong-Chao Zhao¹, Xiao-Lei Yin², Xiao Yu¹, Tian Xue³, Zhi-Gang Xu^{2*}, Jin-Peng Sun^{1*}

¹Shandong University School of Medicine, Jinan, Shandong 250012, China;

²Shandong University School of Life Sciences, Jinan, Shandong 250100, China;

³School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, China

VLGR1, the largest G protein-coupled receptor, is a core component of inner ear hair cell ankle-links. Mutations of *VLGR1* gene affect ankle-links, resulting in hearing loss. However, the mechanism of VLGR1-regulated intracellular signaling and its role in hearing transduction remain elusive. Here, we show that VLGR1 is processed into two fragments after auto-cleavage at the G-protein-coupled receptor proteolytic site (GPS site). The cleaved VLGR1- β subunit constitutively inhibited adenylate cyclase (AC) activity through G_{ai} coupling. R6002A mutation in intracellular loop 2 (ICL2) of VLGR1 abolished G_{ai} coupling, but the pathogenic VLGR1 Y6236fsx1 mutant showed increased AC inhibition. Furthermore, overexpression of PDZD7, a binding partner of VLGR1, decreased the AC inhibition of the VLGR1- β subunit but showed no effect on the VLGR1 Y6236fsx1 mutant. Taken together, we identified an independent G_{ai} signaling of the VLGR1- β subunit and its regulatory mechanisms, which may have a role in the development of hearing loss.

The Role of TRIOBP in Stereocilia Rootlets Formation

Tatsuya Katsuno¹, Kohei Yamahara¹, Tomoko Kita¹, Takeshi Sakamoto³, Kazuya Ono², Kohei Segawa¹, Juichi Ito¹, Shin-ichiro Kitajiri¹

¹Dept. of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine Kyoto University, Kyoto, Japan, ²Lab of Molecular Biology, NIDCD, Bethesda, MD, USA, ³Dept. of Physics and Astronomy, Wayne State University, Detroit, MI 48201, USA.

Inner ear hair cells detect sound through deflection of mechanosensory stereocilia. Each stereocilium is supported by a paracrystalline array of parallel actin filaments that are packed more densely at the base, forming a rootlet extending into the cell body. TRIOBP, a cytoskeleton-associated protein mutated in human hereditary deafness DFNB28, is localized to the stereocilia rootlets in inner ear hair cells. TRIOBP have three major isoforms (abbreviated as T1, T4 and T5). Then, T4/T5 double deficient mice show profoundly deaf. Stereocilia of T4/T5 deficient mice develop normally, but fail to form rootlets and are easier to deflect and damage. It indicates that F-actin of rootlets bundling by TRIOBP provide durability and rigidity for normal mechanosensitivity of stereocilia. Although it has been suggested that each isoforms have their own actin cytoskeleton regulation mechanism, the exact mechanism and function of each isoforms remains obscure. To investigate the role of each TRIOBP isoforms, we generated isoform specific mutant mice. One of them, the T5 deficient mouse became severe hearing loss, but not deaf. It's also suggested that each isoforms have different function. Here we investigate the role of TRIOBP isoform in vivo and in vitro.

We generated T5 specific deficient mice and T1 conditional knockout mice. To evaluate molecular interaction of TRIOBP, we performed biochemical studies such as the fluorescence size exclusion chromatography assay or immune-precipitation.

T5 deficient mice phenotypes are as follow. • Severe Hearing loss. • Stereocilia Rootlets hypoplasia. • Loss of TRIOBP-1 localization at stereocilia rootlets. And from biochemical study, we had the results which indicate that each isoforms make homo oligomer.

We are currently promoting the analysis of molecular mechanisms of TRIOBP. We would like to introduce the results.

Auditory Characteristics in Mouse Model of the Sclerosteosis

Moon IJ¹, Kim KR¹, Cho YS¹, Chung WH¹, Jin DK², Hong SH¹

¹Department of Otorhinolaryngology-Head and Neck Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

²Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

Objectives Sclerosteosis, caused by a mutation of the sclerosteosis gene (SOST, 17q12-21), is a very rare autosomal-recessive disorder characterized by progressive bone overgrowth. Although otolaryngologic manifestations in sclerosteosis patients include progressive hearing loss, the auditory characteristics of sclerosteosis have not been well known. Thus, we aimed to evaluate the auditory characteristics of the sclerosteosis using SOST knock-out (KO) mice.

Methods The SOST-KO mouse model was developed by Dr. Jin DK. Auditory brainstem response, micro-computed tomography (CT) scans of the temporal bone, and histologic analysis of the cochlea were performed in 19 SOST knock-out and SOST wild type (WT) mice (B6/129) from 5 to 25 weeks of age.

Results The SOST-KO mice showed clear discernable phenotype compared with the SOST-WT mice, including short stature, curved spine, and syndactyly. The hearing thresholds for the SOST-KO mice were significantly elevated compared to the SOST-WT mice after 9 weeks of age. Both skull bone thickness and temporal bone volume were significantly increased in the SOST-KO mice at 25 weeks of age. Histologic findings indicated that inner ear structures, such as organ of Corti, spiral ganglions, and stria vascularis, were intact in the SOST-KO mice. However, proliferation of osteoblast in periosteal layer and subsequent new bone formation was observed around the stapes footplate in the SOST-KO mice. Furthermore, cellular proliferations in the malleoincudal joint were also found in the SOST-KO mice.

Conclusions The SOST-KO mouse can be a useful model in understanding auditory features of sclerosteosis. Ossicular fixation caused by increased osteoblastic activities may be responsible for conductive hearing loss in sclerosteosis.

Cochlear pathology contributes to hearing loss in vestibulo-cochlear schwannomas and allows for new diagnostic evaluation and treatment

M. Nue Møller¹, S. Hansen¹, H. Miyazaki¹, P. Caye-Thomasen^{1,2}

1. Dept of oto-rhino-laryngology, head and neck surgery Rigshospitalet/Gentofte, Copenhagen Denmark

2. Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Introduction: The most frequent symptom of vestibulo-cochlear schwannomas (VS) is sensorineural hearing loss. Commonly, this is believed to be caused by a retrocochlear mechanism although indications of contributory cochlear pathology often are present e.g. by lacking otoacoustic emissions in these patients. Only very few studies have assessed this issue by demonstrating cochlear degeneration in temporal bone studies of vestibulo-cochlear schwannomas.

This study aims to present the histo-pathological results of the Rigshospitalet/Gentofte temporal bone collection regarding vestibulo-cochlear schwannomas. By focusing on the cochlear histopathology, it may allow us to better understand the mechanisms of hearing loss in these patients and subsequently develop new strategies for diagnostic evaluation and treatment.

Methods: A retrospective analysis of temporal bone histopathology.

Results: In 19 sporadic unilateral vestibulo-cochlear schwannomas we found that VS caused significantly more inner and outer hair cell loss compared with the contralateral ear. Further we found significant cochlear neuronal loss, typically in a basal to apical gradient. Tumor size and nerve of origin did not correlate with structural changes in the cochlea.

Conclusion: The cochlear degeneration seen in patients with vestibulo-cochlear schwannoma may contribute significantly to sensorineural hearing loss and may be responsible for the initial threshold drop. The recent development of per-operative cochlear monitoring during VS surgery sets a new future scenario, as an electrical stimulation of the cochlea – as opposed to the present acoustic – may prove that multiple patients, who would otherwise be scheduled for standard translabyrinthine transection, are eligible for cochlear implantation.

Evaluation of Hearing Aid's Success Probability Based on Aided Auditory Steady State Response Thresholds

Sardari Sara¹, Sameni Seyed Jalal¹, Jafari Zahra²

¹ Audiology department, Rehabilitation Faculty, Tehran University of Medical Sciences, Tehran, Iran. Email : sardarisara@yahoo.com

² Department of Basic Sciences in Rehabilitation, Faculty of Rehabilitation, Tehran University of Medical Sciences, Tehran, Iran. Email: zahra.acn@googlemail.com

Background and Aim: Some authors believe that aided thresholds can be used to predict hearing aid success and to decide between hearing aid and cochlear implant. The aim of this study was to evaluate if the ASSR could anticipate the hearing aid success; and to compare characteristics of aided and unaided responses in the unifrequency versus simultaneous presentation modes.

Method: 10 normal hearing and 26 severe to profound hearing impaired subjects participated in this study. The subjects underwent acoustic immittance, sound field behavioral audiometry and auditory steady state response, at first without hearing aid and then with it.

Results: Despite observing any response without hearing aid, aided thresholds, however, could be recorded in some frequencies. Aided responses were recorded in fewer frequencies in subjects with poorer speech clarity and speech reading. Multifrequency stimulation did not get the ASSR response affected in the moderate intensities (about 60 dB HL) or in the normal subjects, but it did elevated the thresholds in the higher presentation levels, especially for the higher frequencies. This elevation was more prominent in the aided condition.

Conclusion: The probability of hearing aid success seems to be very poor if auditory steady state responses (especially aided responses) could not be recorded. Simultaneous presentation can make the response of the higher frequency regions of the basilar membrane to be interfered with the lower frequency parts (probably due to unnatural tuning curves in the damaged cochlea); and so, threshold overestimation. In conclusion, special care should be taken about hearing aid's settings and its fitting, testing environment and response repeatability for the aided evaluations.

Phonological loop activation by maintenance of pseudo-words with auditory and visual presentation in functional magnetic resonance imaging

Kanazawa Y¹, Yamazaki H¹, Aso T², Ishii T², Nakamura K², Fukuyama H², Ito J¹

¹Department of Otolaryngology-Head and Neck Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan, ²Human Brain Research Center, Kyoto University Graduate School of Medicine, Kyoto, Japan

Phonological loop, a component of working memory, is a capacity to store verbal information in memory and rehearse in mind for the information not to decay with time. This capacity is important for new long-term phonological learning such as the acquisition of both native and second-language vocabulary. Congenital deafness may affect normal development of normal circuits processing auditory information, which may result in the different use of brain lesions during phonological working memory. Our goal is to clarify the difference in brain activation related to phonological working memory between normal hearing and deaf people. As the first step to the goal, in the present study, we explored this brain activation of normal hearing subjects with functional magnetic resonance image (fMRI). Eight normal hearing subjects were instructed to listen to or read pseudo-word consisting of 9 syllables (e.g., sa-ta-na-mu-ma-yo-se-chi-yu) (encoding phase) and rehearse it in mind during an interval of 9 seconds (maintenance phase), then listen to or read either an identical stimulus (e.g., sa-ta-na-mu-ma-yo-se-chi-yu) or one in which a single syllable was changed (e.g., sa-ta-na-mu-ma-yo-se-ke-yu) and decide a same/different judgment (comparison and decision phase). We evaluated brain activities during the maintenance phase with fMRI to investigate brain regions associated with phonological loop. In both of listening and reading, the left supplementary motor area, left pre-central gyrus, left inferior frontal gyrus, and left superior temporal gyrus were activated. These results suggested that pseudo-words maintenance through listening and reading might similarly activate the phonological loop in normal hearing subjects.

Evaluation of cerebral hemodynamic responses during phonological working memory tasks using functional near-infrared spectroscopy (fNIRS)

Hiroshi Yamazaki, Harukazu Hiraumi, Yuji Kanazawa, Juichi Ito

Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Objectives: Congenital deaf children who underwent cochlear implantation are good targets for studying influence of deafness on maturation of neural circuits processing verbal or non-verbal stimuli, but measuring brain activities is difficult in this population due to restricted use of MRI. The aim of this study is to establish a method to evaluate brain activities during phonological working memory (PWM) tasks with auditory or visual presentation using functional near-infrared spectroscopy (fNIRS) which can be used in deaf children with a cochlear implant.

Methods: 8 adults with normal hearing were recruited. In the PWM trials, combinations of a nine syllabic pseudo-word and the same or 1-syllable mismatched pseudo-word were auditory or visually presented and the subjects decided if the two words were identical or not in a forced two-choice paradigm. Cerebral hemodynamic responses during the PWM tasks were measured using fNIRS and the responses during the decision were compared to baseline signals in the event-related design.

Results: In both PWM tasks, hemodynamic responses in the ventrolateral frontal lobe increased bilaterally during the decision, suggesting activation of the Broca's area in the left hemisphere and its homologue in the right hemisphere. Interestingly, hemodynamic responses in the occipital lobe decreased in the auditory presented task, but increased in the visually presented one.

Conclusion: These data suggested that fNIRS may be useful to monitor brain activities during PWM tasks. The difference in hemodynamic responses in the occipital cortex may be caused by a different neural processing between the auditory and visually presented tasks.

Marlies Knipper

¹Department of Otolaryngology, Head and Neck Surgery, Hearing Research Centre Tübingen, Molecular Physiology of Hearing, University of Tübingen, Elfriede-Aulhorn-Straße 5, 72076 Tübingen, Germany

Hearing disorders (Age- and Noise dependent hearing loss, Tinnitus or Hyperacusis) are expected to increase over the next decades due to demographic changes and altered leisure behavior. We here summarize the current knowledge of different cochlear damage profiles in the context of different brain responses and possible resulting auditory disorders. On the basis of this current knowledge new possible research directions and requests for future medical and industrial research profiles are suggested.

Acknowledgements.

This work was supported by the Marie Curie Research Training Network

CavNET MRTN-CT-2006-035367, the Deutsche Forschungsgemeinschaft DFG-Kni-316-4-1 and Hahn Stiftung (Index AG).

Takashi Ueyama¹, Tomohiro Donishi², Satoshi Ukai³, Kazuhiro Shinosaki³, Muneki Hotomi⁴, Yorihiro Ikeda⁴, Shunji Tamagawa⁴, Masaki Terada⁵, Noboru Yamanaka⁴, Yoshiki Kaneoke²

¹Department of Anatomy and Cell Biology, Graduate School of Wakayama Medical University, Wakayama, Japan

²Department of System Neurophysiology, Graduate School of Wakayama Medical University, Wakayama, Japan

³Department of Neuropsychiatry, Graduate School of Wakayama Medical University, Wakayama, Japan

⁴Department of Otolaryngology-Head and Neck Surgery, Graduate School of Wakayama Medical University, Wakayama, Japan

⁵Wakayama-Minami Radiology Clinic, Wakayama, Japan

Abnormal functionally connected regions in the brain might underlie the pathophysiology of chronic subjective tinnitus. In this study, we used resting-state functional MRI (fMRI) and measured regional mean functional connectivity strength (mean cross-correlation coefficient between a region and all other regions (*rGCa*) to elucidate brain regions related to tinnitus symptoms such as distress, depression and loudness. Positive correlations between the *rGCa* and distress and depressive state were observed in the bilateral rectus gyri and negative correlations were observed in the bilateral anterior and middle cingulate gyri. Positive correlations between the *rGCa* and loudness were found in the bilateral thalamus, the bilateral hippocampus, and the left caudate and negative correlations were found in the left medial superior frontal gyrus and the left posterior cingulate gyrus. These results suggest that each tinnitus symptom is related with distinct brain regions. The regions for distress and depressive state are known to be related to emotion, while the regions for tinnitus loudness are known to be related to the default mode network and integration of multi-sensory information. Neuroimaging will provide a new potential for diagnosis and understanding of tinnitus.

The efficacy of tinnitus counselling compared to Cognitive-Behavioral Therapy on tinnitus

Hee-Jeong Jeong¹, MD, Se-Joon Oh, MD²

¹Department of Psychiatry, Pusan National University School of Medicine, ²Department of Otorhinolaryngology-Head and Neck Surgery, Pusan National University School of Medicine and Medical Research Institute, Busan 602-739, Republic of Korea

Objective : Cognitive-Behavioral Therapy(CBT) is one of the most widely used and accepted psychological strategies for coping with tinnitus. The goal of the therapy is to alter maladaptive cognitive, emotional, and behavioral responses to tinnitus and not to abolish the sound itself. But there are some problems such as a limited counselling time. So we try to use tinnitus counselling as an alternative of CBT.

Method : Prospective comparison between CBT group(n=10) and tinnitus counselling group(n=10). tinnitus counselling group was composed of a teaching paper to change patient's behaviors and beliefs about tinnitus. Its analysis was done by THI score. Treatment duration was 3 months.

Result : THI score of CBT group was from 56 to 31, and simply modified group was from 51 to 30. And there were no statistical difference between them.

Conclusion : tinnitus counselling could be an alternative of original CBT.

Dysfunctional Noise Cancelling of the Rostral Anterior Cingulate Cortex in Tinnitus Patients

Jae-Jin Song¹, Sven Vanneste², Jeong Hun Jang³, Dirk De Ridder⁴

1. Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, Korea, 2. School of Behavioral and Brain Sciences, The University of Texas at Dallas, USA, 3. Department of Otorhinolaryngology, Kyungpook National University College of Medicine, 4. Unit of Neurosurgery, Department of Surgical Sciences, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand.

Objectives: Peripheral auditory deafferentation and central compensation have been regarded as the main culprit of tinnitus generation. However, patient-to-patient discrepancy in the range of the percentage of daytime in which tinnitus is perceived (tinnitus awareness percentage, 0 – 100%), is not fully explicable only by peripheral deafferentation, considering that the deafferentation is a stable persisting phenomenon but tinnitus is intermittently perceived in most patients. Consequently, the involvement of a dysfunctional noise cancellation mechanism has recently been suggested with regard to the perception of tinnitus. By correlating the tinnitus awareness percentage with resting-state source-localized electroencephalography findings, we may be able to retrieve the cortical area that is negatively correlated with tinnitus awareness percentage, and then the area may be regarded as the core of the noise cancelling system that is defective in patients with tinnitus.

Methods: Using resting-state cortical oscillation, we investigated 80 tinnitus patients by correlating the tinnitus awareness percentage with their source-localized cortical oscillatory activity and functional connectivity.

Results: The activity of bilateral rostral anterior cingulate cortices (ACCs), left dorsal- and pregenual ACCs for the delta band, bilateral rostral/pregenual/subgenual ACCs for the theta band, and left rostral/pregenual ACC for the beta 1 band displayed significantly negative correlations with tinnitus awareness percentage. Also, the connectivity between the left primary auditory cortex (A1) and the rostral ACC, as well as between the left A1 and the subgenual ACC for the beta 1 band, were negatively correlated with tinnitus awareness percentage.

Conclusions: These results may designate the role of the rostral ACC as the core of the descending noise cancellation system, and thus dysfunction of the rostral ACC may result in perception of tinnitus. The present study also opens a possibility of tinnitus modulation by neuromodulatory approaches targeting the rostral ACC.

Toward an Objectification of Tinnitus: Machine Learning Approach of Resting-State Cortical Oscillation Pattern Can Detect the Presence of Tinnitus

Sven Vanneste¹, Dirk De Ridder², Jae-Jin Song³

1. School of Behavioral and Brain Sciences, The University of Texas at Dallas, USA, 2. Unit of Neurosurgery, Dept. of Surgical Sciences, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand, 3. Dept. of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, Korea.

Objectives: Non-pulsatile tinnitus, a perception of sound in the absence of an external sound source, is a purely subjective symptom as it can only be observed by the person who suffer from tinnitus. It would be highly desirable to diagnose the presence or absence of tinnitus in an objective way. Recently, scientists have developed machine learning techniques that can learn to recognize patterns by classifying seen data, taking into account their statistical variation. These algorithms can subsequently be applied to unseen data. In other words, based on the known properties learned from the trained data, these algorithms can predict whether the pattern corresponds to the presence or absence of tinnitus. We therefore combined resting-state quantitative electroencephalography (rs-qEEG) with machine learning to develop a brain-based electrophysiological signature for the presence or absence of tinnitus.

Methods: One hundred and fifty-three tinnitus patients and 264 healthy controls underwent rs-qEEG measurements for 5 minutes. These data were used as training sets, and the predictability of the presence of tinnitus was trained using a support vector machine. Regions of interest were the auditory cortex, dorsal- and subgenual anterior cingulate cortex, posterior cingulate cortex, parahippocampus, and insula.

Results: Using the support vector machine, the current yielded better predictive results than using Bayesian inference learning, with a correct predictability of approximately 90%. In other words, presence of tinnitus could be predicted only by rs-qEEG findings with a correct predictability of 90%.

Conclusions: These results suggest that it might become possible to diagnose the presence or absence of tinnitus based solely on an EEG oscillatory signature in the near future.

Modeling of Human Active Cochlea using Finite-Element Method: Simulation of DPOAEs

Takuji Koike¹, Hideyuki Mochizuki¹, Tasuku Sakashita¹

¹The University of Electro-Communications, Chofu, Tokyo, Japan

Otoacoustic emissions (OAEs) are considered to be derived from the active nonlinear behavior of the outer hair cells (OHCs). However, the mechanisms of the OAEs have not been fully clarified, and the relationship between cochlear function and OAEs is still unclear. In this study, a three-dimensional finite-element model of the human cochlea was created considering the non-linear activity of the OHCs, and the mechanism of the development of distortion product otoacoustic emissions (DPOAEs) was investigated.

The cochlear model consists of structural parts and liquid parts. The structural parts are the stapes, the stapedial annular ligament, the round window membrane, the basilar membrane (BM), and the osseous spiral lamina. The liquid parts are the vestibule, the scala vestibuli, the scala tympani, and the cochlear aqueduct, which are all filled with lymphatic fluid. The active cochlear mechanism of amplification related to the electromotility of the OHCs was included by applying excitation force to the BM according to its vibration velocity. Two tones with frequencies of f_1 and f_2 were simultaneously applied to the stapes, and distortion components generated on the BM and the stapes were calculated.

The following results were obtained. The DP component ($2f_1 - f_2$) is observed in a broad area on the BM and is not localized. The portion where the DP component reaches the maximum is located at the more apical side on the BM comparing to the portion characteristic frequency of which is $2f_1 - f_2$. The activities of the OHCs at the more basal part comparing to the maximum DP part has a critical role to generate DPOAEs.

The Use of a Fast Method of Recording Schroeder Phase Masking Function for Measuring Nonlinear Cochlear Function.

Rahmat, S.^{1,2}, O'Beirne, G.A.¹

¹University of Canterbury, Christchurch, New Zealand, ²International Islamic University Malaysia, Kuantan, Malaysia

Background: A difference in masked thresholds at different scalar factors of the Schroeder masker (the "phase effect") can be observed in normal hearing listeners (Lentz & Leek, 2001). This was believed to be due to intact cochlear non-linearity, but recent findings have suggested a possible contribution of a more central mechanism, presumably the medial olivary cochlear reflex (MOCR) (Wojtczak & Oxenham, 2010). We have recently developed a fast method for recording Schroeder phase masking functions which takes 8 minutes of testing time compared to 45 minutes using the conventional 3 AFC method. Our aim was to use this new method to study the mechanism underlying the phase effect.

Methods: 15 normal hearing and 15 sensorineural hearing loss (SNHL) participants were tested. Schroeder phase masking functions were measured at 250 Hz, 500 Hz, 1 kHz, 2 kHz at 45 dB and 75 dB masker levels in all participants, using both on-frequency and off-frequency masking.

Results: Phase effects were significantly reduced at low presentation levels and in participants with SNHL as compared to normal hearing participants ($p < 0.05$), consistent with reduction of cochlear nonlinearity in those conditions. The phase effect was present in off-frequency masking and was significantly larger at 1 kHz and 2 kHz than at 500 Hz ($p < 0.05$), supporting potential involvement of the MOCR which is active in off-frequency listening and shows strong effects at frequencies ≥ 1 kHz.

Conclusion: The findings were consistent with previous studies, suggesting that our fast method is a useful tool for recording Schroeder phase masking functions and investigating cochlear nonlinearity and the potential involvement of the MOCR in creating the phase effect, specifically at higher frequencies.

Otoacoustic Emissions and Hearing Functionality in Patients Affected by Neurodegenerative Diseases

Sisto R.¹, Pisani V.^{2,3}, Moleti A.³, Di Girolamo S.³, Mazzone S.³, Di Mauro R.³, Botti T.¹, Sanjust F.¹, Cerini L.¹, Tirabasso A.¹

¹INAIL Research, Monteporzio Catone, Rome, Italy

²I.R.C.C.S. "Santa Lucia" Foundation, Rome, Italy

³University of Rome "Tor Vergata", Rome, Italy

Objective

Objective of this study is the investigation of cochlear functionality in patients suffering from Central (CNS) and Peripheral Nervous System (PNS) degenerative disorders: Parkinson (PD) and Charcot-Marie-Tooth (CMT). Distortion Product Otoacoustic emissions (DPOAEs) are simple, objective, quantitative and non-invasive biomarkers of healthy cochlear function.

Methods

Neurological patients and age-sex matched control healthy subjects (CTRL), were enrolled. DPOAEs have been recorded alternatively with or in absence of a contralateral suppression stimulus of 80 dB (CAS). High-resolution DPOAE spectra have been measured using an advanced chirp technique. A suitable time-frequency wavelet analysis has been applied to separate DPOAE components.

Results

Statistical differences were found between PD and CTRL groups suggesting a degradation of cochlear functionality at peripheral level in these primarily CNS patients. A reduced activity of a central pathway, the CAS, has been found in patients affected by CMT, a model of PNS degenerative disorder.

Conclusions

Hearing functionality objective analysis can be an important research topic for revealing hidden pathogenetic mechanisms at the basis of CNS and PNS degenerative disorders.

Frequency Tuning and Phase-locking Estimated from Cochlear Potentials in Normal Hearing Human Volunteers

Verschooten E¹, Desloovere C², Joris PX¹

¹Lab. of Auditory Neurophysiology, KU Leuven, Leuven, Belgium, ²Dept of Otorhinolaryngology, Head and Neck Surgery, KU Leuven, Leuven, Belgium

Two fundamental cochlear properties are to separate sounds into their constituent frequencies, and to encode their time waveforms. While some degree of frequency selectivity and phase-locking is present in most cochleas, the limits of these processes vary considerably between species and are disputed for humans. Our aim was to obtain objective, electrophysiological measurements to estimate frequency selectivity and phase-locking in humans with normal hearing.

We developed a technique to record cochlear potentials in healthy volunteers. The eardrum was locally anesthetized, and a needle electrode was placed transtympanically on the cochlear promontory or in the niche of the round window. To estimate frequency selectivity, we measured compound action potentials (CAPs) to tone bursts that were forward-masked with notched-noise. To measure phase-locking, we measured AC potentials ("cochlear microphonics") to tones, and used a combination of forward masking and polarity reversals to disambiguate neural generators from hair cell generators. The stimulus and analysis paradigms were extensively tested in anesthetized experimental animals.

We found that frequency selectivity, quantified with the quality factor Q_{10} , is sharper in humans than in macaque monkeys, cats, and chinchillas. However, the limit of phase-locking is somewhat lower in humans than in the laboratory species studied. The general trend in the data is in accord with our data from single auditory nerve fibers in these same laboratory species.

We conclude that the human cochlea differs from commonly studied species both in the time and frequency domain: the frequency extent of phase-locking is more restricted, while frequency tuning is sharper.

Cochlear Response In A Model Of Middle Ear Surgery

Bergin MJ¹, Bird PA², Vljakovic SM¹, Thorne PR¹

¹University of Auckland, Auckland, New Zealand, ²University of Otago, Christchurch, New Zealand

Permanent cochlear sensorineural hearing loss following middle ear surgery occurs in up to a third of patients. The aetiology of this loss is poorly understood and may involve transmission of supra-physiological forces down the ossicular chain to the cochlea. We are investigating the mechanisms of cochlear injury using guinea-pigs, but evaluating cochlear function with evoked potentials is challenging when middle ear surgery disrupts the normal air-conduction pathway. Traditional bone-conduction is limited by poor transducer output at high frequencies sensitive to trauma in small animals. Here we used a high-frequency magnetostrictive bone-conducting transducer to evaluate the auditory brainstem response (ABR) in guinea pigs before and after middle ear surgical intervention.

The stapes was coupled to a motorized micromanipulator and displaced by varying depths, speeds and iterations to simulate contact with the ossicular chain during middle ear surgery. A second model of injury consisted of a rotating bur brought into contact with the ossicular chain. The influence of bur rotation speed and duration of contact were investigated.

ABR thresholds deteriorated with surgical exposure of the stapes head, particularly in the high frequencies, but showed some recovery over the following hours. Micromanipulator displacements did not appear to cause acute sensorineural injury. Brief ossicular contact with a slowly rotating bur appeared to be tolerated by the cochlea, however, longer contact or a high speed bur caused significant threshold elevation.

The guinea pig cochlea can tolerate frequent and sizable displacements of the stapes footplate without apparent sensorineural reserve impairment. This may be a limitation of the micromanipulator used, as higher energy intensity manipulations with a rotating bur did cause injury. Our ongoing research explores ways in which surgical injury may be reduced and the cochlea protected during middle ear surgery.

Programmed Cell Senescence in Inner Ear Development and Ageing

Isabel Varela-Nieto¹, Alejandro Gibaja¹, Adelaida Celaya¹, Rocío de Iriarte¹, Marta Magariños¹, Jose M Zubeldia^{1,2}, Silvia Murillo-Cuesta¹ and Julio Contreras^{1,3}

¹Institute for Biomedical Research “Alberto Sols”, CSIC-UAM, IdiPAZ, CIBERER. Madrid, Spain

²Hospital GU Gregorio Marañón, Universidad Complutense de Madrid, Madrid, Spain

³Veterinary School, Universidad Complutense de Madrid, Madrid, Spain

Senescence is a cellular program relevant for ageing by which damaged cells in adult tissues stop proliferation. During development senescence has been proposed to serve tissue remodeling as an alternative or complement to apoptotic cell death and to be regulated by the TGF β /SMAD and PI3K/FOXO pathways (Muñoz-Espín et al., 2013). Insulin like growth factor 1 (IGF-1) regulates neurogenesis during development, late neuronal differentiation in the postnatal cochlea and neuronal survival during ageing. IGF-1 levels decrease with ageing being IGF-1 deficiency associated with hearing impairment (Varela-Nieto et al., 2013). Human and mouse embryos present senescence markers at the developing endolymphatic sac. Developmental senescence is strictly dependent on p21 and detectable developmental abnormalities occur in the absence of p21. By using a combination of neurophysiological techniques, immunohistochemistry, Western Blotting, RT-qPCR, ELISA and otocyst cultures, we have shown the presence of senescence during inner ear development in chicken and mouse embryos. The regulation by IGF-1 of cellular senescence during ageing will be discussed.

This work was supported by grants from Spanish SAF2011, FP7 AFHELO and TARGEAR.

References

Muñoz-Espín et al., Cell. 2013, 155(5):1104-18.

Varela-Nieto I et al., *Pediatr Endocrinol Rev.* 2013, 10(4):460-72.

Live Cochlear Explant Imaging Reveals Reorganization Processes Underlying Robust Development of the Organ of Corti

Amir L¹, Hersch M², Bhonker Y³, Chen P⁴, Matsuzaki F⁵, Avraham KB³, Sprinzak D¹

¹Department of Biochemistry and Molecular Biology, Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; ²Department of Medical Genetics and the Swiss Institute of Bioinformatics, CH-1015 Lausanne, Switzerland;

³Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel; ⁴Department of Cell Biology, School of Medicine, Emory University, Atlanta, GA, USA; ⁵Laboratory for Cell Asymmetry, RIKEN Center for Developmental Biology, Japan

During its development, the organ of Corti undergoes a transition from an undifferentiated disordered tissue to the precisely patterned rows of intercalated hair and supporting cells. This transition involves spatially coordinated differentiation of cells as well as dramatic changes in the morphology of the whole tissue and of the individual cells. While some of the regulatory processes involved have been elucidated, we still lack a systems level understanding of how differentiation processes and cellular mechanics coordinate this transition.

Here, we combine live explant imaging with mathematical modeling to elucidate the interplay between tissue mechanics and regulatory processes in the organ of Corti. On the modeling side, we develop a hybrid modeling approach combining regulatory circuits, such as lateral inhibition, with mechanical models describing the position and shape of each cell. We show how a combination of cellular reorganization and differentiation circuits can give rise to precise and robust patterning.

To test the predictions of the model, we developed a mouse cochlear explant setup that allows live 3D imaging of the development of the organ of Corti *ex-vivo*. We use a double transgenic mouse line, Atoh1-GFP/mT-tdTomato to track both cellular boundaries and hair cell differentiation. Using these measurements we show that the cells in the developing organ of Corti undergo local dynamic reorganization processes that sculpt the final pattern of hair and supporting cells. Taken together, our results suggest a unified picture of the patterning of the organ of Corti.

Live Imaging to Explore Dynamics of Stereocilia Formation in the Developing Mammalian Cochlea

Bhonker Y¹, Amir L², Kim SM³, Chen P³, Matsuzaki F⁴, Sprinzak D², Avraham KB¹

¹Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine and Sagol School of Neuroscience, ²Department of Biochemistry and Molecular Biology, Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; ³Department of Cell Biology, School of Medicine, Emory University, Atlanta, GA, USA; ⁴Laboratory for Cell Asymmetry, RIKEN Center for Developmental Biology, Japan

Planar cell polarity (PCP) is the process that determines the uniform orientation of the stereocilia in the organ of Corti. Recent discoveries suggest that PCP is comprised of at least two distinct sub-processes, each with its own molecular pathway: stereocilia orientation is governed by core PCP genes and subsequent morphogenesis is executed by the Gai/Lgn/mInsc pathway, previously associated with mitotic spindle orientation. Lgn, a key gene in this pathway, has been shown to be crucial for proper migration of the kinocilium, a primary cilium whose positioning regulates both PCP processes. Mutations in Lgn lead to deafness in both humans and mice. We hypothesize that kinocilium migration and stereocilia shape formation is a dynamic process mediated by Lgn.

Immunohistochemistry experiments suggest that Lgn affects stereocilia shape but does not affect the localization or expression of core PCP genes. Conversely, the PCP pathway does affect the localization of Lgn. We show that the kinocilium is required to confine Lgn to the lateral side of the hair bundle, suggesting that the Lgn-kinocilium interaction is more complex than previously thought.

In order to follow the dynamics of kinocilium positioning and stereocilia organization during development, we developed a time-lapse microscopy setup using cochlear explants growing *ex-vivo*. Our initial results show that establishment of the organ of Corti involves reorganization of cellular position and identity. We are currently extending this approach to examine the dynamics of stereocilia shape formation and kinocilium positioning in wild type and mutant mice.

Supported by Human Frontier Science Program RGP0012/2012, NIH-NIDCD Grant R01DC011835 and a TEVA Pre-doctoral Neuroscience Scholarship.

Defining Spontaneous Morphological Activity in the Kölliker's Organ of the Developing Cochlea

Dayaratne, N^{1,3}, Vljakovic, S M^{1,3}, Lipski, J^{1,3}, Thorne P R^{1,2,3}

¹Department of Physiology, ²Section of Audiology, ³Centre for Brain Research: The University of Auckland, Auckland, New Zealand

The Kölliker's organ is a transient epithelial structure, present during the critical 'pre-hearing' stages of cochlear development. First described in 1863, it is now believed to be the origin of spontaneous neural activity which retains and refines neural connections during pre-hearing development of the auditory system¹. There is strong evidence that purinergic signaling underlies such rhythmic activity, initiated by ATP release from the Kölliker's organ (KO). ATP also induces morphological changes in the KO in a rhythmic manner that matches the spontaneous neural activity, although the mechanism of these changes has not yet been established. These cells crenate and change their optical properties, allowing optical detection of spontaneous morphological activity in real-time. Using this principle, we explored the origin of these morphological events in the developing cochlea. Apical turns from Wistar rat cochleae (P9-11) were studied, and activity was specific to the Kölliker's organ. Purinergic involvement was explored with acute exposure to ATP (100 μ M; n=6), ATP- γ -S (100 μ M; n=8), and suramin (150 μ M; n=4). Both purinergic P2 agonists (ATP, ATP- γ -S) revealed a synchronous crenation of the Kölliker's organ, reversible with the P2 receptor antagonist, suramin. This activity was faster and more pronounced in the 40 μ m wide region closest to the inner hair cells (IHC) ($p<0.05$). This region also displayed the highest frequency of intrinsic spontaneous activity ($p<0.05$). In contrast to the agonists, suramin caused swelling of this region in the Kölliker's organ ($p<0.05$), suggesting that endogenous ATP regulates epithelial cell crenation. Histological analysis supported these changes and localized these to the supporting 'border cells' adjacent to the IHC. Together, these results further establish the involvement of purinergic signaling in spontaneous morphological activity, and point to a key role played by supporting cells located close to the IHC. ¹Tritsch et al., *Nature*, vol. 450, no. 7166, pp. 50–55, 2007.

76

Inner ear symptoms and quality of life in 1000 observed patients with vestibular schwannomas

P. Cayé-Thomasen^{1,3}, S. Hansen¹, J. Yde¹, S. Stangerup¹, M. Møller¹, C. Workman²

- 1) Department of Otorhinolaryngology Head and Neck Surgery, Rigshospitalet, Copenhagen University Hospital, Denmark.
- 2) Center for Biological Sequence Analysis, Technical University of Denmark, Denmark.
- 3) Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

The Copenhagen Vestibular Schwannoma database includes more than 3200 patients, of which 1133 have been managed by observation only by the end of 2012. The 1133 observed patients were asked to fill in various questionnaires on symptoms and Quality-of-Life via a newly developed website. The questionnaires included Short Form 12 Health Survey (SF-12), the Hearing Handicap Inventory (HHI), Tinnitus Handicap Inventory (THI), Dizziness Handicap Inventory (DHI), The Penn Acoustic Neuroma Quality-of-Life Scale (PANQOL scale), and a socio-demographic questionnaire. The response rate was 88% (994/1133). The lecture will present the results, including the finding that the Quality-of-Life in general is good, that dizziness has major impact on Quality-of-Life, that hearing influences the Quality-of-Life and that tinnitus has an impact on mental Quality-of-Life.

77

The cutting edge of hearing preservation surgery -Using intraoperative monitoring of ABR and Dorsal cochlear nucleus action potential with CE-chirp stimuli-

Hidemi Miyazaki

Faculty of Health and Medical Sciences, University of Copenhagen
Department of Oto-rhino-laryngology, Head & Neck Surgery, Audiology
Copenhagen University Hospital Rigshospitalet (Denmark)

In the past, nobody paid attention to preserve hearing in acoustic neuroma surgery. Most of surgeons focused on preservation of facial function, and thanks to facial nerve monitoring or mapping technique, the preservation rate has been improved obviously. Now, hearing preservation became ultimate goal for Acoustic neuroma treatment. Including all surgery and stereotactic treatment, by comparison with the best result of facial function preservation rate and hearing preservation rate, the former is around 95% and the latter is around 70%. What's the reason for difference of 25% between them? In the CP angle, facial nerve is already differentiated to peripheral nerve, but cochlear nerve is central nerve tissue still. It means cochlear nerve is susceptible to damage. To preserve such a fragile cochlear nerve, we have needed a sensitive and real time cochlear monitoring. Therefore, we invented a new intraoperative continuous cochlear monitoring electrode and developed new monitoring system. By using the electrode we measure the directly auditory evoked action potential from dorsal cochlear nucleus. The voltage of the dorsal nucleus action potential (DNAP) is 10-100 times of ABR, so it takes only 3-7 seconds for averaging. Furthermore DNAP electrode enabled an identification of cochlear nerve by use of monopolar stimulation on the 8th cranial nerve. As a sound stimulation for ABR and DNAP monitoring, we usually use click sound. New sound stimuli CE-chirp was developed by Dr.Claud Elbering to increase peak 5 of ABR. For patient with a severe hearing loss, we have used CE-chirp and got positive results.

The prehabilitation concept (PREHAB) to enhance postoperative recovery in vestibular schwannoma patients

MÅNS MAGNUSSON AND FREDRIK TJERNSTRÖM

Department of otorhinolaryngology, Lund University Hospital, Lund Sweden.

Acute vestibular lesion causes an acute onset of the well known symptoms of a vestibular de-afferentation with malaise, nausea, vomiting. When performing surgery on patients with a vestibular schwannoma where there is an intact or remnant vestibular function, these symptoms may cause further distress in postoperative patients. This may prolong adaptation and perhaps hamper recovery. Secondly, a combined acute vestibular loss together with a cerebellar injury may cause permanent instability and unsteadiness and an uncompensated vestibular loss. A gradual loss of vestibular function, on the other hand, causes few symptoms and may go without notice. We therefore developed a concept where patients train according to a home based vestibular rehabilitation program before and after a vestibular lesion is induced by means of transtympanic gentamicin installments. The vestibular de-afferentation often goes more or less unnoticed and patients have a speedy recovery after surgery. Recently we have also been able to demonstrate that the procedure increase postural control in long term observation, 6 months after surgery and that recovery seem to exceed what rehabilitation per se can achieve. Thus, a PREHAB concept in planned vestibular de-afferentation seem to provide benefits for the patients and may reduce risk of permanent incapacitation when cerebellar structures are in danger to be lesioned by surgery or tumor growth.

Clinical Application for Regeneration of the Skull/Temporal Bone

Kanemaru S, M.D., Ph.D.^{1,2)*}, Kanai R, M.D.¹⁾, Tsuji T M.D.¹⁾, Yamamoto M, M.D.¹⁾, Toda H, M.D., Ph.D.³⁾, Yamashita M, M.D., Ph.D.¹⁾, Maetani T, M.D., Ph.D.¹⁾, Nishida A, M.D., Ph.D.¹⁾.

1) Department of Otolaryngology–Head & Neck Surgery, Medical Research Institute, Kitano Hospital, Osaka, Japan

2) Department of Otolaryngology, The Foundation for Biomedical Research and Innovation, Kobe, Japan

3) Department of Neurosurgery, Medical Research Institute, Kitano Hospital, Osaka, Japan

Objectives/Hypothesis: To establish the new treatment for regeneration of the skull/temporal bone. Cranial bone reconstructive surgery is usually performed after a craniotomy or posttraumatic repair of the skull. In most cases, cranial bone reconstructions have been performed by fixing bone flaps to surrounding bone with artificial devices such as titanium plates or stainless steel wire. These materials interfere with imaging by computed tomography (CT), magnetic resonance (MRI), and X-ray. Because no bone regenerates around the bone flap, it is gradually replaced by fibrous tissues. Deformations of the reconstructed site after surgery cause cosmetic handicaps for patients.

Study Design: Clinical trial

Setting: 3 General hospitals

Patients and methods: 84 patients (Age:1-85,M=37,F=47) were performed this regenerative treatment accompanied by Cochlea Implant(n=33), Decompression of facial nerve(n=23), Endolymphatic sac surgery(n=19) and Resection of Acoustic tumor by Translabyrinthine approach(n=5)/Middle cranial fossa approach(n=4). Materials used for regeneration of the skull/temporal bone were cranial bone flap, bone powder and fibrin glue. After replacing bone flap, the space between bones was filled with bone powder with fibrin glue. One year after the operation, whether the bone was regenerated or not was estimated by CT scan image.

Results: Perfect regeneration of the skull/temporal bone was achieved in 79 patients(94%). Partial bone defects were observed in five patients(6%). No serious sequelae were observed in any patient.

Conclusions: This clinical study demonstrates that a combination of bone flap, bone powder and fibrin glue enables to regenerate the skull without conventional operative procedures. This regenerative treatment is easy, safe, cost-effective, cosmetically-good.

80

Personalized Medicine

Zenner HP¹, Pfister M², Friese N¹, Zrenner E³ and Röcken M⁴

Dept. of Otolaryngology, The University of Tübingen¹, Centre for Ophthalmology of the University Hospital Tübingen³ and University Department of Dermatology⁴, The University of Tübingen, Tübingen, Germany, Otorhinolaryngologie², Sarnen, Schweiz

Objective: To evaluate present options of molecular medicine for the stratification of patients.

Methods: Using „Pubmed“ a literature search was performed. Parameters were „personalized medicine“, „individualized medicine“ and „molecular medicine“.

Results: Personalized medicine using molecular genetics of functional proteins like Otoferlin, Connexin 26, KCNQ4, or of Usher gens play a growing role for the indication of cochlear implants. Determination of HER2/EGFR mutations may be of importance for decision making in head and neck oncology. Mutations in the Hedgehog (PCTH1) and Smoothened (SMO) pathway strongly influence the indication of small molecules in basal cell carcinoma therapy. Analyses of Ckitrezeptor-mutations of the BRAF600Emutation and of NRASmutations, are required for specific molecular therapy of metastasizing melanomas.

Conclusions: Molecular personalized medicine serves for the stratification of patients. This is thought to allow specific indications for cochlear implants or for the therapy of head and neck tumors, melanomas and basalionomas.

81

Development of an *in vitro* bioassay to analyze interface-dependent response profiles of auditory neurons on multi-electrode arrays

Stefan Hahnewald¹, Marta Roccio¹, Emanuele Marconi¹, Carolyn Garnham², Teresa Melchionna², Anne Tschertner³, Jürg Streit³, Julien Brossard⁴, Alexandra Homsy⁴, Herbert Keppner⁴, Hans-Rudolf Widmer⁵, Pascal Senn¹

¹Inner Ear Research Laboratory, Department of Clinical Research, University of Bern and University Department of Otorhinolaryngology, Head & Neck Surgery, Bern, Switzerland, ²MED-EL Elektromedizinische Geräte GmbH (MED-EL), Innsbruck, Austria, ³Department Physiology, University of Bern, Bern, Switzerland, ⁴Haute Ecole Arc Ingénierie, La Chaux-de-Fonds, Switzerland, ⁵Department of Neurosurgery, Bern University Hospital, Bern, Switzerland.

* Corresponding author. E-mail address: stefan.hahnewald@dkf.unibe.ch

Cochlear implants (CIs) have become the gold standard treatment for deafness. Despite all the success, some limitations remain. Our project: “NANOCI” (www.nanoci.org) aims at developing a new generation of CIs where the peripheral processes of the auditory neurons grow towards the electrode array to form a gapless interface in the cochlea. In theory, this strategy should result in *i*) a better auditory resolution and *ii*) lower energy consumption, two of the main limitations of current CI systems.

As a first step towards this ambitious goal, we aim at developing an *in vitro* bioassay based on multi electrode arrays (MEAs) combined with an external stimulation electrode to study auditory neuron activity and to investigate parameters that are relevant in the context of the project. Specifically, how stimulation parameters will need to be adapted in relation to the distance of the CI electrode to fully exploit the theoretical advantages of a gapless interface. Optimum stimulation parameters and distance-related effects are experimentally addressed on a custom-made set up, using murine spiral ganglion neuron cultures on MEAs. The first results confirmed the main hypothesis of the NANOCI project, namely that the smaller the distance between the stimulating electrode and the auditory neurons, the lower the voltage needed to trigger neuronal activity and the larger the dynamic range of responses. Although preliminary, these results are the first of their kind in the auditory field and allow us to address more sophisticated stimulation protocols in the near future.

Keywords: cochlea implants, inner ear, nanotechnology, auditory neurons

Acknowledgements: This work has been sponsored by 7th Framework Programme of the European Union (NANOI, grant agreement no. 281056).

Genetic Background Of Single Nuclear Polymorphism Causing Hearing Loss caused by tobacco Smoking

Pyykkö I¹, Iltanen K², Zou J¹, Juhola M²,

¹Research Unit, Field of Oto-laryngology, School of Medicine, University of Tampere, Tampere, Finland,

²School of Information Sciences, University of Tampere, Finland

Tobacco smoking is associated with age-related hearing loss and in noise-induced hearing loss. It has been suggested that the hearing loss is due to reduced circulation caused by nicotine effect.

We explored the possibility that SNP in known genes would explain at least part of the hearing loss observed among smokers.

From the 5000 subjects collected in ARHI research consortium 2428 were used in this analysis. The marker spacing was initially 0.1 cM yielding 26,876 SNP that were subjected for further analysis and confirmation. We developed a data mining methods in that cluster association rules were applied into subsets of data. We explored the association rules the genetic linkage analysis and phenotype evaluation to evaluate the role of SNP in susceptibility to hearing loss. The program searched efficiently extract rules representing interesting environmental factor-gene or gene-gene interactions.

We found that SNPs in CHD23, GSR, SLC26a4, TRPA1, CHRN2, It8, KCNQ1, TFCP2L3 and TMPRSS3 genes explained significant association with smoking and hearing loss and resulted in 50% of additional age related risk of hearing loss in better hearing ear. Usually the sub-population affected by the each SNP in these genes consisted of 65 to 101 subjects.

Analysis of SNPs explains part of the susceptibility to tobacco smoking related hearing loss. Interestingly SNP related to smoking occurring associated hearing loss in the CHD23 gene was not in the same gene loci as SNP associated with noise susceptibility and these two SNP may jointly potentiate the hearing loss observed in noise exposed workers.

Genetic Background Of Single Nuclear Polymorphism In Causing Noise Susceptibility

Pyykkö I¹, Iltanen K², Zou J¹, Juhola M²,

¹Research Unit, Field of Oto-laryngology, School of Medicine, University of Tampere, Tampere, Finland,

²School of Information Sciences, University of Tampere, Finland

The susceptibility to noise-induced hearing loss is largely unknown. It is demanded in the new European noise directive (2003/10/EC) for noise protection of workers. We explored the possibility that SNP in known genes would explain at least part of the noise susceptibility.

From the 5000 subjects collected in ARHI research consortium 2428 were used in this analysis. The marker spacing was initially 0.1 cM yielding 26,876 SNP that were subjected for further analysis and confirmation. We developed a data mining methods in that cluster association rules were applied into subsets of data. We explored the association rules the genetic linkage analysis and phenotype evaluation to evaluate the role of SNP in susceptibility to noise. The program searched efficiently extract rules representing interesting environmental factor-gene or gene-gene interactions.

We found that SNPs in KCNJ10, EYA4, Catalase, KCNMA1 and CDH23 genes explained significant association with noise susceptibility and resulted in aggravated hearing loss in better hearing ear. Usually the sub-population affected by the each SNP in these genes consisted of 90 to 189 subjects and explained increase risk of hearing loss of on average by 50%.

Analysis of SNPs explains part of the susceptibility to noise-induced hearing. Interestingly some SNPs in the genes were the same that also were involved in causing susceptibility to solvent induced hearing loss and interaction of noise and solvent strongly promoted hearing loss in subjects with solvents and noise exposure.

Genetic Etiology of Presbycusis in Portugal

Chora JR¹, Pereira L¹, Simões-Teixeira H¹, Matos TD¹, Fialho MG¹, Caria MH^{1,2}.

¹BioFIG, Center for Biodiversity, Functional and Integrative Genomics University of Lisbon, Lisbon, Portugal, ²Higher College of Health, Polytechnic Institute of Setúbal, Setúbal, Portugal.

Presbycusis or age-related hearing loss (ARHL) is the most common sensory impairment in older adults. It affects millions of people worldwide, leading to social isolation and exclusion of valid citizens. ARHL is characterized by bilateral progressive hearing loss predominant in the high frequencies. It is a multifactorial condition with etiologic factors both environmental and genetic, the latter dictating an intrinsic susceptibility to presbycusis.

Previous candidate gene studies in different populations found an association between presbycusis and genes involved in hereditary deafness or in oxidative metabolism. Mitochondrial haplogroups U and K in an Australian population, and the *NAT2**6A haplotype in Europe, were found to be significantly associated with ARHL. In addition, three different GWAS have recently been published and a highly significant and replicated SNP in *GRM7* gene was identified.

We have audiotologically assessed a meaningful sample of Portuguese elder individuals and are establishing genotype-phenotype correlations by analyzing *GJB2*, *GJB6*, *GRM7*, *NAT2* and mtDNA genotypes using a variety of methods.

The pattern of variants found in this sample seems to be consistent with those previously described for the general European population. Some associations between presbycusis and certain genotypes are discussed.

In conclusion, this study aims at contributing to the genetic knowledge of ARHL susceptibility factors as well as the audiological characterization of the elderly Portuguese population.

ILDR1 Deficiency Causes Degeneration of Cochlear Outer Hair Cells and Disrupts the Structure of the Organ of Corti: A Mouse Model for Human Deafness DFNB42

Sang Qing^{1,2*}, Li Wen^{3*}, Feng Yi⁵, Xu Yao², Feng Ruizhi², Xing Qinghe², Zhao Xinzhi², Jin Li¹, He Lin^{2,4}, Li Huawei^{3#}, Wang Lei^{1,2#}

¹State Key Lab. of Genetic Eng. & MOE Key Lab. of Contemporary Anthropology, Sch. of Life Sci., Fudan Univ., Shanghai, PR China, ²Inst. of Biomedical Sciences, Fudan Univ, Shanghai, PR China, ³Dept. of Otolaryngology, Eye & ENT hospital, Fudan Univ., Shanghai, PR China, ⁴Bio-X Center, Key Lab. for the Genetics of Developmental & Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong Univ., Shanghai, PR China, ⁵Dept. of Integrative Medicine, Shanghai Medical College, Fudan Univ., Shanghai, China (* These authors contributed equally to this work.) # Correspondence: Dr. Lei Wang, wangleiwanglei@fudan.edu.cn, or Dr. Huawei Li, hwli@shmu.edu.cn

Immunoglobulin-like domain containing receptor 1 (*ILDR1*) is a poorly characterized gene that was first identified in lymphoma cells. Mutations in *ILDR1* are found to be responsible for DFNB42, but the pathogenesis of hearing loss caused by *ILDR1* mutations remains to be elucidated. To explore the role of *ILDR1* in hearing, we created *ildr1* knockout mice. In heterozygous mice, *ILDR1* expression was found in outer hair cells (OHCs), inner hair cells (IHCs), supporting cells, the stria vascularis, and the spiral ligament. *Ildr1*-deficient mice are profoundly deaf by P21. No significant difference was observed in the supporting cells and IHCs of *Ildr1*-deficient mice, but progressive degeneration of OHCs occurred at P15 and disruption of the tunnel running through the organ of Corti was noticeable at P21. By P28, there were no OHCs visible in any of the turns of the organ of Corti, and the tunnel of the organ of Corti was entirely destroyed. The hair cell loss appeared to be mediated by apoptotic cell death. *ILDR1* deficiency affects expression of tricellulin in vivo, which provide a possible explanation to hearing loss. To further elucidate the mechanism of deafness related to *ILDR1* deficiency, we pursued a differential proteomic approach to comprehensively assess differential protein expression in the cochleae of *Ildr1*^{+/-} and *Ildr1*^{-/-} mice at P21. Altogether 708 proteins were up-regulated (fold change >1.5) and 114 proteins were down-regulated (fold change <0.5) in the *Ildr1*^{-/-} mice compared with *Ildr1*^{+/-} mice. Gene ontology classification indicated that a number of differentially expressed proteins are involved in cell adhesion, protein and vesicle-mediated transport, cell death, membrane organization and cellular homeostasis. A few of these proteins are closely related to hearing development. Taken together, our data suggest that *ILDR1* is important for the survival of OHCs and provide novel insights into the pathogenesis of deafness DFNB42.

Novel *MITF* Mutation as a Molecular Etiology of Hereditary Unilateral or Asymmetric Sensorineural Hearing Loss

Kim SH^{1*}, Kim AR^{1*}, Choi HS², Kim MY², Chun EH², Oh SH¹, Choi BY²

¹Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea

²Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea

Waardenburg syndrome type 2 (WS2) is the autosomal dominantly-inherited syndrome with pigmentary abnormalities of skin, hair, and iris and sensorineural deafness. Unilateral sensorineural hearing loss was anecdotally reported in Waardenburg syndrome patients, but there have not been many reports that rigorously describe audiologic phenotypes, precluding any genotype-phenotype correlation.

In this study, we identified a novel mutation of *MITF* to be a molecular etiology of unilateral or asymmetrical sensorineural hearing loss that segregates as a symptom of WS2 in a mid-size Korean family. Here we propose including WS2 as a differential diagnosis of congenital unilateral or asymmetrical sensorineural hearing loss. A comprehensive clinical history and neurotological, ophthalmological, and dermatological examinations with mutational analysis were performed. We found the novel mutation identified in exon 9 of *MITF* in WS2. This was a nonsense mutation (p.Arg356*) observed in affected individuals of family SH136. The four individuals of family SH136 with *MITF* gene mutation show unilateral hearing loss although they have difference on the degree, moreover gray hair and freckled face.

In that of the pattern and severity of hearing loss in our Waardenburg syndrome patient, Waardenburg syndrome can be a cause of single side deafness. Therefore it is important that if the patient has the congenital single side deafness, family history and comprehensive physical exam such as neurotological, ophthalmological, and dermatological examinations should be checked.

Characterization of Expression and Transcriptional Regulation in Short Isoform of the Deafness Gene *Whirlin* in Mice

Yasuda S. P., Kikkawa Y.

Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

The deafness gene *Whirlin* (*Whrn*) is conceptually translated into two isoforms of proteins, the “long” and the “short” isoforms. Previous studies reported that both isoforms show distinct localization on stereocilia of the inner ear hair cell in early developmental stages. The long-isoform localized in the ankle-links of the stereocilia. The short-*Whrn* specifically localized in the stereocilia tips. In this study, we performed molecular biological analyses to investigate the expression and transcriptional regulation of the short-*Whrn*.

The quantitative RT-PCR analysis shows that the expression levels of the long and short isoforms in *Whrn* were significantly different during early developmental stages of the stereocilia. Target RNA-seq analysis for both isoforms from cochlea showed presence of subtypes by alternative splicing. By comparing the depth of coverage among the exons, depth of exon 3 of short-*Whrn* is significantly low level compared with other exons. This result may suggest that the short-*Whrn* has two subtypes, such as presence of the exon 3, and which was confirmed by RT-PCR. However, it seems that all transcripts of short-*Whrn* including exon 3 isolated from the cochlea were pseudogenized by insertion of slight intron sequences. Moreover, we carried out 5' RACE and luciferase assay to identify the promoter region of short-*Whrn*. These analyses predicted that promoter region of the short-*Whrn* is located on 3' end of intron 5 of long isoform. Thus, we propose that the expression of short-*Whrn* is controlled by distinct regulation systems from the long isoform.

The inner ear pharmacokinetics depends on systemic injection dose.

Sho Kanzaki, Masato Fujioka, Yozo Inagaki, Naoki Oishi, Kaoru Ogawa

Department of Otolaryngology, School of Medicine, Keio University, 35 Shinanomachi Shinjuku Tokyo, Japan

OBJECTIVES:

Systemic steroid injections are used to treat sensorineural hearing loss and some inner ear disorders. The inner ear dynamics also changes can be correlated with systemic injection volume. However its time course and dispersion of drugs is unknown. Here, we used a new in vivo imaging system to monitor drug delivery in live mice to compare different volumes over time after systemic injections.

METHODS:

Luciferin delivered into the inner ears of GFAP-Luc transgenic mice reacted with luciferase in GFAP-expressing cells in the cochlear spiral ganglion, resulting in photon bioluminescence. We used the Xenogen IVIS® imaging system and compare the inner ear drug dynamics between different injection volumes.

RESULTS:

There is variation among individuals Our data suggested that systemic injection volume correlated with higher drug dynamics into inner ear although delivery time varied with individuals. Photons were detected higher and longer in inner ear when more volumes are injected.

CONCLUSIONS:

We concluded that inner-ear drug concentration can be maintained longer when more systemic injections volumes

The Regenerative Therapy for Vestibular Disorders with human induced pluripotent stem (iPS) cells.

Taura Akiko¹, Nakashima Noriyuki², Onishi Hiroe¹, Nakagawa Takayuki¹, Ito Juichi¹

¹Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, JAPAN

²Department of Physiology and Neurobiology, Graduate School of Medicine, Kyoto University, Kyoto, JAPAN

Objectives: Lots of patients are suffering from severe balance disorders due to the vestibular dysfunction. Although the mechanisms of the disorders are being revealed, we still have no practical remedies for them. So, it is urgent and important to develop a new therapeutic protocol to treat the vestibular disorders. Here, we searched for the possible utility of human iPS cells to establish the neural reconnection to the damaged hair cells.

Methods: Human iPS cells were induced into neural stem cells (NSCs) and co-cultured with mouse neonatal utricle tissues. We evaluated two co-culture conditions: 1) we simply added (the mass of) NSCs onto the cultured utricle tissues and 2) we injected NSCs into the interstitial part of the utricle tissues with glass pipettes. After one week of incubation period, we examined the differentiation of the co-cultured NSCs morphologically and physiologically.

Results: There was no apparent synapse-like adherence between the utricle hair cells and the differentiated neuron-like cells when we just put NSCs onto utricle tissues. When injected within the utricle, however, the NSCs differentiated to show the elongated axon-like structures that made contact with the hair cells. Furthermore, the differentiated neuron-like cells fired spontaneously.

Conclusions: The injected NSCs showed the signs of morphological and physiological maturation with reconnection to the denervated hair cells; these synaptic contacts might be functional. Thus, the NSC injection method seems promising in treating the neuro-degenerative vestibular disorders.

The Paracrine Effect of Mesenchymal Stem Cells Restored Autoimmune Sensorineural Hearing Loss (hASC)

Zhou, B.¹, Du, X.², Di Girolamo, S.³, Barbieri, M.⁴, Yoo T.J.^{1&5}

Univ of Tennessee¹, Hough Ear Institute², U. of Rome³, U. of Genoa⁴, Italy, StemGen Therapeutics⁵.

The aim of this study was to examine the activities of hASCs on autoimmune hearing loss (EAHL) and how human stem cells regenerated cochlea cells in mice. Hearing loss was induced in mice. Mice with EAHL were given hASCs intraperitoneally once a week for 6 consecutive weeks. ABR was examined over time. The helper type 1 auto-reactive responses and Treg cells were examined. H&E staining or immuno-staining with APC conjugated anti-HLA-ABC antibody were examined. The organ of Corti, stria vascularis, spiral ligament and spiral ganglion in stem cell group were found to be normal. In the control group, without receiving stem cells, the organ of Corti was replaced by a single layer of cells. Systemic infusion of hASCs significantly improved hearing function and protected hair cells in established EAHL. The hASCs decreased the proliferation of antigen-specific Th1/Th17 cells and induced the production of anti-inflammatory cytokine interleukin-10 in splenocytes. The generation of antigen-specific CD4CD25 and Foxp3 Treg cells were also induced. Since newly regenerated mice spiral ganglion cells, not human mesenchymal stem cells given by intra peritoneal transfer were produced, the experiment showed that restoration is due to the paracrine activities of human stem cells.

Auditory brainstem whole mounts promote the differentiation of neuronal stem cells of the cochlear nucleus in co-culture experiments

Rak K., Voelker, J., Voelker C., Schendzielorz P., Radeloff A., Hagen R.

Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery and the Comprehensive Hearing Center, University of Wuerzburg

The cochlear nucleus is the first auditory brainstem nucleus, in which the second auditory neuron arises. Cellular and molecular disorganization of this brainstem nucleus is induced by the deprivation of auditory input. Neuronal stem cells (NSCs) have been identified in the neonatal rat cochlear nucleus recently (Rak et al. 2011). The cells displayed all features of NSCs, particularly the capacity to perform mitosis and differentiation in all cells of the neuronal lineage. In addition persistent NSCs could be demonstrated in this brainstem nucleus until adulthood (Rak et al. 2013).

In the presented experiments the questions whether there is a neurogenic environment in the cochlear nucleus for integration of NSCs and whether cochlear nucleus derived NSCs can differentiate in brain tissue were addressed.

Therefore, cochlear nucleus whole-mounts explants from P6 rats were co-cultured with NSCs, derived from the cochlear nucleus, which were labelled by DiI or Cre Recombinase activated eGFP.

NSCs engrafted well into the brain tissue and differentiated in all cells of the neuronal lineage during co-cultivation. Labelling by Cre Recombinase or eGFP was clearly identified in cultured and transplanted NSCs of the cochlear nucleus.

These experiments demonstrate a neurogenic environment in the cochlear nucleus and the capacity of differentiation of NSCs from the cochlear nucleus in brain tissue. Consequently, the presented results are a first step for a possible application of stem cells in the cochlear nucleus to reorganize the disorganization in this brainstem nucleus after hearing loss.

Toxicity Evaluation of Hydroxyapatite Powder Prepared For Tissue Engineering Using Adipo Derived Stem Cell (ADSC)

Pham TK¹, Do QM¹, Le MD², Pham VP²

¹Department of Ceramic Materials, Faculty of Material Technology, Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, Vietnam

²Laboratory of Stem cell Research and Application, University of Science, VNU-HCM, Ho Chi Minh City, Vietnam

Study's objectives: Hydroxyapatite or HA [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] is widely used in prosthesis, orthopedic, bone implant as biomaterials since its show excellent ability bond to surround bone when implanted. In Vietnam, our research group at HCMUT succeed to prepare HA powder for bone tissue regeneration (phase 1) and in the first step of biocompatibility evaluation by cell toxicity and proliferation. The main object of this study is to evaluate the feasibility to use Vietnam HA powder as biomaterials by toxicity evaluation.

Method: Vietnam HA powder is prepared by precipitation method similar with our previous report. The HA powder is exposed to 150 μL of ADSC medium containing 10% Fetal Bovine Serum (FBS); 1% Penicillin and Strep (PS) and ADSC density of 10.000 cell/well at different concentration such as 0.5; 1; 2; 4 and 8 mg/mL for toxicity evaluation at 37°C using 96-well E-plate. Proliferation and doubling time (DT) were recorded automatically every hour. For statistical analysis, one-way factorial ANOVA were performed. A p-value of < 0.05 was considered statistically significant.

Results: ADSC proliferated well after HA powder immersed in 72 hours regardless of HA concentration, indicate that the HA powder is non toxicity to ADSC cell.

Conclusions: This study suggests that HA powder prepared by HCMUT does not inhibit ADSC growth using DMEM, and thus have ability to use as scaffold for bone tissue regeneration. Further investigation on cell-materials and animal study is awaiting based on this results.

In Vitro Transition of Mouse Embryonic Stem-Cell Differentiation Into Inner Ear Progenitors

Nesrine Abboud¹, Arnaud Fontbonne¹, Jean Michel Brézun¹, Francois Feron², Azel Zine^{1,3}

¹Sensory biophysics, Faculty of Pharmacy, Montpellier I University, Montpellier, France

²Laboratory of Integrative and Adaptive Neurosciences, UMR 7260, AMU, France

³Neurobiology of Cellular Interactions and Neurophysiopathology, UMR 7259, AMU, France

Recent developments have made significant strides in the *in vitro* generation of mouse otic progenitors using different cell culture-based methods of differentiation. However, no clear consensus has been reached so far as the efficiencies of current methods remain unsatisfactory. In order to address these issues, we sought to compare the generation of otic progenitors from mouse ES cells in suspension *vs.* adherent culture systems. In our study, we used ES cell line derived from the *Atoh1*-GFP transgenic mouse model to track the generation of otic progenitors and compare both differentiation systems. Our two-step differentiation method involved an initial period of 5 days during which *Atoh1*-GFP ES cells were cultured in presence of the Wnt inhibitor (Dkk1) and TGF- β /Smad3 inhibitor (SIS3) to suppress mesendoderm and drive the presumptive ectoderm and, IGF1 to promote the anterior ectoderm, which is more competent to otic induction. Embryoid bodies (EBs) were then exposed to bFGF known to play a role in otic cell commitment, for an additional 5-day period. EBs differentiation was performed either in floating or adherent conditions. Upon completion of the differentiation, qPCR analysis and immunostaining were performed on the differentiated EBs. Our results revealed that cells differentiated in floating conditions are more prone to give rise to otic progenitors, with higher efficiencies, when compared to adherent culture conditions. Furthermore, GFP+ cells isolated from the suspension culture can incorporate into cochlear explants previously exposed to an ototoxic drug. Additionally, we observed that a fraction of GFP+ cells expressed the myosin VIIa after transplantation.

Funded by ANR (Agence Nationale de Recherche; ANR-2010-BLAN-1107-01 b)

Differentiation of Human Induced Pluripotent Stem Cells into Glutamatergic Neurons on 3D Scaffolds

Ohnishi H, Skerleva D, Sakamoto T, Yamamoto N, Ito J and Nakagawa T.

Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Objectives: Stem cell transplantation studies for regeneration of spiral ganglion neurons suggested a possibility of functional recovery. However before putting the human induced pluripotent stem cells (iPSCs) into clinical application all safety concerns must be resolved. The risk of tumorigenesis is one of major concerns. To reduce the risk for tumorigenesis, transplantation of terminally differentiated neurons is ideal. On the other hand, detachment of culture neurons from culture dishes causes severe damage to cultured neurons. To resolve these problems, we examined the capability for culture of human iPSC-derived glutamatergic neurons on the 3-dimensional solid scaffolds (3D scaffolds).

Methods: We used a step-wise culture method. Human iPSCs were induced differentiation into neural stem cells (NSCs) using chemically-defined medium (Li et al., 2011). Thereafter, NSCs were seeded on 3D scaffolds composed of collagen, and were cultured in the N2B27 medium. Due to difficulties in cell evaluation by RT-PCR and cell counting by immunocytochemistry in the aforementioned condition, NSCs were also cultured on Matrigel-coated plates as 2-dimensional condition.

Results: NSCs induced from human iPSCs included no undifferentiated cells. After culture in N2B27 medium, approximately 70% of cultured cells were β III tubulin-positive and more than 90% of them were positive for VGLUT1 on 2-dimensional condition. The immunocytochemistry of the cultured cells on 3D scaffolds also showed that the majority of β III TUBULIN-positive neurons were positive for VGLUT1.

Conclusion: The results demonstrate that our differentiation method is highly efficient for the generation of glutamatergic neurons from human iPSCs on 3D-scaffolds.

Transplantation of Neurons Derived from Human Induced Pluripotent Stem Cells into Guinea Pig Cochleae

Masaaki Ishikawa, Hiroe Onishi, Desislava Skerleva, Tatsunori Sakamoto, Norio Yamamoto, Juichi Ito, Takayuki Nakagawa

Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Objectives: Previous studies indicated the efficacy of transplantation of mouse or human ES cell-derived neural progenitors for restoration of spiral ganglion neurons leading to the recovery of functionality (Okano et al., 2005; Chen et al., 2012). However, use of ES cells could involve immune rejection, and use of neural progenitors could cause formation of neuromas. To reduce risk for immune rejection and neuroma generation, use of autografts and terminally differentiated neurons as transplants is required. In this study, we examined the potential of human iPS cell-derived neurons as transplants for functional restoration of the spiral ganglion neurons.

Materials and methods: We prepared human iPS cell-derived neurons cultured on a three-dimensional scaffold (3D scaffold) as transplants, and examined the survival of transplants in the intact cochleae of guinea pigs. Furthermore, we examined the surgical invasion of the transplantation procedures to host cochleae. Surgical invasiveness was assessed by measurements of electrically evoked auditory brainstem responses (eABRs) and SGN densities after implanting a 3D scaffold into the intact cochleae.

Results: Human iPS cell-derived neurons survived into guinea pig cochleae up to two weeks, and over 80% of them expressed VGLUT1, a marker of glutamatergic neuron. No significant alterations in the eABR thresholds or SGN densities were found following surgery.

Conclusion: The findings indicate that our transplantation procedures can be used to deliver neurons derived from human iPS cells into cochleae with low surgical invasiveness.

96

Environmental Demands and Pharmacological Activation of Soluble Guanylyl Cyclase (sGC) interact with the Progression of Age Related and Noise Induced Hearing Loss

Rüttiger L, Varakina K, Möhrle D, Bing D, Knipper M

Department of Otolaryngology, Head and Neck Surgery, Hearing Research Centre Tübingen, Molecular Physiology of Hearing, University of Tübingen, Elfriede-Aulhorn-Straße 5, 72076 Tübingen, Germany

Inner ear physiology and hearing sensation is lost as a consequence of even moderate auditory overexposure. Recent studies showed a close relation between aging, loss of inner hair cell synaptic contacts, and ABR wave amplitudes in the mouse [1] and in the rat [2]. Furthermore, full recovery from TTS after mild noise exposure with full restoration of normal audiometric threshold sensitivity is known to lead to a loss of cochlear function at supra-threshold sensation levels and a loss of IHC synaptic contacts in an age related manner [3].

After noise exposure, the activation of a protective cyclic guanosine monophosphate (cGMP) dependent molecular cascade has been described in the rat and mouse [4]. We therefore tested the protective potential of cGMP increase by pharmacological stimulation of soluble guanylyl cyclase (sGC) activation for age and noise induced progression of hearing deficits. As an alternative approach, we studied the hearing of animals held in tightened environmental conditions (enriched environment), promoting the activation of stress related molecular cascades.

Peripheral and central auditory responses were analyzed and compared to hair cell function (otoacoustic emissions) and quantification of CtBP2 positive staining at the IHC synapse (giving an estimate for the number of afferent contacts). The data will be discussed considering the otoprotective role of the cGMP signalling cascade after noise induced damage of the ear during aging.

This work was supported by DFG (FOR-2060) and Action on Hearing Loss (RNID G54-Ru)

[1]Sergejenco et al., J Neurosci 33:13686-94, 2013

[2]Möhrle et al., IEB 2013

[3]Rüttiger et al., ARO 2014

[4]Jaumann et al., Nat Med. 18:252-9, 2012

97

Noise-induced cochlear F-actin depolymerization is mediated by ROCK2/p-ERM signaling pathway

Su-Hua Sha, Yu Han

Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425, USA

Small GTPases are the major modulators of the actin cytoskeleton. Our previous work has suggested that traumatic noise activates Rho GTPase pathways resulting in cochlear outer hair cell (OHC) death and noise-induced hearing loss (NIHL). Activated Rho GTPases bind to a spectrum of effectors to stimulate downstream signaling pathways. Rho-associated kinases (ROCKs) are the major targets of Rho for reorganization of actin-based cytoskeletons. The ezrin-radixin-moesin (ERM) proteins are the targets of ROCKs and act as cross-linkers between the plasma membrane and the actin cytoskeleton. Here, we investigated the Rho pathway in the regulation of the cochlear actin cytoskeleton using adult CBA/J mice under temporary threshold shift (TTS) and permanent threshold shift (PTS) noise conditions. ROCK2, but not ROCK1, was expressed mainly in the cochlea. The levels of ROCK2 and p-ERM decreased significantly in mouse whole cochlear homogenates 1 hour after either TTS or PTS-noise exposure, but the immunolabeling of ROCK2 and p-ERM antibodies in OHCs decreased significantly only after PTS-noise, not after TTS-noise exposure. Treatment with lysophosphatidic acid (LPA), an activator of the Rho pathway, resulted in significant reversal of the reduction in ROCK2 and p-ERM in OHCs caused by noise exposure and attenuated NIHL and OHC death. In contrast, genetic down-regulation of ROCK2 by pretreatment with ROCK2 siRNA reduced the expression of ROCK2 and p-ERM in OHCs and aggravated TTS to PTS. Finally, noise exposure resulted in changes in the F-actin/G-actin ratio. Our results indicate that a ROCK2-mediated ERM phosphorylation signaling cascade modulates noise-induced hair cell death by targeting the actin cytoskeleton.

The research project described is supported by R01 DC009222 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

Characterisation of Noise-Induced Cochlear Inflammation in a Mouse Model

Tan WJT^{1,2}, Telang RS^{1,2}, Thorne PR^{1,2,3}, Vljakovic SM^{1,2}

¹Department of Physiology, ²Centre for Brain Research, ³Section of Audiology, The University of Auckland, Auckland, New Zealand

Previous studies have shown that exposure to excessive noise induces cochlear inflammation which may contribute to cochlear injury and hearing loss. This study aimed to characterise the noise-induced cochlear inflammatory response in C57BL/6 mice. Cochleae were collected from adult (6-8 weeks) mice at various intervals (0-7 days) after exposure to traumatic noise (100 dB SPL, 8-16 kHz, 24h). Using quantitative RT-PCR and immunohistochemistry, changes in expression levels of proinflammatory cytokines, chemokines and adhesion molecules in the noise-exposed cochlea were studied ($n=8$ per group). We have demonstrated elevated mRNA levels of TNF- α , IL-1 β , CCL2 and ICAM-1 after noise exposure. All transcripts displayed similar dynamics of expression, with an initial up-regulation at 6h and a second peak at 7d after noise exposure. ICAM-1 immunoexpression in the spiral ligament fibrocytes and endothelial cells increased significantly, peaking at 24h post-exposure. We also demonstrated the recruitment of inflammatory cells in the cochlea, with maximum infiltration observed at 24h. These findings suggest that cytokines and adhesion molecules initiate noise-induced cochlear inflammation by mediating the recruitment and extravasation of inflammatory cells, the stage associated with the development of noise-induced cochlear injury. The occurrence of the late peak in expression of inflammatory mediators is not clear, and we postulate that it may be associated with reparative processes. Furthermore, we demonstrated that post-exposure treatment with regadenoson, a selective adenosine A_{2A} receptor agonist, suppressed cochlear inflammation, by significantly ($n=6$, $p<0.05$) reducing ICAM-1 immunoexpression and infiltration of inflammatory cells. This reveals an important role of adenosine receptor signalling in controlling noise-induced cochlear inflammation, and pinpoints A_{2A}Rs as an attractive pharmacological target in this condition.

Cochlear Hair Cell Generation from Lgr5-Positive Supporting Cells

Bramhall, N.F.^{1,2}, Shi, F.^{1,2}, and Edge, A.S.B.^{1,2}

¹Harvard Medical School, Boston, MA, USA, ²Massachusetts Eye and Ear Infirmary, Boston, MA. USA.

In contrast to the limited ability to replace damaged cells in vestibular and auditory organs, cochlear cells showed a capacity for hair cell replacement following ototoxic damage in the neonatal mouse. Based on lineage tracing, we showed that new hair cells, predominantly outer hair cells, arose from supporting cells that expressed Lgr5, a downstream target of the Wnt pathway and a protein that marks intestinal epithelial stem cells. After hair cell loss, supporting cells transdifferentiated to hair cells directly or after cell division. The transdifferentiation and cell division were both seen without pharmacological intervention in the neonatal ears and were thus a spontaneous response to damage. Whereas all supporting cells expressed Sox2, a specific subset expresses Lgr5. Cochlear Lgr5-expressing supporting cells after isolation by flow cytometry gave rise to self-renewing neurospheres that could be induced to differentiate to hair cells. Lgr5-positive cells had distinct phenotypes from other supporting cells and differentiated to hair cells at a higher rate than the total Sox2-positive supporting cells, consistent with these cells playing a role as hair cell progenitors. Hair cells did not differentiate from Lgr5-negative cells. Upregulation of Wnt signaling specifically targeted the Lgr5-expressing cells, leading to proliferation in the postnatal ear, and the cells transdifferentiated to hair cells after increasing expression of Atoh1, which was downstream of Wnt. These data suggest that manipulation of signaling pathways increases regeneration of hair cells and that Lgr5-positive cells act as hair cell progenitors in the cochlea.

DAPT enhances Atoh1 activity to generate new hair cells in situ following neomycin ototoxicity in rat cochleae in vitro

Juan-mei Yang¹#, Wen-wei Luo¹#, Zhao Han¹, Fang-lu Chi¹*

¹ Department of otolaryngology-head and neck surgery; Eye & ENT hospital of Fudan University; 83 Fenyang RD, Shanghai, 200031, # Juan-Mei Yang and Wen-Wei Luo contributed equally to this work.

*Corresponding author: Fanglu Chi, E-mail: chifanglu@126.com

Previous studies showed that adenovirus-mediated Atoh1 overexpression or the pharmacological inhibition of Notch signaling by γ -secretase inhibitors could induce new hair cell formation in mammals. However, both of these methods have limitations for the recovery of hearing loss. The stimulation of in situ formation of hair cells after hair cell loss is a promising approach to hearing recovery. Increasing the viral infection rate and the rate of conversion of supporting cells to hair cells in situ is a difficult problem that needs to be solved. To develop an effective and efficient therapy for hearing recovery, we used a higher concentration of fetal bovine serum (FBS) in the culture medium and prolonged the duration of culture to increase the supporting cell gaps. Simultaneously, we applied a human adenovirus serotype 5 vector that encoded Atoh1 and the reporter gene EGFP (*Ad5-EGFP-Atoh1*) in combination with the γ -secretase inhibitor N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT). Increasing the FBS concentration and prolonging the duration of culture increased the supporting cell gaps and thereby increased the virus infection rate, which, when combined with the application of DAPT and *Ad5-EGFP-Atoh1*, increased the rate of conversion of supporting cells to hair cells and thus produced more hair cells. Thus, Atoh1 overexpression induced in situ formation of hair cells via trans-differentiation, and DAPT enhanced Atoh1 activity, leading to the production of more new hair cells. The concurrent application of DAPT and *Ad5-EGFP-Atoh1* could be an effective and efficient therapy for recovery of hearing loss in the future.

Keywords: Atoh1; supporting cell; transdifferentiation; Notch signaling inhibitor; sensory epithelium region (SER); fetal bovine serum concentration(FBS).

DNA Damage Signaling Regulates Age-Dependent Proliferative Capacity Of The Inner Ear Supporting Cells

Laos M, Pirvola U

Department of Biosciences, University of Helsinki, Helsinki, Finland

Supporting cells (SCs) of the mammalian inner ear show a prominent decline in proliferative capacity at early postnatal life. Our earlier data showed that a large part of the cell cycle reactivated adult utricular SCs fail to proceed into mitosis and accumulate DNA double-strand breaks (DSBs). We suggested that DSB formation is linked with the observed cell cycle arrest (Loponen et al., 2011).

In order to study the possible link between proliferative restrictions and DNA damage response signaling we compared juvenile and adult supporting cells. We used adenoviral-mediated cyclin D1 expression to trigger cell cycle re-entry selectively in SCs in explant cultures prepared from P6 cochleas and utricles, and from adult utricles. We also employed an *in vivo* approach using conditional, inducible *Rb* inactivation (*Rb^{loxP/loxP};Fgfr3-iCre-ER^{T2}* mice) to study the response of auditory SCs.

Our results show that DSBs, as detected by nuclear foci of activated histone H2AX (γ H2AX), accumulate in cell cycle reactivated SCs of both ages. Both juvenile and adult utricular SCs can respond to DSBs by activating the DNA damage response. DSBs can be repaired at both ages, but adult SCs show delayed repair, consistent with the arrest of these cells upon forced cell cycle re-entry and the induction of apoptosis. DSBs accumulate in cell cycle reactivated SCs also *in vivo* in *Rb* mutant mice. Our results underline the importance of DNA damage signaling as a barrier in the attempts to stimulate proliferation as an approach to trigger regeneration in the inner ear. We speculate that there exist differentiation-associated mechanisms, possibly linked to the chromatin status, that modulate DNA damage response and direct the behavior and fate of cell cycle reactivated SCs.

Induction of Auditory Neurons in Cochlear Endogenous Cells in the Mammalian Cochlea

Nishimura, Koji¹, Dabdoub, Alain^{1,2,3}

¹Sunnybrook Research Institute, ²Department of Otolaryngology – HNS, ³Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada

Primary auditory neurons (spiral ganglion neurons) are crucial in hearing as they transmit sound information from the inner ear to the brain. A growing number of evidence suggests that auditory neurons are lost due to disease, excessive noise and aging while hair cells remain intact; and like most neurons, once lost they do not regenerate. Hence, they are a primary target for regeneration since the induction of even a small number of neurons in a damaged ear could have significant impact on hearing restoration.

One approach to hearing loss treatment is the use of gene therapy for the induction of endogenous cells. Two target cell populations in the mouse cochlea for induction are non-sensory epithelial cells and spiral ganglion glial cells. We have used neurogenic transcription factors known to directly reprogram cells and induce neurons in several systems as well as transcription factors required for the generation and survival of auditory neurons. Overexpression of these factors *in vitro* induced neurons at high efficiency at embryonic, postnatal and juvenile stages. The induced neurons expressed neuronal markers including synaptic proteins and were electrophysiologically functional generating action potentials.

Our data indicate that overexpression of transcription factors is sufficient to convert endogenous cochlear cells into functional neuron-like cells. We are investigating *in vitro* combinatorial factors that induce phenotypes that most closely resemble auditory neurons, and examining connectivity to the inner ear in the periphery and the cochlear nucleus in the CNS.

Hearing Regeneration for Severe Sudden Deafness

Hisayoshi Ishizaki,

Ishizaki ENT Clinic, Shizuoka, Japan

Pathogenesis of sudden hearing loss is not well known. The most popular treatment is steroid administration with either intra tympanic instillation, intravenous administration or with oral medication. However the efficacy of steroid treatment is disputable. The therapeutic efforts may influence on preventing of apoptosis but the exact mechanisms are not known. Therefore the possibility that the Insulin like Growth Factor-1 (IGF-1) may play a significant role in sudden hearing loss and that therapeutic efforts may be mediated by the action of IGF-1 was explored.

Eleven patients (mean age 54.2y, range 27-82y, male 6, and female 5) with severe sensori-neural hearing loss with sudden onset were included. The subjects did not show spontaneous recovery in hearing and were hospitalized for the treatment lasting for a week. Their health status was evaluated, common laboratory test were carried out and speech discrimination, pure tone audiometry and MRI scanning were done. The ethical permission was received for this study from local ethical committee.

IGF-1 was dissolved in 3.0ml saline. One injection with 0.3ml solution into the tympanic cavity once a day, successively for 7 days. After injection, patients were kept in supine position for 20 minutes. Mean interval from onset of sudden hearing loss to commencing treatment was 105 days (range 5-360)

The result of Effect on the treatment was evaluated after 6 months. Mean hearing level was 79.9dB before treatment, 42.7dB after 6 months. The eight cases (73%) were gained more than 20dB at the mean hearing level. Three cases with long interval were no effect on hearing improvement.

IGF-1 seems to improve hearing in the patients with sudden deafness hearing improvement when the traditional steroid treatment has failed. The treatment was the safe and no adverse effects were reported.

Conservatively managed sporadic vestibular schwannoma: Audiovestibular factors influencing quality of life

S. Hansen¹, J. Yde¹, S. Stangerup¹, M. Møller¹, C. Workman², P. Cayé-Thomasen^{1,3}

1) Department of Otorhinolaryngology Head and Neck Surgery, Rigshospitalet, Copenhagen University Hospital, Denmark.

2) Center for Biological Sequence Analysis, Technical University of Denmark, Denmark.

3) Faculty of Health Sciences, University of Copenhagen, Denmark.

Introduction: The objective was to evaluate the quality of life (QoL) of patients with conservatively managed Vestibular Schwannomas (VS) and describe their sociodemographic characteristics.

Methods: The questionnaires were answered by patients via a newly developed website using a unique token. Those who did not accept or understood answering via the internet had a second possibility of a paper version, which were sent to them by post. They were asked to return their completed questionnaires in a prepaid envelope.

Results: 87.7% response rate (994/1133). The questionnaires included Short Form 12 Health Survey Version 2 (SF-12v2), the Hearing Handicap Inventory (HHI), Tinnitus Handicap Inventory (THI), Dizziness Handicap Inventory (DHI), The Penn Acoustic Neuroma Quality-of-Life Scale (PANQOL scale), and questions on sociodemographic characteristics. 898 patients reported hearing loss (95.8%). Six hundred eighty four reported tinnitus (72.9%) and 463 reported imbalance (49.4%). Regression analysis showed that DHI score and age were strong predictors of physical component summary. DHI and THI scores were significant predictors of mental component summary.

Conclusion: Dizziness is the most significant audiovestibular predictor of QoL in patients with VS. Tinnitus also has an impact on mental QoL. Hearing loss does also influence QoL significantly. Other factors may have an important role to play in determining QoL.

Otolith Dysfunction Caused By Acoustic Neuroma Affects Head Stability During Gait

Ishikawa K, Itasaka Y, Omi E, Koizumi K.

Department of otorhinolaryngology, Akita Graduate School of Medicine,

1-1-1, Hondo, Akita, 010-8543, Japan

Objective: To elucidate whether or not otolith dysfunction could affect head stability during gait.

Method: Twenty unilateral acoustic tumor patients and nine age and height matched healthy control subjects were enrolled in this study. Subjects were asked to walk freely with comfortable pace with eyes open and eyes closed at a distance of about four meters, and 3-D movement analysis was performed. In this study, the analysis was mainly focused on head movements and peripheral vestibular function. Functional status of the peripheral vestibular nerve was examined by cVEMP & oVEMP.

Results: In AT cases, three cases had normal cVEMP and oVEMP, five cases had abnormal cVEMP with normal oVEMP, three cases had normal cVEMP with abnormal oVEMP, and nine cases had abnormal in both cVEMP and oVEMP. Regarding the relation between those abnormalities and head instability, AT cases had greater horizontal sway movement, especially in cases with oVEMP abnormality. AT cases with cVEMP abnormality had greater pitch and roll movement than that of the control, especially under gait with eyes closed. AT cases with both VEMPs abnormality had the greatest head instability. However, no significant change was found in yaw plane head movement during gait.

Conclusion: Thus peripheral vestibular function plays an important role in stabilizing head during gait, and could have somewhat different contribution between utricular system and saccular system.

Long-term Administration of Vasopressin Can Cause Meniere's Disease in Mice

Masaya Takumida¹, Yoshiaki Katagiri¹, Katsuhiro Hirakawa¹, Matti Anniko²

¹ Department of Otolaryngology, Hiroshima University Hospital, Hiroshima, Japan, and ² Department of Otorhinolaryngology and Head and Neck Surgery, Uppsala University, Uppsala, Sweden

Objective. The purpose of this study was to develop a more suitable animal model, having a closer resemblance to the pathophysiological process in Meniere's disease. **Materials and methods.** Adult CBA/J or ICR mice were treated by subcutaneous injection of 50 µg/100g/day vasopressin for 5 days up to 8 weeks. Morphological analyses were performed of the cochlea, vestibular end organs and endolymphatic sac. **Results.** All experimental animals showed mild to moderate endolymphatic hydrops, increasing in severity as the vasopressin treatment was prolonged. Animals treated with vasopressin for 8 weeks showed severe endolymphatic hydrops with partial loss of outer hair cells and spiral ganglion cells. These animals also had a reversible vestibular dysfunction following intratympanic injection of epinephrine. **Conclusion.** A new murine model of Meniere's disease has been developed, based on long-term administration of vasopressin. Induction of vestibular dysfunction in the present animal model can cause additional stress, by reducing inner ear blood flow.

Psychiatric comorbidity in patients with dizziness and the therapy of psychotropic drugs

Kiyomizu Kensuke^{1) 2)}, Matsuda Keiji²⁾, Torihara Koji²⁾, Yoshida Kensei¹⁾, Tono Tetsuya²⁾

1) Dept. of Psychiatry, Yoshida Hospital MD, Nobeoka, Miyazaki, Japan, 2) Dept. of Otorhinolaryngology, Miyazaki Medical College, University of Miyazaki, Miyazaki, Japan,

[INTRODUCTION] We reported neuro-otological findings in psychiatric patients with nystagmus. In this study, we investigated 3 types of psychogenic dizziness (PsD) and the therapy of psychotropic drugs.

[METHODS] The 746 patients (238 men, 508 women, age range, 7-95; mean age \pm SD 59.0 \pm 18.0 years) with dizziness were classified as otolaryngologic (Otola) disorders (D): dizziness of unknown cause (DUC) in 342 (45.8%), otogenic vertigo (OV) in 141 (18.9%), Meniere's disease (MD) in 106 (14.2%), chronic cerebral insufficiency in 88 (11.8%), BPPV in 39 (5.2%) and other types of diseases in 30 (4%). Patients were diagnosed by designated physicians of mental health or psychiatrists with more than 9 years clinical experience using ICD-10.

[RESULTS] PsD narrow type was revealed in 134 (18%). Psy comorbidity was revealed in 487 (65.3%). Of 487 patients, various types of Psy D were found, such as anxiety or panic D (F41) in 271 (55.6%), mood D (F3) in 89 (18.3%), adjustment D or post-traumatic stress D (F43) in 28 (5.7%), dissociative D (F44) in 6 (1.2%), other neurotic D(F48) in 18 (3.7%), organic mental D (F0) in 38 (7.8%) and schizophrenia (F2) in 26 (5.3%). These patients were not only treated by otolaryngologists, but also received Psy therapy, and 380 (78%) of these patients were prescribed psychotropic drugs. Minor tranquilizer was prescribed in 269 (70.8%), sleeping pills in 126 (33.2%), antidepressant in 94 (24.7%). major tranquilizer in 71 (18.6%), anti-epileptic drugs in 24 (6.3%), lithium carbonate in 6 (4.2%), anti- Parkinson's disease drugs in 16 (4.2%), other drugs in 28 (7.4%).

[CONCLUSIONS] We believe that collaboration between psychiatrists and otolaryngologists in the hospital and/or doctors in local area can improve the mental condition and the quality of life (QOL) of patients who are suffering from dizziness with psychiatric comorbidity.

Hearing Preservation on Intra Tympanic Gentamicin Treatment for Meniere's Disease

Hisayoshi Ishizaki,

Ishizaki ENT Clinic, Shizuoka, Japan

Intratympanic gentamicin has been used for controlling episodic vertigo and Tumarkin attacks in severe form of Meniere's Disease. However this treatment often causes hearing loss. The purpose of this study is to investigate gentamicin induced changes in hearing with pure tone audiometry and extra tympanic electro-cochleography (ECoG).

Thirty eight cases (mean age 55.4y, range 23-86y, male 16, female 22, side rt 17, lt 21) were studied before and after gentamicin treatment with audiogram and click evoked ECoG for more than four years. Summating potential (SP) and action potential (AP) were determined.

Diluted Gentamicin with sodium bicarbonate as a buffer was administered to the middle ear in 1 or 2 times. . Mean hearing level at speech area (500-2000Hz) was 47dB before and 40dB after the treatment. The difference in hearing level was statistically significant between before and after treatment ($p < 0.001$) at the frequency of 125, 250, 500Hz. Mean hearing gains at the frequency of 125, 250, 500Hz and 1 kHz were 12.6, 12.1, 10.5 and 6.2dB. The longitudinal examination 4 years after treatment did not reveal progressive hearing loss and vestibular symptoms. SP/AP ratio (0.49 before and 0.34 after treatment) seemed to be normalized after gentamicin treatment. After gentamicin treatment, normalized SP/AP resulted in gain in hearing at the frequencies of 250Hz and 500Hz. Hearing gain may reflect a reduction in endolymphatic hydrops. SP (0.59 before and 0.3 after treatment) and especially AP (1.23 before and after 0.86 after treatment) amplitudes were reduced after gentamicin treatment indicating hearing loss.

It is suggested that gentamicin treatment is seemingly effective for controlling vertigo and preserving hearing, normalized SP/AP but caused deterioration of hearing that can be revealed in ECoG.

Balance Deficit Enhances Anxiety and Balance Training Decreases Anxiety in Vestibular Mutant Mice

Mintz M¹, Shefer S¹, Gordon C², Avraham KB³

¹School of Psychological Sciences and Sagol School of Neuroscience, Tel Aviv University, ²Department of Neurology, Meir Medical Center, Kfar Saba and Sackler Faculty of Medicine, ³Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

Clinical literature has recognized the high prevalence of comorbidity of balance and anxiety disorders. In spite of the rich clinical literature, it is unclear whether these two disorders are causally related, and what direction this causality may take. Association of imbalance and anxiety has also been demonstrated in animal models. Highly appropriate for the search of causality are vestibular mutant mice with an early and progressive phenotype. In the present study we tested vestibular Headbanger (Hdb) mice in which stereocilia of the hair cells in the vestibular end-organs are abnormally elongated already shortly after birth and this abnormality worsens progressively over time. Two strategies were applied to test causality. First, we followed the progress of balance disorder with behavioral tests of anxiety. Second, we applied balance training and tested whether rehabilitation of balance brings also amelioration of anxiety. Hdb and wild-type (Wt) mice were raised in either balance training or standard cages and were subjected repeatedly at 1, 2 and 3 months of age to balance and anxiety-related tests. Results in untrained groups demonstrated progressive deterioration of balance performance and parallel elevation of anxiety in Hdb vs. Wt mice. Results in Hdb mice demonstrated improved balance performance and decreased level of anxiety in trained vs. untrained mice. These findings confirm that vestibular pathophysiology may be causally related to the development of anxiety and suggest that in some clinical cases of anxiety, the appropriate treatment is physical rehabilitation of balance.

Utilizing the oval window as a route for gentamicin in ablation of the vestibular apparatus, when transtympanic installments fail in patients with Meniere's disease.

Måns Magnusson MD PhD, Mikael Karlberg MD PhD, Fredrik Tjernström MD PhD,
Eva Degerman MD PhD

¹Dept of Otorhinolaryngology, Lund University Hospital, 22185 Lund, Sweden. mans.magnusson@med.lu.se

Objectives: To ablate vestibular function while protecting the cochlea in patients with incapacitating Meniere's disease that failed to respond to transtympanic gentamicin a minor surgical procedure was developed and tested.

Methods: Less than 5-10% of patients have remaining vestibular function and continuous vertigo attacks after repeated transtympanic gentamicin. Although few of these patients have an incapacitating Meniere's disease, these patients will normally be offered a labyrinthectomy or a vestibular nerve section, depending on if there is serviceable hearing or not. As the route to the vestibular sensory end organs are through the oval window while the round window access the scala tympani, it would be preferable to administer an ototoxic agent to the oval window specifically, while protecting the round window. We therefore by means of an endaural exploration of the middle ear, placed gelfoam with gentamicin in the oval window niche and around the stapes, while protecting the round window with gelfoam soaked in saline in 6 patients- 5 with serviceable hearing, PTA <55 dB- not responding to transtympanic gentamicin.

Results: In 10 out of 11 patients, vestibular function was ablated and vertigo attacks disrupted without further deterioration on hearing. The last patient, who before surgery did have PTA >90 dB, did not respond to the procedure and underwent a labyrinthectomy, that although it gave him a complete vestibular loss, did not alleviate him from other problems, that turned out to be less dependent on his Meniere's disease.

Conclusion: The present series suggest that gentamicin penetrates through the oval window to the vestibule enough to cause ablation of the apparatus, when previous transtympanic and hence round window exposure was not enough. Although a small material, this procedure of 'specific oval window gentamicin application' offers a possible option to ablate vestibular function, while saving hearing in those patients that do not respond to transtympanic gentamicin.

Dysfunction of the peripheral vestibular organs may contribute to vertigo in vestibulo-cochlear schwannomas. A human temporal bone histopathology study

M. Nue Møller¹, S. Hansen¹, H. Miyazaki¹, P. Caye-Thomasen^{1,2}.

1. Dept of oto-rhino-laryngology, head and neck surgery Rigshospitalet/Gentofte, Copenhagen Denmark

2. Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Introduction: Vertigo is a common symptom in patients with vestibulo-cochlear schwannoma (VS). Although clinicians have only recently assessed this, several studies have identified vertigo as the single most important symptom affecting quality of life. Clinical and histological observations regarding the concurrent hearing loss in this group of patients, have suggested that the hearing loss may be caused by both retrocochlear and cochlear mechanisms. The same could be true in the case of vertigo, which could potentially modify the therapeutic approach. This study presents a detailed assessment of the vestibular histopathology in patients with VS.

Methods: Retrospective analysis of temporal bone histopathology from patients with uni-lateral sporadic vestibulo-cochlear schwannoma. The material was obtained from the temporal bone collection of Rigshospitalet, Denmark.

Results: VS caused significant atrophy of vestibular ganglions as well as loss of axonal density and atrophy of the neuro-epithelia of the involved vestibular nerve branches and vestibular end organs. However, in cases with small isolated tumors arising from the cochlear nerve or vestibular tumors that affected a singular vestibular branch, we found no evidence of a mass effect or atrophy of remaining vestibular organs.

Conclusion: There is significant degeneration of vestibular organs in patients with VS, where organs are directly affected by tumor mass, which suggests that vertigo in these patients may be caused by deficient vestibular neuroepithelia. In cases of smaller tumors there seems to be a highly localized degeneration that does not affect the remaining vestibular organs. This may lead to a more differentiated clinical investigation and subsequent new therapeutic options.

Nondestructive Observation of The Vestibular Systems Of *Slc26a4* K.O. Mice Using Optical Coherence Tomography

Sakamoto T¹, Tona Y¹, Taura A¹, Nakagawa T¹, Ito J¹

¹Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Objective: Optical coherence tomography (OCT) has been used to visualize cochlear structures *in vivo* (Tona et al., 2014, Otol Neurotol). However, it was difficult to visualize vestibular structures *in vivo* due to the covering of temporal muscles and bones. In this study, we tried to visualize vestibular abnormalities in *Slc26a4* K.O. mice inner ear *ex vivo*.

Methods: OCS1300SS (Thorlabs, NJ) was used for OCT imaging. Inner ears of wild type and *Slc26a4* K.O. adult and postnatal day 1 mice were used. Adult mice were intracaridially perfused with 4% PFA and inner ears were isolated, fixed by 4% PFA and placed under the OCT scanner for image acquisitions. P1 mice inner ears were also handled in the same protocol without intracardial perfusion. For comparisons, samples were decalcified, sectioned at a thickness of 10 µm and stained by hematoxylin and eosin. Some samples from P1 were sectioned without decalcification.

Results: Extensive dilatation of endolymphatic duct was clearly visualized in the *ex vivo* inner ear of *Slc26a4* K.O. mice. Otoconias were visualized in wild type and K.O. mice without decalcification. Signals from otoconias in adult K.O. mice were faint compared to that of wild type mice, which reflected abnormal maintenance of otoconia in the K.O. mice. In P1 K.O. mice, particles were visualized in the endolymphatic space, which resembles giant otoconias in the immature K.O. mice.

Conclusions: Morphological characteristics of *Slc26a4* K.O. mice vestibular systems were visualized using OCT without sectioning and staining. OCT will provide less modified information related to the inner ear function compared to the histological study.

113

On the area motor model of prestin activity

Joseph Santos-Sacchi and Lei Song
Yale University School of Medicine

Outer hair cell (OHC) electromotility is driven by a membrane motor identified as the protein SLC26a5 (prestin). In the 2-state area motor model, developed about two decades ago, the motor resides in either a compact state or an expanded state, each contributing differentially to membrane surface area. Naturally, motor surface area changes alter membrane capacitance. Thus, aside from the nonlinear capacitance (NLC) imparted by prestin's voltage sensor charge movement, linear capacitance (C_{lin}) also displays voltage dependence as motors move between expanded and compact states. Unit linear motor capacitance fluctuation (δC_{sa}) is on the order of 140 zF. In the recent 3-state model of Homma and Dallos (2011) an alternative view proposes that voltage-dependent linear capacitance changes are not real but only apparent. We demonstrate here using simultaneous manipulations of NLC with salicylate and chloride that an enhanced area motor model, including augmented δC_{sa} by salicylate, can accurately account changes in membrane capacitance. We also show that while the 3-state model implicitly avoids measuring voltage-dependent motor capacitance, it registers δC_{sa} effects as a byproduct of its assessment of C_{lin} , which increases during salicylate treatment as motors are locked in the expanded state. The area motor model, in contrast, captures the characteristics of δC_{sa} 's voltage-dependence in support of an area motor model.

114

Biophysical Properties of Mouse Ca^{2+} Channels Alter Before and After Onset of Hearing.

Inagaki, Akira¹, Murakami, Shingo¹

¹Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan

The auditory hair cells are the cells that convert auditory waves into electrical impulses which auditory cortex can percept. Once graded potentials are generated in response to sound waves, voltage dependent calcium channels (VDCC) open by the potentials and release the synaptic vesicles by supplying the calcium ions that trigger the synaptic release which needs to convey the auditory information to the auditory nerve. Due to the level of the temporal precision needed in the system, the biophysical property of the VDCC is extremely important since it directly affects to the rate of the vesicle release and firing rate of the auditory nerve by determining the $[Ca^{2+}]_i$ with its gating property. Previous reports showed $Ca_v1.3$, an isoform of VDCC predominantly expressing in the brain and heart, is almost exclusively expressing as the VDCC in the inner hair cells. However there is a controversy if the calcium sensitivity of the VDCC changes after the onset of hearing. If so, the calcium channel system that includes auxiliary subunits, proteins tethered to the channels changes before and after onset of hearing.

To test if the calcium sensitivity of the VDCC changes before and after the onset of hearing, we recorded calcium current in the immature and mature hair cells. The results showed a developmental decline in the sensitivity of CDI to global elevations in Ca^{2+} , which restricts negative feedback regulation of $Ca_v1.3$ channels to incoming Ca^{2+} ions in mature IHCs.

Rab interacting molecule 2 α (RIM2 α) regulates the number and function of Cav1.3 Ca²⁺ channels at the mouse inner hair cell afferent synapse

T. Oshima-Takago^{1,2,#}, S. Jung^{1,3,4,#}, R. Chakrabarti^{2,5,§}, M.M. Picher^{1,2,§}, A.B. Wong^{1,2,§}, Z. Jing⁶, K. Michel⁷, G. Yamanbaeva⁶, F. Göttfert^{3,8}, F. Predoehl¹, S. Hell^{3,8}, S. Schoch⁷, N. Strenzke^{6,*}, C. Wichmann^{5,*}, T. Moser^{1,3,4*}

¹InnerEarLab, Department of Otolaryngology and Collaborative Research Center 889, University Medical Center Göttingen, Göttingen, Germany, ²Göttingen Graduate School for Neurosciences and Molecular Biosciences, University of Göttingen, Germany

³Center for Nanoscale Imaging and Molecular Physiology of the Brain, University of Göttingen, Germany,

⁴Bernstein Focus for Neurotechnology, University of Göttingen, Germany, ⁵Molecular Architecture of Synapses group, InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Göttingen, Germany,

⁶Auditory Systems Physiology Group, Department of Otolaryngology and Center for Molecular Physiology of the Brain, University of Göttingen Medical Center, Göttingen, Germany, ⁷Institute of Neuropathology, University of Bonn, Medical School, Bonn, Germany, ⁸Department of Nanobiophotonics, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [#], [§]These authors contributed equally to the work, ^{*}Correspondence

Ca²⁺ influx through Ca²⁺ channels triggers synaptic vesicle fusion at the presynaptic active zone (AZ). Here we explored the role of RIM2 α in clustering of Cav1.3 Ca²⁺-channels at the ribbon-type synapses of mouse inner hair cells (IHCs). IHCs of hearing mice expressed RIM2 α and RIM2 β and RIM3 γ . The number of afferent synapses in RIM2 α -deficient IHCs was normal. However, the number of Ca²⁺ channels at the AZs was reduced, resulting in diminished synaptic Ca²⁺ signals and exocytosis. RIM2 α -deficient mice exhibited slightly impaired auditory brainstem responses albeit normal cochlear amplification. Spike rates of individual spiral ganglion neurons (SGNs) were reduced in the absence of sound, and tended to be smaller during sound stimulation. In conclusion, RIM2 α promotes a large cluster of synaptic Ca²⁺ channels at IHC AZs, thereby enabling high rates of spikes in SGNs.

Evaluation of synaptic function of otoferlin at the mouse inner hair cell ribbon synapse by postsynaptic recording

H. Takago^{1,2,3}, T. Moser^{1,2,4}

¹InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Göttingen, Germany

²Bernstein Center for Computational Neuroscience, University of Göttingen, Göttingen, Germany, ³Department of Rehabilitation for Sensory Functions, Research Institute, National Rehabilitation Center for Persons with Disabilities, Saitama, Japan, ⁴Collaborative Research Center 889 Cellular Mechanisms of Sensory Processing, Göttingen, Germany

Introduction: Ca²⁺ signals and subsequent exocytosis at the inner hair cell (IHC) ribbon synapse underlie the encoding of sound. The multi-C₂ domain protein otoferlin, whose mutations cause the autosomal recessive deafness DFNB9, has been assumed to control Ca²⁺-triggered exocytosis at this synapse by means of neuronal SNARE proteins. However, a recent study has argued that the hair cell afferent synapse apparently operates without neuronal SNARE proteins, requesting a detailed evaluation of synaptic function of otoferlin at a single synapse level.

Materials and Methods: Whole-cell voltage-clamp recordings were performed from postsynaptic boutons of type I spiral ganglion neurons in wild-type and otoferlin mutant mice at postnatal day 8 - 11, each of which contacts a single IHC through a single ribbon-type synapse.

Results: We found that deletion of otoferlin decreased frequency of both spontaneous and high potassium-evoked release. Moreover, we found that deletion of otoferlin decreased the amplitude of excitatory postsynaptic currents (EPSCs), but did not abolish large amplitude and amplitude heterogeneity of EPSCs.

Conclusion: These results confirm the role of otoferlin for priming and/or fusion of synaptic vesicles, and further support the hypothesis that unquantal release of synaptic vesicles is a fundamental mechanism at the IHC ribbon synapse.

Supported by a fellowship of MED-EL company (to H.T.) and grants from the German Research Foundation through the Collaborative Research Center 889 (project A2 to T.M.) and from the German Federal Ministry of Education and Research through the Bernstein Center grant 01GQ0433 (to T.M.).

Assessment of efferent control of the cochlea using wide band reflectance measurement

Harada T,

Department of Otolaryngology, International University of Health and Welfare Atami Hospital, Atami, Shizuoka, Japan

Objective: A novel middle ear function assessing method of wide band reflectance measurement was applied to detecting efferent control of the cochlear.

Method: Wide band reflectance was measured in 20 ears of 20 subjects with customized measuring equipment based on otoacoustic emission measuring system. Reflectance was measured from 200 to 6000 Hz without or with 40, 60, 80 dB SPL white noise to the opposite ear. Differences of reflectance between with and without noise to the opposite ear were estimated as change due to efferent control to the cochlea.

Result: Frequency characteristic curve of the reflectance showed trough around 3000 to 4000 Hz as formerly reported. Reflectance decreased around the trough or alternatively decreased and increased with frequency change to the noise to the opposite ear. The level of reflectance change increased as the noise level increased. The patterns of the alternative change of decrease and increase were similar to the fluctuations of the phase of impedance which was simultaneously measured.

Conclusion: The characteristics of reflectance change indicate this was due to decrease reflected sound wave from the cochlea caused by suppressive effect of efferent control. Combined with assessment of middle ear reflex which is known as increase of reflectance in low frequency range, wide band reflectance measurement with contralateral noise is useful for assessing efferent control of the cochlea.

Cochlear Adaptation Underpins Some Aspects of Temporary Threshold Shift

Thorne PR¹., Vljakovic SM¹., Telang RS¹., Ryan AF²., Housley GD³.

¹Department of Physiology and Centre for Brain Research, University of Auckland, Auckland, New Zealand,

²Departments of Surgery and Neurosciences, University of California San Diego and VA Medical Center, La Jolla, CA, USA, ³Department of Physiology and Translational Neuroscience Facility, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia,

There is accumulating evidence that the cochlea adapts to moderate levels of background noise, most likely for it to sustain sensitivity to important incoming sounds. Here we describe a molecular mechanism involving ATP (P2X₂) receptors in cells lining the cochlear endolymphatic space, which enables long-term adaptation to chronic noise exposure but also may explain aspects of Temporary Threshold Shift (TTS) and individual sensitivity to Permanent Threshold Shift (PTS)¹. Comparing *P2rx2*-null and wild type mice we have shown that activation of the P2X₂ receptor by ATP secreted into the endolymph in response to moderately intense sound (85dB SPL) leads to a TTS (assessed by the auditory brainstem response) that recovers within 24hrs. With higher noise levels (>95dB SPL) *P2rx2*-null and WT mice exhibited similar TTS but the *P2rx2*-null mice showed enhanced susceptibility to PTS indicating that this adaptation confers some protection to noise exposure. A mutation in the human *P2rx2* gene leads to the dominantly inherited, progressive sensorineural hearing loss, DFNA41, which is exacerbated by noise exposure². These studies imply that some aspects of TTS may reflect ATP receptor-dependent adaptation rather than cochlear injury and that this confers some protection on the cochlear tissues to excessive sound exposure. However, such an adaptative mechanism has limits which if exceeded may lead to neuronal injury and permanent hearing loss.

1 Housley et al., PNAS, 110: 7494-7499

2 Yan et al., PNAS, 110: 2228-2233

Neurotrophin gene therapy enhances the neural substrate of the deaf cochlea

Takada Y¹, Fukui H¹, Shibata SB¹, Budenz CJ¹, Colesa DJ, Swiderski DL¹, Shivatzki S², Avraham KB², Pfingst BE¹, Raphael Y¹

¹Department of Otolaryngology – Head and Neck Surgery, University of Michigan, Ann Arbor, USA. ²Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Israel.

The objectives were to characterize spiral ganglion neuron survival and growth of neurites induced by neurotrophins in deaf ears with no hair cells, and to assess the influence of the neurotrophin treatment on outcomes of electrical stimulation.

We used guinea pigs and mice. Guinea pigs were deafened either by perilymphatic infusion of neomycin or systemic aminoglycosides and diuretics. Mice were Connexin 26 or Pou4f3 mutants. Neurotrophins (BDNF or NT-3) were provided with viral vectors (adenovirus or adeno-associated virus). Ears were analyzed weeks or months later using whole-mounts and epifluorescence (for sprouting) or plastic sections (for spiral ganglion counts). Both BDNF and NT-3 induced nerve sprouting into the deaf auditory epithelium. Neurons preferred to grow in the direction of cells that were expressing the neurotrophin transgene and advanced to the target between epithelial cells, while traversing the border between the epithelium and the extracellular matrix in the basilar membrane. Areas with a flat epithelium were more permissive for sprouting than regions with remaining supporting cells. Neurotrophin gene therapy also enhanced spiral ganglion survival. These results were seen in both guinea pigs and mouse models for human deafness.

The extent of nerve sprouting and spiral ganglion survival, when correlated with psychophysical and electrophysiological measures (see Dr. Pfingst's presentation), indicates that neurotrophin gene therapy enhances the performance with cochlear implants.

Support: R01 DC-010412, R01-DC011835, T32-DC005356 and P30 DC05188, MED-EL, the UM Center for Organogenesis, the Hirschfield Foundation and the Williams Professorship.

Protein transduction using arginine-rich cell penetrating peptides into the inner ear through the round window

Takeda H¹, Minoda R¹, Yamada T¹, Ise Momoko¹, Yumoto E¹

¹Departments of Otolaryngology-Head and Neck Surgery Kumamoto University, Graduate School of Medicine, Kumamoto city, Japan

It is a crucial issue to develop effective and safe drug delivery methods into the inner ear in order to establish treatment modalities of inner ear diseases. We herein reported on the successful protein transduction into the inner ear utilizing Cell Penetrating Peptides (CPPs).

To induce protein transduction, we placed Spongel soaked in EGFP or EGFP-9R (EGFP was coupled with nine arginines) in the round window niche of guinea pigs and we periodically assessed the intensity of EGFP expression utilizing Labeling Index (H A Lehr, et al. J. histochem. cytochem. 1997). Additionally, we assessed auditory threshold, vestibular function and morphologies at day 28 after the treatments.

Consequently, EGFP-9R transduction induced significant EGFP expression at the cochlea, which reached at peak at 12 and 24 hours and disappeared by 72 hours after the treatment. The both groups showed no significant deterioration of auditory and vestibular functions. Next, to elongate the protein expression period, we tried to perform second protein transduction at 24 hours after the 1st protein transduction through the round window; after removing the existing Spongel we placed Spongel soaked in EGFP or EGFP-9R in the round window niche again. Consequently, 2 successive EGFP-9R transduction induced protein transduction at 48 hours and 72 hours after the 1st transduction comparing with a single administration. These findings suggest that repeated or continuous protein transduction utilizing 9R through the round window may be a promising drug delivery method into the inner ear.

How long should patients remain in the supine treatment position after intratympanic dexamethasone injection?

Soon Hyung Park,MD¹, Changhun Park,MS²,Jin Young Seo,BA³, In Seok Moon,MD,PhD³

¹Department of Otorhinolaryngology, Keimyung University College of Medicine, Daegu, ²Clinical Trials Center, Yonsei University Health system, Seoul, ³Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea

Objectives: Intratympanic dexamethasone injection (ITDI) is a widely accepted treatment for patients with sudden hearing loss. We investigated the appropriate patient wait time in the supine treatment position after ITDI. **Study Design:** Prospective study

Methods: In an *in vivo* animal study, 24 mice were injected intratympanically with dexamethasone. Perilymphatic fluid was sampled at 5, 10, 15, 20, 25 and 30 min post-injection. The dexamethasone concentration was analyzed using high-performance liquid chromatography. In a separate prospective clinical study, 79 patients with refractory sudden hearing loss underwent intratympanic injection. After the injection, patients remained in the supine position with the head rotated 45° to the unaffected side. Patients were divided into two groups according to the wait time in this treatment position postinjection: 30 min ($n = 47$) and 10 min ($n = 32$). Final hearing assessments were conducted 2 months after salvage treatment.

Results: In the *in vivo* animal study, the perilymphatic concentration of dexamethasone showed no significant increase after 10 min. In the clinical setting, hearing improvement according to Siegel's criteria was similar in the 30-min (14/47) and 10-min (10/32) groups ($p = 0.999$). No significant differences in relative hearing gain was observed between the two groups (13.80 ± 19.9 dB and 12.57 ± 14.9 dB, respectively; $p = 0.766$).

Conclusion: We suggest that 10 min is a sufficient time to remain in the supine treatment position after ITDI in patients with sudden hearing loss.

Round window membrane vibration may increase the effect of intratympanic dexamethasone injection

Soon Hyung Park,MD¹, In Seok Moon,MD,PhD²

¹Department of Otorhinolaryngology, Keimyung University College of Medicine, Daegu, Korea, ²Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea

Objectives: We investigated whether round window membrane (RWM) vibration can facilitate dexamethasone perfusion via the RWM in patients with sudden hearing loss.

Study Design: Prospective study.

Methods: We first performed an *in vitro* study using a semipermeable membrane. In the subsequent *in vivo* study, 20 mice were randomized into two groups: an intratympanic dexamethasone injection (ITDI)-only group, and an ITDI with RWM vibration group. Concentration of dexamethasone was investigated using high performance liquid chromatography. Third, we performed a prospective clinical study. Fifty-five refractory sudden hearing loss patients were divided into two groups: those who received ITDI only ($n=36$), and those who received ITDI with RWM vibration ($n=19$). Final hearing assessments were conducted 2 months after salvage treatment.

Results: In the *in vitro* study, the concentration of dexamethasone increased with vibration time with the peak concentration observed at 3 minutes of vibration. In the *in vivo* study, ITDI with RWM vibration resulted in a significantly higher perilymph concentration of dexamethasone ($7.68 \pm 3.13 \mu\text{g/ml}$) than that in the ITDI-only group ($2.66 \pm 1.73 \mu\text{g/ml}$). In a clinical setting, the overall improvement in hearing was similar between the two groups. However, when we compared the speech discrimination score between the two groups, we found that the relative discrimination gain in the ITDI with RWM vibration group ($18.11 \pm 23.54\%$) was higher than that in the ITDI-only group ($7.00 \pm 15.54\%$) ($p=0.042$).

Conclusion: RWM vibration can enhance the effect of intratympanic dexamethasone injection and is a viable treatment option for SHL.

Virally-mediated Gene Therapy to Restore Hearing for the Most Common Types of Human Congenital Deafness Caused by Null Mutations in *Gjb6*, *Gjb2*, *Slc26a4* and *Kcnq1* Show Promising Therapeutic Effects in Mouse Models

Wang J.J.¹, Chang Q.¹, Li Q.¹, Zhou B.F.¹, Wall S.² and Lin, X.¹

Depts. of Otolaryngology¹ and Internal Medicine², Emory University School of Medicine, Atlanta, GA, USA

Objectives: Mutations in the gap junction proteins (coded by *GJB2*, *GJB6* genes) and membrane transporter/channel (*SLC26A4* and *KCNQ1* genes) are causes of the most common human congenital non-syndromic and syndromic deafness, respectively. Currently no treatment is available.

Methods: We used *Gjb6*^{-/-}, conditional *Gjb2*^{-/-}, *Slc26a4*^{-/-} and *Kcnq1*^{-/-} mice to test a virally-mediated gene therapy approach. The genes used in the replacement therapy was inserted and packaged into AAV2/1 viruses. Virus solution of high titers was micro-injected into the scala media of the left cochlea of either newborn mice (P0-P2) or mouse embryos (~E12), while the other cochlea was used as a control. Cochlear morphology and auditory brainstem responses were examined to determine the morphological and hearing thresholds restoration, respectively. Immunostaining and Western blots were performed to study cellular expression of the viral-mediated gene expressions in the cochlea.

Results: We observed: (1) high efficiency viral-mediated expressions of exogenous genes in cells lining the endolymphatic space; (2) the injection procedure didn't harm normal hearing sensitivity; (3) ectopic virally-mediated expressions didn't affect normal hearing; (4) nearly-full (for *Gjb6*^{-/-} & *Kcnq1*^{-/-} mice) or partial (for *cGjb2*^{-/-} & *Slc26a4*^{-/-} mice) restoration of cochlear morphology and hearing thresholds. The effect was generally long-lasting, with the exception of *Kcnq1* treatment which probably lasted for about 2-3 months.

Conclusions: Virally-mediated gene transfer of exogenous gene fully or partially restored hearing in four types of mouse models representing the most common forms of human congenital deafness. Timing, delivery route and titer of AAV virus were found to be key factors determining the outcomes. The human implications of such therapeutic approach will be discussed.

Behavior of tricellular junctions in temperature-sensitive mouse cochlear precursor hair cells

Kakuki.T, Kojima.T, Takano.K, Miyata.R, Kohno.T, Kaneko.Y, Himi.T

Departments of Otolaryngology and Cell Science, Sapporo Medical University, Sapporo, Hokkaido, Japan

The epithelial cell polarity of sensory hair cells in the mammalian cochlea are important in the function. It is known that mutation of gap and tight junctions which maintained epithelial cell polarity causes non-syndromic hearing loss. Recently, it is reported that tight junction molecules, tricellulin and Lipolysis-stimulated lipoprotein receptor (LSR) which regulated tricellular junctions, are closely associated with hearing loss. However, the behavior of tricellular junction molecules in development and regeneration of the cochlear hair cells, remain yet unknown. In the present study, we investigate the changes of tricellular junction molecules by using temperature-sensitive mouse cochlear precursor hair cells. Furthermore, we examined the effects of γ -secretase inhibitor (which can induce regeneration of the cochlear hair cells) and metformin (which can prevent apoptosis of the cochlear hair cells induced by gentamicin).

We used three type of immortalized cochlear precursor hair cells (UB/OC-1, UB/OC-2, E36) which were separated and cultured from temperature-sensitive SV 40 large T antigen transgenic mouse fetuses (gifted from Dr. Holley). We performed RT-PCR, Western blotting and immunofluorescence staining for tricellulin and LSR. In the differentiated cells induced by changing culture temperature, expression of tricellulin and LSR was increased. In the cells treated with γ -secretase inhibitor and metformin, expression of tricellulin and LSR were also increased.

In conclusion, the dynamic changes of tricellular junction molecules were observed by using temperature-sensitive mouse cochlear precursor hair cells and treatment with γ -secretase inhibitor and metformin. The study of regulation in tricellular junction molecules by using cochlear precursor hair cells may be important in therapy for non-syndromic hearing loss.

Primary cilia in temperature-sensitive mouse cochlear precursor hair cells

Takano K¹, Kojima T², Kakuki T^{1,2}, Miyata R^{1,2}, Ninomiya T³, Konno T², Kaneko Y¹, Himi T¹

¹Department of Otolaryngology, Sapporo Medical University School of Medicine, Sapporo, Japan

²Department of Cell Science, Research Institute of Frontier Medicine, Sapporo Medical University School of Medicine; Sapporo, Japan.

³Department of Anatomy, Sapporo Medical University School of Medicine, Sapporo, Japan

The planar cell polarity of sensory hair cells in the mammalian cochlea are important in signaling function. The formation of nonmotile primary cilia which play a crucial role in planar cell polarity, have a function as sound sensor and primary ciliary dyskinesia causes hearing loss. However, the behavior of primary cilia in development and regeneration of the cochlear hair cells, remain yet unknown. In the present study, we investigate the changes of primary cilia by using temperature-sensitive mouse cochlear precursor hair cells. Furthermore, we also examined the effects of metformin which can prevent apoptosis of the cochlear hair cells induced by gentamicin.

We used three type of immortalized cochlear precursor hair cells (UB/OC-1, UB/OC-2, E36) which were separated and cultured from temperature-sensitive SV 40 large T antigen transgenic mouse fetuses (gifted from Dr. Holley). We performed immunofluorescence staining for acetylated-tubulin and γ -tubulin, scanning electron microscope (SEM) and Western blotting for signal transduction. In the differentiated cells induced by changing culture temperature, the length and the number of primary cilia were significantly decrease. In the cells treated with metformin, not only the length and the number of primary cilia but also the number of basal body were markedly increased. Furthermore, phosphorylation of AMPK, mTOR and LKB1 was increased by treatment with metformin.

In conclusion, the dramatic changes of primary cilia were observed by using temperature-sensitive mouse cochlear precursor hair cells and treatment with metformin. The primary cilia may play a crucial role in signaling function which induces development and regeneration of the cochlear hair cells.

The Expression of PTEN in the Developmental Spiral Limbus

Dong YY^{1,2}, Sui L², Tokuda M², Hu BH¹

¹The Center for Hearing and Deafness, State University of New York at Buffalo, Buffalo, NY, USA

²Department of Cell Physiology, Kagawa University, Miki-Cho, Kagawa, Japan

Objectives: Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene that regulates various cell processes including differentiation, neural stem/progenitor cell renewal. Our previous studies have found that PTEN can regulate the differentiation of the sensory cells in the inner ear. However, it is not clear whether PTEN plays a role in the development of spiral limbus (SL). Here, we examined the expression pattern of PTEN in the fibrocytes and the interdental cells of SL in mouse developing cochlea. **Methods:** RT-PCR, Western blotting and immunohistochemistry were used in this study. **Results:** PTEN expression was found in the fibrocytes and the interdental cells at postnatal day (P) 4 when SL began to differentiate. At P7, the immunoreactivity of PTEN was significantly increased in the fibrocytes and the interdental cells. At P14, the expression of PTEN was down-regulated in the fibrocytes, but the expression remained at a high level in the interdental cells. In the mature cochlea, the expression of PTEN became undetectable in the fibrocytes, but the expression in the interdental cells was still present. **Conclusions:** The dynamic change in PTEN expression suggests that this gene may play a role in the differentiation of SL.

Dominant Negative Connexin26 Mutation R75W Causing Severe Hearing Loss Influences Normal Programmed Cell Death In Postnatal Organ Of Corti.

Inoshita A, Karasawa K, Funakubo M, Miwa A, Ikeda K, Kamiya K

Department of Otorhinolaryngology, Juntendo University Faculty of Medicine, Bunkyo-ku, Tokyo, Japan

[Objectives] The greater epithelial ridge (GER) is a developmental structure in the maturation of the organ of Corti. Situated near the inner hair cells of neonatal mice, the GER undergoes a wave of apoptosis after postnatal day 8 (P8).

[Methods] We evaluated the GER from P8 to P12 in transgenic mice that carry the R75W + mutation, a dominant-negative mutation of human *gap junction protein, beta 2, 26 kDa (GJB2)* (also known as *connexin 26* or *CX26*). Cx26 facilitate intercellular communication within the mammalian auditory organ.

[Results] In both non-transgenic (non-Tg) and R75W + mice, some GER cells exhibited apoptotic characteristics at P8. In the GER of non-Tg mice, both the total number of cells and the number of apoptotic cells decreased from P8 to P12. In contrast, apoptotic cells were still clearly evident in the GER of R75W + mice at P12. In R75W + mice, therefore, apoptosis in the GER persisted until a later stage of cochlear development. In addition, the GER of R75W + mice exhibited morphological signs of retention, which may have resulted from diminished levels of apoptosis and/or promotion of cell proliferation during embryogenesis and early postnatal stages of development.

[Conclusions] Here we demonstrate that Cx26 dysfunction is associated with delayed apoptosis of GER cells and GER retention. This is the first demonstration that Cx26 may regulate cell proliferation and apoptosis during development of the cochlea.

Glutamatergic Signaling is Required for Maintenance of Inner Hair Cell, but Not Outer Hair Cell Innervation During Early Postnatal Development.

Barclay, M¹, Robertson, IP¹, Montgomery, JM¹, Thorne, PR^{1,2}

¹Department of Physiology and Centre for Brain Research, ²Section of Audiology, University of Auckland, Auckland, New Zealand

The innervation of sensory hair cells is established during the first 2 weeks of postnatal development in the rodent cochlea; type I spiral ganglion neurons (SGN) withdraw their branches from the outer hair cells (OHC) and adjacent inner hair cells (IHC) to innervate a single IHC and type II dendrites extend their innervation along the 3 rows of OHC. We examined whether hair cell-SGN synaptic neurotransmission plays a role in establishing this neural circuitry by blocking glutamatergic transmission via the α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Intact organ of Corti and SGN tissue from P0 mice cochleae were cultured for 96 hours, with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an AMPA receptor antagonist, added to half of the cultures during the latter 72 hours. Tissue was fixed (4% PFA) and immunohistochemistry and confocal microscopy used to examine the consequences of loss of neurotransmission via the GluAR receptors. Myosin7a immunoreactivity confirmed the maintenance of hair cell architecture in the organ of Corti. Immunostaining for β -tubulin, a marker for SGN, showed the loss of radial fibres and innervation of the IHC, however the outer spiral bundle innervation of OHC remained intact. RIBEYE and GluAR4 antibody labeling of presynaptic ribbon structures and postsynaptic glutamate receptors respectively indicated a loss of post-, but not presynaptic terminals with AMPA receptor blockade. Thus it seems that there is a differential dependence of afferent fibres on glutamatergic transmission at IHC vs OHC synapses. This could arise from differences in spontaneous activity in IHC vs OHC during this developmental period.

FGFR1 regulates the Kinocilia-specific Transport during Hair Cell Development

Honda A.¹, Kita T.¹, Misaki K.², Nakagawa R.³, Yonemura S.², Ladher R. K.¹

¹Laboratory for Sensory Development, ²Electron Microscope Laboratory, ³Proteomics Laboratory: RIKEN Center for Developmental Biology, Kobe, Japan

The mechanosensory cells of the inner ear, the hair cells, detect sound and balance information through its distinctive apical architecture, the hair bundle. The hair bundle consists of the kinocilium and stereocilia, which register mechanical stimuli when deflected. The formation of the kinocilia can be considered a modification of primary cilia, however the mechanisms for specialization are not known. We find that the fibroblast growth factor receptor 1 (FGFR1) is localized to forming kinocilium, but not to primary cilia of neighboring cells. FGFR1 colocalizes with intraflagellar transport (IFT) components that contain protocadherin-15 (PCDH15), a kinocilia-specific protein involved in establishing the morphology of hair bundle. Inhibition of FGFR causes redistribution of PCDH15, and malformation of hair bundle, suggesting that FGFR1 is instrumental in kinocilia specialization. We also find disabled-homologue 2 (DAB2), a clathrin-associated adaptor protein, in the PCDH15-containing IFT particle. DAB2 interacts with FGFR1 and PCDH15 and thus we propose that FGFR1, DAB2 and PCDH15 are incorporated into the IFT particle where they function for kinocilia specialization.

Expression of TRPM4 in the Mouse Cochlea and its Implicated Functions in the Depolarization of Inner Hair Cells and the Endolymph Formation

Sakuraba M¹, Murata J¹, Teruyama R², Kamiya K¹, Yamaguchi J³, Okano H⁴, Uchiyama Y³, Ikeda K¹

¹Department of Otorhinolaryngology, Juntendo University Graduate School of Medicine, Bunkyo-ku, Tokyo 113-8421 Japan, ²Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803,

³Department of Cellular and Molecular Neuropathology, Juntendo University Graduate School of Medicine, Bunkyo-ku, Tokyo 113-8421 Japan, ⁴Department of Physiology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160-8582 Japan

We tried to elucidate the presence of the transient receptor potential cation channel, subfamily M, member 4 (TRPM4), in the mouse inner ear.

TRPM4 immunoreactivity (IR) was found in: (a) the cell body of inner hair cells (IHCs) in the Organ of Corti, (b) the apical side of marginal cells of the stria vascularis, (c) the apical portion of the dark cells of the vestibule, and (d) a subset of the type II neurons in the spiral ganglion. Subsequently, changes in the distribution and expression level of TRPM4 in the inner ear during embryonic and postnatal developments were also evaluated. Immunohistochemical localization demonstrated that the emergence of the TRPM4-IR in IHCs occur shortly before the onset of hearing, while that in the marginal cells happens earlier at the time of birth coinciding with the onset of endolymph formation.

Furthermore, semi-quantitative real-time PCR assay showed that *TRPM4* transcripts in the Organ of Corti and in the stria vascularis increased dramatically at the onset of hearing. Because TRPM4 is a Ca²⁺-activated monovalent selective cation channel, these findings imply that TRPM4 contributes in potassium ion transport essential for the signal transduction in IHCs and the formation of endolymph by marginal cells.

We are now evaluating the auditory function of the *TRPM4* knockout mice by auditory brain-stem response (ABR) and planning to show a part of the results in the IEB workshop 2014 in Kyoto.

Distribution Of TBC Proteins In The Sensory Epithelium Of The Cochlea

Juergens L¹, Rak K^{1,2}, Mlynski R³

¹Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery, University of Wuerzburg, Germany

²Comprehensive Hearing Center, University of Wuerzburg, Germany

³Department of Otorhinolaryngology, Head & Neck Surgery, University Medical Center, Rostock, Germany

Introduction: In a previous study it was shown that a point mutation in the TBCE (tubulin-specific chaperone E) gene encoding for the protein Cofactor E leads to degeneration of the auditory nerve and the outer hair cells. Cofactor E is one of five cofactors (A -E) of tubulin biosynthesis acting as cascading chaperones in the folding of tubulin monomers and dimers. Dysfunction of Cofactor E illustrates the important role of these cofactors in maintaining the sensory epithelium of the cochlea. Aim of this study was to investigate the local and temporal expression of all the five TBC cofactors in the organ of Corti during the first four postnatal weeks.

Methods: Mice (NMRI) were transcardially perfused and the cochleae prepared for cryoembedding. After sectioning, cochleae were immunohistologically stained by primary antibody against Cofactor A, B, C, D and E and secondary Alexa antibody.

Results: An age- and cell-specific expression of the cofactors was detected. Cofactors A, B, C and D co-localized with supporting cells whereas Cofactor E only stained outer hair cells.

Conclusion: All cofactors investigated showed different, cell-specific patterns of expression. It is yet to be solved how these differences are linked to the specific function of cells in the cochlea. Consecutively mutations of single cofactors might cause different cochlear pathologies and age-dependent occurrence of hearing loss.

Shaping the mammalian inner ear sensory organs by the vertebrate planar cell polarity pathway

Sun Myoung Kim, Rida Padmashree, Cristy Tower-Gilchrist, Lindsey Knapp, Chonnetia Jones, Dong Qian, Shuangding Li, Ping Chen

Department of Cell Biology, Emory University, Atlanta, GA 30322, USA

The vertebrate planar cell polarity (PCP) pathway regulates the coordinated orientation of sensory hair cells in the mammalian inner ear, which is essential for the sensitivity and resolution of mechanotransduction of the inner ear. Collective studies including ours demonstrated a conserved mechanism of polarized partition of membrane PCP proteins in coordinating the polarity among neighboring cells, and the involvement of cilia genes in directing the intrinsic polarity of each individual cell. Many key issues of the mammalian PCP regulation remain unknown. It is not clear how the polarized partition of membrane PCP complexes is achieved and how the membrane PCP complexes communicate with the intrinsic polarity determinants. To further explore the mechanisms underlying vertebrate PCP regulation, we undertook a 2-hybrid screen with the cytoplasmic domain of a membrane PCP protein, Vangl2. We identified proteins with roles in protein trafficking and membrane targeting, in ciliogenesis, and potentially in cytoskeleton regulation. Functional studies of these genes revealed a molecular network that act together for PCP regulation in vertebrates. We will present the new results of these studies and discuss the implications of these studies in the understanding of the vertebrate PCP pathway that play essential roles in gastrulation, neurulation, and organogenesis of many tissues during development.

Regulation of Inner Ear Hair Cell Fate: Transcription Factor Combination and Epigenetics

Ryouchi Ikeda^{1,2,3,4}, Kwang Pak^{1,2}, Chavez Eduardo^{1,2}, Sho Hashimoto³, Yukio Katori⁴, Allen F Ryan^{1,2}

¹University of California San Diego and ²VA Medical Center, La Jolla, CA, USA, ³Sendai Medical Center, Sendai, Japan, ⁴Tohoku University, Sendai, Japan.

OBJECTIVES: inner ear Atoh1 expression is thought to be the earliest determinant of hair cell (HC) fate. Atoh1 absence results in a complete HC loss, and forced over-expression in nonsensory inner ear cells can induce ectopic HCs. However, Atoh1 induces alternative cell fates in several other tissues. Cell fate is determined by many factors, including the presence/activity of transcription factors (TFs), cell signaling networks, and epigenetic mechanisms. We evaluated the role of TFs and epigenetics in Atoh1 HC fate determination.

METHODS: Bioinformatic comparison across four mammalian species widely separated by evolution was used to identify conserved TF binding sites near those for Atoh1 on the *pou4f3* gene, an early regulatory target of Atoh1. Expression plasmids for identified TFs were introduced into nonsensory cells of neonatal mouse greater epithelial ridge (GER) by electroporation, followed by evaluation of POU4F3 and MYO7A expression. In a separate experiment, neonatal GER transfected with Atoh1 was treated with DNA methylation inhibitors (5-Aza, zebularine and BIX01294) or histone deacetylation inhibitors (HDACi) (TSA and VPA).

RESULTS: Out of 25 TFs with highly conserved binding sites in the *pou4f3* gene, we identified five that enhance the ability of Atoh1 to induce a HC phenotype and three that inhibit Atoh1 HC induction. In contrast, neither DNA methylation inhibitors (5-Aza, zebularine or BIX01294) nor HDACi (TSA or VPA) influenced the ability of Atoh1 to induce HCs in the GER.

CONCLUSIONS: The results suggest that TF combinatorial coding may be a more important determinant of HC fate than epigenetics.

Supported by grants from the VA and the NIH/NIDCD.

The role of planar cell polarity pathway in the ectopic regenerated hair cells regulated by the testosterone treatment

Ren Dong-Dong¹, Yang Xiao-Yu¹, Jin Kai¹, Ma Rui¹, Luo Wen-Wei¹, Yang Juan-Mei¹, Liu Xiang¹, Chi Fang-Lu¹

¹ Dept. of Otolaryngology, Eye, Ear, Nose and Throat Hospital, Fudan University, Shanghai, China.

Objectives: Planar cell polarity (PCP) signaling regulates cochlear extension and coordinated orientation of sensory hair cells in the inner ear. Studies have shown retrovirally-mediated introduction of Atoh1 transcription factor is capable of causing some mature supporting cells to transdifferentiate into hair cells. Testosterone is a gonadal sex steroid hormone that plays an important role on neuroprotection and regeneration in CNS development. However, the role of PCP pathway in ectopic regenerated hair-cell like cells (HCLCs) regulated by testosterone treatment is still unknown.

Methods: Ad5-EGFP-math1/Ad5-math1 was used to over-express math1 in the cochlea of neonatal mice in vitro. We investigated the establishment of ectopic hair cell polarity. We also observed the proliferation, differentiation, cell-cell adhesion, epithelial PCP in the lesser epithelial ridge (LER) after testosterone-3-(O-carboxymethyl) oxime bovine serum albumin (Testosterone-BSA) treatment in vitro.

RESULTS: It showed that there are some ectopic regenerated Hair cell like cells (HCLCs) in the LER after over-expressing Atoh1. After ectopic regenerated HCLCs developed actin-rich stereocilia, their basal body moved from center to the distal side, suggesting the underlying PCP establishment in ectopic regenerated HCLCs. After testosterone-BSA treatment 9 days in vitro, more Edu(+) cells and HCLCs cells were observed in LER with the down-regulation of E-cadherin. Interestingly, the CE of Ad5-EGFP-math1 infected LER is affected but the cell polarity of is not changed obviously.

CONCLUSION: Our results indicate that PCP signaling exists in the development of ectopic HCLCs and the convergent extension (CE) of the ectopic sensory region regulated by the testosterone-BSA through down-regulation of the cell-cell adhesion. Testosterone-BSA could promote more HCLCs in LER through proliferation. These data also suggest that testosterone maybe not essential for hair cell polarity while it plays roles in cell-cell adhesion, proliferation and differentiation of cochlear LER regions.

The Effects of Surface Patterning in Cell Expansion of Auricular Chondrocytes

Oh JH¹, Hwang SH¹, Park SH¹, Kim DS² and Kim SW¹

¹ Department Otolaryngology-Head and Neck Surgery, The Catholic University of Korea, Seoul, Korea,

² Department of Mechanical Engineering, Pohang University of Science and Technology, Pohang, Korea

Background and Purpose: The function of living cells is exquisitely sensitive to the local micro- and nano-scale topographic and biomolecular patterns constituting complex and hierarchical adhesive microenvironment of extracellular matrix (ECM) in three dimensions. Recent progress in developing techniques for fabrication of biocompatible materials at nano-scale help address how cellular interactions with engineered ECM on nanoscale coordinate diverse cellular processes. The synthesis and fabrication of structural and functional materials can enhance mechanical properties and structural features of the extracellular milieu. The aim of this study is to observe the surface effects of nano-patterning in cellular proliferation of auricular chondrocytes and to investigate the potential for fabricating advanced scaffolds with nanopillar and nanopore patterns, which may be beneficial for tissue regeneration versus flat surface scaffolds.

Materials and Method: Human auricular chondrocytes were harvested and seeded onto the wells of micro-pillar, micro-hole and flat types of nanoscale plate. The rate of cellular proliferation was compared between each type of the plate at the time of 0, 3, 5 and 7 days by means of DNA and mRNA synthesis and expression of Aggrecan and type II collagen using a cell counting kit (CCK)-8 and RT-PCR. Histologic analysis was also performed with a scanning electron microscopy and a confocal microscope.

Results: The proliferation rate of the cells cultured on micro-pillar and micro-hole type nanoscale plates were significantly higher than that of the cells on the flat type plate. The expression of Aggrecan and type II collagen was increased on nanopillar and nanopore plates. The morphology of the cells cultured on nanoplates resembled that of the native auricular chondrocytes.

Conclusion: The surface effects of nano-patterning in cellular proliferation of auricular chondrocytes was significant to fabricate advanced scaffolds with nanopillar and nanopore patterns, which may be beneficial for tissue regeneration.

Gap Junctional Coupling Is Essential For Epithelial Repair In The Chicken Cochlea

Jagger, D.¹, Nickel, R.¹, Forge, A.¹

¹UCL Ear Institute, University College London, London, United Kingdom

The loss of auditory hair cells triggers extensive repair responses within the population of non-sensory supporting cells. When hair cells are irreversibly lost from the mammalian cochlea, supporting cells migrate and expand to fill the resulting lesions in the sensory epithelium, a repair process which is dependent on gap junctional intercellular communication (GJIC; Forge et al, *J Cell Sci* 2013). In the chicken cochlea (the “basilar papilla”, BP) dying hair cells are extruded from the epithelium and supporting cells expand to fill the lesions, and supporting cells then replace the hair cells via mitotic and/or conversion mechanisms. Here we investigated the involvement of GJIC within the epithelial repair process in the aminoglycoside-damaged BP. Gentamicin-induced hair cell loss was associated with a decrease of chicken connexin43 (cCx43) immunofluorescence, yet cCx30-labeled gap junction plaques remained evident. FRAP experiments confirmed that GJIC remained robust in gentamicin-damaged explants. Dye injections in slice preparations from undamaged BP explants identified morphologically distinct, but electrophysiologically unspecialized cell types. In gentamicin-damaged BP, supporting cells expanded to fill hair cell lesions and displayed more variable electrophysiological phenotypes. Dye injections also identified cell-pairs that were morphologically suggestive of damage-activated asymmetric division. When GJIC was inhibited during the aminoglycoside damage paradigm the repair response was halted. Dying hair cells were retained within the sensory epithelium and supporting cells remained unexpanded. These observations suggest repair of the auditory epithelium shares common mechanisms across vertebrate species, and emphasize the importance of functional gap junctions in maintaining a homeostatic environment permissive for hair cell regeneration.

Inner Ear Neuron Culture In 3-D Gradient.

Edin F¹, Li H¹, Liu W¹, Rask-Andersen H¹

¹Department of Surgical Sciences; Otolaryngology and Head & Neck Surgery, Uppsala University, Uppsala, Sweden

Background: Since its introduction 50 years ago the cochlear implant (CI) has provided people afflicted with profound hearing loss a sense of hearing. Speech recognition in noisy environments remains a challenge for CI users as well as enjoying music. Closing the gap between CI and spiral ganglion (SG) neurons could improve the hearing sensation and increase frequency discrimination but requires potent guidance compounds and nerve stimulating gels.

Objectives: Develop a technique to culture inner ear neurons in a gradient maintained in a 3-D gel.

Methods: Human superior vestibular ganglion can be isolated during translabyrinthine surgery removing Vestibular Schwannoma. SG neurons were isolated from neonatal mice. After pre-culture in Neurobasal media explants sprouting neurons were embedded in Matrigel inside a microfluidic gradient chamber, applying a gradient of BDNF or Netrin-1. Time-lapse video microscopy (TLVM) was used to track neural outgrowth. Neurons were identified using nerve specific class III β -tubulin (Tuj1).

Results: Inner ear neurons can be maintained inside the gradient chamber and sprout neurons into the Matrigel. Length, elongation speed and directional changes in the growth cones and axons could be analyzed from TLVM videos.

Conclusions: This technique could prove useful in the search for neural guidance compounds and nerve stimulating gels that could be used to close the gap between CI and SG.

Defining the Role of Integrins in the Repair and Regeneration of Hair Cells in the Human Vestibular System

Hussain K¹, Forge A¹, Saeed SRS¹, Dawson S¹, Taylor R¹.

¹UCL Ear Institute, London, UK

Integrins are cell adhesion receptors that play important roles in physiological and pathological processes throughout the body. Integrins comprise alpha and beta subunits and the 24 $\alpha\beta$ heterodimeric members mediate the interaction of cell – cell signalling as well as communication between the cell and the extracellular matrix.

The lesion created by the death of a hair cell is closed by supporting cells in a manner that maintains the permeability barrier at the luminal surface of the epithelium. This controlled process relies on cell shape changes and spreading that likely involves integrins. Furthermore, the supporting cells are thought to remove these dead hair cells by a phagocytic process. Certain integrin receptors recognise apoptotic cells mediating phagocytosis of the apoptotic body.

Following hair cell loss in the vestibular system of mammals there is limited regeneration which may be due to the direct phenotypic conversion of supporting cell into hair cells without an intervening mitotic event. We hypothesise that integrins play an integral role in the repair and recovery process. We are able to test this hypothesis with human vestibular tissue obtained from patients undergoing trans labyrinthine procedures for acoustic neuromas and provided by a National consortium of surgeons.

Real time PCR has been used to demonstrate which integrins are present in human vestibular explants at different time points. Human utricles have been cultured for 14 days following ototoxic injury and complete hair cell loss. The integrin profile at specific time points during the regeneration phase will be presented. We shall also show their location and further elucidate their role in the repair and regeneration of hair cells.

Effect Of Netrin-1 For Spiral Ganglion Afferent Dendrite And Synapse Regeneration

Nakarin Asaka¹, Kazuhiro Shiina¹, Kohei Yamahara², Norio Yamamoto², Takayuki Nakagawa², Juichi Ito², Ishikawa Kazuo¹

¹ Akita University, Akita, Japan, ² Kyoto University, Kyoto, Japan

Netrin-1(Ntn1) is a member of a family of secreted laminin-related proteins involved in axon guidance and cell migration. Promotion of neurite outgrowth in spiral ganglion cells by exogenous Ntn1 has been reported. Previously, we have demonstrated the effect of insulin-like growth factor1 (IGF-1) for afferent dendrites of spiral ganglion neurons and synaptic contacts between spiral ganglion neurons (SGNs) and inner hair cells (IHCs). Ntn1 has been identified as one of the IGF1 signaling downstream effectors therefore we hypothesized that Ntn1 may also induce regeneration of afferent dendrites and their synaptic contacts.

Objective: To identify the role of Ntn1 for spiral ganglion afferent dendrite and synapse regeneration.

Method: Cochlear explants obtained from P2 mice were used in this study. Degeneration of afferent dendrites between spiral ganglion neurons and inner hair cells was induced by application NMDA and kainite. After intoxication by NMDA and kainite, Ntn1 was applied to the culture media at different concentrations. We then assessed therapeutic effects of Ntn1 by quantified the numbers of afferent dendrites attaching to inner hair cells and synaptic vesicles using immunostaining for neurofilament, myosin VIIa, CTBP2 and Shank1.

Results and Conclusion: The therapeutic effect of Ntn1for spiral ganglion afferent dendrites and synapse regeneration were comparable to the IGF1, particularly in the afferent dendrites and post synapse restoration. Accordingly, Ntn1 may have intimate role for the afferent dendrites and post synaptic regeneration.

Acknowledgements: This work has been supported by the grant from the Japanese Ministry of Health, Labor & Welfare and the Ministry of Education, Science, Sports, Culture and Technology of Japan.

The activation of stem cell homing factors highly induces the cochlear invasion of bone marrow mesenchymal stem cells.

Ichiro Fukunaga¹, K Karasawa¹, K Hatakeyama, O Minowa², K Ikeda¹, Kazusaku Kamiya¹,

¹ Juntendo University School of Medicine, Department of Otorhinolaryngology, Tokyo, Japan, ifukunag@juntendo.ac.jp

² BioResource Center, RIKEN, Tsukuba, Japan

Objectives: Congenital deafness affects about 1 in 1000 children and more than half of them have genetic background such as Connexin26 (Cx26) gene mutation. The strategy to rescue such heredity deafness has not been developed yet. We have previously developed a new strategy for sensorineural deafness, mesenchymal stem cell (MSC) transplantation targeting cochlear fibrocyte. In this study, we examined the new treatment to enhance the MSC induction to cochlear tissue to improve this strategy.

Methods: We applied the inner ear MSC transplantation to Cx26 deficient mice which we developed as a model for hereditary hearing loss. We transplanted the MSCs to the lateral semicircular canal after the induction of stem cell homing factors (stromal cell-derived factor-1: SDF-1, monocyte chemotactic protein-1: MCP-1) in the host cochlear tissue, and their receptors in transplant MSCs.

Results: To enhance MSC invasion to cochlea tissue, we developed a novel transplant strategy by induction of SDF-1 /MCP-1 expression in host cochlear tissue and enhanced expression of their receptors, chemokine (C-C motif) receptor 2 (CCR2) and C-X-C chemokine receptor type 4 (CXCR4) in MSC.

Conclusions: With this strategy, we induced efficient invasion of MSC to inner ear tissue and differentiation to form gap junctions with Cx26 among transplanted MSCs in Cx26-deficiente mouse inner ear.

From Hair Follicles In The Human Ear Canal To Neuron-Like Cells

Li H¹, Edin F¹, Hayashi H^{1,2}, Rask-Andersen H¹

¹Department of Surgical Sciences; Otolaryngology and Head & Neck Surgery, Uppsala University, Uppsala, Sweden, ²Department of Otolaryngology, Gifu University, Gifu, Japan

Background: Damages on auditory hair cells in the human organ of corti are irreversible and often lead to degeneration of auditory nerve. Intense researches have been focused on stem cells either as an option of treatment or as a tool to screen for compounds with regenerative effects. However, the adult human inner ear tissues are very limited and often not accessible.

Objectives: The aim is to locate, isolate and expand stem cells from the human ear canal, and evaluate the possibility to differentiate these cells into auditory neurons and eventually auditory hair cells.

Methods: Human skin in the ear canal was harvested during translabyrinthine surgery for removal of Vestibular Schwannoma. Immunohistochemistry was performed to locate adult human stem cell markers, and neural progenitor cell markers. Single hair follicles were dissected and cultured in gamma secretase inhibitor (Y-27632) containing medium. Differentiation was started using BDNF, GDNF and NT3 supplemented Neurobasal medium.

Results: Hair follicles in the human ear canal stained positive for Lgr5, Nestin, SOX2 and CD34. Spheres were formed in Y-27632 supplemented medium 1 week after seeding and 9-10 days without Y-27632. After 2 weeks of differentiation, Neuronal Class III β -Tubulin (TUJ1) positive neuron-like cells started to appear. ENStemA sphere induced differentiation of hair follicle spheres also resulted in neuron-like cells in Matrigel.

Conclusions: The human ear canal provides an excellent source of primary adult stem cells. It contains more stem cells, is more accessible, and easier to work with than human inner ear tissues. The possibility of differentiating hair follicles into auditory hair cells remains to be studied.

Differentiation Of Human Embryonic Stem Cells Towards Sensory Neuronal Phenotype

Ali RQ¹, Cheng YF¹, Kostyszyn B¹, Ährlund-Richter L², Ulfendahl M¹.

¹Department of Neuroscience, Karolinska Institutet, Retzius väg 8, SE-171 77, Stockholm, Sweden, ²Department of Women's and Children's Health, Karolinska Institutet, CCK R8:00, SE-171 76, Stockholm, Sweden.

Sensorineural hearing loss, caused by the degeneration of inner ear sensory hair cells or afferent auditory neurons, is a major problem in clinical medicine. In birds and lower vertebrates, regeneration of these cells may occur but in mammals, the loss is irreversible. Neuronal progenitors were derived from human embryonic stem cells in the form of neural tube-like rosettes. Such neuronal progenitors were harvested enzymatically after 4 days (NS4), 7 days (NS7) or 11 days (NS11) and allowed to further differentiate on laminin-coated plates for 1 or 3 weeks in neural induction medium without bFGF.

Cells in all cultures (NS4, NS7 and NS11) were found immunoreactive for nestin, a common neuronal progenitor marker, as well as for Tuj1, an early marker for neurons. The induced presence of the markers TrkC (20%) and peripherin (11%) indicated the NS7 protocol as the most favorable of the three tested for the induction of sensory neurons. NS7+1 is a straightforward protocol compared to other published protocol where different growth factors were required. These results may contribute to the further exploration of novel approaches for *in vitro* generation of sensory neurons, and treatment of sensory hearing impairment using cell transplantation.

Natural pathway of stem cells within the cochlear nucleus of rats

Voelker J¹, Rak K¹, Schendzielorz P¹, Radeloff A¹, Hagen R¹

¹Department of Oto-Rhino-Laryngology, plastic, aesthetic and reconstructive head and neck surgery, comprehensive hearing center, University of Wuerzburg, Josef-Schneider-Strasse 11, 97080 Wuerzburg, Germany

In 2011 neural stem cells could be identified in the cochlear nucleus of rats (Rak et al.). In order to get more information on the function of these cells, the further natural pathway of these stem cells was in the focus of the present investigations. Therefore the influence of exogenous soluble factors on these stem cells, particularly the modulators of the beta-catenin-pathway was examined. In a first series the modulatory capacity of SB-216763, an ATP-competitive inhibitor of glycogen synthase kinase 3, was evaluated.

Cochlear nuclei of P6 rats were dissected microscopically, cultured for 4 weeks in stem cell medium (Neurobasal, GlutaMAX, B27, EGF, FGF-2), and expanded by formation of neurospheres. After dissociation of the spheres single cells were plated in differentiation medium (Neurobasal, GlutaMAX, B27). SB-216763 (10 µM), retinoic-acid (10 µM) or DMSO as negative control were added to the differentiation medium. After a period of 1 week, the cells were fixed by PFA. Neuronal and glial markers were identified immunocytochemically in the matured cells and quantified by fluorescence microscopy.

A significantly higher number of neurons, stained by β-III tubulin antibody, could be detected in the SB-216763 group compared to the retinoic-acid- and the control group. Additionally the number of astrocytes, stained by GFAP antibody, was significantly lower in the SB-216763 group. The highest rate of astrocytes could be identified in the retinoic-acid group

The present study shows that the beta-catenin-pathway is a key component in the natural course of stem cells of the cochlear nucleus. The differentiation of these cells can be modulated exogenously by soluble factors. These insights possibly can be used for the development of neurobionic electrode coatings in future.

Characterization of differentiating neural stem cells from the rat cochlear nucleus by spontaneous calcium activity analysis

Voelker C¹, Rak K¹, Voelker J¹, Schendzielorz P¹, Radeloff A¹, Hagen R¹

¹Department of Oto-Rhino-laryngology, plastic, aesthetic and reconstructive head and neck surgery, comprehensive hearing center, University of Wuerzburg, Josef-Schneider-Strasse 11, 97080 Wuerzburg, Germany

In 2011 neural stem cells could be identified in the cochlear nucleus of rats (Rak et al.). In order to achieve more information on the function of these cells, the capacity of differentiation of these stem cells was the focus of the present investigations. Therefore the stem cell differentiation was characterized by calcium imaging, because the spiking activity of calcium is an indirect method to measure spontaneous depolarization and action potentials within neural cells.

Cochlear nuclei of P5 rats were dissected microscopically, cultured for 4 weeks in stem cell medium (Neurobasal, GlutaMAX, B27, EGF, FGF-2), and expanded by formation of neurospheres. After dissociation of the spheres single cells were plated in differentiation medium (Neurobasal, GlutaMAX, B27 with retinoic-acid (10 μ M). Subsequently the cells were loaded with the calcium-sensitive fluorophore Oregon-Green (OG) subsequently. Measurements were performed on day 0, 4, 8, 12, 16 of differentiation. Finally measured in-vitro cultures were fixed with paraformaldehyde and analyzed immunocytochemically.

The results show that the potential of spiking activity during differentiation is highest at a critical period of cell development. Cells, coupled in a network, showed an increase of activity between day 0 and day 4 of differentiation. After the peak the frequency of spontaneous oscillations decreased continuously until running off on day 12 to 16. These results display, that cochlear nucleus derived NSCs show different activity during differentiation in cell culture. It will lead to a better insight of the developmental features of the auditory system. Thus might in new therapeutically approaches for hearing loss in the future.

Hypoxia Induces A Metabolic Shift And Enhances The Stemness Of Cochlear Spiral Ganglion Stem/Progenitor Cells

Wang CH, Chen HC, Li SL, Chen HK, Shih CP, Lin YC

Department of Otolaryngology-Head and Neck Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, Republic of China.

Previously, we demonstrated that hypoxia (1% O₂) enhances stemness and expands the cochlear stem/progenitor cells (SPCs). In this study, we further investigated the effect of hypoxia on stemness and the bioenergetic status of cochlear spiral ganglion SPCs cultured at different low oxygen tensions.

Cochlear spiral ganglion SPCs were obtained from postnatal day 1 CBA/CaJ mouse pups. The morphology, stemness-related protein expression, and distribution of side population (SP) of spiral ganglion SPCs cultured at different oxygen tensions (1%, 5%, and 20% O₂) were assessed following long-term cultivation. The measurement of oxygen consumption rate, extracellular acidification rate (ECAR), and intracellular ATP levels corresponding to different oxygen tensions were determined using a Seahorse XF Extracellular Flux Analyzer.

After low oxygen tension cultivation for 21 days, the mean size of the hypoxia-expanded neurospheres was significantly increased at 5% O₂; it correlated with high-level expression of Hif-1 α , PCNA, cyclin D1, Abcg2, Nestin and Nanog but downregulated expression of p27 compared to that in a normoxic condition. Hypoxia tended to increase the SP fraction, with a significant difference found at 5% O₂ compared to that at 20% O₂ cultivation. In addition, low oxygen tension induced a metabolic shift of cells toward higher basal ECARs, higher maximal mitochondrial respiratory capacity, and higher mitochondrial reserve capacity than under normoxia. The ATP content showed no difference at 5% O₂ compared to normoxia.

Hypoxia was demonstrated to expand SPCs in a more efficient way than normoxia by leading to an increase the neurosphere size and numbers, and switching of SPCs metabolism toward glycolysis.

H₂O₂ treatment accelerates autophagy and cellular senescence in auditory cell line.

Tsuchihashi Nana Akagi^{1,2}, Hayashi Ken¹, Goto Fumiyuki¹, Yasuyuki Nomura³, Takeshi Masuda³, Fujioka Masato¹, Kanzaki Sho¹, Ogawa Kaoru¹

¹ Otorhinolaryngology, Keio University, Tokyo, Japan ² Otorhinolaryngology, Kyushu University, Fukuoka, Japan, ³ Otorhinolaryngology, Nihon University, Tokyo, Japan

Objectives: Age-related hearing disorder is known to be elicited by mitochondrial dysfunction caused by various stresses including reactive oxygen species. Recent studies have indicated that autophagy plays an important role in maintenance of cellular function sequestering damaged, oxidized or dysfunctional intracellular components and organelles, and is also associated with aging. However, the role of autophagy on cellular senescence of inner ear and age-related hearing loss remains unclear. In this study, we investigated the molecular mechanism of autophagy and cellular senescence in auditory cells.

Methods: We used auditory cell line, HEI-OC1. After treatment with H₂O₂, cell viability and population doubling were counted. We detected autophagy related proteins with western blotting. SA-β-gal staining was performed for detecting cellular senescence. Atg7 and AMPKα siRNA were transfected into HEI-OC1 cells with electrophoration.

Results: The cell doubling time was significantly delayed in low dose H₂O₂-treated HEI-OC1, while cell viability didn't change. The proportion of SA-β-gal-positive cells was significantly increased in treated cells. The expression levels of Atg7 and LC3-II were elevated with its peak at 6h, followed by activation of Lamp2 with its peak at 24h. The protein level of p-AMPK, known as a key gene of life span elongation, also was increased at 24h in treated cells. The premature senescence with characteristic increase in SA-β-gal staining was significantly induced in H₂O₂-treated AMPK and Atg7 KD cells, compared with control cells.

Discussion: Our results suggested that autophagy and AMPK activation plays a crucial role in cellular senescence in auditory cell under oxidative stress.

Inhibition of histone deacetylases attenuates noise-induced hearing loss and outer hair cell death

Kayla Hill, Jun Chen, Su-Hua Sha

Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425, USA

Post-translational modification of histones alters their interaction with DNA and nuclear proteins, influencing cell fate. Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDAC inhibitors have been studied for the treatment of cancer and neurodegenerative diseases. We investigated noise-induced histone modification in outer hair cell (OHC) nuclei of adult CBA/J mice. One hour after noise exposure, the acetylation of histone (histone H3 lysine 9) was decreased in OHC nuclei in the basal region of the cochlea. Consistent with these results, levels of histone deacetylase 2 (HDAC2), an enzyme that removes acetyl groups, were increased in noise-exposed OHC nuclei in the basal region, while histone acetyltransferase (HAT) p300, which adds acetyl groups, was unchanged. On the other hand, methylation of histone H3 lysine 9 (di-meH3 K9) was unaffected by noise exposure. Finally, treatment with the HDAC inhibitor SAHA (suberoylanilide hydroxamic acid) altered the distribution of histone acetylation in OHC nuclei and attenuated noise-induced hearing loss and OHC death. These findings suggest that histone acetylation is involved in the pathogenesis of noise-induced OHC death. Pharmacological targeting of histone deacetylase may protect against noise-induced hearing loss.

The research project described is supported by R01 DC009222 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

Expression of insulin and cAMP signaling components in hair cells and supporting cells of the human inner ear-implications for diabetes and Menieres disease.

Eva Degerman, Uwe Rauch, Anna Hultgårdh, Sven Lindberg, Per Cajé-Thomasen, Måns Magnusson

Introduction: A number of studies have demonstrated an association between diabetes and inner ear morphological as well as functional alterations as well as between cAMP signaling and Menieres disease. In two previous studies (1,2) we have started to map insulin and cAMP signaling components and targets in the human inner ear. In the present study we continue this work.

Material and Methods: Under an operating microscope, human saccules were dissected from the vestibule during the removal of vestibular schwannoma via the translabyrinthine approach and analysed using immunohistochemistry.

Results: We show hair cell localization of the cAMP and cGMP degrading enzymes PDE3B, PDE1B, PDE1C, PDE4D, PDE8A, the cAMP targets aquaporin 2 and TORC2 (a regulator of CREB, cAMP response element binding protein) and the insulin signaling components mTOR and also for proteins of relevance for exocytosis, SYT7, STX1 possibly involved in AQ2 translocation. We show that supporting cells express a number of insulin target proteins; the NKATPase, the insulin receptor, the IGF1 receptor and protein kinase B as well as another set of exocytotic proteins including SNAP25, SYT11 possibly involved in GLUT4 translocation. Selected targets will be tested functionally in mouse models for endolymphatic hydrops and diabetes using MR as read-out.

Discussion: Insulin and cAMP signaling components identified in the human saccule provide a new platform for the understanding of mechanisms of disease pathophysiology and for drug development.

1.Degerman E et al. Expression of insulin signalling components in the sensory epithelium of the human saccule. *Cell Tissue Res.* 2013 Jun;352(3):469-78

2.Degerman E et al. , Identification of new signaling components in the sensory epithelium of human saccule. *Front Neurol.* 2011 Aug 5;2:48.

Arc Gene Expression Difference In The Adult Rat Brain Between Unilateral And Bilateral Deafness

Park MH¹, Lee HS²

¹Department of otorhinolaryngology, Boramae Medical Center, SMG-Seoul National University,

²Department of Otorhinolaryngology, Seoul National University Hospital, Seoul, Korea.

Deafness induces many plastic changes in the neural auditory system. Arc is one of the immediate early genes and a regulator of synaptic plasticity. It is also induced hearing loss in the auditory cortex. The object of this study is to evaluate the Arc expression in deafened adult rat brain and to distinguish the difference between unilateral and bilateral deafness. Three groups of adult male Sprague-dawley rats were prepared for control, unilateral deafness and bilateral deafness. Deafness induced using cochlear ablation method. After deafening, 2, 4, 6 and 12 weeks later, auditory cortices were harvested for RT-qPCR test. In the bilateral deafened group, mRNA expression of Arc was decreased at 2, 4, 6 weeks and normalized at 12 weeks. In the unilateral deafened group, expression was much decreased at 4 weeks and increased at 6 and 12 weeks. This pattern was shown as same manner in both sides of auditory cortex. Western blot testing showed same results. This results suggest that a possibility of some regulatory pathway between both auditory cortex so they response same pattern after unilateral deafness.

Two Novel Causative Proteins for Hereditary Hearing Loss, ATP6B1 and PENDRIN, co-expressed in the Inner Ear Lateral Wall of Common Marmoset

Hosoya Makoto¹, Fujioka Masato², Okano Hideyuki³ and Ogawa Kaoru²,

¹Department of Otolaryngology Head and Neck surgery, KEIO university school of medicine, Shinjuku-ku, Tokyo, Japan ²Department of Physiology, KEIO university school of medicine, Shinjuku-ku, Tokyo, Japan

Mutations in ATP6B1 (also called V-ATPase B1) in human being cause autosomal recessive distal renal tubular acidosis and deafness, yet its knock-out mouse does not suffer hearing loss. Mutations in PENDRIN (also called SLC26A4) in human being cause Pendred Syndrome, an autosomal recessive disease characterized by progressive hearing loss combined with thyroid goiter, yet its knockout mouse caused congenital profound hearing loss. Common marmoset (*Callithrix jacchus*) is a new world non-human primate which has recently been used by biomedical researchers. Here, we report expression patterns of ATP6B1 and PENDRIN in the inner ear of common marmoset for understanding the two novel causative proteins for hereditary hearing loss with distinct different phenotypes between human patients and mouse knock-out model. <Methods> Temporal bones of young adult common marmoset and mouse were dissected, fixed and decalcified and made into cryosections. Immunohistochemistry was done with antibodies for V-ATPase B1 and PENDRIN. <Results> As reported previously, immunoreactivity for ATP6B was not detected in the mouse inner ear lateral wall, while robust signal was observed in the inner ear of marmoset, restricted in the outer sulcus cells and co-expressed with PENDRIN. <Conclusion> Knowledge in the inner ear field is largely dependent on the experiments performed in rodent models, but the phenotypes of human beings are not always recapitulated in the rodents presumably due to the large species differences. Our result suggests gaps in biochemical and functional characters of outer sulcus cells between mouse and primates (at least Common Marmoset, probably Human).

Assembly and Disruption of Cochlear Gap Junction Macromolecular Complex are Regulated by Connexin 26

Hatakeyama, K¹, Fukunaga, I¹, Ikeda, K¹, Kamiya, K¹

¹Department of Otorhinolaryngology, Juntendo University School of Medicine, Tokyo, Japan,

Objectives: The mutations in connexin26 (Cx26), a cochlear gap junction protein, represent a major cause of pre-lingual, non-syndromic deafness, as they are responsible for as many as 50% of such cases in certain population. Recently, we reported that Cx26-dependent gap junction plaque (GJP) disruption occurred as the earliest change during embryonic development, results in a drastic reduction in the GJP area and the protein levels (Kamiya et al., J Clin Invest, 2014). To elucidate the mechanism of this biochemical change, we developed the molecular live imaging system targeting GJP composed of Cx26 and Cx30. Our final goal is to screen the chemicals to stabilize the cochlear GJPs at the cell borders.

Methods: The cells with the transient expression and stable expression of human wild type Cx26, mutant Cx26 (R75W) and wild type Cx30 tagged with GFP or mCherry were generated with HEK293 and HeLa cell lines.

Results: With the connexin expressing cells which we newly generated, we observed various types of GJPs. Our system enabled us to analyze the formation, trafficking, membrane integration and degradation of GJPs composed of Cx26 and Cx30 in live cell monitoring.

Conclusions: This imaging system will enable the large scale drug screening targeting GJP stabilization for most typical hereditary deafness, Cx26 associated deafness.

Tricellulin deficiency causes deafness due to cochlear rapid hair cell degeneration.

Toru Kamitani^{1,3,4}, Hirofumi Sakaguchi¹, Takenori Miyashita², Ryuhei Inamoto², Reitaro Tokumasu³, Atsushi Tamura³, Nozomu Mori², Sachiko Tsukita³, Yasuo Hisa¹

¹Department of Otolaryngology-Head and Neck Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan, ²Department of Otolaryngology, Faculty of Medicine, Kagawa University, Kagawa, Japan,

³Laboratory of Biological Science, Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University, Osaka, Japan, ⁴Department of Otolaryngology, Saiseikai Kyoto-fu Hospital, Kyoto, Japan

Tricellulin is a protein which localizes mainly at tricellular tight junctions. It is reported that the tricellular junction was disorganized and that the barrier function was decreased in *tricellulin* knock-down cell lines. However, the function of tricellulin *in vivo* has not been elucidated. On the other hand, mutation of *tricellulin* causes human nonsyndromic deafness DFNB49.

We generated *tricellulin* knockout (*Tric*^{-/-}) mice to investigate the pathophysiology of DFNB49. *Tric*^{-/-} mice looked normal, but ABR revealed severe hearing loss. Immunofluorescence microscopy revealed rapid cochlear hair cell generation from P14, although any other apparent alterations were not observed by scanning and transmission electron microscopy. Many Hair cells from *Tric*^{-/-} cochlea survived *in vitro*, as well as hair cells from *Tric*^{+/+} cochlea. The EP of *Tric*^{-/-} cochlea was maintained normally.

As a conclusion, *Tric*^{-/-} mice showed severe hearing loss without any other abnormalities. *Tric*^{-/-} mice is a good model of DFNB49. Hair cells of *Tric*^{-/-} cochlea degenerated from P14. The degeneration coincides with the rapid elevation of EP. Although any other structural alteration was found, it is assumed that the hair cell degeneration is due to the increased K⁺ ion permeability of the reticular lamina.

Three Dimensional Organization Of Inner Hair Cell Membrane Systems And Their Relationship To Synaptic Structures

Bullen, A.¹, West, T.¹, Fleck, R.², Ashmore, J.¹, MacLellan-Gibson, K.², Moores, C.³, Forge, A.¹

¹Centre for Auditory Research, UCL Ear Institute, London, United Kingdom, ²National Institute for Biological Standards and Control, Hertfordshire, United Kingdom, ³Institute of Structural and Molecular Biology, Birkbeck College, London, United Kingdom

The organization of the inner hair cell (IHCs) allows for rapid, precise and apparently indefatigable release of neurotransmitter to encode complex auditory stimuli. How the overall organization of the cell contributes to its unique properties is not well understood. Previous work in our lab identified and modeled a membrane network that appeared to be a consistent structural feature of IHCs. The aim of the current study was to further characterize this network and its relationship to synaptic structures within the cell.

3 month old C57/Bl6 mice and adult guinea pig cochlea were examined using serial block face scanning electron microscopy, electron tomography and immunoelectron microscopy.

The membrane network was comprised of putative membrane sheets including a large single sheet that traversed the cell from the apical region to the base. The distribution of the network appeared morphologically related to the positioning of afferent terminals around the cell. Previous work identified vesicles and mitochondria bound to the membrane sheets within IHCs. Examination of these vesicles showed them to have characteristics in common with synaptic vesicles, and vesicles were assessed for the presence of the glutamate transporter VGLUT3. Links between vesicles at the synaptic ribbon were also examined.

The shape and membrane sheet distribution of the IHC membrane network suggests that the network may be dynamic and multifunctional, with potential roles in vesicle biosynthesis, trafficking and calcium regulation. Links close to the synaptic ribbon may have a role in orientation of vesicles for docking.

Acoustic overstimulation activates 5'-AMP-activated protein kinase through a temporary decrease in ATP level in the cochlear spiral ligament prior to noise-induced hearing loss

Masanori Yoneyama,¹ Taro Yamaguchi,¹ Nobuyuki Kuramoto,² and Kiyokazu Ogita,¹

¹Laboratory of Pharmacology, ²Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka, Japan, m-yone@pharm.setsunan.ac.jp

Inner ear disorders are known to be elicited by mitochondrial dysfunction, which decreases the ATP level in the inner ear. 5'-AMP-activated protein kinase (AMPK) is a serine/threonine kinase activated by metabolic stress and by an increase in the AMP/ATP ratio. To elucidate the involvement of AMPK-derived signals in noise-induced hearing loss, we investigated whether *in vivo* acoustic overstimulation would activate AMPK in the cochlea of mice. Std-ddY mice were exposed to 8 kHz octave band noise at a 90-, 110- or 120-dB sound pressure level (SPL) for 2 h. Exposure to the noise at 110 or 120 dB SPL produced outer hair cell death in the organ of Corti and permanent hearing loss. Exposure to the noise at 120-dB SPL elevated the level of the phospho-AMPK α -subunit (p-AMPK α), without affecting the protein level of this subunit, immediately and at 12-h post-exposure in the lateral wall structures including the spiral ligament and stria vascularis. In the hair cells and spiral ganglion cells, no marked change in the level of p-AMPK α was observed at any time post-exposure. Noise exposure significantly, but temporarily, decreased the ATP level in the spiral ligament, in an SPL-dependent manner at 110 dB and above. Likewise, elevation of p-AMPK α and p-JNK levels was also observed in the lateral wall structures post-exposure to noise at an SPL of 110 dB and above. Taken together, our data suggest that AMPK and JNK were activated by ATP depletion in the cochlear spiral ligament prior to permanent hearing loss induced by *in vivo* acoustic overstimulation.

Keywords: Acoustic overstimulation, 5'-AMP-activated protein kinase, ATP, c-Jun N-terminal kinase, Hearing loss, Spiral ligament

The Effect of Mutation of the ATP-gated P2Y₂ receptor on hearing and susceptibility to noise

Park M¹, Moon JH¹, Suh MW¹, Lee JH¹

¹Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, Korea

Objectives

P2Y₂ receptor is known to be expressed widely in the cochlea. The objective is to examine the hearing of P2Y₂ receptor knock-out mice (Y₂ KO) compared to wild type (Y₂ WT) littermates and the response to exposure of loud sound.

Methods

Ten C57BL/6 Y₂ KO and 10 WT mice were investigated. Hearing was analyzed with auditory brainstem response (ABR) in response to click stimuli at 4 weeks after birth in quiet environment. And then the mice were exposed to 120 dB SPL white noise for 60 minutes. The ABR was measured soon after noise exposure and then 3 weeks later.

Results

The hearing threshold showed no difference in between the Y₂ KO group (48.2 ± 11.0 dB SPL) and Y₂ WT group (40.0 ± 10.5 dB SPL) at quiet environment (p = 0.78). The hearing immediate after noise exposure showed no difference in between the Y₂ KO group (65.0 ± 12.0 dB SPL) and Y₂ WT group (57.5 ± 10.1 dB SPL) (p = 0.77). The hearing 3 weeks after noise exposure showed no difference in between the Y₂ KO group (60.6 ± 14.4 dB SPL) and Y₂ WT group (52.5 ± 17.7 dB SPL) (p = 0.84)

Conclusions

P2Y₂ KO and WT mice showed no significant difference in hearing at quiet condition and in response to noise exposure. It suggests that P2Y₂ function is not essential for normal hearing and for protection from exposure to noise.

Notch Signaling Negatively Regulates Formation and Growth of the Stereocilia Bundle

Yi Li^{1,2}, Shuping Jia², Shusheng Gong¹, David Z. Z. He²

¹Department of Otolaryngology, Beijing Tongren Hospital, Beijing, P.R.China; ²Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, Nebraska, USA

Our goal is to determine whether the differentiation and growth of stereocilia and the mechanotransduction apparatus are regulated by Notch signaling. Hair cells are polarized neuroepithelial cells characterized by stereocilia bundles on their apical surface. The molecular mechanisms that regulate morphogenesis of the stereocilia bundle are still elusive. We questioned whether Notch signaling regulates hair-bundle morphogenesis. The cultured organ of Corti from neonatal gerbils was used for the experiment. Notch pathway was pharmacologically blocked by adding 5 μ M DAPT. Morphology and function of the ectopic hair cells and their stereocilia were examined using immunohistochemistry (with confocal microscopy), scanning electron microscopy, and electrophysiology. We shows that pharmacological inhibition of the Notch pathway in the cultured organ of Corti of neonatal gerbils induced formation of stereocilia bundles in the supporting cells and supernumerary stereocilia in the existing hair cells. Despite the fact that the mechanotransduction apparatus of the newly emerged hair bundles is functional, the ectopic hair cells lacked other specializations of a typical hair cell, suggesting suppression of Notch signaling is insufficient to make a fully functional hair cell. Thus, it appears that Notch signaling regulates the pattern of hair cell and supporting cell differentiation by suppressing morphogenesis of stereocilia in supporting cells and maintaining stereocilia dynamics in hair cells. (Supported by NIH Grant DC 04696 to DH and by the Major State Basic Research Development Program of China (973 Program) (No. 2011CB504503) to SHG.

Regulation of KCNQ1/KCNE1 Channels by Sphingomyelin Synthase 1.

Wu MK^{1,2}, Takemoto M¹, Okazaki T³, Song W-J^{1,2}

¹Department of Sensory and Cognitive Physiology, Graduate School of Life Sciences, Kumamoto University, Kumamoto, Japan, ²HIGO Program, Kumamoto University, Kumamoto, Japan, ³Department of Hematology and Immunology, Kanazawa Medical University, Ishikawa, Japan.

Sphingomyelin synthase (SMS) catalyzes the conversion of phosphatidylcholine and ceramide to sphingomyelin and diacylglycerol. Two isoforms of SMS, SMS1 and SMS2, have been identified and exhibit differential subcellular localization. We have previously shown that SMS1 knock-out mice develop hearing impairment in a low frequency range. As a cause for the hearing impairment in SMS1 knock-out mice, we have demonstrated reduced and aberrant expression of the potassium channel KCNQ1 in the marginal cells of the stria vascularis of these mice (Lu et al., 2012). It is not known, however, whether SMS1 affects the expression of KCNQ1 directly in the marginal cells or via indirect effects. Here, we tested if manipulation of SMS1 activity affects KCNQ1 current in individual cells. To this end, we expressed KCNQ1/KCNE1 channels in HEK293-T cells which express little endogenous potassium currents, and evaluated current density with whole-cell recordings after SMS1 manipulations. Application of D609, a non-specific inhibitor of SMSs, significantly reduced current density and altered channel kinetics and voltage-dependence as well. Down-regulation of SMS1 by a shRNA, however, only reduced current density. Further, over-expression of SMS1 increased the current density without changing channel properties. These results suggest that SMS1 regulates KCNQ1/KCNE1 channel density in individual cells, and provide further evidence for the importance of SMS1 in inner ear function.

The Oxidative Stress Role in the Pathophysiology of Otosclerosis

Rudic M^{1,*}, Wagner R², Wilkinsson E^{2,3}, Kiros N⁴, Ferrary E^{5,6}, Sterkers O^{5,6}, Bozorg Grayeli A^{5,7}, Zarkovic K⁸, Zarkovic N⁹

¹ENT Department, General Hospital Zadar, Croatia; ²Global ENT Outreach, USA; ³House Ear Clinic, LA, USA; ⁴Megbare Senayi General Hospital, Addis Ababa, Ethiopia; ⁵UMR-S 867 Inserm / University Paris 7, France; ⁶AP-HP, La Pitié Salpêtrière Hospital, University Paris 6, France; ⁷Present address: ENT Department of Dijon, Bourgogne University, France; ⁸University Medical School, Clinical Hospital Centre Zagreb Division of Pathology, Croatia; ⁹Institute Ruđer Bošković, Zagreb, Croatia; *Present address: ENT Department, University Hospital Galway, Ireland

Objective: Otosclerosis is a complex disease characterized by an abnormal bone turnover of the otic capsule resulting in conductive and in advanced stages sensorineural hearing loss. Oxidative stress has been linked to hearing loss in different disorders such as noise and age induced hearing loss, Menière's disease, or Pendred syndrome. We investigated the role of oxidative stress, namely its secondary messengers (4-hydroxynonenal, HNE) in the pathophysiology of otosclerosis. **Methods:** The appearance of protein adducts of the HNE, in otosclerotic bone has been analyzed. In the first study we analysed 21 stapedial specimens in Caucasian population (15 otosclerotic, 6 controls), and in the second study, we analyzed 59 bone specimens in African population (25 otosclerotic stapedial, 34 control temporal bone). **Results:** Immunohistochemical analysis of HNE-modified proteins in tissue samples of the stapedial bones in the first series revealed regular HNE-protein adducts present in the subperiosteal parts of control bone specimens, whereas irregular areas of a pronounced HNE-protein adduct presence were found within otosclerotic samples. In the second series, the presence of HNE was found in almost all bone samples, without particular difference in the HNE distribution pattern between the otosclerotic and respective control bone specimens. Moreover, there was no obvious association between HNE and otosclerotic bone outgrowth observed. **Conclusion:** The results of the study indicate that HNE and oxidative stress might act in the regulation of bone cell growth and in the pathophysiology of otosclerosis. The obtained differences in the expression of oxidative stress product are most likely related to the different ecological and genetic influences in the studied population groups.

The $\alpha_2\delta_3$ Ca²⁺ Channel Subunit Is Essential For Normal Function Of Auditory Nerve Synapses And Is A Novel Candidate For Auditory Processing Disorders

Kurt S^{2,3,#}, Pirone A^{1,11,#}, Zuccotti A¹, Rüttiger L¹, Pilz P⁴, Brown DH^{5,12}, Franz C¹, Schweizer M⁶, Rust MB⁷, Rübsamen R⁵, Friauf E⁸, Knipper M¹, Engel J^{9,10}

¹Univ. of Tübingen, Dept. of Otolaryngology, Tübingen Hearing Research Centre (THRC), Molecular Physiology of Hearing, Tübingen, Germany, ²Cluster of Excellence "Hearing4all", Inst. of Audioneurotechnology & Hannover Medical School, Dept. of Experimental Otolaryngology, ENT Clinics, Hannover, Germany, ³Univ. of Ulm, Institute of Neurobiology, Ulm, Germany, ⁴Univ. of Tübingen, Inst. of Neurobiology, Tübingen, Germany, ⁵Univ. of Leipzig, Inst. of Biology II, Leipzig, Germany, ⁶Univ. of Hamburg, Center for Molecular Neurobiology, Hamburg, Germany, ⁷Univ. of Kaiserslautern, Dept. of Biology, Neurobiology/Neurophysiology Group, Kaiserslautern, Germany, ⁸Univ. of Kaiserslautern, Dept. of Biology, Animal Physiology Group, Kaiserslautern, Germany, ⁹Univ. of Tübingen, Dept. of Physiology II & Tübingen Hearing Research Centre (THRC), Tübingen, Germany, ¹⁰Saarland Univ., Dept. of Biophysics, Homburg/Saar, Germany, ¹¹present address: Tufts Medical School, Dept. of Neuroscience, Boston, MA, USA, ¹²present address: Program in Neuroscience, Dept. of Psychology, Florida State Univ., Tallahassee, FL, USA

([#] These authors contributed equally to this work)

The auxiliary subunit $\alpha_2\delta_3$ modulates the expression and functional properties of voltage-gated calcium channels. We found $\alpha_2\delta_3$ mRNA expression in spiral ganglion neurons and midbrain auditory nuclei. Genetic deletion of $\alpha_2\delta_3$ in mice led to mildly increased hearing thresholds in both click (5 dB) and frequency (10-15 dB) auditory brainstem response (ABR) measurements. ABR waveforms showed reduced amplitudes of wave II and distortion of waves III and IV in mutants, indicating impaired signal transmission along the auditory pathway. Further, expression of Ca_v2.1 channels was reduced at both somata of spiral ganglion neurons and bushy cells of the ventral cochlear nucleus. Using light and electron microscopy, we found significantly smaller sizes of auditory nerve fibre synaptic boutons, which terminate at bushy cell somata. We propose that the combination of reduced bouton size and smaller numbers of presynaptic Ca²⁺ channels accounts for the acoustic impairments of $\alpha_2\delta_3^{-/-}$ mice. *In vivo* recordings at the auditory nerve – bushy cell synapses revealed increased first spike latencies and reduced spike rates as a function of stimulus level in $\alpha_2\delta_3^{-/-}$ mice, indicating malfunction of the endbulb of Held synapse. In a behavioural task, auditory learning was assessed by training wildtype and $\alpha_2\delta_3^{-/-}$ mice to discriminate different simple and complex sound signals. As a result, $\alpha_2\delta_3^{-/-}$ mice showed pronounced deficits in discriminating complex acoustic signals. In conclusion, $\alpha_2\delta_3^{-/-}$ mice might represent a model for an auditory processing disorder.

Effect of prestin on cell volume regulation

Takashi Nabekura, M.D., Ph.D., Keiji Matsuda, M.D., Ph.D.,

Takeshi Nakamura, M.D., Tetsuya Tono, M.D., Ph.D.

Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, University of Miyazaki

Even under anisotonic conditions, most cells can regulate their volume by mechanisms called regulatory volume decrease (RVD) and increase (RVI) after osmotic swelling or shrinkage, respectively. In contrast, the initial processes of necrosis and apoptosis are associated with persistent swelling and shrinkage. Necrotic volume increase (NVI) is initiated by uptake of osmolytes, such as Na⁺, Cl⁻, and lactate, under conditions of injury, hypoxia, ischaemia, acidosis, or lactic acidosis. Apoptotic volume decrease (AVD) is triggered by activation of K⁺ and Cl⁻ conductances following stimulation with a mitochondrion-mediated or death receptor-mediated apoptosis inducer. The volume-sensitive outwardly rectifying (VSOR) anion channel is most prominently activated and ubiquitously expressed. This channel is known to be involved in a variety of physiological processes, including cell volume regulation, cell proliferation, differentiation, and cell migration, as well as cell turnover involving apoptosis. In the present study, we studied whether prestin, which is the motor protein attributed to the voltage-dependent contractility of OHCs, affects cell volume regulation. We measured cell volume with a DS camera. Hypotonic challenge induced transient swelling in HEK cells and HEK cells overexpressed with prestin, which is typically followed by RVD. In contrast, hypotonic challenge with salicylic acid induced persistent cell swelling in HEK cells overexpressed with prestin, without accompanying RVD; HEK cells failed to respond to hypotonic stress with RVD, and VSOR anion currents were inhibited.

We concluded that prestin affects cell volume regulation by coordinating VSOR anion currents.

Evaluation of the mouse vestibulo-ocular reflex with video-oculographyYasumitsu Takimoto^{1,2}, Takao Imai¹, Atsuhiko Uno¹, Yusuke Ishida², Tadashi Kitahara³, Takefumi Kamakura¹, Tomoko Okumura¹, Arata Horii¹, Hidenori Inohara¹ and Shoichi Shimada²¹ Department of Otorhinolaryngology - Head and Neck Surgery, Osaka University Graduate School of Medicine, Osaka, Japan² Department of Neuroscience and Cell Biology, Osaka University Graduate School of Medicine, Osaka, Japan³ Department of Otolaryngology - Head and Neck Surgery, Nara Medical University School of Medicine, Nara, Japan

The vestibulo-ocular reflex (VOR) is a reflex eye movement that stabilizes images on the retina during head movement by producing an eye movement in the direction opposite to head movement. Generally, VOR test has been well known to be useful model for studying the assessment of the vestibular function in animal. However, evaluation of diminutive animal eye movements is technically difficult. We developed a new system for analyzing the function of VOR during rotation with video-oculography in mouse.

Male C57BL/6J mice adult mice were included in this study. A custom-built head holder was cemented to the skull of animals. Animals were placed in a custom-built Plexiglas tube at the center of a turntable and then connected to the head holder. The animal's head was fixed 35° nose down to align the horizontal semicircular canals with the horizontal plane in darkness. Eye movements were recorded using a high resolution 240-Hz camera. The recorded images were analyzed by our own algorithm. The turntable was rotated sinusoidally at 0.5Hz, 1.0Hz or 2.5Hz. We calculated the gain and phase of VOR. This method makes it possible to evaluation the vestibular function of pharmacologically-treated, transgenic or knockout mice.

Whole-Mount Fluorescent Imaging of the Endolymphatic Sac using Prox1-GFP Transgenic Mice

Takeori Miyashita¹, James L. Burford², Young-Kwon Hong³, Haykanush Gevorgyan², Lisa Lam², Hiroshi Hoshikawa¹, Nozomu Mori¹, Janos Peti-Peterdi²

¹ Department of Otolaryngology, Faculty of Medicine, Kagawa University, Kagawa, Japan

² Departments of Physiology and Biophysics and Medicine, Keck School of Medicine, University of Southern California, Los Angeles, U.S.A.

³ Department of Surgery and Department of Biochemistry and Molecular Biology, Keck School of Medicine, University of Southern California, Los Angeles, U.S.A.

The transcription factor Prox1 belongs to the family of homeobox transcription factors. Prox1 is critical for organ development during embryogenesis and is involved in neurogenesis and in a variety of cancer cells. Prox1 was reported to have a cell-autonomous function in neurons in the cochlea. Although Prox1 expression is well investigated in the cochlea and the vestibule, expression in the endolymphatic sac has not been established. In this study, we examined Prox1 expression in the endolymphatic sac in adult mice, and established a whole-mount imaging system of the endolymphatic sac.

A strong Prox1-GFP fluorescence signal was observed in epithelial cells of the endolymphatic sac in adult mice. In the intermediate portion of the endolymphatic sac, mitochondria rich cells did not express Prox1.

The use of Prox1-GFP mice made possible the whole-mount imaging and three-dimensional observations of the endolymphatic sac epithelial cells. Using this whole-mount imaging technique, the endolymphatic sac was observed. This whole-mount imaging technique of the endolymphatic sac will be a useful method for endolymphatic sac research.

A Cochlear Origin for Binaural Time and Phase Delay?

Karino S^{1,2}, Joris PX¹

¹ Lab. of Auditory Neurophysiology, KU Leuven, Leuven, Belgium, ² Dept of Otorhinolaryngology, University of Tokyo, Japan

Low-frequency neurons in various nuclei in the brainstem and midbrain, including the inferior colliculus (IC), display sensitivity to interaural time differences (ITDs). A general feature of this sensitivity is that it seems to reflect a mixture of a pure *time* difference between inputs from the two ears (e.g. as would be generated by an axonal delay line) and a constant *phase* difference (i.e. a phase-difference between the two ears which is not frequency-dependent). These two aspects of ITD-responses are referred to as the characteristic delay (CD) and characteristic phase (CP) and are derived by graphing interaural phase as a function of stimulus frequency. In several binaural studies, an inverse relationship between CD and CP has been reported, where large CDs are associated with small, negative CPs and large CPs with small, negative CDs. The basis for this relationship is unclear.

We studied the effects of differences in characteristic frequency (CF) on CD and CP with a coincidence analysis. Responses of cat auditory nerve (AN) fibers to tones were obtained for a range of frequencies. We then counted coincidences between spike trains of pairs of fibers with similar but not identical CF, and subjected the resulting trains of coincidences to a CD-CP analysis. We compared the outcome with responses to binaural tones recorded in the IC.

In the AN, random mismatches in CF of pairs of fibers generate time and phase differences in coincidence patterns. CD and CP are inversely related with a pattern consistent to that found in the IC, and a similar pattern is obtained in response to broadband noise.

These results are consistent with CF mismatches as a mechanism for the generation of CD and CP, and suggest the possibility that the inverse relationship between CD and CP reflects cochlear mechanics.

Evidence of the K⁺-circulation Current that Controls the Electrochemical Properties of the Mammalian Cochlea.

Sato M^{1,2}, Yoshida T^{1,3}, Nin F¹, Ogata G¹, Doi K², Hibino H¹

¹Molecular Physiology, Niigata Univ, Niigata, Japan, ²Otolaryngology, Kinki Univ, Osakasayama, Japan,

³Otolaryngology, Kyushu Univ, Fukuoka, Japan

The endolymph of the mammalian cochlea exhibits 150 mM [K⁺] and a highly positive potential of +80 mV. These unique electrochemical properties, which sensitize hair cells, have been hypothesized to be maintained by the K⁺-circulation current that unidirectionally flows between the hair cells and the lateral cochlear wall. The lateral wall contains two epithelial layers; the outer layer containing fibrocytes and basal cells of the stria vascularis and the inner layer composed of other stria cells. We previously developed the computational model based on the measurements of the electrochemical properties of the lateral wall and theoretically demonstrated that the K⁺-circulation current would be driven by various K⁺-transport molecules and determine the endolymphatic properties by controlling [K⁺] and potential inside the lateral wall. **Objectives:** We attempted to show this phenomenon actually occurs *in vivo*. **Methods:** As for simulation, the model was operated under the condition that the fibrocytes' Na⁺,K⁺-ATPases, which seems to be critically involved in establishment of the K⁺-circulation current, was blocked. In the electrophysiological assays, the cochleae of deeply anesthetized guinea pigs (200-400 g) were inserted with the double-barreled electrodes monitoring [K⁺] and potential. **Results:** The model described that inactivation of the ATPases impairs the circulation current and decreases [K⁺] in the extracellular space between the two layers, accompanied by loss of the endolymphatic potential. Similar results were observed with the electrophysiological experiments during the ATPases were pharmacologically inhibited. **Conclusions:** These observations provide the strong evidence that the K⁺-circulation current mediated by the ATPases exists *in vivo* and contributes to maintaining the endolymphatic properties.

The Susceptibility to Noise Injury Is Increased in Streptozotocin-induced Diabetic Mice.

Fujita T¹, Yamashita D¹, Katsunuma S¹, Hasegawa S¹, Tanimoto H¹, Nibu K¹

¹Department of Otolaryngology-Head and Neck Surgery, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan

Objective: To investigate the pathophysiology of diabetes-associated hearing impairment in type1 diabetes using streptozotocin-induced diabetic mice.

Methods: Thirty-eight C57BL/6J mice (8 weeks old, male) were used. Hearing function was evaluated 1, 3 and 5 months after induction of diabetes (5 diabetic and 5 control animals per time point) using auditory evoked brainstem responses (ABR). Mice (4 diabetic and 4 control) were exposed to loud noise (105 dB) 5 months after induction of diabetes. ABR were measured before and after noise exposure. Cochlear blood flows were measured by laser-Doppler flowmeter. Spiral ganglion cells (SGCs) were counted. Vessel endothelial cells were observed by CD31 immunostaining.

Results: Chronologic changes in the ABR threshold shift were not significantly different between the diabetic group and controls. However, vessel walls in the modiolus of the cochleae were significantly thicker in the diabetic group than the control group. Additionally, recovery from noise-induced injury was significantly impaired in diabetic mice. Reduced cochlea blood flows and SGC loss were observed in diabetic mice cochleae after noise exposure.

Conclusion: The present study suggests that diabetic cochleae are more susceptible than controls to loud noise exposure. Long-term diabetic status leads to thickening of modiolar vessel walls and reduced cochlear blood flow after noise exposure. Disrupted microcirculation may cause loss of SGCs and irreversible hearing impairment.

Acknowledgements: This work was supported by Grant-in-Aid for Young Scientists (B) (No. 23791896 to T.F) (No. 20791191 to H.T) and Grant-in-Aid for Scientific Research (C) (No.23592482 to D.Y) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT).

Adrenergic and Cholinergic Stimulation-Mediated Changes of Transepithelial Current from Human Endolymphatic Sac Epithelium

Kim SH¹, Kim BG², Kim JY³, Jeong J⁴, Choi JY¹

¹Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea, ²Department of Otorhinolaryngology-Head and Neck Surgery, Soonchunhyang University College of Medicine, Bucheon, Korea,

³Research Center for Human Natural Defense System, Yonsei University College of Medicine, Seoul, Korea,

⁴Department of Otorhinolaryngology, Seoul Medical Center, Seoul, Korea

Objectives: Endolymphatic sac (ES) epithelium consists of heterogeneous cell types and various ion transports occurred through the epithelium to maintain the inner ear ion homeostasis and fluid volume. So far, there has been no functional study about the ion channel activity of human ES epithelium.

Methods: We harvested human ES during acoustic tumor surgery via translabyrinthine approach. And then, we measured the net physiologic current from human ES epithelium through specific ion channels and investigated the effect of adrenergic and cholinergic stimulation on the transepithelial current using electrophysiologic and pharmacologic methods. Ion channel expression was also investigated using microarray and proteomics.

Results: The vector of net transepithelial current was various and the net current from ES epithelium was very tiny. The cation secretion current was inhibited by 100μM and 1mM Ba²⁺, 1mM 4-AP, 10 μM DIDIS, 100nM apamin, and 10μM amiloride in the cystic portion. But the current from the ductal portion was inhibited only by 10μM amiloride. The current of the cystic portion was significantly changed by the application of adrenergic stimulator and/or cholinergic stimulator. Various K⁺ channels, ENaC, Cl⁻ channels, adrenergic receptors, and cholinergic receptors were detected in the microarray and proteomic analysis.

Conclusion: These results suggest that transepithelial current from ES epithelium of cystic portion is nearly neutral in physiologic condition, which means cation secretion and absorption is balanced, however, ductal portion has more cation secretion activity than cystic portion. The ion transport in human ES epithelium of cystic portion was regulated by adrenergic and cholinergic stimulation.

Developmental increase in hyperpolarization-activated current regulates intrinsic firing properties in rat vestibular ganglion cells.

Ryoichi Yoshimoto¹, Shinichi Iwasaki², Hideki Takago³, Toshiaki Nakajima⁴, Yoshinori Sahara⁵, Ken Kitamura¹

¹Department of Otolaryngology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan, ²Department of Otolaryngology, Faculty of Medicine, University of Tokyo, Tokyo, Japan, ³Department of Rehabilitation for Sensory Functions, Research Institute, National Rehabilitation Center for Persons with Disabilities, Saitama, Japan, ⁴Department of Circular Physiology, Faculty of Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan, ⁵Department of Physiology, Iwate Medical University School of Dentistry, Iwate, Japan

The primary vestibular neurons convey afferent information from hair cells in the inner ear to the vestibular nuclei and the cerebellum. The intrinsic firing properties of vestibular ganglion cells (VGCs) are heterogeneous to sustained membrane depolarization. The firing properties of VGCs undergo marked developmental changes from phasic to tonic types during the early postnatal period. In the present study, we explored the developmental changes in the properties of hyperpolarization-activated current (I_h) in rat VGCs and the role played by I_h in determining the firing properties of VGCs. Tonic firing VGCs showed larger current density of I_h as compared to phasic firing VGCs, indicating that I_h controls the firing pattern of VGCs. The amplitude of I_h increased and the activation kinetics of I_h became faster during the developmental period. Analysis of developmental changes in the expression of HCN channels revealed that expression of HCN1 protein and its mRNA increased during the developmental period. Our results suggest that HCN1 channels are critical in determining the firing pattern of rat VGCs and that developmental up-regulation of HCN1 transforms VGCs from phasic to tonic firing phenotypes.

Measuring Glutathione Content In The Organ of Corti Using Live Imaging

Majumder, P¹, Duchen, MR², Gale, JE^{1,2}

¹UCL Ear Institute, London, UK, ²Department of Cell and Developmental Biology, UCL, London, UK

Reactive Oxygen Species (ROS) are natural bio-products of mitochondrial metabolism. Enhanced production of ROS has been implicated in ototoxicity, noise-induced, and age-related hearing loss. One of the key intracellular molecules that neutralizes ROS is glutathione (GSH). We used live confocal imaging to measure GSH content in organ of Corti cells at different stages of cochlear maturation. Auditory bullae from C57BL/6 mice were opened at the apex and incubated with monochlorobimane (MCB, which forms fluorescent GSH-adduct) for 40 minutes. MCB-GSH, proportional to the reduced GSH concentration, was detected using multiphoton confocal microscopy. GSH was evaluated in inner-hair-cells (IHCs), inner sulcus, outer-hair-cells, and Deiters' cells from postnatal day (P)4, P15, P30 and P365 mice. At P30 there was a significant increase of GSH in IHCs ($p < 0.05$). Since we were able to access the apical turn of the acute bullae preparation we used P5 cochlear cultured cochlear explants to determine whether there was an apex to base GSH-gradient. Initial data suggested no significant difference between turns. To test the effect of lowering GSH content on aminoglycoside toxicity, P5 explants (basal-middle turns) were incubated in either buthionine-sulfoximine (BSO, inhibitor of gamma-glutamylcysteine-synthetase, crucial for GSH synthesis) or control media, for 16-18 hours. Subsequently explants were incubated in 1mM neomycin for 6 hours prior fixation and immunostaining to determine hair-cell survival. Lowering GSH levels did not affect hair cell survival after neomycin-treatment. One interpretation is that ROS may play a lesser role during aminoglycoside-induced hair cell death but further experiments are required to prove this.

Quantal Release At The Auditory Hair Cell Synapse In The Turtle.

Schnee M. E.¹, Ricci A.J.^{1,2}

¹Departments of Otolaryngology, ²Molecular and Cellular Physiology Stanford University, Stanford, CA United States

Auditory hair cell ribbon synapses are specialized to respond to graded changes in receptor potential with varying levels of vesicle release. Ribbon synapses differ from conventional synapses in their ability to maintain a constant release of multiple vesicles from each active zone to ensure the precise transmission of sound signals to the cochlear nucleus. Does the primary auditory ribbon synapse follow classical quantal release properties? Here we show in paired recordings of turtle auditory hair cells and primary afferent fibers that distinct unitary or quantal events are detectable. Small hyperpolarized voltage steps evoked EPSC populations with Gaussian distributions whose size varies from 40 to 70 pA with a mean of 53 ± 26 pA $N=15$. Amplitude histograms generated from depolarization around the hair cell's resting potential, show multiple peaks for small event numbers that is masked with increased sampling, suggesting an increased variance unmasked with larger sampling. Release at these potentials was low so single events could be resolved. With steps that evoked Ca currents greater than 50% maximal, single events were resolved due to the increased release frequency. EPSC amplitude histograms varied in peak number from 3 to 9 with a mean of 5 ± 2 . EPSC amplitude did not change with increasing depolarization rather the frequency increased similar to results obtained with afferent recordings and high K^{+1} . Quantal analysis of release at this auditory synapse shows that release can be predicted by classical quantal analysis but the probability of multi-quantal events is much higher than at conventional synapses.

This work was funded by NIDCD Grant DC009913 and core grant P30 44992.

References ¹M. E. Schnee, M. Castellano-Muñoz and A. J. Ricci *J Neurophysiol* 110:204-220, 2013.

Identification Of Factors Underlying Spiral Ganglion Neurons' Dynamic Range

Mamiko Niwa and Anthony J. Ricci

Department of Otolaryngology, Stanford University, Stanford, CA, USA

Type-I spiral ganglion neurons (SGNs), representing the first neuronal processing step in the auditory system, can be divided into at least two subgroups; (1) fibers with high spontaneous rate (SR) that detect low intensity sounds and saturate to high intensity sounds and (2) low SR fibers with detection thresholds at higher intensities, showing non-saturating response to high intensity. Low SR fibers contact the modiolar side of IHCs while high SR fibers contact the pillar side. Given that IHCs' dynamic range is thought to be largely homogeneous, it remains unclear how individual fibers acquire heterogeneous dynamic ranges. Our goal is to identify physiological differences between low vs. high SR fibers by examining the synaptic and action potential (AP) generation mechanisms of pillar vs. modiolar fibers. Intracellular recordings from acutely dissected cochlea sections from postnatal day 9 - 12 rats were made from SGN boutons, using whole cell current- and voltage-clamp techniques. We determined the current threshold (minimum current necessary for AP generation), EPSC frequency, and median EPSC amplitude for each bouton. We found that the pillar-side fibers had a slightly lower current threshold compared to the modiolar fibers, but this difference was not significant ($p = 0.208$ by rank sum test). Similarly, there was no significant difference in EPSC amplitude between modiolar vs. pillar fibers nor in EPSC frequency. We speculate that those physiological differences observed by single-unit recordings in adult whole animals may require a further post-natal development after the onset of hearing (P11-12).

Metabolome Analysis of Inner Ear Fluid in Guinea Pigs Cochlea after Intense Noise

Yamashita Daisuke¹, Fujita Takeshi¹, Shinomiya Hitomi¹, Inokuchi Go¹, Hasegawa Shingo¹, Nibu Ken-ichi¹

¹Department of Otolaryngology-Head and Neck Surgery, University of Kobe, Hyogo, Japan

The metabolome analysis is to analyze metabolites resulting from all cellular activity to have of the organism cyclopedically. The intracellular dynamics which are not understood by expression of mRNA and proteome analysis are found by the profile of the metabolome analysis. Various clinical conditions such as cancer and immune disease have been recently apparent by metabolome analysis with many organs and tissues. This time we examined metabolome analysis of inner ear fluid in guinea pigs cochlea using gas-chromatography/mass-spectrometry (GC/MS), and also detected changes after intense noise. As for the metabolite which was specific for inner ear fluid of guinea pig cochlea, it was detected 29 kinds in total. Also, six kinds of metabolites changed significantly in inner ear fluid after intense noise. Based upon our these results, it might be effectiveness to elucidate the mechanism of metabolic pathway. And it seems that these results lead to protect against the sensorineural hearing loss. Furthermore, as clinical application to human, it is thought to become a diagnostic tool for perilymph fistula and treatment therapy.

Type I IFN is Produced in Supporting Cells against Virus Infection of the Cochlear Sensory Epithelium via RIG-I Like Receptor Signaling Pathway

Hayashi Y¹, Onomoto K^{2,3}, Narita R², Yoneyama M^{2,3}, Kato H², Taura A², Nakagawa T², Ito J², Kaga K¹, Fujita T²

¹Institute of Clinical Research of National Hospital Organization Tokyo Medical Center, Tokyo, Japan

²Kyoto University, Kyoto, Japan, ³Chiba University, Chiba, Japan

Objectives: RIG-I like receptors (RLRs) including RIG-I and MDA5 were identified as a sensor of viral dsRNA. Activation of RLRs results in the up-regulation of transcription factors like IRF-3, which leads to transcription of type I IFN. The aim of this study was to investigate whether the cochlea possesses immunocompetence against viruses through RLR signaling.

Methods: We used ex vivo system of the cochlear sensory epithelium infected with Theiler's murine encephalomyelitis virus (TMEV). The expression change of RLRs and type I IFN induced by virus infection was estimated using immunohistochemistry, western blotting and qRT-PCR.

Results: RIG-I and MDA5 were expressed weakly in Hensen's and Claudius' cells which are supporting cells in the uninfected cochlear sensory epithelium, but their expression was absent in auditory hair cells. When infected with TMEV, dsRNA of viruses became detectable in the area of Hensen's and Claudius' cells. In these dsRNA-positive cells, the expression of RIG-I and MDA5 was dramatically increased as compared with uninfected cells. Results of histochemical analysis showed that IRF-3 is also detectable in Hensen's and Claudius' cells of the normal cochlea. Virus-infected cells exhibited nuclear accumulation of IRF-3, suggesting that TMEV infection activates IRF-3 in the cochlea. TMEV infection induced high levels of gene expression of IFN α 4 and IFN β 1 which are type I IFNs, suggesting that the cochlea recognizes viral infection and triggers an antiviral program.

Conclusions: Not auditory hair cells but supporting cells like Hensen's and Claudius' cells play an important role in innate immunity against virus infection.

Interferon Gamma Activity Research In Case Of Acute Sensorineural Hearing Loss

Petrova Natalya N., Perevozchikova Vera N., Maslova Yulia A., Petrova Ekaterina V.

North - Western State Medical University I.I. Mechnikov, St.-Petersburg Russia, E-mail: nnp.62@mail.ru

Introduction. According to the literature immune mechanisms take part in the pathogenesis of sensorineural hearing loss (SHL). Understanding of the processes occurring in the immune system in case of SHL helps to prescribe correct treatment.

Purpose: to study the levels of interferon-gamma (IFN γ) in the serum of patients suffering from acute SHL.

Patients and methods. We observed 60 patients, which were formed in 2 groups: primary (36 people) and control group (24 people). The criterion for inclusion in the first group was a diagnosis of acute SHL. A second group was made up of people without pathology of the auditory system. The concentration of IFN γ was determined in the serum of patients by ELISA test systems.

Results. Unilateral SHL was observed in 72,2% of cases, while a bilateral - in 28,2% of cases. Our studies have found that patients with acute SHL have disorders in the cytokine profile. The average level of IFN γ in control group was $15,058 \pm 5,5$ pg / ml. In primary group 4 patients showed very high values of IFN γ - more 940pg/ml. Other individuals of the first group had the average value of IFN γ $54,9 \pm 10,5$ pg/ml, which is 3.6 times higher than healthy ones had.

Conclusions. Our studies show that imbalance of the immune system takes place in the pathogenesis of the acute period of the disease. This fact proves the necessity for more studies of the cytokine profile of patients with acute SHL. Also it can be use in the future as a basis for developing new recommendations for the treatment of this pathology.

ALH-L1005 Attenuates Endotoxin Induced Inner Ear Damage

Jang CH¹, Jo SY¹, Cho YB¹, Park BY², Kim MY²

¹Department of Otolaryngology, Chonnam National University, Gwangju, South Korea

²AngioLab, Daejeon, South Korea

Objective: To assess whether this compound (ALH-L1005) is conceivably an effective agent in protecting against cochlear damage induced by LPS.

Materials and methods: Tube formation using human umbilical vein endothelial cell (HUVEC) and matrix metalloproteinase (MMP)-9 inhibition assay was performed. 24 guinea pigs were randomly divided into three groups. Intratympanic instillation of LPS (n = 8) as negative control, instillation of oxytetracycline 1 h after LPS as positive control (n = 8), and intratympanic instillation of ALH-L1005 (n = 8) 1 h after LPS were considered experimental group. Evaluation by auditory brainstem response (ABR) measurement, cochlear blood flow, and blood-labyrinth barrier (BLB) permeability were performed. Cochlear hair cells were observed by field emission-scanning electron microscopy (FE-SEM). MMP-9 activation was measured by gelatin zymography.

Results: For HUVEC, the tube formation was suppressed in a dose dependant manner. ALH-L1005 inhibited the MMP-9 activity prominently. It also attenuated the elevation of LPS-induced hearing threshold shift and recovery of CBF. By FE-SEM, cochlear hair cells could be preserved in experimental group. ALH-L1005 significantly reduced the BLB opening compared to LPS group. Active MMP-9 expression could be detected in the LPS group. In contrast to ALH-L1005 group, active MMP-9 expression was not detected.

Conclusion: Our results conclude that ALH-L1005 showed a protective effect in the cochlear lateral wall damage induced by LPS.

Activation Of MiR-34a/SIRT1/p53 Signaling Contributes To Cochlear Hair Cell Apoptosis: Implications For Age-related Hearing Loss

Xiong H¹, Pang J¹, Lai L¹

¹Department of Otolaryngology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China

Objective: We aimed to evaluate the miR-34a/Sirtuin 1 (SIRT1)/p53 signaling in cochlear hair cell of C57BL/6 mice during aging and in the inner ear HEI-OC1 cell line. **Methods:** Auditory brainstem response and hair cell counts were conducted on young (1-2 months) and old (12-16 months) C57BL/6 mice. Q-PCR, immunochemistry, western blotting, TUNEL, FACS were performed in mice and HEI-OC1 cell. **Results:** Elevated hearing thresholds and increased hair cell loss were found in old C57BL/6 mice compared with young mice. Apoptotic hair cells were detected in the old C57BL/6 mice. MiR-34a, acetylated p53 and apoptosis increased in the cochlea of C57BL/6 mice with aging, whereas an age-related decrease in SIRT1 was observed. In the inner ear HEI-OC1 cell line, miR-34a overexpression inhibited SIRT1, leading to an increase in acetylated p53 and apoptosis. Knockdown of miR-34a increased SIRT1 expression and diminished acetylated p53 and decreased apoptosis. Moreover, resveratrol, a SIRT1 activator, significantly rescued HEI-OC1 cell death induced by miR-34a overexpression. **Conclusions:** Our results support a link between cochlear hair cell apoptosis and miR-34a/SIRT1/p53 signaling, specifically modulated by aging and position it as a potential target for treatment of age-related hearing loss.

Absence Of Serpinb6 Causes Sensorineural Hearing Loss With Multiple Histopathologies In The Mouse Inner Ear: Implications For Non-syndromic Hearing Loss In Humans

Tan J.¹, Kaiserman D.², Prakash M.², Bird P. I.²

¹Department of Otolaryngology, University of Melbourne, East Melbourne, Victoria, Australia.

² Department of Biochemistry & Molecular Biology, Monash University, Clayton, Victoria, Australia.

Serpins are inhibitors of serine and cysteine proteases. The first case of a protease inhibitor associated with hearing loss in humans was reported in 2010 and this inhibitor was identified as SERPINB6 (Sirmaci et al. 2010). Hearing loss is manifested in adults with homozygous deficiency of SERPINB6. Affected individuals noticed a progressive loss in hearing. It is unknown how SERPINB6 deficiency causes hearing loss.

We use mouse models to help us understand this deafness syndrome in humans by analysing mutant mice in which the orthologous *Serpinb6a* gene is replaced by enhanced green fluorescent protein. We exposed mice to tones varying from 4 to 32 kHz to determine hearing thresholds. Cochleae of these mice were dissected and decalcified to produce frozen sections. Representative sections of each cochlea were stained with eosin and haematoxylin and examined for pathological changes. Remaining sections were incubated with an anti-serum against SERPINB6.

SERPINB6 is present in the neurosensory epithelium, lateral wall and spiral limbus, with highest levels in the inner and outer hair cells of the organ of Corti, cells lining the inner sulcus, and supporting cells distributed along the epithelial gap junction layer to the outer sulcus. Measurements of hearing thresholds in these mice demonstrated age-related hearing loss in all homozygous null, but not heterozygous, mice. The defect is associated with progressive cellular degeneration within the cochlea.

SERPINB6 is essential for protecting cochlear cells. We have generated a mouse model that can be used to screen drugs targeting the cognate protease of SERPINB6.

This work is supported by the National Health and Medical Research Council of Australia Program Grant # 490900, Project Grant #1019941.

Vacuolar Formation In Intermediate Cells of Stria Vascularis From Rat With Vasopressin-induced Hearing Loss

Naganuma H.¹, Kawahara K.², Sato R.², Ochiai A.³, Tokumasu K.³ and Okamoto M.³

¹Division of Neurotology, Department of Advanced Medicine, Research and Development Center for New Medical Frontiers, Kitasato University School of Medicine. Sagamihara Kanagawa, Japan. ²Department of Physiology, Kitasato University School of Medicine. Sagamihara Kanagawa, Japan. ³Department of Otorhinolaryngology, Kitasato University School of Medicine. Sagamihara Kanagawa, Japan.

The precise etiology in Menier's disease and the cause of endolymphatic hydrops remain unclear. The antidiuretic hormone vasopressin (AVP) causes rapid increase in the water permeability of the inner ear as well as the mammalian collecting duct via stimulation of AQP2.

To examine the involvement of AVP on both hearing and stria vascularis in the cochlea, we investigated the effects of AVP for 60 (or 90) min with the specific V2- and V1a-receptor antagonists (OPC-31260 and OPC-21268, respectively).

The initial threshold of auditory brain stem responses (ABR) displayed six typical clear peaks in both control and AVP groups. Their averages were 18 ± 1.3 (SE) dB SPL and 16 ± 2.2 dB SPL, respectively, which were not significantly different each other ($P = 0.405$). When AVP (0.02 unit/g ip) was injected, the threshold increased and peaked at 60 min (27 ± 2.6 dB SPL, $P < 0.05$ vs. time 0, Steel test), and, then decreased. Morphological analysis of stria vascularis at 60 min with the electron microscope showed increase in the size of both the intermediate cells and their cytoplasmic vacuoles. The mean ratio of the vacuoles to the whole stria vascularis significantly increased from 0.011 ± 0.006 (control) to 0.03 ± 0.014 (AVP) ($P < 0.01$, Mann-Whitney U test). More importantly, the size of AVP-induced vacuoles was significantly attenuated in the presence of OPC-31260, but not OPC-21268.

In conclusion, external addition of AVP may cause acute hearing loss in conjunction with vacuole formation in stria vascularis via stimulation of V2R.

Indicators Acoustic Reflex In Persons Employed In The Furniture Industry

Petrova Natalya N., Panshina Viia S.

North - Western State Medical University I.I. Mechnikov, St.-Petersburg, Russian Federation

Introduction. The main factors of the working environment of the enterprises of furniture industry are noise, organic dyes and pigments. It is known that each of these factors can cause sensorineural hearing loss.

The aim of the research was the study of auditory function in persons, employed in the furniture industry and exposed to noise, organic dyes and pigments.

Patients and methods. We examined 80 patients with sensorineural hearing loss in aged 24-45 years. Auditory function was evaluated using a tone threshold audiometry and acoustic reflexometry.

Results. In all workplaces noise exceeded the permissible level at 2-8 dB. Maximum permissible concentration of chemical substances was also raised: phenol - 0.3 mg/m³, butyl alcohol - 5.5 mg/m³. In patients of the main group according to the tonal audiometry identified hearing loss 1 degree in 18,75% of cases, 2 degree 3.75% of cases, 3 and 4 degrees - in 0% of cases. In the study of amplitude characteristics of the acoustic reflex (AR) revealed a decrease since 30 years of age. A decrease in the amplitude of the AR was detected with the frequency of 4000 Hz, and with increasing age and length frequency range is expanding. Changes temporal characteristics defined, since 40 years.

Conclusion. The combined effect of noise, organic dyes and pigments leads to dysfunction of the auditory analyzer in the form of reduced amplitude AR, beginning with the age of 30. The primary changes in the amplitude are recorded at a frequency of 4000 Hz. With increasing age and work experience a decrease in the amplitude AR progresses and changes temporal characteristics.

Accumulated Caveolins in the models of Connexin26 associated deafness

Anzai T., Kamiya K, Ikeda K,

Juntendo University School of Medicine, Department of Otorhinolaryngology, Tokyo, Japan

Introduction and Purpose: The mutations in connexin26 (Cx26), a cochlear gap junction protein, represent a major cause of pre-lingual, non-syndromic deafness. Recently, we showed that Cx26 mutation resulted in a drastic reduction in the gap junction plaque (GJP) and caveolins associated with the GJP disruption. (Kamiya et al. J. Clin. Invest. 2014). Caveolins are integrated plasma membrane protein and structural component of caveolae membranes. Recent studies showed that the over expression or abnormal localization of caveolins associated with delayed wound healing or cellular aging in several organs. The purpose of this study is to investigate the association of caveolins in the pathology of Cx26 related hearing loss.

Method: We analysed the expression and localization of Caveolin-1 or Caveolin-2 in two types of the models of GJB2 associated deafness, Cx26R75W-Tg (dominant negative) and Cx26cKO (protein deficient).

Conclusion: Although, only diffused labeling of caveolins were observed in the control mice, there were accumulated caveolins in the organ of Corti in both Cx26 mutant mice. Especially, these accumulations were notably observed in the outer hair cells (OHCs), Deiter's cells and pillar cells. The Cells with abnormal accumulated caveolins were significantly increased in the both Cx26 mutant mouse. In this study, we suggested that caveolins in cochlea may play a crucial role in the pathogenesis of GJB2 associated deafness.

Disruption of ion-trafficking system in the cochlear spiral ligament prior to noise-induced hearing loss

Taro Yamaguchi, Masanori Yoneyama and Kiyokazu Ogita

Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka, Japan, t-yamagu@pharm.setsunan.ac.jp

The endocochlear potential (EP), which is maintained by various K^+ -transport apparatuses including Na^+ , K^+ -ATPase and gap junction in the lateral wall structures, is essential for the hearing ability of the inner ear. Noise-induced hearing loss is at least in part due to disruption of the EP and excess oxidative stress. In this study, we examined the changes in the ion trafficking-related proteins in the cochlear spiral ligament fibrocytes (SLFs) following *in vivo* acoustic overstimulation or *in vitro* exposure of cultured SLFs to 4-hydroxy-2-nonenal (4-HNE), which is a mediator of oxidative stress. Connexin (Cx)26 were ubiquitously expressed throughout the spiral ligament, whereas Na^+ , K^+ -ATPase $\alpha 1$ was predominantly detected in the stria vascularis and spiral prominence (type 2 SLFs). One-hour exposure of mice to 8 kHz octave band noise at a 110 dB sound pressure level produced an immediate and prolonged decrease in the Cx26 level and in Na^+ , K^+ -ATPase activity. The noise-induced hearing loss and decrease in the Cx26 protein level and Na^+ , K^+ -ATPase activity were abolished by a systemic treatment with a free radical-scavenging agent, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl or with a nitric oxide synthase inhibitor, N^G -nitro-L-arginine methyl ester hydrochloride. *In vitro* exposure of SLFs in primary culture to 4-HNE produced a decrease in protein levels of Cx26 and Na^+ , K^+ -ATPase $\alpha 1$, as well as in Na^+ , K^+ -ATPase activity, and resulted in dysfunction of the ion-trafficking system between the SLFs. Taken together, our data suggest that disruption of the ion-trafficking system in the SLFs is caused by an oxidative stress-induced decrease in the levels of Cx26 and Na^+ , K^+ -ATPase, and is at least in part involved in permanent hearing loss induced by intense noise.

Keywords: Cochlear spiral ligament fibrocytes; Connexin; Gap junction intercellular communication; Hearing loss; 4-Hydroxy-2-nonenal; Ion-trafficking system; Na^+ , K^+ -ATPase; Oxidative stress

Contract grant sponsor and COI: Grants-in-aid for scientific research (to K.O.) from the Ministry of Education, Science, Sports, and Culture of Japan. The authors declare that they have no conflicts of interest in the research.

Analysis of Sirt3 Gene and Mitochondrial Genome in Japanese Patients with Presbycusis

Hisashi Ohtsuka¹, Kirito Shimamoto¹, Koji Fukuda¹, and Hiroaki Sato¹

¹Department of Otolaryngology, Head and Neck Surgery, Iwate Medical University, School of Medicine, Morioka, Japan

Objectives:

We speculated that mutations in the Sirt3 gene and mitochondrial SNPs were associated with hearing impairment in patients with presbycusis. Our goal was to analyze mutations in the Sirt3 and the mitochondrial genome, consisting of several polymorphic patterns, and determine if any of the polymorphisms were associated with presbycusis.

Methods:

Japanese individuals whose ages more than 65 years old (67 men and 93 women; mean age: 75.8 years; age range: 65-92 years) were enrolled. Pure-tone audiometry (PTA) was performed in all the subjects. To track disease-related genes in the present study, 13 SNPs on the SIRT3 gene scanned in the subjects with and without age-associated hearing loss. Furthermore, we scanned SNPs of mitochondrial genome by direct sequencing.

Results:

Fourteen SNPs in the mitochondrial genome and four SNPs in SIRT3 were associated with presbycusis ($P < 0.05$; Fisher's exact test).

Conclusion

We found statistically significant associations in patients with presbycusis and in individuals with normal hearing. These data suggest that some of SNPs in mitochondrial genome and/or SNPs in SIRT3 have the potential to be predisposing factors that can lead to presbycusis in the Japanese population.

Novel *TECTA* Mutations Identified in Stable Sensorineural Hearing Loss and Their Clinical Implication

Chang, Mun Young¹, Kim, Ah Reum¹, Ja-Won Koo², Seung Ha Oh¹, Choi, Byung Yoon^{2§}

¹Dept. of Otorhinolaryngology, Seoul National Univ. Hospital, Seoul national University College of Medicine, Seoul, South Korea, ²Dept. of Otorhinolaryngology, Seoul National University Bundang Hospital, Seongnam, South Korea

TECTA is a causative gene of autosomal dominant (DFNA8/A12) and autosomal recessive (DFNB 21) nonsyndromic sensorineural hearing loss (NSHL). Mutations in *TECTA* account for 4% of all autosomal dominant NSHL cases in some populations and are thus thought to be one of the major causes of autosomal dominant NSHL. A genotype–phenotype correlation for autosomal dominant mutations in the *TECTA* gene has been proposed. Two families (SB146 and SB149), which segregated moderate NSHL in an autosomal dominant fashion, were included in this study. We performed targeted resequencing of 134 known deafness genes (TRS-134) and bioinformatics analyses to find causative mutations for NSHL in these two families. Through TRS-134, we detected two novel mutations, c.3995G>T (p.C1332F) and c.5618C>T (p.T1873I), in the *TECTA* gene. These mutations co-segregated with NSHL in the studied families and were not detected in normal controls. The mutations c.3995G>T and c.5618C>T reside in the von Willebrand factor type D3-D4 (vWFD3-D4) interdomain of the zonadhesin (ZA) domain and the zona pellucida (ZP) domain, respectively. p.C1332F is the first mutation detected in the vWFD3-D4 interdomain of the ZA domain. The mutations p.C1332F and p.T1873I were associated with stable high-frequency and mid-frequency hearing loss, respectively. Notably, the cysteine residue mutated to phenylalanine in SB146 was not related to progression of sensorineural hearing loss, which argues against the previous hypothesis. Here we confirmed a known genotype–phenotype correlation for the ZP domain and propose a hypothetical genotype–phenotype correlation which relates mutations in vWFD3-D4 to stable high-frequency NSHL in Koreans. This clinical feature makes subjects with the missense mutation in the vWFD3-D4 interdomain of *TECTA* potentially good candidates for middle ear implantation.

Key words. Nonsyndromic sensorineural hearing loss; *TECTA*; mutation; mid-frequency hearing loss; high-frequency hearing loss

Characterization of Pathogenic Mechanism of a Nonstop Extension Mutation of *EYA1* Detected in BOR Syndrome

Kim Ah Reum¹, Choi Hyun Suk², Koo Ja Won², Jeon Eun Hee², Kim Min Young², Choi Byung Yoon²

¹Department of Otorhinolaryngology-Head and Neck Surgery: Seoul National University Hospital, Seoul, Korea, ²Department of Otorhinolaryngology-Head and Neck Surgery: Seoul National University Bundang Hospital, Seongnam, Gyeonggi, Korea,

Objectives: A pathogenic potential of a nonstop extension mutation has not been clearly elucidated in many genes. We have recently detected a nonstop extension variant of *EYA1* that leads to only six amino acid extension of EYA1 protein from a BOR family. We intended to confirm a pathogenic potential of this variant and to characterize the pathogenic mechanism of this variant.

Materials and methods: We performed Sanger sequencing of the *EYA1* gene, based upon the BOR phenotype of the family and detected a nonstop extension variant (p.X560GlnextX6) of *EYA1*. After checking the segregation of this variant, we generated a wild type and mutant pcDNA3-*EYA1* expression vectors by site directed mutagenesis. We expressed these wild type and mutant *EYA1* in COS7 cells and extracted wild type and mutant EYA1 mRNA and protein, respectively. We performed a Western blot to see if the protein stability was affected by this variant.

Results: The mutant EYA1 protein showed a marked decreased amount compared with wild type EYA1 protein, while mRNA EYA1 revealed a comparable expression to that in wild type in COS7 cells, revealing instability of the translated product of this nonstop extension variant.

Conclusion: Here we implicate a pathogenic mechanism underlying a mutation that extends the EYA1 protein C-terminally only by six amino acids to the instability of the protein products. Substances that would attenuate this instability may relieve or rescue the phenotype caused by this nonstop extension mutation.

Down Regulated Connexin26 At Different Postnatal Stage Displayed Different Types Of Cellular Degeneration And Formation Of Organ Of Corti

Chen Sen¹, Sun Yu^{1,*}, Lin Xi³, Kong Weijia^{1,2,*}

¹Department of Otolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China

²Institute of Otorhinolaryngology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China

³Department of Otolaryngology, Emory University School of Medicine, 615 Michael Street, Atlanta, GA 30322-3030, USA

* Correspondence

Connexin26 (Cx26) mutation is the most common cause for non-syndromic hereditary deafness. Different congenital Cx26 null mouse models revealed a profound hearing loss pattern and developmental defect in the cochlea. Our study aimed at establishing a Cx26 knocking down mouse model at different postnatal time points and to investigate the time course and pattern of the hearing loss and cell degeneration in these models. Morphologic changes were observed for five months to detect long-term diversities among these models. Depending on the time point when Cx26 expression was reduced, mild to profound hearing loss patterns were found in different groups. Malformed organ of Corti with distinct cell loss in middle turn was observed only in early Cx26 reduction group while mice in late Cx26 reduction group developed normal organ of Corti and only suffered a few hair loss in the basal turn. These results indicated that Cx26 may play essential roles in the postnatal maturation of the cochlea, and its role in normal hearing at more mature stage may be replaceable.

Targeted Exome Sequencing Identified A Novel Truncation Mutation Of *EYA4* In Moderate Degree Hearing Loss

Hyun Seok Choi^{1,2}, Ah Reum Kim², Shin Hye Kim², Ja-Won Koo¹, Byung Yoon Choi^{1,3}

¹Dept. of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, College of Medicine, Seoul National University, Seongnam, Korea, ²Dept. of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Hospital, Seoul, Korea, ³Sensory Organ Research Institute, Seoul National University Medical Research Center, Seoul, Korea

Background: The mutations of *EYA4* gene are known to cause postlingual and progressive sensorineural hearing loss (SNHL) either as a nonsyndromic hearing form (DFNA10) or syndromic hearing loss according to degree of truncation.

Method: We have recruited one mid-sized family segregating moderate degree SNHL as a dominant inheritance pattern. Thorough medical history taking and physical examination was performed to rule out any syndromic feature. Mutational analysis was performed by targeted exome sequencing (TES) of known 134 deafness genes (TES-134) from a proband. Candidate variants were pursued by basic filtering step, further segregation analysis, and checking Korean normal hearing control samples.

Result: After basic filtering of the variants detected from TES-134, we identified a novel truncation mutation, c.1194delT (p.Met401TrpfsX3) of the *EYA4* gene from the proband manifesting slightly downsloping moderate degree SNHL with progressive nature. The variant perfectly co-segregated with the deafness phenotype and was not detected in none of 276 normal hearing control chromosomes, further supporting its pathogenic potential. This variant was supposed to generate protein products that truncated at just downstream to the eya-VR domain. None of the three affected members in this family (SH117) showed any syndromic feature including cardiac problems, indicating that SNHL in this family was DFNA10.

Conclusion: Our identification of a novel *EYA4* truncation mutation associated with DFNA10, non syndromic hearing loss, supports previously reported genotype-phenotype correlation in this gene. *EYA* should be included in the main candidate gene list when we encounter a dominant type slightly downstream SNHL with moderate degree.

MARVELD2 (DFNB49) Mutations In The Hearing Impaired Central European Roma Population: Prevalence And Founder Effect

Varga L^{1,2}, Mašindová I², Šoltýsová A³, Matyáš P⁴, Ficek A³, Hučková M², Sůrová M², Anwar S⁵, Bene J⁴, Janicsek I⁴, Seeman P⁶, Riazuddin S⁵, Melegh B⁴, Klimeš I², Kádasi L³, Profant M¹, Gašperíková D²

¹Dept of ORL-HNS, Comenius University, Bratislava, Slovakia, ²Inst Exp Endocrinology, SAS, Bratislava, Slovakia, ³Dept of Mol Biol, Comenius University, Bratislava, Slovakia, ⁴Dept of Med Genet and Szentagothai Research Centre, University of Pécs, Pécs, Hungary, ⁵Dept of ORL, College of Medicine and Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA, ⁶Dept of Pediatric Neurology, Charles University 2nd Medical School, Prague, Czech Republic

Objectives: 1) To establish the prevalence of DFNB49 mutations in the deaf Roma from 2 Central European /CE/ countries (Slovakia /SK/ and Hungary /HU/), and 2) to perform the haplotype analyses to detect a possible common origin of the c.1331+2T>C mutation among Roma as well as Pakistani /PK/ mutation carriers identified in previous studies. **Methods:** We tested 228 unrelated deaf Roma patients and 702 healthy Roma controls for the presence of MARVELD2 mutations. **Results:** The mutation c.1331+2T>C was found in 12 (5.3%) deaf subjects in homozygous state and in 4 (1.8%) in heterozygous state. In normal hearing controls we identified 14 (2%) heterozygotes. The identified common haplotype defined by 18 SNP markers (3.89 Mb) was shared among all SK, Czech /CZ/ and HU Roma patients in homozygous state suggesting the common ancestor for this mutation in CE Roma subjects. Fourteen common SNPs were shared among PK patients and all Roma patients with biallelic c.1331+2T>C mutation. In addition, we found one yet unpublished probably pathogenic variant (c.950G>A) in 1 subject in heterozygous form. **Conclusions:** Testing for c.1331+2T>C mutation may be recommended in GJB2 negative Roma cases with early-onset hearing loss. Haplotype analysis of the c.1331+2T>C mutation supported the hypothesis of common ancestry of SK, CZ and HU Roma patients as well as the common origin of the mutation in Roma and PK population. Supported by APVV-0148-10, OTKA K103983, NIDCD/NIH R01 DC011748 and DC011803.

Identification of Deafness Genes in Middle Eastern and Brazilian Families Using Exome Sequencing

Martins FTA^{1,2}, Brownstein Z¹, Abu-Rayyan A³, Birkan M¹, Sokolov M¹, Farran ND⁴, Isakov O⁵, Shomron N⁵, Shalev S⁴, Kanaan M³, Sartorato EL², Avraham KB¹

¹Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; ²Human Molecular Genetics Lab, Molecular Biology and Genetic Engineering Center, University of Campinas, Campinas, Brazil; ³Hereditary Research Lab, Department of Life Sciences, Bethlehem University, Bethlehem, Palestinian Authority; ⁴Genetics Institute, Ha'Emek Medical Center, Afula, Israel and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; ⁵Department of Cell and Developmental Biology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

So far, about 70 genes are involved in human hearing loss, many of which have at least 20 mutations, and the estimate is that this number may reach 300 genes. The conventional molecular techniques do not have the ability to affordably and easily diagnose these mutations. Thus, high-throughput molecular platforms were created to help fill this gap, among them Next Generation Sequencing (NGS) that allows the sequencing of multiple genes simultaneously of the whole genome. Some of the most powerful platforms available today are based on NGS, including Whole Exome Sequencing (WES), which allows for the complete sequencing of the exons of an individual and Targeted Genomic Capture (TGC) associated with Massive Parallel Sequencing (MPS), which allows for sequencing of the exons of genes related to the disease.

We employed a TGC approach, capturing the exons of 284 genes associated with deafness in humans and mice, followed by MPS. In other cases, WES was performed. After alignment of the obtained sequence, subsequent bioinformatics analysis included variant calling, prioritization of allele frequency, variant location, variant effect, and prediction tool scores to evaluate pathogenicity. The segregation of a subset of variants was examined in each family and when relevant, functional analysis was performed.

Overall, 150 families were evaluated from the hearing impaired Israeli Jewish and Palestinian Arab populations and one extended Brazilian family using this approach, allowing us to identify new variants in over 20 genes, as well as variants previously associated with deafness. While some genes and mutations overlapped between the groups, others were unique. The results of this work demonstrate that the discovery of the molecular basis of deafness is an important key to the molecular diagnosis and better understanding of the mechanisms of hearing loss and may provide an entry point into the development of therapeutics.

Analysis of a conserved *GJB2* intronic region in hearing impaired monoallelic Portuguese patients

Matos TD¹, Simões-Teixeira H¹, Gonçalves AC¹, Chora JR¹, Rosa H², Monteiro L³, O'Neill A^{4,5}, Dias ÓP⁶, Andrea M⁶, Caria H^{1,7}, Fialho G¹

¹ BioFIG, Univ. of Lisbon, Lisboa, Portugal, ² Dep. ORL, Hospital Garcia de Orta, Almada, Portugal, ³ Dep. ORL, Hospital D. Estefânia, Lisboa, Portugal, ⁴ Dep. Head and Neck, Hospital Egas Moniz, Lisboa, Portugal, ⁵ Dep. Anatomy, Fac. Medical Sciences, New Univ. of Lisbon, Lisboa, Portugal, ⁶ Dep. ORL, Hospital Santa Maria, Lisboa, Portugal, ⁷ Higher College of Health, Polytechnic Institute of Setúbal, Setúbal, Portugal.

The mutations in the *DFNB1* locus are a major cause of nonsyndromic sensorineural hearing loss (NSSHL), being responsible for over 1/5 of the Portuguese cases. However, more often than expected, some patients are monoallelic, suggesting the presence of an accompanying *DFNB1* mutation. We have noticed a conserved region in the *GJB2* intron, which might be a location of pathogenic mutations. We have thus investigated that conserved region in 22 NSSHL Portuguese patients harbouring one pathogenic (c.35delG, p.Trp24X, p.Val37Ile, p.Lys224Glu and p.Asn206Ser; n=15), controversial (p.Met34Thr, p.Arg127His and p.Val153Ile; n=5) or unclear (p.Gly160Ser; n=2) *GJB2* mutation, and none of the two common *GJB6* deletions (del1830 and del1854). Mutations in *GJB2* basal promoter or the donor splice site had been previously excluded by sequencing. Previous sequencing of the 3'UTR of *GJB2* had revealed only five known SNPs.

In this study, the referred intronic region was sequenced in the 22 patients and 42 controls. A total of seven known variants (c.-22-1207G>C, c.-22-1203A>G, c.-22-1198G>A, c.-22-1157A>T, c.-22-1153G>A, c.-22-1108G>A, c.-22-1073A>G) and one novel variant (c.-22-1415A>G) were identified among patients and controls. No intronic variant was identified exclusively in the patients, or was noticeably overrepresented in either patients or controls here analysed. However, this region should not be discarded in other patients with only one *GJB2* mutation, since it might play a role in the regulation of the gene.

Vascular endothelial growth factor polymorphisms and hearing impairment in Japanese aged population

Uchida Y^{1,2}, Sugiura S², Nakashima T², Ueda H¹, Otsuka R³, Ando F^{4,3}, Shimokata H^{5,3}

¹Department of Otolaryngology, Aichi Medical University, Aichi, Japan, ²Department of Otorhinolaryngology, National Center for Geriatrics and Gerontology, Aichi, Japan, ³Section of NILS-LSA, National Center for Geriatrics and Gerontology, Aichi, Japan, ⁴Department of Health and Medical Sciences, Aichi Shukutoku University, Aichi, Japan, ⁵Graduate School of Nutritional Sciences, Nagoya University of Arts and Sciences, Aichi, Japan

Objectives: Vascular endothelial growth factor (VEGF) plays a major role in the regulation of angiogenesis, vasodilation, differentiation, anti-apoptosis, proliferation, and vascular permeability in endothelial tissues. It also has neurotrophic and neuroprotective effects in non-endothelial tissues. This study aimed to assess the contribution of the VEGF polymorphisms to age-related hearing impairment.

Methods: Data were collected in the Longitudinal Study of Aging surveyed biennially between 1997 and 2010. The participants without any missing information at baseline were 1,959 individuals. Two hearing impairment criteria were taken as the better ear pure-tone average at frequencies of 0.5, 1, 2, and 4 kHz (PTABE) and at 2, 4, and 8 kHz (high PTABE) greater than 25 dB. We analyzed the gross accumulated number of 8,663 subjects (40-89 years of age) using generalized estimating equations to investigate the association of 3 polymorphisms, namely, C-2578A (rs699947), C936T (rs3025039), and G-1154A (rs1570360).

Results: The odds ratio for the hearing impairment risk (high PTABE > 25dB) under additive genetic model was significant in C936T (rs3025039), which was 1.241 (95% confidence interval: 1.054-1.462) with one minor allele increase after adjustment, especially in individuals with smoking history.

Conclusions: VEGF and its receptors are present in the cochlea, and have been shown to play significant roles in the maintenance of cochlear homeostasis. The present observation implied the contribution of the VEGF to age-related hearing impairment.

Massively Parallel DNA Sequencing Successfully Identifies New Causative *TMPRSS3* Mutations in Patients with EAS

Miyagawa M¹, Nishio S¹, Tsukada K¹, Moteki H¹, Usami S¹

¹Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan

Genetic factors, the most common etiology in severe to profound hearing loss, are one of the key determinants of Cochlear Implantation (CI) and Electric Acoustic Stimulation (EAS) outcomes. Satisfactory auditory performance after receiving a CI/EAS in patients with certain deafness gene mutations indicates that genetic testing would be helpful in predicting CI/EAS outcomes and deciding treatment choices. However, because of the extreme genetic heterogeneity of deafness, clinical application of genetic information still entails difficulties. Target exon sequencing using massively parallel DNA sequencing is a new powerful strategy to discover rare causative genes in Mendelian disorders such as deafness. We used massive sequencing of the exons of 58 target candidate genes to analyze 24 Japanese EAS patients, who did not have mutations in commonly found genes including *GJB2*, *SLC26A4*, or mitochondrial 1555A>G or 3243A>G mutations. We successfully identified four rare causative mutations in the *TMPRSS3* gene in three patients who showed relatively good auditory performance with EAS, suggesting that genetic testing may be able to predict the performance after implantation.

Major susceptibility gene (s) on chromosome 10 for congenital hearing loss in NOD/Shi mice

Suzuki S^{1,2}, Obara Y¹, Yasuda SP¹, Takada T³, Shiroishi T³, Kikkawa Y¹

¹Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, ²Tokyo University of Agriculture, Abashiri, Hokkaido, Japan, ³National Institute of Genetics, Mishima, Shizuoka, Japan

The NOD/Shi mouse congenitally develops profound hearing loss due to stereocilia defects in the cochlear hair cells. The *ahl2* locus that contributes to the hearing loss of NOD/Shi mice was previously mapped to chromosome 5 by linkage analysis of (NOD/Shi × C57BL/6J) × NOD/Shi backcross mice. Although we recently mapped QTLs on chromosome 5, including the *ahl2* locus, using the same genetic cross, we found susceptible QTLs in other regions on the mouse chromosome. To define the major QTL, we performed genetic analysis of F₂ mice using MSM/Ms mice, which have good hearing ability in inbred mice. First, we assessed the ABR to tone-pip 4, 8, 16, and 36 kHz stimuli in F₁ and F₂ mice produced by crosses between NOD/Shi and MSM/Ms mice. The average ABR thresholds (37.4 dB) of the F₁ mice tested at 1 month of age were statistically different from the ABR thresholds of NOD/Shi (96.4 dB) and MSM/Ms (23.9 dB) mice. The distribution of ABR thresholds to the stimuli of all tested frequencies in the F₂ mice clearly showed a bell shaped normal distribution, suggesting that NOD/Shi mice develop hearing loss by a combination of one major and one minor QTL. Employing QTL analysis, we identified the major QTLs on chromosome 10 that significantly affected the ABR threshold. In particular, a marked influence of the region on ABR thresholds of F₂ mice enabled us to refine the candidate gene interval to a 30 Mb region.

Comprehensive Genetic Testing in a Japanese Hearing-Loss Population using Targeted Genomic Enrichment and Massively Parallel Sequencing

Moteki H^{1,2}, Azaiez H¹, Booth KT¹, Shearer AE¹, Sloan CM¹, Kolbe DL¹, Usami SI², Smith R JH¹

1: Molecular Otolaryngology & Renal Research Laboratories, Department of Otolaryngology-Head and Neck Surgery, University of Iowa Hospital and Clinics, Iowa, USA

2: Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan

Non-syndromic hearing loss (NSHL) affects at least 1 in 500 newborns, with genetic causes accounting for 50-70% of all childhood hearing loss. Recent advances in targeted genomic enrichment with massively parallel sequencing (TGE+MPS) have made comprehensive genetic testing for non-syndromic hearing loss possible.

We examined DNA from 198 probands with NSHL collected through 33 otolaryngology clinics and hospitals across Japan. After excluding mutations in *GJB2* and the mitochondrial DNA (mtDNA) 1555A>G variant by Sanger sequencing, we applied TGE+MPS to the genomic DNA. Data analysis was performed on a local installation of Galaxy and variant annotation was completed using a customized workflow.

Causative mutations were identified in 26% of probands, with solve rates of 33%, 33% and 19% for dominant, recessive and sporadic NSHL, respectively. Mutations in *MYO15A* and *CDH23* follow *GJB2* as the second and third most common causes of recessive NSHL; with copy number variations (CNVs) in *STRC* a major cause of mild-to-moderate NSHL.

In a *GJB2*/mtDNA1555A>G-negative population, comprehensive genetic screening could identify the genetic cause of hearing loss in 26% of Japanese patients with NSHL. To facilitate variant calling, ethnic-specific filtering by allele frequency is essential for the optimization of variant interpretation, and CNV analysis should be included in the data analysis pipeline.

Microarray Analysis of Tonotopic Gene Expression Patterns in the Mouse Cochlea

Hidekane Yoshimura¹, Shin-ya Nishio¹, Yutaka Takumi¹, Yoh-ichiro Iwasa¹ and Shin-ichi Usami¹

¹Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, JAPAN

The audioprofile in autosomal dominant nonsyndromic hearing loss can be distinctive; however, the mechanism responsible for the distinct audiograms remains unclear. In addition, tonotopy is one of the most fundamental principles of auditory function. Although gradients in the morphological and physiological characteristics of the inner ear in different species have been reported, few reports are available on genetic gradient analysis. We speculated that tonotopic gradients of genes within the cochlea account for the distinctive audiograms.

We examined and compared the expression profiles of genes in the organ of Corti and spiral ganglion neurons between the apical, middle, and basal turns of the mouse cochlea by microarray technology and quantitative RT-PCR. Of 24,547 genes, 784 were differentially up-regulated or down-regulated by more than two-fold. In particular, *Emilin-2*, *Tectb* and *Slc26A5* were expressed in a gradient that increased from the basal to apex turn. The most remarkable finding was a gradient of genes whose mutations cause autosomal dominant deafness (*Pou4f3*, *Slc17a8*, *Tmc1* and *Crym*). All of these genes were expressed in a gradient that increased from the basal to apex turn.

This study provides baseline data of the normal relative levels and patterns of gene expression in the mouse cochlea, particularly for *Emilin-2*, *Tectb*, *Slc26A5* and genes whose mutations cause autosomal dominant deafness (*Pou4f3*, *Slc17a8*, *Tmc1* and *Crym*). This data may help to explain a number of findings from previous reports.

Clinical Genetic Testing Based on Massively Parallel DNA Sequencing

Shin-ya Nishio¹, Maiko Miyagawa¹, Takehiko Naito¹, Yoichiro Iwasa¹, Aya Ichinose¹, Shin-ichi Usami¹.

¹Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan

Congenital hearing impairment is one of the most common sensory disorders, with 50 to 70% of cases attributable to genetic causes. Although recent advances in the identification of deafness genes have resulted in more accurate molecular diagnosis, leading to the better determination of suitable clinical interventions, difficulties remain with regard to clinical applications due to the extreme genetic heterogeneity of deafness. Approximately one hundred genes are estimated to cause hereditary hearing loss, so effective genetic testing is required.

Toward more effective genetic testing, we are now applying Massively Parallel DNA Sequencing (MPS) of target genes using an Ion PGM™ system and an Ion AmpliSeq™ panel in order to diagnose common mutations responsible for deafness and discover rare causative gene mutations. Prior to its clinical application, we investigated the accuracy of MPS-based genetic testing. We compared the results of Invader assay-based genetic screening, the accuracy of which has already been verified in previous studies, with those of MPS-based genetic testing for a large population of Japanese deafness patients. We confirmed that the Ion PGM™ system had sufficient uniformity, sensitivity and specificity for the clinical diagnosis of common causative mutations, and efficiently identified rare causative mutations and/or mutation candidates. Our data suggest that targeted exon re-sequencing of selected genes using the Ion PGM™ system is suitable for clinical diagnosis, enables the identification of rare gene mutations responsible for hearing loss in individual patients and improves molecular diagnosis in a clinical setting (Miyagawa et al., 2013).

Difference In Ototoxicity Of Acetic Acid And Maturity Of The Guinea Pigs.

Yamano Takafumi^{1,2}, Higuchi Hitomi², Ueno Tetsuko², Nakagawa Takashi², Morizono Tetsuo³

¹Department of Otorhinolaryngology, Fukuoka Dental College, Fukuoka, Japan

²Department of Otolaryngology, Fukuoka University, Fukuoka, Japan

³Nishi Fukuoka Hospital, Fukuoka, Japan

Objectives: The objective of this study is to elucidate the difference in Ototoxicity of acetic acid in the matured guinea pigs and less matured guinea pigs.

Methods: We used Hartley guinea pigs with normal Preyer's reflex. Matured animal group had 400grams body weight and less matured animal group had 200 grams body weight. After Nembutal anesthesia and local infiltration with Xylocain, middle ear cavity was opened and the round window membrane was visualized with operating microscope. After compound action potential of the eighth cranial nerve (CAP) was measured, the middle ear cavity was filled with pH 5 acetic acid. After 30 minutes, the middle ear cavity was thoroughly cleaned with wicks of tissue paper, and the CAP was measured and compared with original CAP. Sound stimuli consists of click sound, 8kHz and 4kHz tone bursts.

Results: In less matured animal groups, statistically significant elevation of the CAP threshold was noted for click sounds ($p < 0.05$). For tone bursts of 4kHz and 8kHz, elevation of the CAP threshold was noted in less matured group, but it was not statistically significant.

Conclusions: Less matured animal group was found more vulnerable to the acetic acids. Presumably, it indicates more penetration of the acetic acid through the round window membrane. Previously, no study reported difference in ototoxicity in the experimental animals with different maturity.

Pyrroloquinoline quinone protects vestibular hair cells against the aminoglycoside ototoxicity

Kazuma Sugahara¹, Yoshinobu Hirose¹, Makoto Hashimoto¹, Hiroaki Shimogori¹, Hiroshi Yamashita¹

¹Department of Otolaryngology, Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi 755-8505, Japan

Introduction: Pyrroloquinoline quinone (PQQ) is an organic molecule that was discovered as a redox cofactor. The molecule was reported as the new vitamins (Nature 2003). It is to be involved in mitochondrial function, and the animals with PQQ deficiency showed development disorder, immunodeficiency and infertility. In addition, the animals which received the molecules showed myocardial protective effect and neuroprotective effect from ischemia for a strong antioxidant activity. In the present study, we evaluated the protective effect on the inner ear sensory cells against neomycin ototoxicity.

Materials and Methods: Cultured utricles of CBA/N mice were used. Cultured utricles were divided to three groups (Control group, Neomycin group, Neomycin + PQQ group). In the Neomycin group, utricles were cultured with neomycin (2 mM) to induce hair cell death. In Neomycin + PQQ group, utricles were cultured with neomycin and PQQ (100 – 1 µM). Twenty-four hours after exposure to neomycin, the cultured tissues were fixed with 4% paraformaldehyde. To label hair cells, immunohistochemistry were performed using anti-calmodulin antibody. The rate of survival vestibular hair cells was evaluated with the fluorescence microscope. In addition, immunohistochemistry against 4-hydroxy-2-nonenal was performed to evaluate the product of hydroxy radical.

Results: The survival rate of hair cells in Neomycin + PQQ group was significantly more than that in Neomycin group. The signals of 4-hydroxy-2-nonenal were inhibited in neomycin + PQQ group. The results indicated that PQQ protects sensory hair cells against neomycin-induced death in mammalian vestibular epithelium. PQQ can be used as the protective drug in the inner ear.

Ototoxic effect of Ultrastop anti-fog solution applied to the guinea pig middle ear

Kazuhiro Nomura¹, Hidetoshi Oshima¹, Daisuke Yamauchi¹, Hiroshi Hidaka¹, Tetsuaki Kawase¹, Yukio Katori¹

¹Department of Otolaryngology-Head and Neck Surgery, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Corresponding author: Kazuhiro Nomura, MD, Dept. of Otolaryngology-Head and Neck Surgery, Tohoku University Graduate School of Medicine, 1-1 Seiryō-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan

E-mail address: kazuhiroie@gmail.com

Objective. Recent advances in endoscopic technology have allowed application to middle ear surgery. Anti-fog agent is necessary for endoscopy because moisture and blood may cause obscured vision. Ultrastop® is one of the most commonly used anti-fog agents. The current study examined the ototoxic effect of topical application of Ultrastop® in the guinea pig ear.

Study Design. A preliminary experimental animal study.

Setting. University hospital.

Subjects and Methods. Eighteen male Hartley guinea pigs (weight, 480–620 g) were divided into 3 groups to be treated with Ultrastop®, gentamicin (50 mg/mL, positive control), or saline solution (negative control). After auditory brainstem responses were measured, topical solutions of 0.2 mL were applied through a small hole made at the tympanic bulla. Post-treatment auditory brainstem responses were obtained 14 days after the treatment. The extent of middle ear damage was investigated and scored.

Results. The saline-treated group showed no deterioration in auditory brainstem response threshold. The Ultrastop®-treated and gentamicin-treated groups showed severe deterioration in auditory brainstem response threshold. Middle ear examination revealed extensive changes in the Ultrastop®-treated group and medium changes in the gentamicin-treated group.

Conclusion. Ultrastop® applied topically to the guinea pig middle ear caused significant middle ear inflammation and hearing impairment.

Cochlear lesion of the animal model of eosinophilic otitis media

Naomi Kudo, Atsushi Matsubara, Hisanori Nishizawa, Junko Takahata and Akira Sasaki.

¹Department of Otorhinolaryngology, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan

Background: Eosinophilic Otitis Media (EOM), which is first reported by Tomioka et al., is an intractable otitis media with eosinophil-enriched middle ear effusion (1993). As some researchers reported, EOM is known to be a high risk disease often involving severe sensory hearing loss, therefore it is important to clarify its etiology for the purpose of establishing the effective therapy for it. Although thorough mechanism of EOM is still obscure, we recently have reported about the model animal in which eosinophils infiltrate to the middle ear (Nishizawa ACTA 2012, Matsubara ACTA 2014) .

Objectives: The purpose of the present study was to elucidate the inner ear damage of the animal model of EOM by observing the histological sections. We also tempted to detect allergic inflammation by immunohistochemical analysis.

Methods: We constructed the model animals of EOM by intraperitoneal and intratympanic injection of Ovalbumin (OVA). We examined the infiltrating cells in the inner ear and the extent of inner ear damage by histological study.

Results: In the inner ear of 7-day stimulation side, a few eosinophils were seen in the scala tympani of the organ of Corti, and the dilation of capillaries of the stria vascularis was observed. In the 14-day antigen stimulation side, some eosinophils and macrophages were seen in not only the scala tympani but also the scala vestibule. In the 28-day stimulation side, we observed severe morphological damage of the organ of Corti and many cells infiltrating the perilymph, containing eosinophils, red blood cells, and plasma cells

Keywords: Eosinophilic otitis media, organs of Corti, scala tympani, scala vestibule, stria vascularis, round window membrane, ovalbumin

p53 plays a key role in cisplatin-induced cochlear cell death: toward the new therapeutic strategy

Hideaki Ogita^{1,2}, Jean-Luc PUEL^{1,2} and Jing WANG^{1,2}

¹INSERM - UMR 1051, Institut des Neurosciences de Montpellier, Montpellier, France,

²Université Montpellier 1&2, Montpellier, France,

Background and Aims: Cisplatin (CDDP) is a widely used anti-cancer drug. Unfortunately, it presents a significant toxicity to the cochlea. It is well known that the CDDP cytotoxicity in cancer cells is mediated by the activation of the DNA damage response pathways, but it is not known about whether and how the DNA damage response pathways are activated in nonmalignant, cochlear cells. Understanding of the role of DNA damage response pathways in cochlear cells after CDDP challenging would aid in developing effective therapeutics to prevent hearing loss in CDDP treated patients.

Methods: Here, we investigated DNA damage response in CDDP intoxicated p3 mouse cochlea in culture and adult mice *in vivo*.

Results: Our results confirm the greater vulnerability of the outer hair cells to CDDP ototoxicity. OHCs apoptosis coincided with early robust DNA damage response involving ATM and Chk2 that is largely responsible for CDDP-induced p53 activation and cochlear cell apoptosis. Targeting the downstream of ATM signaling pathway through genetic or pharmacological invalidation of p53 attenuated cochlear cell apoptosis, preserving hair cell and hearing function in cisplatin treated mice *in vivo*.

Conclusion: These results suggest that p53 is potential therapeutic target to prevent CDDP ototoxicity in human cancer therapy.

The effect of sphingosine-1-phosphate receptor antagonists on gentamicin-ototoxicity of the rat cochlea

Masahiro Nakayama¹, Keiji Tabuchi¹, Bungo Nishimura¹, Mariko Nakamagoe¹, Akira Hara¹

¹ Department of Otolaryngology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

Sphingosine-1-phosphate (S1P) is a sphingolipid metabolite that regulates various critical biological processes, such as cell proliferation, survival, migration, and angiogenesis. The action of S1P is exerted by its binding to 5 specific G protein-coupled S1P receptors (S1PR), S1PR₁-S1PR₅. Aminoglycoside antibiotics including gentamicin induce cochlear hair cell loss and sensorineural hearing loss. Apoptotic cell death is considered to play a key role in this type of cochlear injury. S1P acts as a cochlear protectant against gentamicin ototoxicity. In the present study, expression of S1PRs in the cochlea was examined. In addition, the effects of S1PR antagonists on gentamicin ototoxicity were investigated using tissue culture techniques. Cochleas were dissected from Sprague-Dawley rats on postnatal days 3 to 5. Basal turn organ of Corti explants were exposed to 35 μ M gentamicin for 48 hours with or without S1PR antagonists. S1PR₁₋₃ were expressed in the organ of Corti and spiral ganglion. The S1PR₂ antagonist increased gentamicin-induced hair cell loss, while the S1PR₁ and S1PR₃ antagonists did not affect gentamicin ototoxicity. Additionally, the activation of the intrinsic apoptotic pathway was examined by measuring cleaved caspase 3 and 9, using western blotting. The cleaved caspase 3 and 9 were detected after gentamicin exposure. Furthermore, an S1PR₂ antagonist enhanced the cleavage of caspase 3 and 9 induced by gentamicin. These results indicate that S1P acts as a cochlear protectant against gentamicin ototoxicity via activation of S1PR₂.

Redox Imbalance In Styrene Ototoxicity And Acoustic Trauma: In Vivo Protective Effect Of Q-ter

Fetoni AR¹, Rolesi R¹, Paciello F¹, Eramo SML², Paludetti G¹, Troiani D²

¹Institute of Otorhinolaryngology Catholic University, School of Medicine, Rome, Italy, ²Institute of Human Physiology, Catholic University, School of Medicine, Rome, Italy.

Introduction: Styrene is an industrial solvent. Styrene ototoxicity has been reported in industrial workers. Styrene exposure causes a reduction of the GSH levels and an increase of lipid peroxidation. Because GSH is an important antioxidant, it has been speculated that reduction of GSH level would induce oxidative stress. As well as in styrene-induced ototoxicity, a progressive increase of ROS have been demonstrated to be involved in NIHL. Namely, several studies have suggested a synergistic interaction between noise and styrene, but the combined exposure to noise+styrene on redox imbalance in the cochlea is not been reported. The aim of this study is to investigate the mechanisms involved in styrene ototoxicity and the effect of the antioxidant Q-ter on the styrene-induced cochlear injuries and on the combined effect of noise+styrene.

Methods: Rats were exposed to styrene by gavage (400mg/Kg) and to chronic noise exposure (97 dB SPL, 10 kHz, 60 min/day, 3 weeks, 5 days/week). Two groups were simultaneously treated with the Q-ter (100 mg/Kg) over the same period. Functional evaluation was performed by ABR and DOPAE. We studied the immunostaining for redox imbalance in the cochlea.

Results: Our results demonstrate that hearing loss and cochlear damage by styrene exposure are increased by the concomitant exposure to noise and Q-ter treatment can reduce damage caused by chronic exposure to noise+styrene.

Conclusion: We speculate that the association between noise and styrene exposure represent a risk factor for the health workers and the antioxidant treatment provides a promising preventive approach.

Protective effects of curcumin against cisplatin induced hearing loss in vitro and in vivo studies.

Fetoni AR¹, Mezzogori D², Eramo SML², Paciello F¹, Rolesi R¹, Paludetti G¹, Troiani D²

¹Institute of Otorhinolaryngology, Catholic University, School of Medicine, Rome, Italy, ²Institute of Human Physiology, Catholic University, School of Medicine, Rome, Italy.

Hypothesis: To investigate whether curcumin may have protective effects against cisplatin ototoxicity by its direct antioxidant activity by using in vitro and in vivo studies.

Background: Cisplatin-induced ototoxicity is a major dose-limiting side effect in anticancer chemotherapy. A protective approach to decrease cisplatin ototoxicity without compromising its therapeutic efficacy remains a critical goal for anticancer therapy. Recent evidences indicate that curcumin exhibits antioxidant, anti-inflammatory, and chemosensitizer activities.

Methods: In vitro studies were conducted in head and neck squamous cell carcinoma treated with cisplatin and curcumin at different doses. In Wistar rats, a curcumin dose of 200 mg/kg, selected from a dose-response curve, was injected 1 hour before cisplatin administration and once daily for the following 3 days. A single dose of cisplatin (16 mg/kg) was administered intraperitoneally. In vitro the viability of cancer cell and apoptotic pathway were studied. In vivo, Rhodamine-phalloidin staining, 4-HNE and HO-1 immunostainings, and Western blot analyses were performed to assess and quantify OHC loss, lipid peroxidation and the endogenous response to cisplatin-induced damage and to curcumin protection.

Results: Curcumin significantly reduced tumor growth of squamous cell carcinoma activating the apoptotic pathway and attenuated hearing loss induced by cisplatin, increased OHC survival, decreased 4-HNE expression, and increased HO-1 expression.

Conclusion: Taken together, our results suggest that curcumin is a potent anti-tumor agent and it can be used to overcome cisplatin chemotherapy. In addition in vivo study demonstrates that systemic curcumin attenuates ototoxicity and provides molecular evidence for a role of HO-1 as an additional mediator in attenuating cisplatin-induced damage.

In vivo overexpression of X-linked inhibitor of apoptosis protein protects against neomycin-induced hair cell loss in the apical turn of the cochlea during the ototoxic-sensitive period

Shan Sun¹, Yanping Zhang¹, Mingzhi Sun², Cheng Cheng³, Muhammad Waqas³, Huiqian Yu², Yingzi He², Bo Xu⁵, Lei Wang^{4,6}, Shankai Yin⁷, Renjie Chai^{3#}, Huawei Li^{2,4#}

¹Research Center, Affiliated Eye & ENT Hospital of Fudan Univ., Shanghai, China, ²Dept. of Otorhinolaryngology, Affiliated Eye & ENT Hospital of Fudan Univ. Shanghai, China, ³Key Lab. for Developmental Genes & Human Disease, Ministry of Education, Institute of Life Sciences, Southeast Univ., Nanjing, China ⁴Inst. of Stem Cell & Regeneration Medicine, Inst. of Biomedical Science, Fudan Univ., Shanghai, China, ⁵Anesthesiology Dept., Xin Hua Hospital Affiliated to Shanghai Jiao Tong Univ. School of Medicine, Shanghai, China, ⁶State Key Lab. of Genetic Engineering & MOE Key Lab. of Contemporary Anthropology, School of Life Sciences, Fudan Univ., Shanghai, China ⁷Dept. of Otolaryngology, the Sixth Hospital Affiliated to Shanghai Jiao Tong Univ., Shanghai, China

#Correspondence: Huawei Li, M.D. lihuawei63@gmail.com & Renjie Chai, Ph.D. renjiechai@seu.edu.cn

Aminoglycoside-induced cochlear ototoxicity causes hair cell (HC) loss and results in hearing impairment in patients. Previous studies have developed the concept of an ototoxicity sensitive period during which the cochleae of young mice are more vulnerable to auditory trauma than adults. Here, we compared neomycin-induced ototoxicity at four developmental ages in mice – postnatal day (P)1–P7, P8–P14, P15–P21, and P60–P66 and found that when neomycin was administered during from P8 to P14, the auditory brainstem response threshold increase was significantly higher at low frequencies and HC loss was significantly greater in the apical turn of the cochlea compared to neomycin administration during the other age ranges. qPCR data revealed that the expression of apoptotic markers, including *Casp3* and *Casp9*, was significantly higher when neomycin was injected from P8 to P14, while the expression of X-linked inhibitor of apoptosis protein (XIAP) gene was significantly higher when neomycin was injected from P60 to P66. Because XIAP expression was low during the neomycin-sensitive period, we overexpressed XIAP in mice and found that it could protect against neomycin-induced hearing loss at low frequencies and HC loss in the apical turn of the cochlea. All together, our findings demonstrate a protective role for XIAP against neomycin-induced hearing loss and HC loss in the apical turn of the cochlea during the ototoxic-sensitive period, and suggest that apoptotic factors mediate the effect of neomycin during the ototoxic-sensitive period.

CTP detection test and Idiopathic sudden sensorineural hearing lossShibasaki O¹, Ikezono T², Matsuda H², Matsumura T³, Saito S³, Ito A¹¹Department of Neuro-Otology, ²Otolaryngology, Saitama Medical University, Saitama, Japan, ³Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan

Background: Idiopathic sudden sensorineural hearing loss (ISSHL) remains a diagnosis of exclusion, and known causes should be excluded. CTP (Cochlin tomo-protein, an isoform of Cochlin) is a newly identified perilymph specific protein, which is not expressed in the blood, CSF or saliva (*). The detection of CTP in the MEL (Middle Ear Lavage) is one of the Japanese diagnostic criteria of PLF.

Object: To evaluate if PLF is one of the cause for ISSHL.

Method: MEL was tested for CTP by ELISA. The cut off value was set by ROC analysis and AUC was above 0.9 indicating that CTP test has high diagnostic accuracy.

Result: We have tested acute HL patients and excluded cases with any preceding events such as trauma. CTP values were higher than normal range in 4 cases out of 23 ISSHL cases. One had above cutoff value.

Conclusion: PLF is one of the causes for the ISSHL, and should be diagnosed properly for specific treatment such as PLF repair patching surgeries. (*)Ikezono T, et al. The performance of CTP detection test for the diagnosis of perilymphatic fistula. *Audiol Neurotol* 2009

Protective effect of resveratrol against cisplatin-induced ototoxicity in HEI-OC1 auditory cells

Se Hee Lee, Hyung Sub Kim, Yun Suk An, Jiwon Chang, June Choi, Gi Jung Im*

Department of Otolaryngology – Head and Neck Surgery, Korea University College of Medicine, Inchon-ro 73, Seongbuk-Gu, Seoul, Korea

*Corresponding author: Gi Jung Im, E-mail: logopas@korea.ac.kr

Objective: Cisplatin is an effective chemotherapeutic drug, but it generates reactive oxygen species (ROS) that induce severe adverse effects such as ototoxicity. Resveratrol reportedly prevents oxidative stress-induced cell death. Thus, we hypothesized that the anti-oxidative effect of resveratrol could protect against cisplatin-induced ototoxicity. The present study examined the protective effect of resveratrol against cisplatin-induced ototoxicity in HEI-OC1 auditory cells.

Methods: HEI-OC1 cells were pretreated with resveratrol at 1 μ M for 24 h and then exposed to 15 μ M cisplatin for 48 h. Resulting cytotoxicity was measured by the MTT method, and intracellular ROS was measured using flow cytometry.

Results: Pretreatment with resveratrol 1 μ M protected HEI-OC1 auditory cells against cisplatin-induced cytotoxicity and significantly reduced a cisplatin-induced increase in ROS. Resveratrol provided significant protection against 15 μ M cisplatin applied for 48 h (50.8% cell viability in the cisplatin group vs. 57.6% in the cisplatin-plus-resveratrol group), and there was a 9% decrease in cisplatin-induced ROS associated with resveratrol.

Conclusion: This is the first study investigating the protective effects of resveratrol against cisplatin-induced ototoxicity in an auditory cell line. Resveratrol significantly reduced a cisplatin-induced increase in ROS and thereby inhibited cisplatin-induced cytotoxicity.

Keyword: Resveratrol, Cisplatin, Reactive oxygen species, Ototoxicity

Clinical Characteristics and Course of Recurrent Vestibulopathy Following Diuretics

Ahn Seong-Ki, Hur Dong Gu, Ko Joon-Seok, Won Seong-Joon

Department of Otolaryngology, School of Medicine, Gyeongsang National University, Jinju, Korea

Objectives: Recurrent vestibulopathy (RV) is a disease that displays recurrent symptoms of episodic vertigo that last for several minutes to several hours without auditory or neurological signs or symptoms. The purpose of this study is to investigate the clinical characteristics and course of RV with diuretics medication.

Material and Methods: During the period January 2008 to December 2010, we reviewed the clinical records of 30 patients diagnosed with RV. All patients were given hydrochlorothiazide medication at least 3 months, approached by telephone and using a questionnaire to make a long term follow-up. The analysis included age, sex distribution, natural history, pure tone audiometry, caloric response, age at onset, and the characteristics of vertigo.

Results: Median follow-up was 29 months (range, 27-37 months). Patients had a mean age at onset of 48.2 years and a mean duration of 2.75 years. An obvious female predilection was found, and unilateral caloric paresis ($\geq 25\%$) was seen in 23.3%. Of the 30 patients, symptoms resolved in 80% but were unchanged in 20% (Figure 1). No patient with RV developed a central nervous system disease or benign paroxysmal positional vertigo during follow-up.

Conclusion: The present study shows that in the majority of cases, vertigo resolved following diuretics medication. In cases of the patients with severe or disabling RV, the diuretics medication may be effective in reducing the frequency of vertigo attacks.

Changes In Eye Movements Elicited By A Vestibular Prosthesis Following Ototoxic Lesioning

Phillips C¹, Shepherd SJ⁴, Nowack A³, Nie K¹, Rubinstein JT^{1,2}, Ling L^{1,3}, Phillips JO^{1,3}

¹Otolaryngology – Head and Neck Surgery, ²Physiology and Biophysics, ³Washington National Primate Research Center: University of Washington, Seattle, WA, USA, ⁴Vanderbilt University Medical Center: Nashville, TN, USA

Background: Unilaterally implanted vestibular neurostimulators have been demonstrated to be effective in driving the vestibulo-ocular reflex (VOR) in monkeys and humans. However, results to date have illustrated that a number of challenges associated with this approach – such as low elicited eye velocities, incorrectly directed eye movements, or unilateral eye velocity bias – will have to be overcome through adaptive mechanisms present in the VOR. It is possible that these adaptive mechanisms in the vestibular periphery, in the secondary vestibular neurons, or both, can alter the effects of vestibular stimulation.

Methods: We performed intratympanic gentamicin perfusions in 4 rhesus macaque monkeys implanted with long-standing functional vestibular neurostimulators to induce peripheral vestibular loss. Over the duration of the perfusions, we measured rotational VOR, vestibular evoked compound action potentials (vECAPs), electrically elicited eye movements, and electrode impedances.

Results: Gentamicin injection was effective in producing a reduction in natural VOR, especially when it was performed in the non-implanted ear. Injection of the implanted ear produced a reduction in the vECAP responses in that ear. Finally, injection of the contralateral ear produced central plastic changes, which resulted in a dramatically increased slow phase velocity nystagmus elicited by electrical stimulation.

Conclusions: These results suggest that natural VOR adaptive mechanisms can affect the behavior elicited by vestibular changes. In this case, the increase in behavioral response was not correlated with an increase in peripheral responsiveness to electrical stimulation, but followed more closely the central adaptive changes that were induced by decreasing natural VOR gain.

Control of vestibular symptoms in patients with Meniere's disease using middle ear pressure applications

Takasi Futaki¹, Takasi Fukaya¹

¹ Futaki & Fukaya's ENT clinic, Tokyo, Japan

Pressure chamber treatment has shown, in 1970's from Scandinavia, that some patients respond well to the treatment with diminishing inner ear symptoms and also some hearing improvement. Pressure treatment has a role on the treatment staircase between pharmacological treatment and surgical procedures.

Comparing these chambers, a portable apparatus (Meniett^R) has developed by Odkvist LM et al. and many papers have reported as a useful treatment for the patient with Meniere's disease.

Instead of these Norway apparatus, we have introduced the tympanic membrane massagers as a pressure treatment apparatus.

On to three patients with Meniere's disease, we performed pressure treatment using with massagers and compared mainly vestibular symptom before and after these treatment during six months, and yielded rather good effect in these troublesome symptoms. And we discussed mechanisms of these pressure treatments.

The endolymphatic sac is the primary immunological organ of the human inner ear

Martin Nue Møller¹, Svend Kirkeby², Jonas Vikeså³, Finn Cilius Nielsen³, Per Cayé-Thomasen^{1,4}

¹ Dept. Of Oto-rhino-laryngology, head and neck surgery, Rigshospitalet DK – 2100 Copenhagen Denmark

² Department of Oral Medicine, Dental School, Panum Institute, University of Copenhagen, Copenhagen, Denmark

³ Center for Genomic Medicine, University of Copenhagen, Rigshospitalet, DK - 2100 Copenhagen, Denmark

⁴ The Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

OBJECTIVES/HYPOTHESIS: The purpose of the present study is to explore, demonstrate and describe the expression of genes related to the innate immune system in the human endolymphatic sac. It is hypothesized, that the endolymphatic sac is the primary immunological organ of the human inner ear

STUDY DESIGN: DNA micro-arrays and immuno-histochemistry were used for analyses of fresh human endolymphatic sac tissue samples.

METHODS: Twelve tissue samples from the human endolymphatic sac were obtained during translabyrinthine surgery for vestibular schwannoma. Microarray technology was used to investigate tissue sample gene expression, using adjacent dura mater as control. The expression of genes specific for the innate immune system was determined and results for selected key molecules verified by immuno-histochemistry.

RESULTS: A comprehensive overview of expressed genes of the innate immune system was obtained. Multiple key elements of both the cellular and humoral innate immune system were expressed, including Toll-like receptors 4 and 7, as well as beta-defensin and lactoferrin.

CONCLUSIONS: The present data provides the first direct evidence of an immunological capacity of the human endolymphatic sac. At the molecular level, the endolymphatic sac is capable of antigen recognition and processing for initiation of an immune response. In addition, potent molecules directly toxic to invading pathogens are expressed by the sac epithelium.

The evidence strongly supports the endolymphatic sac as the primary immunological capacity of the inner ear.

Influence of Tinnitus on Auditory Spectral and Temporal Resolution, and Speech Perception Ability in Tinnitus Patients

Kim DH¹, Kang HW¹, An YH¹, Won JH², Shim HJ¹

¹Dept. of Otorhinolaryngology, Eulji Medical Center, Eulji University School of Medicine, Seoul, KOREA, ²Dept. of Audiology and Speech Pathology, University of Tennessee Health Science Center, Knoxville, TN 37996, USA

Aims: To investigate 1) the influence of tinnitus upon the auditory spectral and temporal resolution and 2) the effect of tinnitus on speech perception ability in noise.

Methods: To exclude the effect of hearing impairment, tinnitus-affected ears (TAEs) and non-tinnitus ears (NTEs) of 19 unilateral tinnitus subjects with symmetric hearing thresholds (binaural difference < 10 dB at 0.25-8 kHz) were compared. Nineteen normal hearing subjects without tinnitus as a normal control group, and 10 bilateral tinnitus subjects with symmetric hearing thresholds as a positive control were enrolled. Four different psychoacoustic measurements were performed: 1) spectral-ripple discrimination, 2) temporal modulation detection, 3) Schroeder-phase discrimination, and 4) speech recognition threshold (SRT) in noise.

Results: There were no significant differences in spectral-ripple thresholds, temporal modulation detection thresholds, and Schroeder-phase discrimination scores between TEs and NTEs of unilateral tinnitus subjects. On the contrary, TEs showed poor SRT in noise compared to NTEs ($p < 0.05$). For the bilateral tinnitus subjects, there was no difference in SRT in noise between both TEs ($p > 0.05$).

Conclusion: The spectral ripple discrimination data suggests that the TEs do not have broader auditory filters compared to NTEs with the same hearing thresholds. However, the difference in SRT suggests that tinnitus might play a role as a masker not in cochlear level but in central auditory pathway. These results imply that the occurrence of tinnitus does not depend upon the degree of cochlear damage, but upon the change of central auditory pathway by deafferentation.

Acknowledgement: The psychoacoustic test materials were provided by the Rubinstein Lab at Virginia Merrill Bloedel Hearing Research Center.

A population-based cohort study of tinnitus in Japan

Sugiura S¹, Nakashima T¹, Yasue M², Uchida Y^{3,1}, Otsuka R⁴, Ando F^{5,4}, Shimokata H^{6,4}

¹Department of Otorhinolaryngology, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan

²Department of Otorhinolaryngology, Kamiida daiichi General hospital, Nagoya, Aichi, Japan

³Department of Otorhinolaryngology, Aichi Medical University, Nagakute, Aichi, Japan

⁴Section of NILS-LSA, Center for Gerontology and Social Science, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan

⁵Department of Health and Medical Sciences, Aichi Shukutoku University, Nagakute, Aichi, Japan

⁶Graduate School of Nutritional Sciences, Nagoya University of Arts and Sciences, Nisshin, Aichi, Japan

The prognosis of tinnitus is of great importance to patients suffering from this pathology. We epidemiologically investigated the course of tinnitus.

Data were collected from Japanese community dwellers aged 40 or above who participated in the biennial-cycled Longitudinal Study of Aging by the National Institute for Longevity Sciences (NILS-LSA). The present analyses were conducted using questionnaire responses on tinnitus, obtained in the investigations from the 4th wave (2004 to 2006) to the 7th wave (2010 to 2012).

Of the 1626 participants, 536 (33%) felt tinnitus and 304 (19%) had annoyance of tinnitus in the 4th wave investigation. Of the 536 participants who reported having tinnitus in the 4th wave, 105 persons (19%) conveyed disappearance of tinnitus in the 7th wave. Of the 304 participants who reported annoyance of tinnitus in the 4th wave, 111 persons (37%) reported no annoyance of tinnitus in the 7th wave.

The disappearance of tinnitus as long-term prognosis is not rare in community dwellers. The disappearance of tinnitus annoyance seemed to occur at nearly double the rate for disappearance of tinnitus.

Difference of autoantibody between Meniere's disease and bilateral sudden hearing loss : Is there a different autoimmunity mechanism?

Hyun Ji Kim¹, Kyung Jin Roh¹, Bo Gyung Kim², Jae Young Choi¹, Sung Huhn Kim¹

Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea¹

Department of Otorhinolaryngology, Soonchunhyang University College of Medicine, Bucheon, Korea²

Objectives: This study was performed to investigate the difference of autoantibodies and their target tissues between Meniere's disease and bilateral sudden hearing loss.

Materials & Methods: Ten patients with definite Meniere's disease, seven patients with bilateral sudden hearing loss, and ten controls were enrolled in this study. To identify serum circulating autoantibodies, Protoarray® analysis with patients' and controls' sera were performed. To detect Antigen-antibody reaction between circulating antibody and each cochlear and vestibular tissue, western blotting using mouse inner ear and patients' sera was performed. In addition, difference of clinical features according to the presence of antigen-antibody reaction was investigated.

Results: Eighteen and twelve proteins had more than 2-fold greater signal intensity in the Protoarray® analysis of Meniere's disease and bilateral sudden hearing loss. The signal intensity of 8 and 2 proteins was more than 10-fold higher in the patients than in the controls in each disease entity. Western blotting showed multiple Ag-Ab reaction between patients' sera and mouse inner ear tissues, and the band distribution was different between Meniere's disease and bilateral sudden hearing loss. Vertigo spells were more severe and treatment response was poorer in the Meniere's disease patients with the presence of Ag-Ab reaction, whereas hearing threshold was not significantly different between the sudden hearing loss patients' with or without Ag-Ab reaction.

Conclusion: Main pathologic mechanism of Meniere's disease and bilateral sudden hearing loss can be autoimmunity. There were multiple autoantibodies for each disease but their target antigen could be different between Meniere's disease and bilateral sudden hearing loss. Autoimmune reaction was correlated with severity of vertigo symptoms and represented poor response to conventional medical treatment.

Results on video head impulse test differ from those on caloric testing in the patients with Meniere's disease.

Toru Seo, Takamitsu Kobayashi, Mie Miyashita, Kazuya Saito, Katsumi Doi

Dept. of Otolaryngology, Kinki University Faculty of Medicine

Osaka-sayama, Osaka, Japan

Objective

Video head impulse test (vHIT) was established in 2009 and has been widely performed for clinical examination. It was reported that the results of vHIT were different from those of the caloric testing in some cases with Meniere's disease. The aim of this study was to clarify whether the discrepancy exist.

Subjects and Method

The subjects consisted of 6 cases of unilateral Meniere's disease, 5 cases of vestibular neuritis and a case of Ramsay Hunt syndrome. The vHIT were examined using ICS Impulse. When VOR gain was lower than 0.8, we defined result as abnormal. CP% wase calculated from monothermal caloric testing. When CP% exceeded 25%, we defined a result to be abnormal.

Result

While abnormal results in caloric testing were shown in 5 cases of vestibular neuritis, vHIT showed abnormality except one case of them. One case of Ramsay Hunt syndrome showed abnormality on both caloric testing and vHIT. On the other hand, abnormal results on caloric testing were shown in 4 cases of Meniere's disease and vHIT showed abnormal result on 1 case among them.

Conclusion

Discrepancy between the results on vHIT and those on caloric testing was confirmed. We suggested the following mechanism: endolymphatic hydrops causes the enlarged ampula portion, and then convection flow during caloric stimulation decrease. When sensory cell or cupula are not damaged, vHIT shows normal even in enlarged ampula portion.

Increased Risk Of Stroke And Post-stroke Mortality In Patients With Meniere's Disease: Two Nationwide Studies

Chien-Chang Liao¹, Ta-Liang Chen¹

¹School of Medicine, Taipei Medical University, Taipei, Taiwan

Background: The association between Meniere's disease and stroke remains known. The aim of this study is to investigate whether patients with Meniere's disease have an increased risk of stroke or post-stroke mortality.

Methods: Using Taiwan's National Health Insurance Research Database, we conducted a retrospective cohort study of 9,547 patients with new Meniere's disease and 38,188 persons without Meniere's disease between 2000 and 2004. The risk of stroke was compared between two cohorts through December 31, 2008. To investigate the association between in-hospital mortality after stroke and history of Meniere's disease, we conducted a case-control study of 7751 patients with newly diagnosed stroke between 2005 and 2008.

Results: During the follow-up period, the incidences of stroke for people with and without Meniere's disease were 10.0 and 18.9 per 1000 person-years. The cohort with Meniere's disease had an increased stroke risk (hazard ratio 1.94, 95% confidence interval [CI] 1.81-2.08) after adjustment. The association between Meniere's disease and stroke risk was observed in both sexes and every age group. Among patients with stroke, those with a history of Meniere's disease had a higher risk of poststroke mortality compared with those without Meniere's disease (odds ratio 1.57, 95% CI 1.13-2.19) after adjustment.

Conclusions: Meniere's disease was associated with risk of stroke and post-stroke mortality. The relationship between Meniere's disease and post-stroke mortality seem to transcend all age groups.

Triple Combined Intravenous and Intratympanic Steroid, Hyperbaric Oxygen Therapy for Severe Idiopathic Sudden Sensorineural Hearing Loss

Toshiro Kawano¹, Junichi Ishitoya², Yasunori Sakuma², Tomoki Matsuura¹, Ryo Ikoma³, Masahiro Takahashi⁴, Nobuhiko Oridate⁴

¹Department of Otorhinolaryngology, Nishi-Yokohama International Hospital, Yokohama, Japan

²Department of Otorhinolaryngology, Yokohama City University Medical Center, Yokohama, Japan

³Department of Otorhinolaryngology, Minami-kyosai Hospital, Yokohama, Japan

⁴Department of Otorhinolaryngology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Objective: The purpose of this study was to compare the efficacy of the triple combined intravenous steroid (IVS) and intratympanic steroid (ITS), hyperbaric oxygen (HBO) therapy with the double combined IVS and HBO therapy for severe idiopathic sudden sensorineural hearing loss (ISSHL) with a pure tone average (PTA) ≥ 70 dB.

Methods and Patients: This was a retrospective case review. 137 patients who met the inclusion criteria for ISSHL were included in this study. These 137 patients were divided into two subgroups: the severe ISSHL group with a PTA (70-89 dB) (85 patients), and the profound ISSHL group with a PTA (≥ 90 dB) (52 patients). And, 85 patients with a PTA (70-89 dB) were also divided into two subgroups according to therapy: group A1 (40 patients) received double combined therapy and group B1 (45 patients) received triple combined therapy. And, 52 patients with a PTA (≥ 90 dB) were divided into two subgroups according to therapy: group A2 (24 patients) received double combined therapy and group B2 (28 patients) received triple combined therapy. These two groups were compared. **Results:** There was not a significant difference ($p > 0.05$) in a recovery rate between A1 and B1 groups with a PTA (70-89 dB). But, there was a significant difference ($p < 0.05$) in a recovery rate between A2 and B2 groups with a PTA (≥ 90 dB). **Conclusions:** We concluded that the triple combined therapy (B2 group) gave better hearing improvement than the double combined therapy (A2 group) for ISSHL patients with a PTA ≥ 90 dB.

Controlled Release of Dexamethasone Protects TNF alpha Apoptosis of Auditory Hair Cells

Rie T. Horie¹, Takashi Saito¹, Junichiro Jo¹, Yasuhiko Tabata¹

¹Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

The use of high dose steroid is a standard treatment for inner ear disorder containing acute sensorineural hearing loss. Drug delivery technology is one of the promising methods to enhance the curative effect on inner ear disorders at present. However some properties of materials for drug delivery should be improved, such as the biodegradability and biocompatibility. The degradation process of materials and their remaining often cause material-induced inflammatory problems. Therefore, as a trail to resolve these problems, we have demonstrated that biodegradable gelatin micelles prepared by simple mixing of water-insoluble steroid and L-lactic oligomer-grafted gelatin allowed the sustained release of drug. It is well known that the materials of water-containing and hydrophilic properties induced less inflammatory reaction rather than those of non-water-containing and hydrophobic ones. A gelatin sample with an isoelectric point of 5.0 was used. A L-lactic acid oligomer (LAO) with the number-average of molecular weight of 1000 was synthesized from L-lactic monomer by ring-opening polymerization. The Lao was covalently grafted to gelatin to obtain the LAO-grafted gelatin. Recently, it is reported that tumor necrosis factor-alpha (TNF- α) is associated with trauma-induced hearing loss and dexamethasone protects organ of corti against TNF- α ototoxicity in an explant culture. This study is aim to examine the potential of releasing low-dose dexamethasone to protect auditory hair cells by drug delivery system with the LAO-grafted gelatin and how the delivery technology protects the TNF- α apoptosis.

Translational Research of Meniere's Disease; Morphological Changes of the Stria Vascularis in Animal Model

Masahiko Nishimura¹⁾, Akinobu Kakigi²⁾, Taizo Takeda³⁾, Setsuko Takeda⁴⁾, Teruhiko Okada⁵⁾, Hiroaki Sato¹⁾

1) Iwate Medical University, Iwate, 2) The University of Tokyo, Tokyo,

3) Nishinomiya Municipal Central Hospital, Hyogo, 4) Nishinomiya, Hyogo, 5) Muko, Kyoto, Japan

Objectives: Water homeostasis is essential for maintaining a function of the inner ear normally. Discovery of aquaporin (AQP) water channels revealed that AQP2 water regulation system mediated by vasopressin (VP), and many lines of evidence lead to the novel view that endolymphatic hydrops, which is the morphological characteristic of Meniere's disease, reflects the malregulation of the VP- AQP2 system in inner ear fluid. In order to develop the new treatments of Meniere's disease, we investigated a time course of changes of the stria vascularis after VP injection and the influence of Vasopressin type 2 receptor (V2) antagonist on experimentally induced enlargement of the intrastrial space caused by VP injection.

Materials and methods: In the time course study, Wistar rats were injected with 50 mg/kg of VP subcutaneously. The stria vascularis specimens were harvested at 10, 20, 30, and 60 min after VP injection. For V2 antagonist administration, animals were administered 100 mg/kg of V2 antagonist orally 60 min before receiving 50 mg/kg of VP subcutaneously. The specimens were harvested 20 min after VP injection. These specimens were observed using transmission electron microscopy.

Results: In the time course study, the mean intrastrial space enlargement was 3.2%, 16.2%, 1.4%, and 0% for 10, 20, 30, and 60 min, respectively. In the study with V2 antagonist administration, the enlargement of the intrastrial space was reduced.

Conclusion: The intrastrial space was enlarged remarkably at 20 min after VP injection, and this enlargement of the intrastrial space was reduced by administration of V2 antagonist before VP injection. These results suggest that VP increases the influx of water from the perilymph to the basal cells via AQP 2 and causes the formation of endolymphatic hydrops.

Otoprotective Treatments: Pharmacokinetic properties of Adenosine Amine Congener (ADAC) in Rat Plasma and Cochlea

Chang H¹, Telang RS¹, Sreebhavan S², Tingle M³, Thorne PR^{1,4}, Vlajkovic SM¹

¹Department of Physiology and Centre for Brain Research, ²Auckland Cancer Society Research Centre, ³Department of Pharmacology and ⁴Section of Audiology, The University of Auckland, New Zealand

We have previously shown that Adenosine Amine Congener (ADAC), a selective A₁ adenosine receptor agonist, can ameliorate noise- and cisplatin-induced cochlear injury. More recently, we have demonstrated the dose-dependent rescue effects of ADAC on noise-induced cochlear injury in a rat model and established the time window for treatment (unpublished data). The present study investigated pharmacokinetic properties of ADAC in rat plasma and cochlea after systemic (intravenous) administration using reverse phase high pressure liquid chromatography (RP-HPLC) and liquid chromatography-tandem mass spectrometry (LCMS/MS). The studies were performed on male Wistar rats (6-8 weeks) exposed to 8-16 kHz octave band noise for 2 hours at 110 dB SPL. The pharmacokinetic properties were analysed using PKSolver program for pharmacokinetic data analysis. Our results show that ADAC remains stable in plasma without detection of degradation products. The peak value in plasma was 0.48 µg/ml. The ADAC concentration curve follows a one-compartment bolus model with first-order output. The short half-life (5 minutes) of ADAC in plasma after intravenous injection is most likely due to rapid distribution in tissues, and it may explain a lack of side effects reported previously. ADAC was detected in cochlear perilymph four minutes after administration, followed by a rapid accumulation phase. The peak cochlear levels of ADAC detected following intravenous administration were consistent with pharmacological action, given the high affinity of A₁ receptors for ADAC. Our pharmacokinetic study suggests that systemic administration of ADAC has a potential to be developed as a clinical otological treatment for acute hearing loss caused by exposure to traumatic noise.

Liposome-Encapsulated Hemoglobin Alleviates Hearing Loss After Transient Cochlear Ischemia

Masahiro Okada¹, Akira T. Kawaguchi², Nobuhiro Hakuba¹, Jun Hyodo³, Naohito Hato¹, Kiyofumi Gyo¹

¹Ehime University Graduate School of Medicine : Otolaryngology Head and Neck Surgery, Toon, Ehime, Japan, ²Tokai University School of Medicine: Cell Transplantation and Regenerative Medicine, Isehara, Kanagawa, Japan, ³Takanoko Hospital : Otolaryngology, Matsuyama, Ehime, Japan

The effects of liposome-encapsulated hemoglobin (LEH), an artificial oxygen carrier, were experimentally investigated in gerbils in the context of alleviation of hearing loss after transient cochlear ischemia. Animals were randomly assigned to receive 2 mL/kg of either LEH (P₅₀O₂ = 15 mmHg) or saline 1 h after the experimental induction of 15 min of ischemia. Sequential recordings of auditory brainstem response (ABR) showed that administration of LEH prevented hearing loss due to cochlear ischemia. The mean ABR threshold at 32 kHz on day 1 was 21 ± 7 dB in the LEH group (n = 6) and 45 ± 6 dB in the saline group (n = 6). Thereafter, hearing impairment gradually improved up to day 7 in both groups. The animals were then subjected to histological study, which revealed that there was more substantial loss of the inner hair cells, but not the outer hair cells, in the saline group as compared to the LEH group. These results suggest that LEH is an efficient agent with regard to protection against hearing loss and underlying hair cell damage due to ischemic insult.

Screening For Protective Effect In Kampo (Herbal Medicine) Using The Zebrafish Lateral Line Hair Cell

Hirose, Y, Sugahara, K, Yamashita, H,

Yamaguchi University Graduate School of Medicine, Ube-City, Yamaguchi, Japan,

Introduction: The zebrafish lateral line is a powerful system for studying hair cells and hair cell death. Hair cells can be easily labeled and imaged in vivo with fluorescence microscopy. We have previously described a screening system to rapidly assess drugs for possible ototoxic effects in anti-cancer drugs (Hirose et al., 2011). Also it is possible to screen protective effect against some ototoxic drugs.

Objectives: We have screened the herbal medicines for protective effects against aminoglycoside.

Methods: 5–7 dpf Zebrafish embryos were used in this study. Zebrafish larvae were exposed to herbal medicine (1–1000 µg/ml) for 1 hour before 200 µM Neomycin for 1 hour. After that, they were fixed in 4% paraformaldehyde, incubated with anti-parvalbumin, Alexa 594, and hair cell damage was assessed by fluorescent microscope. Also, we evaluate an antioxidative effect. 5-dpf alive zebrafish were used in this experiment. We made control group, Neomycin group, and Shimotsuto+Neomycin group. Zebrafish larvae were exposed to Shimotsuto (1000 µg/ml) for 1 hour before 200 µM Neomycin for 5 minutes. H2DCF was used as marker of oxidative stress.

Results: We made dose-response curves to evaluate protective effect. All of the Kampo medicine shows protective effects against Neomycin hair cell damage. In oxidative stress experiment, Neomycin enforces H2DCF staining and Shimotsuto reduce. This suggests Neomycin generates strong oxidative stress, and Shimotsuto weakens.

Conclusions: In conclusion, herbal medicine shows protective effect against zebrafish hair cell, this study shows that can be a powerful protective drug against inner ear damage.

Enhancing the cochlear protection of X-linked inhibitor of apoptosis protein by using arginine-rich cell-penetrating peptide

Takaomi Kurioka¹, Takeshi Matsunobu¹, Ryosei Minoda², Toru Miwa², Takao Yamada², Kunio Mizutani¹, Katsuki Niwa¹, Atsushi Tamura¹, Akihiro Shiotani¹

¹Department of Otolaryngology, National Defense Medical College, Tokorozawa, Saitama, Japan,

²Department of Otolaryngology, Kumamoto University, Kumamoto, Kumamoto, Japan

Exposure to intense noise induces apoptosis in hair cells in the cochleae, and that antiapoptotic therapy will be effective in helping to prevent cochlear damage that can occur as a result of acoustic trauma. Intracellular delivery using membrane-permeable peptide vectors is a recently developed methodology that has been employed to transport various molecules into cells. Arginine-rich peptides are categorized into one of the most frequently used peptide vectors.

In this study, we examined the transduction effects of enhanced green fluorescent protein fused to a nine-arginine peptide (EGFP-9R) into cochleae and the highly therapeutic effects of X-linked inhibitor of apoptosis protein fused to a nine-arginine peptide (XIAP-9R) to protect against acoustic trauma. First, we found that EGFP-9R placed on the round window membrane (RWM) could deliver the protein into the entire cochleae efficiently. The basal turn tended to show higher immunoreactivity than the apical turn. The EGFP expressions were remained for 12–24 h after application. When we treated with XIAP or XIAP-9R before noise exposure, XIAP-9R significantly reduced the extent of hearing loss, hair cells death, cleaved caspase-3 activation.

These findings indicate that XIAP-9R topically applied on the RWM can enter the cochleae by diffusion and effectively prevent noise-induced apoptosis of cochleae by suppressing the caspase-3 pathway.

Intra-tympanic Injection of Isosorbide for Endolymphatic Hydrops in Guinea Pig

Kim M, Do KH and Kim KS

Department of Otorhinolaryngology, Inha University College of Medicine, Incheon, Korea

Objectives. The aims of this study were to investigate intracochlear isosorbide concentration in perilymph after round window perfusion (RWP) and feasibility of intra-tympanic injection (IT) of isosorbide for endolymphatic hydrops in guinea pig model.

Methods. Twenty-four male guinea pigs were used. Isosorbide was administered via RWP or PO. Perilymph sampling was performed after RWP for 15, 30, or 60 min. To compare the drug concentration after RWP versus that after PO, perilymph was aspirated in 3 and 6 h. And concentration was checked after IT of isosorbide with different concentration. Finally, improvement of hydrops was observed histologically after IT in animal model with surgically induced hydrops.

Results. After RWP for 15, 30, and 60 min, mean isosorbide concentrations in perilymph were 116.27 ± 44.65 , 245.48 ± 112.84 , and 279.78 ± 186.32 mM, respectively. RWP for 30 min shows higher concentration than RWP for 15 min ($P = 0.043$). At 3 and 6 h after PO, isosorbide concentrations after RWP were 117.91 ± 17.70 and 75.03 ± 14.82 mM at 3 and 6 h, respectively. Isosorbide concentrations in perilymph following RWP were significantly higher than those following PO administration at both 3 and 6 h ($p = 0.025$ and 0.034). Similar concentration was shown between 100% and 50% isosorbide injection, however, significantly lower after 25% isosorbide. In animal model, IT of isosorbide reduced endolymphatic hydrops histologically.

Conclusion. Isosorbide can rapidly pass through the round window membrane after RWP, and RWP can deliver higher concentrations of isosorbide than PO. In the animal model, improvement of hydrops was observed after IT.

The change of Vocal Performance according to Cochlear Implantation: for 1 year

Se-Joon Oh¹, Soo-Keun Kong¹, Il-Woo Lee¹, Eui-Kyung Goh¹

¹Department of Otorhinolaryngology-Head and Neck Surgery, Pusan National University School of Medicine and Medical Research Institute, Busan 602-739, Republic of Korea

Objectives : To investigate change of vocal performance between pre-op and post op in cochlear implantation(CI).

Methods : The subjects were 10 patients with profound and over who were diagnosed from otolaryngologist. All subjects had been cochlear implantation.

Results : The age ranged from 15 to 46, average 33.8. And hearing loss period ranged from 1 to 23 years. According to language acquisition time, subjects were divided pre-lingual and post-lingual. Change of vocal performance was measured with VRP of CSL4500 system. Recording was using headset microphone. The results of this study follows. First, for change pitch characteristic according period of CI, there was significant differences in F₀-L, ST-M, ST-R. Second, for change SPL characteristic, there was significant difference in I-L, I-R. Third, for change VRP characteristic according period of hearing loss, there was significant difference in F₀-R_{POp6}, ST-R-R_{P6P12}, I-L_{P6P12}23, F₀-R_{POp12}, ST-M_{POp12}, ST-R_{POp12}, ST-M_{POp12}, ST-R_{POp12}. Fourth, for change VRP characteristic according timing of hearing loss, there was significant differences between pre-lingual and post-lingual in ST-M, ST-R. And there were significant differences both in I-L.

Conclusions : VRP shows apparent aspect of vocal performance. This study provides basic material useful in CI study, suggesting a guideline for voice treatment using VRP

Cochlear implantation in patients with eosinophilic otitis media

Takahashi M¹, Sudo N¹, Isono Y¹, Arai Y¹, Sakuma N¹, Oridate N¹, Iwasaki S²

¹ Dept of Otorhinolaryngology Head and Neck surgery, Yokohama City University School of Medicine, Yokohama, Japan

² Dept. of Otorhinolaryngology, International University of Health and Welfare, Mita Hospital, Tokyo, Japan

Objective: Eosinophilic otitis media (EOM) is intractable middle ear disease, characterized as eosinophil-enriched secretion. The deterioration of the high-frequency bone conduction is more severe in EOM patients than that in chronic otitis media patients. It is said that 6% become profound sensorineural hearing loss among EOM patients. We reported that cochlear implantation in two patients with eosinophilic otitis media.

Patients

Case 1 53 y.o. F : She was diagnosed as bilateral chronic otitis media 30 years ago. She was also diagnosed as chronic sinusitis without nasal polyposis. The pathology of the middle ear mucosa: content numerous eosinophils. Therefore, she was diagnosed as EOM. She had been suffering from progressive bilateral sensorineural hearing loss, then bilateral profound sensorineural hearing loss was detected at 49 y.o. We performed minimum invasive cochlear implantation using flex electrode arrays since no ossification of the cochlea.

Case 2 73 y.o. F : She was diagnosed as bilateral EOM 10 years ago. The pure tone audiogram showed bilateral profound hearing loss. The electrode array was inserted into the scala vestibule since ossification of the scala tympani was found. After surgery, the progresses were satisfactory with wearing of the implant although the middle ear effusion was seen.

Conclusion: Cochlear implantation in patients with eosinophilic otitis media (EOM) is safe and not complicated. The early implantation should be considered for deaf patients with eosinophilic otitis media, since there is a possibility of cochlear ossification.

Evaluation of intraoperative electrically evoked auditory brainstem response findings in patients with bilateral cochlear implants

Kishimoto I¹, Yamazaki H², Fujiwara K¹, Shinohara S¹, Kikuchi M¹, Suehiro A¹, Tona R³, Harada H¹, Kuwata F¹, Moroto S¹, Yamamoto R¹, Naito Y¹

¹Department of Otolaryngology, Kobe City Medical Center General Hospital, Kobe, Japan, ³Department of Otolaryngology, Institute of Biomedical Research and Innovation, Kobe, Japan

²Department of Otolaryngology-Head and Neck Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

Objectives: To assess the functional status of the brain stem auditory pathway and its relationship with hearing in profoundly deafened children with bilateral cochlear implants (CI).

Patients: Twelve prelingually deafened children with bilateral cochlear implants were included in the current study. Subjects with inner ear malformation were excluded.

Methods: Intraoperative EABR testing was performed in both ears at the time of the second cochlear implantation. The presence or absence of wave eV, its latency as well as the right-left difference were assessed in channel 3, 12 and 20 electrodes.

Results: Wave eV was observed in all channels in all twelve cases. The eV wave latency was shorter for the first CI ear than for the second CI ear in channel 3, but the difference was not significant for channels 12 and 20. No definite correlation was observed between the period of cochlear implant use and the right-left latency difference.

Conclusion: Unilateral CI may result in functional asymmetry of the brain stem auditory tract, some part of which might be corrected by using additional CI on the opposite ear. Further studies are needed to understand the overall effects of the functional asymmetry of the auditory tract in CI users on the development of spoken language acquisition and its critical period.

Reversed internal magnet of cochlear implant after magnetic resonance imaging

Se-Joon Oh¹, Hee-Jeong Jeong²

¹Department of Otorhinolaryngology-Head and Neck Surgery, Pusan National University School of Medicine and Medical Research Institute, Busan 602-739, Republic of Korea

²Department of Psychiatry, Pusan National University School of Medicine, Busan 602-739, Republic of Korea

Cochlear implants (CI) have now become a standard method of treating severe to profound hearing loss. Recently, the number of patients with CI has been rapidly increasing as the big benefits of CI become more widely known. Magnetic resonance imaging (MRI) has also become a routine diagnostic imaging modality, used in the diagnosis of common conditions, including stroke, back pain, and headache. We report our recent experience with a case in which internal magnet of the cochlear implant was reversed after 1.5T lumbar spine MRI. This complication is managed successfully by reversing the orientation of the external magnet in the head coil.

Outcome of bilateral cochlear implantation for children with SNHL

Matsui T¹, Ogawa H², Yamada N³, Baba Y¹, Suzuki Y³, Nomoto M¹, Omori K¹

¹Department of Otolaryngology Fukushima Medical University: Fukushima, Japan,

²Department of Otolaryngology Aizu Medical Center: Fukushima, Japan,

³Fukushima Rehabilitation Center for Children: Fukushima, Japan

Objective: New criteria of indication and selection of cochlear implantation for children was made by the Oto-Rhino-Laryngological Society of Japan in 2014. Therefore, the number of bilateral cochlear implantation at early age has increase further. The purpose of this report is to evaluate the speech and language skills and sound localization of children who underwent bilateral cochlear implantation.

Methods: Six Japanese children with profound sensorineural hearing loss underwent bilateral cochlear implantation in our hospital.

Speech and language development were evaluated post-implantation using The Japanese MacArthur Communicative Development Inventory.

Sound localization was tested in an anechoic sound chamber with multiple speakers. Four equidistant speakers at intervals of 90 degrees were located in a circle of 2 meter diameter around the room in a horizontal plane approximately 1 meter above the floor.

Results: The Japanese MacArthur Communicative Development Inventory scores of the children continued to increase after implantation.

Sound localization ability showed a tendency to improve with bilateral cochlea implantation.

Conclusions: The present result suggested that the bilateral cochlear implantation was effective for speech and language development and sound localization.

The Basal Turn: Variability Of An Ideal Insertion Vector For Cochlear Implantation

Breinbauer H.A.^{1,3}, Plinkert P.K.¹, Praetorius M.J.¹

¹Division of Otolaryngology and Neurotology, Department of Otolaryngology, University of Heidelberg, Heidelberg.

²Department of Otolaryngology, Clínica Alemana de Santiago, Santiago. ³Department of Otolaryngology, Hospital San Juan de Dios, Universidad de Chile, Santiago.

Hypothesis: The orientation of the basal turn of the cochlea – which determines the ideal electrode insertion vector during cochlear implantation – may vary more than previously considered among candidates, particularly under a three-dimensional analysis.

Background: Whereas through cochleostomy or round window approaches, the vector – of insertion after this first entry point appears to be related to intracochlear damage, which in turn may correlate with anatomical and functional results in cochlear implants.

Methods: Three dimensional reconstructions of temporal bones of 50 cochlear implant candidates (for a total of 100 ears) were assessed. The spatial orientation of an ideal insertion vector for both a cochleostomy and a round window approach were estimated.

Results: A difference as large as 60° was found between subjects for an ideal insertion vector. From an intraoperative perspective, this variability involves pushing the electrodes “as near from the buttress” or “as near from the emergence of the corda tympani” as possible, depending on the case.

Conclusion: The orientation of the basal turn, and the corresponding ideal electrode insertion vector, varies largely among subjects. A proper pre-operative estimation on a case to case scenario for this feature may lead to technique adaptation during insertion, which may contribute to minimizing electrode insertion trauma and thus optimizing anatomical and functional results.

Real-time impedance measurement for intracochlear electrode insertion

Chin-Tuan Tan¹, Mario Svirsky¹, Abbas Anwar¹, Shaun Kumar², Bernie Caessens², Claudiu Treaba², Paul Carter², and J. Thomas Roland Jr¹

¹New York University School of Medicine, New York, NY, USA

²Cochlear Ltd., Sydney, NSW, Australia

Objectives: This pilot study describes the use of a software tool that measures electrode impedance continuously during intracochlear electrode insertion, with the eventual potential to assess and optimize electrode placement and reduce insertional trauma.

Methods: A prototype program to measure electrode impedance and display it graphically in real time has been developed. The software was evaluated in human cadaveric temporal bones and in two live surgeries during intracochlear electrode insertion. Electrode position was crossevaluated with real time fluoroscopic analysis.

Results: Several electrode designs were evaluated with the new software system and data were obtained during electrode insertion with fluoroscopic guidance. Impedance changes were observed with various scalar positions. Using Contour Advance™ electrodes, impedance values increased after stylet removal, particularly when the electrodes are stimulated in the monopolar mode.

Conclusion: Impedance values seem systematically affected by electrode position, with higher values being associated with proximity to the cochlear wall. The new software is capable of acquiring impedance measurements during electrode insertion and this data may be useful to guide surgeons to achieve optimal and atraumatic electrode insertion, to guide robotic electrode insertion, and to provide insights about electrode position in the cochlea.

This study was supported by Cochlear Ltd. (PI: Roland). Dr. Tan's participation in the study was supported by NIH grant K25-DC010834 (PI: Tan), and Dr. Svirsky's participation was supported by NIH grant R01-DC003937 (PI: Svirsky).

Observation of Cortical Activity During Speech Stimulation in Prelingually-Deafened Adolescent and Adult Patients With Cochlear Implantation by PET-CT

Yoshida H¹, Kanda Y², Usui T³, Miyamoto I³, Tiba K³, Takahashi H¹

¹Department of Otolaryngology-Head and Neck Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, ²Nagasaki Bell Hearing Center, Nagasaki, Japan, ³Nishiisahaya Hospital, Nagasaki, Japan

Objective: To evaluate the cortical activity of prelingually-deafened adolescent and adult patients with cochlear implant (CI).

Methods: Brain activities examined by positron emission tomography (PET)-CT using 18-fluoro-deoxy-glucose (FDG) of 6 prelingually-deafened CI users were compared with those of 10 normal individuals. Changes in regional cerebral blood flow were measured during auditory language stimuli while listening to a story. The mean age at CI of the 6 patients was 19.7 years old, and the mean postoperative observation time was 26.7 months. Ten normal volunteers were observed as age-matched controls (mean age = 27.1 years old; 6 male and 4 female).

Results: In the successful prelingually-deafened adult cases, there were much increased FDG uptake regions in the adult cases than in the adolescent cases. This means that these adult cases pay more attention and make more an effort when communicating with CI than adolescent cases. In the unsuccessful prelingually-deafened adolescent users, the hypermetabolism was seen in the primary auditory cortex and Broca's area, not in the auditory association area. Activation in these areas can be related to such bad performances with CI in prelingually-deaf adolescent and adult patients.

Conclusion: Brain activities examined by PET-CT can show the useful presurgical information for prelingually-deaf adolescent and adult patients with CI. Despite the limits imposed by the small sample size, this study yielded insights on the nature of the brain plasticity in prelingually-deafened CI users.

The effect of the cochlear implantation in teenagers with progressive hearing loss

Kitaoka K¹, Takahashi H¹, Kanda Y²,

¹Otolaryngology Head and Neck, Nagasaki University Hospital, Nagasaki City, Japan

²Nagasaki Bell Hearing Center, Nagasaki City, Japan

Objective: Although the importance of the newborn hearing screening examination is well known, management of children with progressive hearing loss remains a problem. We investigated the outcome of cochlear implantation (CI) in teenagers with progressive hearing loss.

Methods: The teenager group consisted of 9 children who underwent CI because of progressive hearing loss from February 1999 to July 2013. All of them had received auditory verbal education. All patients were operated in Nagasaki University hospital.

Results: Pure-tone threshold level and speech discrimination score are compared. Almost all the children had good performance; the average threshold level was 29dB, and the speech discrimination scores were more than 70 percent in all but one child, whose threshold level was 40dB and speech discrimination score was 5 percent.

Conclusion: Most of teenagers with progressive hearing loss were found to get good CI performance. One child who showed insufficient result indicated the importance not only of the treatment and education but also the timing of the CI.

The Strategy of Cochlear Implantation in Patients with Chronic Otitis Media

Kanai Rie, Shin-ichi Kanemaru, Nishida Akiko, Misaki Yamamoto, Maetani Toshiki, Yamashita Masaru, Furuta Ichiro, Kitada Yuji
Tazuke Kofukai Foundation, Medical Research Institute, Kitano Hospital, OSAKA, JAPAN

Purpose: Cochlear implantation (CI) in patients with chronic otitis media (COM) is not performed commonly because of the risks of complication; meningitis or extrusion of electrode. We performed CI for fifteen patients with COM. The purpose of this report is to introduce our management strategy for CI patients with COM.

Patients and Methods: Fifteen patients (M : F = 9 : 6, mean age: 73.7) were enrolled in this study. All patients had COM and underwent CI between April 2009 and April 2014. We researched surgical procedure, post-operative courses about each patient.

Results: Five patients with active infection underwent two-staged surgeries. We performed canal wall up (N=3) or revision canal wall down (N=2) tympanomastoidectomy to control infection, followed by CI 3 to 6 months after the initial surgeries. Other ten patients without active infection underwent CI at single-staged surgery. Eight patients had radical cavity or cavity after tympanomastoidectomy. These patients required additional procedure to prevent electrode extrusion. Electrode array were housed in grooves drilled on mastoid surface and fixed by bone tips, bone putty, cartilage, and fibrin glue. Eleven patients were uneventful post-operatively. In four patients, minor complications (myringitis, wound dehiscence, tympanic membrane perforation, and defect of epithelium on mastoid cavity) occurred, which were managed by local treatments or re-operation. All patients continue to use CI without electrode extrusion and serious infection.

Conclusions: COM is not contraindication for CI, however, infection control by middle ear surgery and stabilization of electrode array are important.

Localization of Kainate Receptors in Inner and Outer Hair Cell Synapses

Fujikawa T^{1,2}, Petralia RS², Fitzgerald TS², Wang YX², Millis B², Kitamura K¹, Kachar B²

¹Department of Otolaryngology, Tokyo Medical and Dental University, Tokyo, Japan, ²National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, USA

Glutamate plays a role in hair cell afferent transmission, but the receptors that mediate neurotransmission between outer hair cells (OHCs) and type II ganglion neurons are not well defined. A previous study using *in situ* hybridization showed that several kainate-type glutamate receptor (KAR) subunits are expressed in cochlear ganglion neurons. To determine whether KARs are expressed in hair cell synapses, we performed X-gal staining on mice expressing *lacZ* driven by the *GluK5* promoter, and immunolabeling of glutamate receptors in whole-mount mammalian cochleae. X-gal staining revealed *GluK5* expression in both type I and type II ganglion neurons and OHCs in adults. OHCs showed X-gal reactivity throughout maturation from postnatal day 4 (P4) to 1.5 months. Immunoreactivity for *GluK5* in IHC afferent synapses appeared to be postsynaptic, similar to *GluA2* (*GluR2*; AMPA-type glutamate receptor (AMPA) subunit), while *GluK2* may be on both sides of the synapses. In OHC afferent synapses, immunoreactivity for *GluK2* and *GluK5* was found, although *GluK2* was only in those synapses bearing ribbons. *GluA2* was not detected in adult OHC afferent synapses. Interestingly, *GluK1*, *GluK2* and *GluK5* were also detected in OHC efferent synapses, forming several active zones in each synaptic area. At P8, *GluA2* and all KAR subunits except *GluK4* were detected in OHC afferent synapses in the apical turn, and *GluA2*, *GluK1*, *GluK3* decreased dramatically in the basal turn. These results indicate that AMPARs and KARs (*GluK2*/*GluK5*) are localized to IHC afferent synapses, while only KARs (*GluK2*/*GluK5*) are localized to OHC afferent synapses in adults. Glutamate spillover near OHCs may act on KARs in OHC efferent terminals to modulate transmission of acoustic information and OHC electromotility.

A Rare Case of Meningeal Carcinomatosis with Multiple Nerve Palsy, Deaf and Facial Palsy

Ida Yutaro^{1,2}, Yasuda Mamiko², Sasaki Yuko², Sugo Nobuo³, Edamatsu Hideo²

¹ Department of Otorhinolaryngology, International Goodwill Hospital, Yokohama, 245-0006 Japan

² Department of Otorhinolaryngology, Toho University School of Medicine, Tokyo, 143-8541 Japan

³ Department of Neurosurgery, Toho University School of Medicine, Tokyo, 143-8541 Japan

Meningeal carcinomatosis has been reported to cause inner ear dysfunction, hearing loss or dizziness. Our case is a 67-year-old man with right acute sensorineural hearing loss at first. Then, he showed multiple neural problems, bilateral deaf and facial palsy. However, DPOAE was pass even in both deaf ears. His clinical course seemed to be central nervous disease and so brain MRI was studied. A tumor was detected in bilateral cerebellopontine angle, and a craniotomy biopsy was performed promptly by neurosurgeon. Diagnosis was unexpectedly poorly differentiated adenocarcinoma. Pathological findings suggested metastasis of pancreatic cancer, but primary tumor was not clear yet by further examinations. He still has above neural symptoms after irradiation and chemotherapy, but he can maintain activities of daily living.

Our conclusion is as follows, 1) there is a rare case of brain tumor mimicking inner ear disease of hearing loss or facial palsy, 2) DPOAE and brain MRI are important and useful to make an exact diagnosis, 3) cooperation between otologist and neurosurgeon is necessary for this kind of complicated case.

Audiologic Tests Results Of Patients Referred From Newborn Hearing Screening Program

Chan Il Song (Song CI)¹, Ho Seob Kang (Kang HS)², Joong Ho Ahn (Ahn JH)²

¹ Department of Otolaryngology, Jeju National University Hospital, Jeju, Korea, ² Department of Otolaryngology-Head and Neck Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Objectives: To analyze the audiologic tests results of referred patients from newborn hearing screening program.

Methods: Neonates were screened by automated auditory brainstem response (AABR) or automated evoked otoacoustic emissions (AOAE). Subjects whose result of AABR or AOAE was “refer” were tested auditory brainstem response (ABR), auditory steady-state response (ASSR) and distortion product otoacoustic emissions (DPOAE). Final results from audiologic tests and correlation between these tests were analyzed.

Results: Of 237 babies who were “referred”, 116 patients had normal hearing, 121 showed ABR threshold over 30 dB nHL in at least one ear. One hundred fifty-six ears underwent ASSR tests and mean ASSR threshold had a strong correlation with ABR threshold ($r=0.905$, $p<0.001$). Sensitivity and specificity of ASSR to ABR were 89.6% and 93.3%. DPOAE tests were performed to 180 ears, with sensitivity of 85.9% and specificity of 82.8%.

Conclusion: ASSR and DPOAE had relatively high sensitivity and specificity. In addition, ASSR can be considered as substitute of ABR.

Effect Of Edaravone On Acute Brainstem-cerebellar Infarction With Vertigo And Sudden Hearing Loss

Yuta Inoue^{1,2}, Takao Yabe², Kazunari Okada²

¹Department of Otolaryngology, Faculty of Medicine, The University of Tokyo, Tokyo, Japan

²Department of Otolaryngology, Tokyo Metropolitan Hiroo Hospital, Tokyo, Japan

Objective: We report 2 cases with acute brainstem and brainstem-cerebellar infarction showed improvement of their signs and symptoms after administration of edaravone.

Methods: We used 30 mg of edaravone was infused twice a day for 14 days to 2 cases. Case 1, a 74-year-old woman who experienced sudden vertigo, also had dysarthria and left hemiplegia. Case 2, a 50-year-old man who experienced sudden vertigo and sensorineural hearing loss (SNHL), developed dysarthria after admission.

Results: Case 1, Magnetic resonance imaging (MRI) showed an abnormal region in the right ventrolateral medulla oblongata. The patient's vertigo and hemiplegia improved completely after treatment. Case 2, MRI revealed acute infarction in the right cerebellar hemisphere. Magnetic resonance angiography revealed dissection of the basilar artery and occlusion of the right anterior inferior cerebellar artery. The patient's vertigo and hearing remarkably improved.

Conclusion: We have described 2 patients whose early symptoms were vertigo and sudden SNHL, but who were later shown to have ischemic lesions of the central nervous system. Edaravone is neuroprotective drug with free radical-scavenging actions. Free radicals in the ear are responsible for ischemic damage. Edaravone, a free radical scavenger, may be useful in the treatment of vertigo and SNHL.

Analysis of the input/output functions of different components of otoacoustic emissions

Moleti A.¹, Sisto R.²

¹University of Roma Tor Vergata Physics Department, Roma, Italy, ²INAIL Research, Monte Porzio Catone, RM, Italy

Otoacoustic Emissions (OAEs) provide, in principle, sensitive, non-invasive, objective diagnostics of the cochlear function, because, for each frequency, the OAE levels are directly related to the local basilar membrane (BM) displacement, i.e., to the auditory perception level. Their clinical use is limited by the complexity of their generation mechanisms. Indeed, OAE responses are the vector superposition of backward cochlear waves associated with different generation mechanisms and different generation regions.

Objective: The objective of this study is to highlight how disentangling the different OAE components helps understanding the cochlear physiology and improves their diagnostic power.

Methods: DPOAEs, TEOAEs and SFOAEs are recorded with high frequency resolution in a sample of normal hearing human ears, at different stimulus levels. Nonlinear cochlear models are used to predict the expected time-frequency behavior of the OAE response, and time-frequency filtering is used to separate the different latency components.

Results: The comparison between the I/O curves of the different OAE components confirms the theoretical predictions about their different cochlear generation mechanisms, and permits to get independent information on the influence of additional parameters, such as the middle ear transmission and the basal reflectivity, on the OAE output, eventually improving their diagnostic power.

Conclusions: This study shows an example of how advanced OAE acquisition techniques, time-frequency analysis methods, cochlear modeling considerations may be used together to improve the clinical use of otoacoustic emissions.

New otolith and semicircular functional test using eccentric center rotation

Okumura Tomoko¹, Imai T¹, Takimoto Y¹, Inohara H¹

¹Department of Otolaryngology, Osaka University Medical School, Osaka, Japan

Objective: We report a new modified eccentric rotation (mECR) test that can investigate both otolith and semicircular canal function in the same time.

Methods: During mECR, rotational stimulation evokes vestibulo-ocular reflex by semicircular canal (sVOR) and tangential linear acceleration evokes VOR by otolith (oVOR). 7 healthy volunteers were faced to the ground (90deg nose-down) and rotated 100cm away from the center of rotation at a frequency of 0.1, 0.3, 0.5, 0.7Hz with a maximum angular velocity of 50°/s. With this situation, as the direction of eye movement induced by sVOR are torsional, and the direction of eye movement induced by oVOR are horizontal or vertical, it is possible to distinguish the eye movement induced by oVOR from that induced by sVOR. The eye movement during mECR were analyzed three-dimensionally with our own algorithm (Imai et al *acta oto-laryngol* 1999).

Results and conclusion: The gain of sVOR and oVOR elevates by the frequency. Especially, the gain of oVOR elevated dramatically at more than 0.5Hz. In conclusion, mECR at 0.5Hz is very useful stimulation for the evaluation of the function of semicircular canal and otolith simultaneously.

Ex vivo MR histology of nerve fibers in a human temporal bone by high-resolution diffusion tensor imaging using 9.4 T MRI

Fujioka M^{1,2}, Hikishima K³, Oishi N¹, Ohta H⁴, Kawai Y⁵, Kawaura M², Ogawa K¹ and Okano HJ⁶

¹Dpt. of Otolaryngology- Head and Neck Surgery, School of Medicine, Keio University, ²Dpt. of Otorhinolaryngology, Keiyu Hospital, ³Okinawa Institute of Science and Technology, ⁴Dpt. of Vascular Surgery, ⁵Dpt. of Anatomy and ⁶Div. of Regenerative Medicine, Jikei Medical School

MR histology is a novel entity finely visualizing tissue/organs at any plane in 3D imaging, of which quality is guaranteed by high magnetic field MRI. Internal auditory canal (IAC) in the temporal bone contains four nerves, cochlear nerve, superior vestibular nerve, inferior vestibular nerve and facial nerve, all of which are derived from different nucleus, gather at the CP-angle, run through the canal and project to their target organs. Using a 9.4 T MRI (Bruker Biospec®), we were able to visualize fine structures of human temporal bone including Reissner membrane, scala tympani, media and vestibule, and nerve fibers in the IAC. With a diffusion tensor imaging (DTI) analyzed by an algorithm for tracking fibers (TrackVis®), we successfully distinguished four nerves separately and track individual fibers to the end organ (cochlea, vestibules and main trunk of facial nerve at the mastoid tip). We were able to separate auditory nerve fibers from apical and basal turn of the cochlea and, interestingly, nerve fiber from the mid-turn contacted to the inferior vestibular nerve was observed. The result suggests the feasibility of autopsy imaging in the temporal bone histopathology without dissection by using high field MRI scan.

Three-dimensional Analysis of the Eye Movement of a Patient Whose Lateral Semicircular Canal Has Morphological Malformation

Higashi-Shingai K¹, Imai T², Kitahara T³, Ohta Y², Morihana T² and Inohara H².

¹Department of Otolaryngology, Suita Municipal Hospital, Suita, Osaka, Japan, ²Otolaryngology-Head and Neck Surgery, Osaka Graduate School of Medicine, Suita, Osaka, Japan, ³Department of Otolaryngology-Head and Neck Surgery, Nara Medical University, Nara, Japan

Inner ear malformations without symptom are occasionally found in image inspections. We have experienced a patient with Meniere Disease whose lateral semicircular canal of the non-affected side has morphological malformation. The canal was cystoid. In this study, we investigated which the cystic lesion was endolymphatic or perilymphatic space with inner ear gadtrinium contrast-enhanced MRI, and also investigated the function of the canal using three-dimensional analysis of eye movement during caloric test.

The patient was 75 year old woman and her chief complaint was vertigo. She had sensorineural hearing loss on her left side. In addition to the hearing test or the nystagmus inspection, we performed inner ear gadtrinium contrast-enhanced MRI to the patient to detect endolymphatic hydrops and to rule out the acoustic tumor. We confirmed the patient's left ear had endolymphatic hydrops, and diagnosed her as left Meniere disease. Furthermore, her right semicircular canal was extremely enlarged. The MRI showed us the cystic lateral semicircular canal was filled with perilymph space. The patient underwent the caloric test and we analyzed the induced nystagmus three-dimensionally. The result of both ear had poor response, and she was not diagnosed as canal paralysis by caloric test. When hot water was injected in her right ear, the axis of eye movement during slow phase of nystagmus was perpendicular to the plane of right lateral semicircular canal. This result means that the cystic canal worked as lateral semicircular canal.

In conclusion, the cystic lateral semicircular canal worked as lateral semicircular canal because the cystic portion was perilymphatic space and as a result endolymphatic space was normal.

Temporal changes of c-Fos expression in medial vestibular nuclei after unilateral chemical labyrinthectomy in rats

Hwnaho Lee¹, Hyun Kim²

¹Department of Otolaryngology-Head and Neck Surgery; ²Department of Anatomy, Kosin University College of Medicine, Busan, South Korea

Objectives: The expression of c-Fos has been well documented as a useful marker for the neuronal activation induced by pharmacological, electrical and physiological stimuli to the central nervous system. This study investigated spatio-temporal changes in c-Fos protein expression in the brain stem following lidocaine and gentamicin-induced chemical unilateral labyrinthectomy (UL) and evaluated the different course of vestibular compensation between lidocaine and gentamicin UL.

Materials and Methods: Twenty female Sprague-Dawley rats were used. Sham operation (n=4) and the left intratympanic soaking of 10% lidocaine (n=8) or gentamicin (n=8) were performed in rats.

c-Fos protein expression were investigated in the medial vestibular nuclei at 3, 6 and 12 hours (after lidocaine and gentamicin-induced UL.

Results: c-Fos protein expression was appeared in the bilateral medial vestibular nuclei after UL. But, lidocaine and gentamicin UL produced significant asymmetry of c-Fos protein expression between the bilateral medial vestibular nuclei, with marked expression in the contralateral medial vestibular nuclei than in the ipsilateral medial vestibular nuclei to the injured side. Lidocaine induced c-Fos expression appeared in 3 hours and c-Fos expression increased as time goes on. Gentamicin UL produced c-Fos protein expression reached peak at 6 hours, and c-Fos protein expression decreased at 12 hours after UL.

Conclusions: The author observed that the spatio-temporal expression of c-Fos protein following lidocaine UL differed from that of gentamicin UL. It suggests that vestibular compensation mechanism in the brain stem nuclei differs between lidocaine and gentamicin UL

Clearing of the mouse temporal bone using a modified SeeDB protocol

Rebecca Cook, BS, Naoki Shimizu, MD, PhD, Atsushi Tamura, MD, Adrian Perachio, PhD, Tomoko Makishima, MD, PhD.

Department of Otolaryngology, University of Texas Medical Branch, 301 University Blvd., Galveston, Texas 77555-0521, USA.

Background: Histological and anatomical studies of the mouse inner ear are technically difficult due to its small size and its location embedded in the temporal bone. A recently published protocol, SeeDB (*Nat Neurosci* 16:1154-1161, 2013), allows the clearing and the visualization of a whole brain in mice in a 3D fashion. Our goal was to modify the SeeDB protocol to achieve 3D visualization of inner ear organs encased in the temporal bone. We sought to establish the optimal protocol for clearing bone and cartilage in the mouse temporal bone to be suitable for immunofluorescent labeling.

Method: Temporal bones were dissected from mice at either postnatal day 1 – 2 (P1-2) or at P30. The SeeDB protocol was modified for the mouse temporal bone. Briefly, the adult temporal bones were decalcified before starting the process. The cartilaginous temporal bone of younger mice was processed directly without decalcification. After fixation in 4% paraformaldehyde in PBS, the samples were labeled with hair cell-specific markers. Then the samples were incubated in a series of fructose solutions with increasing concentrations. The cleared samples were labeled with fluorescent markers including Neurotrace Fluorescent Nissl Stain, Phalloidin, and Hoechst. Samples were mounted and viewed using fluorescent microscopy.

Results: Successful clearing of cartilaginous temporal bone and partial clearing of decalcified temporal bone was achieved.

Conclusion: Our modified version of the SeeDB protocol was low-cost using readily available materials. Using this protocol, we were able to clear and visualize immature and mature mouse temporal bones. We hope to optimize the protocol for immunofluorescent labeling that is specific to the mouse inner ear.

Evaluation of the Endolymphatic Hydrops of Guinea Pigs Using Optical Coherence Tomography

Kakigi A¹, Takubo Y², Egami N¹, Matsumoto Y¹, Sakamoto T¹, Yamashita S², Yamasoba T

¹Department of Otolaryngology, Faculty of Medicine, The University of Tokyo, Tokyo, Japan, ²Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

Background: Structural observation of the cochlea has primarily relied on histological methods, which require chemical fixation followed by dissection of the tissues or embedment in mold such as paraffin and sectioning. However, these preparations of tissue samples introduce significant changes in tissue integrity and organization, which may induce misinterpretation, limiting the generality and overall value of the results. To innovate visualization of intracochlear structure, optical coherence tomography (OCT), an emerging noninvasive imaging modality, was applied.

Methods: Guinea pigs were used and underwent the electro-cauterization of the endolymphatic sac and were fed for 4 weeks. To observe the cochleae with OCT in vivo, the bulla was opened via ventral approach. The animal was placed to obtain the optical sections including the mid-modiolar section. Then we obtained images of the cochleae by using Santec OCT system (Santec Co., Aichi, Japan). After the in vivo observation, the temporal bones were immediately excised following fixation and kept them in 10% formalin solution for 1 week. Subsequently, the specimens underwent decalcification in EDTA for 14 days. Then we obtained images of the cochleae by OCT.

Results: In the preliminary data obtained, we visualized the internal structures of the limited number of cochlear turns in vivo. OCT could demonstrate endolymphatic hydrops shown as the distention of the Reissner's membrane. These findings were consistent with the images of the decalcified cochlea. By decalcification, we could clearly and widely visualize the internal structures of the cochlea.

Conclusion: These results indicate that observing the cochlea of live animals by using OCT would be of great value of examining the cochlear pathology, prior to or without histological examination.

Highlighting Small Contrast Agent Variations in Cochlear DCE-MRI using VIBE Sequences

Plumat JM¹, Rooney WB², Nuttall AL², Vlajkovic SM¹, Thorne PR¹

¹Department of Physiology, University of Auckland, Auckland, New Zealand, ²Advanced Imaging Research Center, Oregon Health and Science University, Portland, Oregon, USA

Dynamic Contrast Enhanced MRI (DCE-MRI) is a methodological framework that is useful for deducing the permeability of blood vessel barriers based on a set of MRI capture sequences. A physical and mathematical based model enables estimation of the concentration and rate of uptake of the Gadolinium-based contrast agent (GBCA, Dotarem, dose: 0.2ml/kg, intravenous injection) in the studied organ over time and has an advantage over qualitative relative signal intensity approaches. We used this framework to approximate the permeability of the blood labyrinth barrier (BLB) on control subjects with a 3T scanner (Siemens Skyra).

Studying the BLB is very challenging: it is a relatively tight barrier and only a small amount of contrast agent reaches the inner ear compartments. Thus, the signal is weak and the volume of interest is small. Additionally, further information is needed to calculate the GBCA concentration as the pixel enhancement is not a direct measure of this concentration: the DCE-MRI framework usually recommends a T1-weighted sequence to construct its models. We have investigated various sequences to overcome some of these obstacles. Here we report on the use of a particular VIBE sequence (TR/TE=20/3.7 [ms]). This T1-weighted sequence has the advantages of being sensitive to small enhancements and both a high spatial resolution (spacings=[0.3,0.3], slice thickness=0.9mm) and a fast acquisition (5 min). We also use a reference GBCA signal to balance internal signal variations during acquisition.

The proposed protocol allows us to deduce approximations of the amount of GBCA inside different compartments of the inner ear up to 4h post GBCA injection. The MRI sequence allows us to reach accurate measurements (higher enhancement – at least 30%, better spatial and temporal resolution) compared to other available sequences (3D-FLAIR, standard T1-weighted sequences).

Preliminary Results of TEN(HL) Test for Patients With Sensorineural Hearing Loss in South Korea

Lee HY¹, Park SK², Cho CS¹

Eulji University Medical Center, Eulji University, Daejeon, South Korea

Objectives: To assess clinical applicability of TEN(HL) test in order to find the possibilities of cochlear dead regions and confirm the relevance to various clinical factors.

Methods: Puretone audiometry, tympanometry, and threshold equalizing noise hearing level (TEN(HL)) was performed in 52 patients (Male: 29, Female: 23, aged 25-81) with sensorineural hearing loss (86 ears). Mean puretone thresholds were 40.23±27.33 dBHL (Ranged 4.17-110dB). Positive TEN(HL) test was defined as masked threshold ≥10 dB above the quiet threshold and this case was considered as having cochlear dead regions. Inconclusive results was defined as TEN(HL) level could not be made to elevate the absolute threshold more than 10dB. Patient characteristics including age, sex, the affected side, mean hearing level, the audiometric patterns, tinnitus, accompanying diabetes and hypertension were assessed.

Results: 8 ears (9.3%) were found to have a cochlear dead region for at least one frequency. Of these, 5 ears showed positive test results at one frequency alone. For audiogram pattern, 7 patients with flat-type hearing loss (36.8%) showed positive results, followed by high tone hearing loss. Age, presence of Tinnitus, accompanying diabetes and hypertension were not associated with TEN test results. (p<0.05). The difference between masked threshold and quiet threshold at 0.5 Hz, 1000 Hz, 2000 Hz, and 4000 Hz showed moderately correlated with the hearing threshold at each frequency (p<0.05). 18 ears (20.9%) showed inconclusive results.

Conclusion: Audiogram patterns and hearing thresholds seemed to be associated with TEN(HL) test results.

3D-imaging of Optical Coherence Tomography for Endolymphatic Hydrops Model

Jeong Hun Jang¹, Nam Hyun Cho², Woonggyu Jung³, Jeehyun Kim², Sang Heun Lee⁴

¹Department of Otorhinolaryngology, Kyungpook National University College of Medicine; ²Department of Electronic Engineering, Kyungpook National University College of Information Technology, Daegu; ³School of Nano-Bioscience and Chemical Engineering, School of Electrical and Computer Engineering Ulsan National Institute of Science and Technology, Ulsan Korea; ⁴Department of otorhinolaryngology-Head and Neck Surgery Daegu Veterans Hospital, Daegu, Korea;

Objective: OCT, an emerging noninvasive imaging modality, has been applied to better visualize the internal structure of the cochlea. The decalcification of the cochlear bony wall using ethylenediamine tetra-acetic acid (EDTA) has been applied. We could clearly visualize internal structures of the cochleae and identify endolymphatic hydrops (EH) and 3D image was reconstructed. **Methods:** Guinea pig (Slc: Hartley strain, 5weeks, 180g) was used in this study. EH model was induced using surgical procedure described by Megerian et al(2010). Hearing level was estimated using ABR was measured (System 3, Tucker-Davis Technologies, USA) at 1 week and 2 weeks postoperatively. **Results:** The image of cochlea was acquired by OCT and the structural change of whole cochlea was evaluated by 3D reconstruction. The structural and functional changes of EH were identified by OCT image and ABR, respectively. **Conclusion:** By decalcifying the bony wall of the cochlea, we could clearly and widely visualize the internal structures of pathological cochleae. These findings indicate that imaging the decalcified cochlea by using OCT would be of great value when examining cochlear pathology, especially EH, prior to or without histological examinations. These findings indicate that observing the decalcified cochlea by using OCT would be of great value when examining cochlear pathology, especially EH, prior to or without histological examinations.

Morphofunctional Alterations In The GASH:Sal Induced By Audiogenic Kindling

Hernández-Noriega, S.¹, Sánchez-Benito, D.¹, Côrtes de Oliveira J.A.², Garcia Cairasco, N.², López D.E.¹, Sánchez C.¹ and Castellano, C.¹

¹Institute for Neuroscience of Castilla y Leon/IBSAL, University of Salamanca, Salamanca, Spain. ²Neurophysiology and Experimental Neuroethology Laboratory (LNNE) of the Ribeirão Preto School of Medicine - University of São Paulo. Brazil.

Animal models are important for the study of neural substrates involved in epileptic seizures phenomena. The GASH:Sal exhibits generalized tonic-clonic seizures of genetic origin in response to sound stimulation and is currently being validated as a reliable model of epilepsy.

Objectives: The main focus is to understand the nuclei involved in the development of a seizure and which of them are related to repetitive seizures.

Methods: We evaluated behavioral sequences before, during, and after a seizure. We analyzed each behavioral item, measuring its frequency, duration, and their interactions with others through a validated neuroethological program called Ethomatic. This protocol of 20 stimulation sessions, during 10 consecutive days (at 09:00 and 19:00 h) is known as “audiogenic kindling”. We correlated the behavioral data with histological quantitative study of the *c-fos* expression as a marker of neuron activation.

Results: We observed behavioral and morphological changes during kindling. Seizures activated nuclei of the auditory pathway, as cochlear nuclei and inferior colliculus. The increase of the number of crisis is correlated with differential involvement of limbic areas, as amygdala, periaqueductal gray, hippocampus or median eminence, demonstrated by the behavioral performance of animals and by the increased expression of *c-fos* in the recruited nuclei.

Conclusions: We provide useful information to go forward on the comprehensive characterization of a model of audiogenic epilepsy and the recruitment of brain areas during the kindling. This study has been sponsored by USAL-USP, Program for the Promotion of the Bilateral Cooperation in the Field of Research and JCyL (SA023A12-2)

Espin-like is a Novel Myosin III Cargo, which Participates in the Regulation of Stereocilia Length

Ebrahim S¹, Grati M¹, Windsor A¹, Dong L², Millis BA¹, Cui R¹, de Sousa A¹, Salles FT¹, Raval M³, Yengo CM³, Kachar B¹

¹ National Institute on Deafness and other Communication Disorders, NIH, Bethesda MD, USA

² National Eye Institute, NIH, Bethesda MD, USA

³ College of Medicine, Penn State University, Hershey PA, USA

The precise regulation of stereocilia length is essential for normal hearing and balance. Myosin IIIa (MYO3A) localizes to stereocilia tips and regulates stereocilia-length via an actin-regulatory cargo, Espin1 (ESPN). MYO3A is associated with late-onset hearing loss (DFNB30), and ESPN with hearing and vestibular deficits. Myosin IIIb (MYO3B), a MYO3A paralog, is thought to partially compensate for MYO3A function, delaying the onset of DFNB30. To investigate the distinct and overlapping roles of each protein in this complex, we generated mice lacking Myo3a, Myo3b and Espn1 respectively. Neither *Myo3a* ^{-/-} nor *Myo3b* ^{-/-} mice presented abnormalities in stereocilia morphology or auditory function, but mice lacking both genes were embryonic lethal. *Espin1* ^{-/-} mice had stereocilia with misregulated lengths, but only in extrastricular hair cells of the otolithic organs. We show here that espin-like (EspinL), a structural homolog of Espn1, also localizes to stereocilia tips, albeit in an inverse length-dependent concentration. Remarkably, EspnL expression is enhanced in the striola. Heterologous expression assays show that both Myo3a and Myo3b transport EspinL to the tips of filopodial actin protrusions, and an observed inverse correlation between EspnL concentration and Myo3 tip localization provides evidence for a new cargo-mediated myosin regulation. Biochemical analyses using Myo3 tail fragments suggest the region of interaction with EspnL is distinct, with some overlap to that of Espn1. Together, these data suggest that EspnL is a novel member of the Myo3/Espn1 complex, with both complementary and compensatory function to Espn1, providing additional insights into mechanisms underlying stereocilia regulation and late onset of DFNB30.

Alterations In The Efferent System In The Epileptic Hamster GASH:Sal

Sánchez-Benito, D.(1), Hernández-Noriega, S.(1), Hyppolito, M.A.(2), Gómez-Nieto, R.(1), Castellano, O.(1), Muñoz, L.J.(1), Batista Murashima, A. (2), Garcia-Cairasco, N. (3), Morujo, A.(1) and López, D. E. (1).

1. Institute for Neuroscience of Castilla y Leon/IBSAL, University of Salamanca, Spain. 2. Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil. 3. Neurophysiology and Experimental Neuroethology Laboratory (LNNE). Ribeirão Preto School of Medicine - University of São Paulo. Brazil

Objectives: A hamster strain from the University of Salamanca, the Genetic Audiogenic Seizure Hamster (GASH:Sal), is being validated as a new model of epilepsy. As a part of this process, we are studying its auditory nuclei to determine the anatomical substrate involved in the mechanism of inducing audiogenic seizures.

Material and Methods: Four control hamsters and six adult GASH:Sal were used in this study. Accordingly, we labeled the OC neurons projecting to one cochlea by means of retrograde axonal transport of FluoroGold. In four animals, all labeled OC neurons were counted and digital images of the labeling were captured and analyzed morphometrically. In one case, a 3D computer reconstruction of the bilateral distribution of OC neurons was made and also of the LSO. In addition, we study the activity of the efferent system by the measuring of distortion-product of otoacoustic emissions (DPOAEs).

Results: The GASH:Sal shows morphological changes in the nuclei origin of the olivocochlear system, more pronounced in the LSO, with a 30% of volumen reduction comparing with controls. The analysis of the DPOAEs reveals functional alterations in the medial efferent system of this strain, with a functional asymmetry between both ears. Further studies are needed to test if the changes in the efferent system could be involved in the genesis or the maintenance of the audiogenic epilepsy that exhibit these animals.

Acknowledgements: This study has been sponsored by USAL-USP, Program for the Promotion of the Bilateral Cooperation in the Field of Research and JCyL (SA023A12-2).

Labyrinthine Fistulae Secondary To Middle Ear Cholesteatoma

Kumiko Gyo, Makoto Miura, Hiroki Ikeda, Shinji Takebayashi, Satoshi Ohno,
Akiyoshi Yasumoto, Yasuyuki Hayashi
Japanese Red Cross Wakayama Medical Center, Wakayama shi, Wakayama ken, Japan

The incidence of labyrinthine fistula secondary to cholesteatoma has been frequently reported to be 5-10 % of cases. Although the recent development of imaging study has improved the preoperative diagnosis of labyrinthine fistulae, we often encounter unexpected fistulae in ear surgery. This retrospective study reviews 19 patients who underwent surgery in our hospital between April 2009 and March 2014 and confirmed 20 labyrinthine fistulae secondary to cholesteatoma. The location of the fistula was in the lateral semicircular canal in 16 cases, in the vestibule in 1 case, in the cochlea in 1 case, and in the combined lateral semicircular canal and vestibule in 1 case. Although the staging of the fistula by Dornhoffer and Milewski was various, no case had type III fistula. The size of fistula was larger than 2 mm in 8 cases. Temporal bone computed tomography (CT) enabled us to predict the presence of fistula in 15 of 20 sites (75%). All other 5 of 20 fistulae that were not detected by CT (25%) were less than 1 mm in size. However, we were able to predict up to 18 of 20 fistulae (90%) by using nystagmus findings obtained from infrared CCD camera with CT findings. No case showed any bone conducted hearing deterioration after 1 year of surgery. On the basis of our data, we discuss clinical issues with regard to the diagnosis and management of this difficult complication.

Hearing Loss in Xeroderma Pigmentosum (XP) and Mechanism of Inner Ear Disorder

Yamashita Daisuke¹, Fujita Takeshi¹, Shinomiya Hitomi¹, Inokuchi Go¹, Hasegawa Shingo¹, Nibu Ken-ichi¹
¹Department of Otolaryngology-Head and Neck Surgery, University of Kobe, Hyogo, Japan

Xeroderma pigmentosum (XP) is a rare autosomal recessive inherited disease characterized by photosensitivity and proneness to skin cancers of the sun-exposed skin. The deficiency in the repair of UV-induced DNA damage results in development of skin cancers. There are eight subgroups in XP (XP-A through XP-G genetic complementation groups and XP variant type) and all responsible gene for the subgroups have been cloned. The responsible gene for XP-A, *XPA* is considered to play an important role in DNA repair process. The incidence of the skin cancer in XP-A is considered to be approximately 2,000 times of that in healthy subject because of the deficiency in DNA repair for the UV-induced DNA damage. Also, in addition to skin symptoms, various neurologic symptoms including an intellectual impairment and the movement disorder develop from progressive neuropathy and hearing impairment, and the medical treatment for life is required. Though the responsible genes are apparent, the basic therapy is not yet established. About the hearing impairment, the clinical courses of the onset time and degree and progress of hearing loss are various. Therefore we have examined clinical features in the hearing impairment of the XP-A patients whom we experienced this time. Also, we investigate the mechanisms of the inner ear disorder using the *XPA* knock out mice, by assessing hearing function and morphological changes in the cochlea.

Effects of caffeic acid on cisplatin-induced hair cell damage in HEI-OC1 auditory cells

June Choi¹, Sung Won Chae¹, Jong Dae Lee², Byung Don Lee², and Moo Kyun Park³

¹ Dept. of Otorhinolaryngology-Head and Neck Surgery, Korea Univ. College of Medicine, Seoul, Korea, ²Dept. of Otorhinolaryngology-Head and Neck Surgery, Soonchunhyang Univ. College of Medicine, Seoul, South Korea, ³Dept. of Otorhinolaryngology-Head and Neck Surgery, Seoul National Univ. College of Medicine, Seoul, South Korea
Corresponding author: aseptic@snu.ac.kr

Introduction: Cisplatin is a widely used anticancer chemotherapeutic agent. However, it is notorious for its ototoxicity and nephrotoxicity due to induction of reactive oxygen species (ROS). Caffeic acid is a naturally occurring polyphenol present in honey that is known to reduce the generation of oxygen-derived free radicals. The objective of the present study was to evaluate the protective effects and mechanism underlying the effect of caffeic acid on cisplatin-induced ototoxicity in HEI-OC1 auditory cell lines.

Methods: Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Apoptosis was determined by Hoechst 33258 staining and Annexin V-fluorescein isothiocyanate/propidium iodide double staining. Cell cycle stages were analyzed by flow cytometry. The radical-scavenging activity of caffeic acid was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The expression levels of caspase-3, -8, and -9, as well as the activity of caspase-3, were evaluated.

Results: Caffeic acid showed a protective effect against cisplatin-induced HEI-OC1 cell damage as demonstrated by the MTT assay. Caffeic acid decreased cell death by apoptosis and necrosis. Caffeic acid showed strong scavenging activity against the radical DPPH and decreased intracellular ROS production. Caffeic acid decreased the expression of caspase-3 and -8 and increased the activity of caspase-3.

Conclusions: Caffeic acid attenuated cisplatin-induced hair cell loss in HEI-OC1 cell lines; these effects were mediated by its radical scavenging activity and inhibition of apoptosis.

Keywords: Caffeic acid, Cisplatin, Ototoxicity, Reactive oxygen species

The Effect of Microgravity on mRNA Expression in the Vestibular Endorgans: Comparison of the 90- day and 15-day Space Flight.

Yutaka Takumi¹, Tomohiro Oguchi¹, Nobuyoshi Suzuki¹, Shin-ya Nishio¹, Richard Boyle², Shin-ichi Usami¹

¹ Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, 390-8621, JAPAN.

² BioVIS Technology Center, Mail Stop 239-11, Ames Research Center, NASA, Moffett Field, CA 94035.

<Objective> Significant decrease of vestibular inputs during the space flight is one of the major reasons for space motion sickness. In space, astronauts adapt normally to the microgravity environment within a few days. Indeed the central nervous system has a central role of adaptation, but the vestibular endorgans are believed to also show adaptation. Previous space flight study revealed the obvious change of synaptic arrangement in the rat vestibular endorgans [1]. Therefore, it is interesting and important to investigate the gene expression patterns in the vestibular endorgans during space flight. In this study, mRNAs from the mouse vestibular endorgans in two different space flight groups were analyzed.

<Methods>

• 90- day flight group: The samples were provided by Biospecimen Sharing Program (TSP) of Mice Drawer System (STS-128/129). Only three mice survived for 90 days in the mission to the International Space Station. The vestibular endorgans were dissected out from the temporal bones under RNA later solution and mRNAs were extracted.

• 15- day flight group: The samples were provided by BSP of STS-131. Tissues were extracted same as above.

Each extraction was quality checked and applied for DNA microarray.

<Results and Conclusion> Each extraction was quality checked and applied for DNA microarray. In each sample genes were selected from the gene profile that exhibited either an up (>2-fold) or down (<0.5) regulation. In 90-day flight group, 424 up-regulated and 306 down-regulated genes were detected. In contrast, the gene expression patterns were quite different in the 15-day flight group. These genes might be key molecules altered by microgravity and these analyses might shed light on the time course and underlying mechanisms driving vestibular adaptation during the space flight.

[1] Ross M.D.(2000) Acta Otolaryngol 120, 490-499.

The Rho GTPase Cdc42 Regulates Patterning and Polarization of Hair Cells in the Embryonic Organ of Corti

Kirjavainen A, Laos M, Anttonen A, Pirvola U

Department of Biosciences, P.O. Box 65, Viikinkaari 1, 00014 University of Helsinki, Finland

Cell polarization, establishment and modulation of cytoskeleton together with the formation and rearrangement of intercellular junctions characterize embryonic and postnatal development of epithelial cells of the organ of Corti. In addition to apico-basal polarity, cells of the organ of Corti exhibit planar cell polarity (PCP), defined by the orientation of cells within the plane of the sensory epithelium. The most visible readout of PCP is the uniform orientation of the hair cell stereociliary bundles.

The Rho GTPase, Cdc42, regulates cytoskeletal dynamics, cell polarity and junctional integrity of epithelial cells. In the present study, we were interested in Cdc42's role during embryonic development of the auditory sensory epithelium.

In order to study the role of Cdc42 *in vivo*, we used tamoxifen-inducible *Fgfr3-iCre-ER^{T2}* mouse line crossed with *Cdc42^{loxP}* mice to inactivate *Cdc42* in outer hair cells and supporting cells during late-embryogenesis. *Cdc42* was inactivated between E13 and E14, after the formation of the progenitor cell population but before the onset of cell type-specific differentiation programmes. We found that *Cdc42* inactivation leads to patterning defects of the auditory sensory epithelium, most distinctly to polarity defects in hair cells. The organ of Corti showed a PCP phenotype, based on random orientation of the stereociliary bundles of outer hair cells. In addition, hair cells showed impaired hair bundle morphology and misplacement of the kinocilium. PCP defects were accompanied by defects in outer hair cell shapes. However, global development of the cochlea was unaffected. Our data suggest that Cdc42 acts downstream or parallel of core PCP components to regulate the establishment of polarity of outer hair cells.



Speaker Index

Speaker names and presentation number

Ahn S-K	207	Harada T	117
Ali RQ	142	Hatakeyama K	151
Anttonen T	19	Hayashi K	14
Anzai T	179	Hayashi Y	172
Asai Y	10	He DZZ	9
Asaka N	139	Hernández-Noriega S	248
Avraham KB	KL2	Higashi-Shingai K	241
Barclay M	128	Hill K	147
Bergin M	71	Hirose Y	221
Bhonker Y	74	Honda A	129
Bodmer D	13	Horie RT	217
Breinbauer HA	229	Hosoya M	150
Bullen A	153	Hu BH	8
Canlon B	12	Hu L	30
Chai RJ	204	Hussain K	138
Chang H	219	Ida Y	235
Chang J	17	Ikeda R	133
Chang MY	182	Im GJ	206
Chen P	132	Inagaki A	114
Choi BY	SS2-2	Inoshita A	127
Choi HS	185	Inoue Y	237
Choi J	4	Ishikawa K	105
Chora JR	84	Ishikawa M	95
Dabdoub A	29	Ishizaki H	103, 108
Dayaratne N	75	Ito J	PL
Degerman E	148	Jagger D	136
Doi K	45	Jahan I	32
Dong YY	126	Jang CH	174
Ealy M	SS1-4, 36	Jang JH	247
Ebrahim S	249	Jeong H-J	64, 227
Edge ASB	SS1-3, 99	Jeong J	166
Edin F	137	Joris PX	70
Fetoni AR	202, 203	Juergens L	131
Fuchs PA	KL5	Kachar B	KL4
Fujikawa T	234	Kakigi A	244
Fujioka M	240	Kakuki T	124
Fujita T	165	Kalinec F	51
Fukunaga I	140	Kamitani T	152
Futaki T	209	Kamiya K	22
Gale JE	KL1, 25	Kanai R	233
Gleiser C	24	Kanazawa Y	61
Gyo K	251	Kanemaru S	79
Hahnewald S	81	Kanzaki S	88
Hansen S	104	Karino S	163

Katsuno T	56
Kawano T	216
Kikkawa Y	11
Kim AR	183
Kim DH	211
Kim HJ	213
Kim M	223
Kim SH	86
Kirjavainen A	255
Kishimoto I	226
Kita T	33
Kitajiri S	49
Kitaoka K	232
Kiyomizu K	107
Knipper M	62
Koike T	67
Kudo N	199
Kulkarni A	LS3
Kurima K	47
Kurioka T	222
Kurt S	159
Ladher R	34
Laos M	101
Lee H	242
Lee HY	246
Li H	141
Li Y	156
Liao C-C	215
Lin SCY	5
Lin X	123
Liu W	50
Lowenheim H	SS1-1
Lysakowski A	53
Magnusson M	78, 110
Majumder P	168
Makishima T	243
Martins FTA	187
Matos TD	188
Matsui T	228
Mintz M	109
Miyagawa M	190
Miyashita T	162
Miyazaki H	77
Miyoshi T	26

Moleti A	238
Moon IJ	57
Moon IS	121
Moteki H	192
Murata J	130
Mustapha M	20
Nabekura T	160
Naganuma H	177
Nakagawa T	SS1-5
Nakayama M	201
Navaratnam D	54
Neef J	SS2-3
Nevoux J	27
Nishimura K	102
Nishimura M	218
Nishio S	194
Niwa M	170
Nomura K	198
Nue Moller M	52, 58, 111, 210
Ogita H	200
Oh JH	135
Oh SH	44
Oh S-J	224
Ohnishi H	94
Ohtsuka H	181
Okada M	220
Okano H	KL3
Okano T	28
Okumura T	239
Oshima-Takago T	115
Panshina VS	178
Park M	155
Park MH	149
Park MK	253
Park SH	122
Patrick J	MS1-2, LS2-2
Petit C	SS2-1
Petrova NN	173
Pfingst BE	39
Pham TK	92
Phillips C	208
Plumat JM	245
Pyykkö I	38, 82, 83
Rah YC	42



Rahmat S	68	Tani K	3
Rak K	91	Tateya T	31
Raphael Y	119	Taura A	89
Ren DD	134	Thomasen PC	21,76
Rivolta M	SS1-2	Thorne PR	118
Rudic M	158	Tona Y	46
Ruettiger L	96	Torii H	37
Ryan AF	7	Treaba C	MS1-1
Sakaguchi H	48	Tsuchihashi NA	146
Sakamoto S	35	Uchida Y	189
Sakamoto T	112	Ueyama T	63
Sanchez-Benito D	250	Usami S	SS2-4
Sang Q	85	van de Water TR	LS1
Santos-Sacchi J	113	Varela-Nieto I	72
Sardari S	59	Varga L	186
Sato M	164	Verhoeven K	MS1-3
Schacht J	1	Vlajkovic SM	15
Schnee ME	169	Voelker C	144
Seo T	214	Voelker J	143
Sha S-H	97	Volkenstein S	41
Shepherd RK	LS2-1	Wang CH	145
Shibasaki O	205	Wu MK	157
Shirai K	43	Xiong H	175
Sisto R	2, 69	Xu Z-G	55
Song Cl	236	Yamaguchi T	180
Song J-J	65, 66	Yamahara K	6
Sprinzak D	73	Yamanaka S	SL
Sugahara K	197	Yamano T	196
Sugiura S	212	Yamashita D	171, 252
Sun Y	184	Yamazaki H	61
Suzuki S	191	Yang J-M	100
Sziklai I	40	Yang TH	16
Takago H	116	Yasuda SP	87
Takahashi H	ES	Yoneyama M	154
Takahashi M	225	Yoo TJ	90
Takano K	125	Yoshida H	231
Takeda H	120	Yoshida T	23
Takeda N	MS2	Yoshimoto R	167
Takimoto Y	161	Yoshimura H	193
Takumi Y	254	Zenner HP	80
Takumida M	106	Zine A	93
Tan C-T	230	Zou J	18
Tan J	176		
Tan WJT	98		

Acknowledgement

Inner Ear Biology Workshop 2014 in Kyoto is supported by many organizations, foundations, companies and individuals that have understanding of the Workshop.

Public organizations and foundations

The Kyoto University Foundation

The Uehara Memorial Foundation

Daiichi Sankyo Foundation of Life Science

The Tokyo Biochemical Research Foundation

The Federation of Pharmaceutical Manufacturers' Associations of JAPAN

Japan Hearing Instruments Manufacturers Association

Companies

Advanced Bionics Japan

Astellas Pharma Inc.

Bayer Yakuhin, Ltd.

Daiichi Sankyo Co., Ltd.

J.Morita Mfg. Corp.

Johnson & Johnson K.K.

Kowa Pharmaceutical Co. Ltd.

MED-EL Medical Electronics

MED-EL Japan Co., Ltd.

Medtronic Japan Co., Ltd.

NAGASHIMA MEDICAL INSTRUMENTS CO., LTD.

Nihon Cochlear Co., Ltd.

Nihon Kohden Corporation

Olympus Medical Science Sales CO., LTD.

ONO PHARMACEUTICAL CO., LTD.

Otsuka Pharmaceutical Factory, Inc.

TERUMO CORPORATION