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ID: IP231**middle ear mucosal regeneration by nasal mucosal epithelial cell sheets transplantation**Presenting Author: **Yuichiro Yaguchi**Yuichiro Yaguchi¹, Kazuhisa Yamamoto²,
Tsunetaro Morino², Hiromi Kojima²¹*St. Marianna University School of Medicine,*²*Jikei University School of Medicine**Learning Objectives:*

Postoperative regeneration of the middle ear mucosa and pneumatization of the middle ear cavity are of great importance after middle ear surgery. This study developed a new method to transplant autologous nasal mucosal epithelial cell-sheets into the damaged middle ear cavity. The aim of this study was to evaluate postoperative healing after the transplantation of the cell sheets in rabbits. Rabbit nasal mucosal epithelial cell-sheets were fabricated from a temperature-responsive culture dish and transplanted into the damaged middle ear of rabbit, which was surgically created. The healing of middle ears was evaluated with histological methods and computed tomography findings at 8 weeks after transplantation. Functional evaluation was performed by measuring the maximum middle ear total pressure reflecting a trans-mucosal gas exchange function. Two control groups were used: the normal control group and the mucosa-eliminated control group. Transplantation of nasal mucosal epithelial cell-sheets suppressed the bone hyperplasia and the narrowing of pneumatic space in the middle ear cavity more clearly than the mucosa-eliminated control group. The mucosal gas exchange function was also found to be good in the cell sheet-transplanted group. These results suggested that posttransplanted middle ear cavity was not only morphologically but also functionally similar to the normal middle ear cavity. Nasal mucosal epithelial cell-sheet was confirmed to be useful as an effective graft material after middle ear surgery and hopefully become a novel therapy in the future.

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ID: IP232**KGF controls on the epithelial stem/progenitor cell proliferation in external auditory canal**Presenting Author: **Tomomi Yamamoto-fukuda**Tomomi Yamamoto-fukuda¹, Haruo Takahashi²,
Hiromi Kojima¹¹*Department of Otorhinolaryngology Jikei University School of Medicine,* ²*Department of Otolaryngology-Head and Neck Surgery, Department of Translational Medical Science, Nagasaki University Graduate School of Biomedical Sciences**Learning Objectives:*

Introduction: The epidermal basal stem/progenitor cell maintains homeostasis of epidermis under development, self-renewal and differentiation. In many cases of adult basal stem/progenitor cell regulation, the importance of extracellular signals provided by the surrounding cells are well recognized. Keratinocyte growth factor (KGF) is a mesenchymal-cell-derived paracrine growth factor that specifically participate in tissue development as well as wound repair. In this study, we investigated the effects of over-expressed KGF during epithelial cell proliferation and differentiation by using a cell labeling system.

Methods: After anesthetized ICR mouse Flag-hKGF cDNA driven by a CMV14 promoter was transfected into ear skin with electroporation. The ears with empty vector transfection were used as controls. 5-bromo-2'-deoxyuridine (BrdU) and 5-ethynyl-2' deoxyuridine (EdU) were administered at different time points before or after KGF expression vector transfection to identify stem cells or progenitor cells, which are believed to divide slowly or to segregate chromosomes asymmetrically. At 1, 4 and 7 days after vector transfection, 3 mice at each time-point were sacrificed. The paraffin sections were used for H&E and immunohistochemistry for Flag, KGF, BrdU and cytokeratin (CK)14. EdU staining was performed according to the manufacturer's protocol (Life Technologies).

Results: Each plasmid was transfected into the epithelial and subepithelial cells, successfully. After KGF transfection, keratin accumulations were observed 3 of 3 ears at 4 days. BrdU(+)EdU(+) cells (stem/progenitor cell) were detected in the upper layer of thickened CK14 positive epithelium in KGF transfected specimens at 4 days.

Conclusions: These findings indicated that KGF overexpression may possibly increase stem or progenitor cell proliferation and block terminal differentiation, resulting in epithelial hyperplasia and stratification.

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ID: IP233**Middle Ear Mucosal Regeneration by tissue-engineered cell sheets transplantation**Presenting Author: **Kazuhisa Yamamoto**Kazuhisa Yamamoto, Tsunetaro Morino, Takanori Hama,
Yuichiro Yaguchi, Hiromi Kojima*Jikei University School of Medicine**Learning Objectives:*

Introduction: Recurrence of cholesteatoma is mainly caused by poor mucosal regeneration in the middle ear cavity and mastoid cavity. If middle ear mucosa can be preserved and the rapid postoperative regeneration of mucosa on the exposed bone surface can be achieved after middle ear surgery, surgical treatment for otitis media including

cholesteatoma can be potentially improved. Conventional canal wall up tympanoplasty often results in a lack of mucosal regeneration in the resected area of the mastoid cavity. We developed a novel method combining canal wall up tympanoplasty and autologous epithelial cell sheet transplantation for postoperative regeneration of the middle ear mucosa.

Methods: We obtained the approval of the ethics committee of our institution and the Ministry of Health, Labor, and Welfare. We endoscopically removed an approximately $10 \times 10\text{-mm}^2$ nasal mucosal tissue from her inferior concha. Tissue-engineered autologous nasal mucosal epithelial cell sheets were fabricated by culturing the harvested cells using temperature-responsive culture dishes for 26 days in an aseptic environment in a good manufacturing practice (GMP)-compliant cell processing center (CPC). The cultivated cell sheets were transplanted, during canal wall up tympanoplasty, onto the exposed bony surface of the attic of the tympanic and mastoid cavities where the mucosa was lost.

Results: During the cultivation, the sterile environment in the CPC was confirmed. Autologous cell sheets were successfully transplanted to human middle ear. We have already clinically applied cell sheets to treat 5 patients of middle ear cholesteatoma. All patients showed a favorable postoperative course, with no adverse events or complications.

Conclusion: This is a first-in-man study in the world that the cultured cells were transplanted to the human ear. This novel technology of transplantation might be an effective alternative to the surgical operation on intractable otitis media in the near future.

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ID: IP234

Practicality Analysis of JOS Staging System for Cholesteatoma Secondary to a Pars tensa Perforation: Japan Multicenter Study (2009–2010)

Presenting Author: **Yutaka Yamamoto**

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Learning Objectives:

Introduction: Primary cholesteatoma generally arises from retraction of the squamous epithelium of the tympanic membrane (TM). However, in rare cases, epithelial invasion occurs from the edge of the TM perforation and migrates to the medial surface of the TM. In such cases, a thick

TM, blunt perforation edge, and discharge of debris from the medial side of the TM are often observed. In this paper, the clinical features of the cholesteatoma secondary to a pars tensa perforation were evaluated and the pathogenesis of the disease was discussed.

Methods: A total of 599 ears that underwent surgery for fresh cholesteatoma between 2009 and 2010 at 6 institutions in Japan were recruited and cases with cholesteatoma secondary to a pars tensa perforation were selected. The criteria of the disease were defined as follows; a TM perforation in the pars tensa, continuous epithelial invasion from the perforation edge to the back side of the TM, and no adhesive lesion directly between the TM and promontrium. Incidence of the disease and clinical characteristics were evaluated retrospectively.

Results: Twenty-three ears of 23 patients with cholesteatoma secondary to a pars tensa perforation were identified. Incidence of the disease was 4.1 % of all of the cholesteatoma cases or 5.2 % of all of the acquired cholesteatoma cases. Characteristics of the disease were represented as following; high incidence in elder women, low rate of undeveloped mastoid air cell system, severe destruction of the stapes, and complex extension pathway.

Conclusions: The pathogenesis of cholesteatoma secondary to a pars tensa perforation is very different from that of other types of cholesteatoma. This disease should be clearly categorized as a different type of cholesteatoma and we need to recognize the nature and behavior of this disease. Additional storage of the data and detailed analysis by the multicenter study should be continued.

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ID: IP235

Auditory test battery for the ear surgery and the postoperative evaluation

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Learning Objectives: Auditory test battery.

Hearing result of the ear surgery is important for the quality of life of the patients. Pure tone audiometry, speech audiometry, and the Eustachian tube function test are done for cholesteatoma patients and chronic otitis media ear patients in our hospital.

We studied the preoperative and postoperative hearing result of the patients from the view point of quality of life.

Case: 75 year-old female. She had the drained ear on both side from her childhood. She noticed hearing impairment when she was 74 years old. She also had discharge of the ear and visited ENT doctor. She was diagnosed that she had chronic otitis media ear on both side and ENT doctor recommended her surgical intervention.

She visited our hospital. She had large perforation on both side and the pure tone audiometry showed mixed hearing