Expression of Anion Exchangers in Cultured Human Endolymphatic Sac Epithelia

Won Sun Yang

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Jae Young Choi

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Won Sun Yang

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This certifies that the Doctoral Dissertation

of Won Sun Yang is approved.

Thesis Supervisor : Jae Young Choi

Thesis Committee Member #1 : Hee Nam Kim

Thesis Committee Member #2 : Jinwoong Bok

Thesis Committee Member #3 : Joo Young Kim

Thesis Committee Member #4: Sung Huhn Kim

The Graduate School

Yonsei University

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ABSTRACT

Expression of Anion Exchangers in Cultured Human Endolymphatic Sac Epithelia

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The Graduate School, Yonsei University

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The endolymphatic sac (ES) is part of the membranous labyrinth in the inner ear that plays an important role in maintaining homeostasis of the endolymphatic fluid system. However, the exact mechanism of fluid volume and pH regulation is not fully understood yet. We aimed to demonstrate the expression of various anion exchangers (AE), including pendrin, in cultured human endolymphatic sac epithelial (HESE) cells.

Endolymphatic sac specimens were harvested during acoustic neuroma surgery (*n*=24) using the translabyrinthine approach and then subcultured with high-epidermal growth factor (EGF) (25 ng/mL) media and differentiated using low-EGF (0.5 ng/mL) media. The cultured cells were classified according to the morphology on TEM. The Cl^-/HCO_3^- exchanger activity was assessed by *pHi* measurement using *pH* sensitive dye 2′, 7′-bis (2-carboxyethyl)-5(6) –carboxyfluorescein (BCECF/AM). We performed RT-PCR and immunohistochemical staining for AEs.

We determined that 7.3 ± 6.7% of cells differentiated into mitochodria-rich cells and 50.2 ± 15.1 of cells differentiated into

ribosome-rich cells. bAE3, AE4, SLC26A4, SLC26A6, and SLC26A11 were also expressed in cultured HESE cells. The cultured cells had Cl^-/HCO_3^- and Cl^-/formate exchange activity on the luminal membrane, which is sensitive to anion channel inhibitors (DIDS 500 μM). Furthermore, we showed that pendrin (SLC26A4) was expressed in cultured HESE cell membranes.

Our results suggest that AEs, including pendrin, are expressed in epithelia of ES and may have role in maintaining ionic homeostasis, and the HESE culture system are useful for uncovering the functional role of ES epithelial cells.

Keywords: endolymphatic sac; pendrin; pH; cell culture; inner ear

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I. INTRODUCTION

The endolymphatic sac (ES) belongs to the non-sensory portion of the membranous labyrinth in the inner ear. The ES is filled with endolymph with a relatively high Na^+ and low K^+ concentration compared to that of the cochleovestibular endolymph[1-2]. It is commonly believed that the ES regulates endolymph volume and pH by various ion transporters[3]. However, the exact mechanism by which the ES regulates fluid homeostasis is still unclear. Anion exchangers (AEs) translocate anions such as Cl^- and HCO_3^- across plasma membranes and are involved in the regulation of intra- and extracellular pH. Anion exchangers are also expressed in the inner ear. They have been suggested to play a role in maintaining the shape and mobility of hair cells[4]. Pendrin, an anion exchanger of the SLC family, is expressed in the cochlea, the vestibular labyrinth, and the ES epithelia of the inner ear[5]. Human mutation of pendrin leads to the enlargement of ES and prelingual deafness. It is assumed that pendrin acts as a Cl^-/HCO_3^- exchanger and is responsible for endolymphatic fluid volume and pH homeostasis[6]. Failure of fluid absorption due to lack of pendrin expression appears to initiate cochlear duct enlargement in mice[7]. Pendrin may also be expressed in ES tissue and appears to have a critical role in developing normal hearing phenotype, but the functional expression and exact role of pendrin in human ES is not yet clear[8].

The ES is covered with an epithelial monolayer composed of heterogeneous

cells. Ultrastructural characteristics indicative of cellular function suggest that ES mitochondria-rich cells are predominantly involved in the transepithelial transport of water and ions. In contrast, ES ribosome-rich cells may contribute to endolymph homeostasis by secretion and/or absorption of osmotically active protein components[9-14] . However, no definitive criteria exist for dividing epithelial cells into these two types and the concrete function of these cells remains to be elucidated. Because of the location of the endolymphatic duct and sac deep inside the petrosal bone, physiological experiments are difficult to perform. An *in vitro* culture system for ES epithelial cells would provide a useful tool for research on the molecular pathogenesis of various inner ear diseases. A number of investigators have established primary culture systems for ES epithelial cells from laboratory animals[14-16]. Although it is difficult to collect sufficient human tissue specimens, Linder et al. tried to culture cells from humans and succeeded in establishing a primary culture system for human endolymphatic sac epithelial (HESE) cells. They demonstrated that the cultured HESE cells have the characteristics of epithelial cells, but they did not differentiate into various cell types as they do *in vivo*[17]. The experimental data with these undifferentiated cells can provide only limited information about the physiological and pathological roles of endolymphatic epithelial cells. We also established a primary culture system for HESE cells. With this technique we showed the functional and molecular expression of ion transporters such as epithelial sodium channels (ENaCs) and Na^+-H^+ exchangers[18-19]. These cultured cells need to be further characterized, including the proportion of mitochondrial-rich cells and ribosomal-rich cells.

In this study, we demonstrated that the cultured HESE cells have mitochondrial-rich and ribosomal-rich phenotypes similar to the *in vivo* tissue. We also demonstrated that cultured HESE cells express pendrin and exhibit anion exchanger activity.

II. MATERIALS AND METHODS

1. Tissue harvest

Patients were enrolled for the study after they provided written informed consent. We obtained approval from the local ethics committee for the use of patient specimens. Endolymphic sacs were excised from patients during acoustic neuroma surgery (*n=24*) using the translabyrinthine approach. The extrasosseous portion of the ES was exposed at the posterior cranial fossa and the external half was cut with a pair of micro scissors and the epithelial cells were scraped with a No. 15 blade (Fig. 1). Undesirable connective tissue including the dura mater was removed and dissected from the surface epithelium as much as possible under a wide stereoscopic microscope at 25x magnification with lateral illumination via a flexible, fiber-optic illuminator (Model 190, Dolan-Jenner Industries; Lawrence, MA, USA).

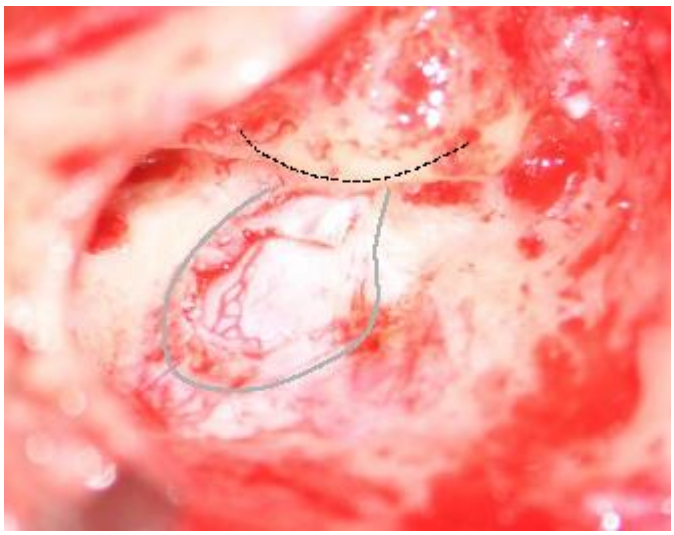

Figure 1. Surgical view of the endolymphatic sac (ES). The external half of the ES (gray line) was excised and the opened internal half with vessel remained. The dashed line indicates the posterior semicircular canal.

2. HESE cell cultures

Epithelial cells were further isolated from the tissue by treatment with 1% Pronase (type XIV protease; Sigma, St. Louis, MO, USA) for 18 hours at 4℃. To remove fibroblasts and endothelial cells, isolated cells were placed in a plastic dish and cultured for 30 minutes at 37℃. Epithelial cells were subcultured in a 1:1 mixture of bronchial epithelial cell basal medium and Dulbecco's modified Eagle's medium containing insulin (5.0 μg/mL), hydrocortisone (0.5 μg/mL), epinephrine (0.5 μg/mL), triiodothyronine (6.5 μg/mL), transferrin (10 ng/mL), gentamicin (50 μg/mL), and amphotericin (50 ng/mL), all supplied by Clonetics Corp., and further supplemented with epidermal growth factor (EGF, 25 ng/mL; Collaborative Res., Bedford, MA, USA), all-trans retinoic acid (50 nM, Sigma), and bovine pituitary extract (1% v/v Pel Freez). The culture medium was changed every other day. Cells were dissociated with 0.25% trypsin:EDTA (Clonetics) when they reached 50–60% confluence and 2000 cells/cm^2 were seeded for subsequent passage. For differentiation, passage-2 HESE (10^5 cells per culture) were seeded onto semi-permeable membranes in each well (0.45 ㎛ pore size, Transwell-clear; Costar Co., Cambridge, MA, USA). The medium used for differentiation was the same as that used for the subculturing, except that it included a lower concentration of EGF (0.5 ng/mL). The medium was changed twice a week and cultures were maintained at 37℃ in a humidified atmosphere of 5% CO_2. Cultured cells were examined daily using a phase contrast light microscope(Vanox-S type; Olympus, Japan) to check cell confluency and fibrocyte contamination. The cells usually form a confluent sheet in seven days after seeding.

3. Transmission electron microscopy (TEM)

Because cultured cells attached to the membrane can easily break during the process of transmission electron microscopy (TEM) preparation, we dissociated the confluent cells by treatment with 0.25% trypsin:EDTA (Clonetics), and fixed for 6h in 2% glutaraldehyde/paraformaldehyde solution.

The cells were washed with cacodylate buffer followed by postfixation in 1.% OsO4. After washing three times in 0.12 M sodium phosphate buffer, pH 7.3, cells were dehydrated by passing through each of 30%, 50%, 70%, 80%, 90%, 95%, 100% ethanol. Then, cells were embedded overnight in EPON mixture (EPON 812, MNA, DDSA, DMP30) and Propylene oxide. Semi-thin sections (300nm) were stained with uranyl acetate and lead citrate and examined with transmission electron microscope (JEM-1011, JEOL / Megaview Ⅲ).

4. Immunohistochemical characterization

To characterize the cultured cells and confirm the expression of pendrin, cytospin slides were made for immunostaining, and the cells were were washed in PBS 3 times and pretreated with 0.3% H_2O_2 for 20 minutes at room temperature. The cells were fixed with a chilled 1:1 mixture of methanol and acetone for 5 minutes. Slides were treated with 1:600 normal rabbit serum for 20 minutes to block non-specific reactions and then permeabilized by incubation in 1% Triton-X-100 for 10 minutes. Cells incubated with monoclonal mouse antibodies for human pan-cytokeratin (1:100; DAKO, Denmark) and vimentin (1:50; DAKO) at 37℃ for 2 hours along with the secondary antibody with avidin-biotin peroxidase complex. Specimens were incubated in diaminobenzidine tetrahydrochloride. To confirm pendrin expression, we used polyclonal goat anti-PDS (G-19) (diluted 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for primary antibody, and then fluorescently labeled as secondary antibodies. Fluorescent images were obtained with a Zeiss LSM 510 confocal microscope. FITC fluorescence was induced at an excitation wavelength of 488 nm using an argon laser, and emitted fluorescence was detected with a 505–530 nm band-pass filter.

5. Measurement of $[pH]_i$

Passage-2 HESE cells were incubated in HEPES solution containing 1 ml 2’-7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF/AM, Molecular Probes, Eugene, OR) for 15–20 min at room temperature. The cells were then

mounted in a miniature Ussing chamber (AKI Institute, University of Copenhagen, Denmark), which consisted of top and bottom half-chambers (volume = 250$\mu\ell$ each). A transwell wafer containing the HESE cells monolayer is mounted between the two half-chambers and the two half-chambers are tightly sealed together. A glass coverslip was affixed to the bottom of the serosal chamber with dental sticking wax (model Deiberit-502; Ludwig Bohme). The mucosal chamber is open to the atmosphere, and both half-chambers have inlet and outlet ports to allow solution to flow. The BCECF/AM fluorescence ratio was recorded from an area in the center of the epithelium at excitation wavelengths of 440 and 490 nm. Fluorescence emission intensity was recorded at 520 nm[20].

6. RT-PCR

Total RNA was isolated from cultured HESE cells using TRIzol (Invitrogen, San Diego, CA, USA), and cDNA was synthesized with random hexamers (PerkinElmer, Roche, Branchburg, NJ, USA) using MMLV reverse transcriptase (RT; PerkinElmer Life Sciences). Oligonucleotide primers were designed according to published sequences as follows. (bAE3; 5' ATC TGA GGC AGA ACC TGT GG 3' TTT CAC TAA GTG TCG CCG C 418 bp) (AE4; 5' AGC GCT TGG ACT GCC TTG GTA TGT 3' AGG GGG AAG ATG ATG GCT GCA GGG GTA GAC 431bp) (SLC26A4; 5' GTT TAC TAG CTG GCC TTA TAT TTG GAC TGT 3' AGG CTA TGG ATT GGC ACT TTG GGA ACG 330bp) (SLC26A6; 5' TAG GGG AGG TTG GGC CAG GGA TGC 3' TGC CGG GAA GTG CCA AAC AGG AAG AAG TAG AT 456bp) (SLC26A7; 5' CAC TGT GTC TGG GAT AAT GTT GG 3' CCA GTT GCA GCA CAA ACA TG 353bp) (SLC26A9; 5' TCC AGG TCT TCA ACA ATG CCA C 3' CGA GTC TTG TGC ATG TAG CGA G 400bp) (SLC26A11; 5' ATC CCG CCC TTC TCA GTG AC, 3' TAG TCC AGA GAC AGC AGC ACC AG 329bp). PCR parameters used involved 23 cycles as follows: denaturation at 94 ℃ for 30 s, annealing at 55 ℃ for 30 s, and polymerization at 72 ℃ for 30 s. The PCR products were run in a 1.5% agarose gel and

visualized with ethidium bromide under a transilluminator.

7. Solutions and chemicals

The HCO_3^--buffered solutions contained (in mM) 120 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 glucose, 5 Hepes, 25 $NaHCO_3$ and was continuously gassed with 95% O_2 and 5% CO_2 to maintain solution pH. For the Cl^- free solution, all Cl^--containing salts were replaced with gluconate salts. For formate-containing solutions, 1 mM KCl^- was replaced with 1 mM K-formate. The osmolarity of all solutions was adjusted to 310 mOsm with NaOH prior to use. BCECF-AM was purchased from Molecular Probes (Eugene, OR). 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) were purchased from Sigma(St. Louis, MO, USA).

8. Statistical analysis

Data are presented as the original recording and as the mean value and SD from n observations. Differences were tested with the ANOVA test and p-value < 0.05 was considered statistically significant.

III. RESULTS

1. Characterization of cultured HESE cells

To characterize the cultured cells, we performed immunohistochemical staining for cytokeratin and vimentin. All cultured HESE cells expressed cytokeratin, an epithelial cell marker. Interestingly, some cells expressed vimentin as well as cytokeratin. Vimentin is an intermediate-sized filament, which is usually detected in mesenchymal cells (Fig. 2).

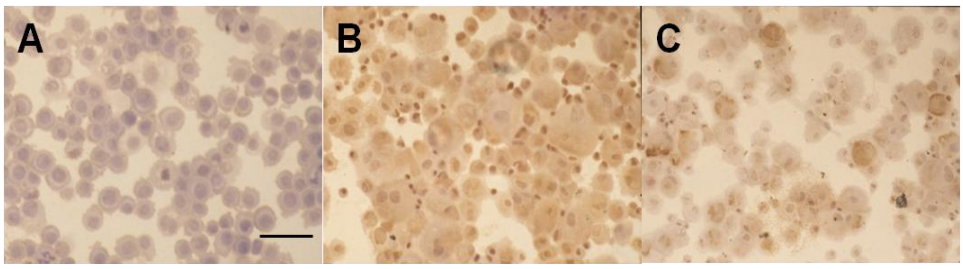

Figure 2. Immunocytochemical staining of the cultured cells. (A) Negative controls show no immunoreactivity. (B) Cytokeratin is expressed in all cultured cells. (C) Vimentin is co-expressed in some cells. Bars indicate (X 200).

Cell Type	Proportion (%, Mean±SD)
Mitochondria-rich cell	7.2±6.7
Ribosome-rich cell	50.2±15.1
Intermediate cell	42.2±18.02

Table 1. Classification of cultured HESE cells based on histological features.

2. Morphological classification of cultured HESE cells

Using TEM, the cultured cells appeared heterogeneous in shape, size, electron density, and cell surface. We divided the HESE cells into three categories (mitochondria-rich, ribosome-rich, and intermediate cells) based on histological features such as the number of mitochondria, shape of microvilli, and the distribution of ER (Fig. 3, Table 1). Some cultured HESE cells (7.3±6.7%) had the characteristic features of mitochondria-rich cells previously observed *in vivo*. These cells frequently had numerous predominantly supranuclear-localized mitochondria in their cytoplasm and the apical surfaces of the mitochondria-rich cells were covered with long microvilli. They also had few ER or Golgi complexes (Fig. 3A). The ribosome-rich cells were the most frequent cell type (50.2±15.1%) among the cultured HESE cells. These cells contained very well-developed ribosome-studded rough endoplasmic reticulum (rER) and dark cytoplasm as well as several mitochondria scattered throughout the cytoplasm (Fig. 3B). The remaining HESE cells (42.2±18.02) had characteristic features of both

mitochondria- and ribosome-rich cells (Fig. 3C).

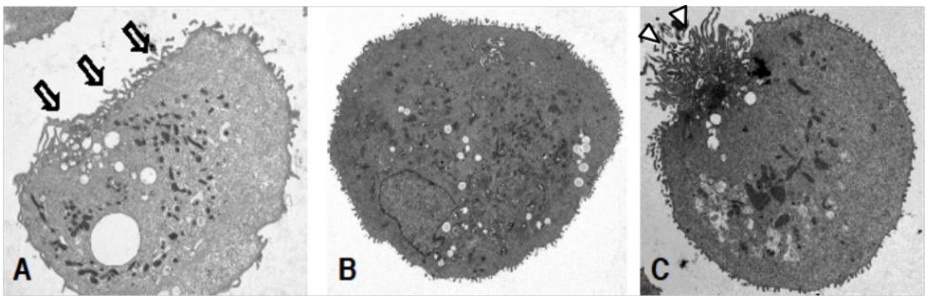

Figure 3. TEM images of cells (×5000). (A) Mitochondria-rich cells possess numerous microvilli on their apical surface(arrows) along with mitochondria in the cytoplasm, with very few endoplasmic reticulum and Golgi complexes. (B) Ribosome-rich cells have a relatively electro-dense cytoplasm with short microvilli. (C) An intermediate cell has short microvilli, but well-developed cytoplasmic processes (filopodia) on the apical surface(arrowheads). They contain some cytoplasmic vacuoles, and a moderate number of mitochondria and endoplasmic reticulum.

3. Expression of anion exchangers in cultured HESE cells

We examined the presence of various anion exchanger isoforms. Two isoforms of the SLC4 family (bAE, AE4) are expressed in cultured HESE cells. Three isoforms of SC26 (A4and A11) were also expressed in cultured HESE cells. However, the mRNA of SLC26A6, SLC26A7 and SLC26A9 were not expressed. We used human kidney tissue (AE4, SLC26A7) or human nasal epithelial cells (other AE isoforms) as a positive control (Fig. 4).

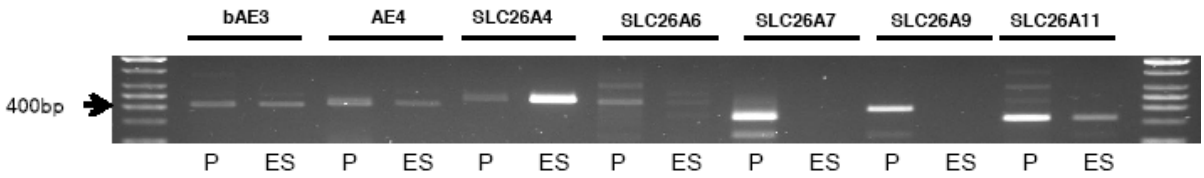

Figure 4. Expression of anion exchangers in HESE cells. bAE3, AE4,

SLC26A4, and SLC26A11 mRNA were expressed in HESE cells. Human kidney (for AE4, SLC26A6, SCL26A7) and human nasal epithelial cells (for others) were used as a positive control; P: positive control, ES: endolymphatic sac epithelia.

4. Anion exchanger activity in cultured HESE cells

Previous results suggest that the defect in Cl^-/HCO_3^- exchange activities at the apical membrane of inner ear epithelial cells is the key factor triggering deafness. Cl^-/HCO_3^- exchange activity in the apical membranes of cultured HESE cells (n=7) was estimated from the initial rate of pHi change caused by the removal and addition of Cl^- to the perfusing solution while the other membrane was bathed in Cl^- free medium. As demonstrated in Fig. 5, removal of Cl^- from the perfusing solution induced an intracellular pH increase (2.28 ± 0.72 unit/min), which indicated Cl^-/HCO_3^- exchange activity. This activity was suppressed by treatment with DIDS (500 μM), a non-specific anion exchanger inhibitor, to 0.98 ± 0.84 unit/min. ($p < 0.05$). The Cl^-/formate activity was also checked in the presence of 1mM formate (n=3). The Cl^-/formate activity (1.21 ± 0.76 unit/min) is smaller than Cl^-/HCO_3^- exchange activity (Fig 5 B & C).

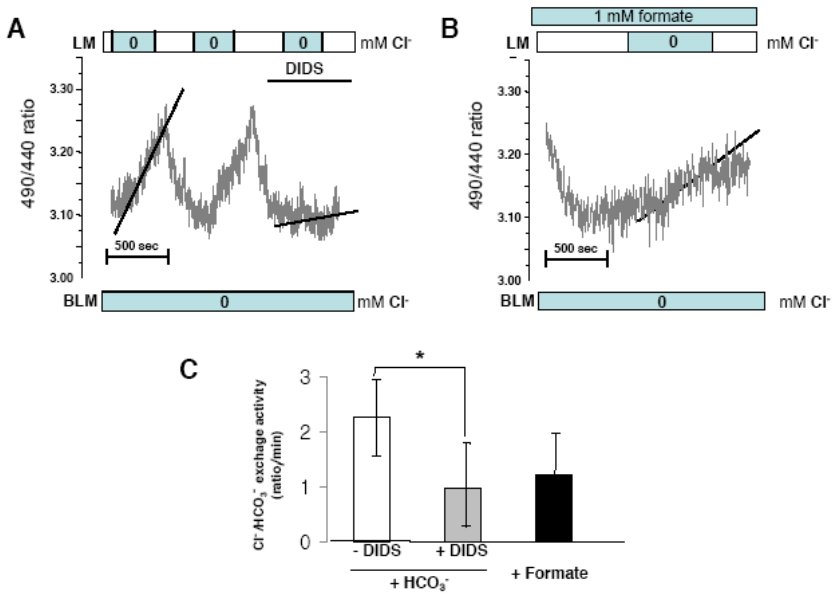

Figure 5. Measurements of Cl-/HCO3- and Cl-/formate exchange activity in cultured human endolymphatic sac epithelial cells. (A) Representative traces of pH change caused by Cl^- removal and addition and the effect of DIDS in HCO_3^- containing solution. (B) Representative tracings demonstrating the effect of Cl^- removal and readdition on *pHi* in the presence of 1mM formate. (C) Summary of data from separate experiments(n=4 to 7). The data are means ± SDs. An asterisk indicates statistical significance ($P < 0.05$). AM; apical membrane, BLM;basolateral membrane

5. Expression of pendrin in cultured HESE cells

We examined the presence of pendrin in cultured HESE cells. Pendrin is expressed in the membrane of some cultured HESE cells while in other cells, pendrin is diffusely stained in the cytoplasm (Fig. 6).

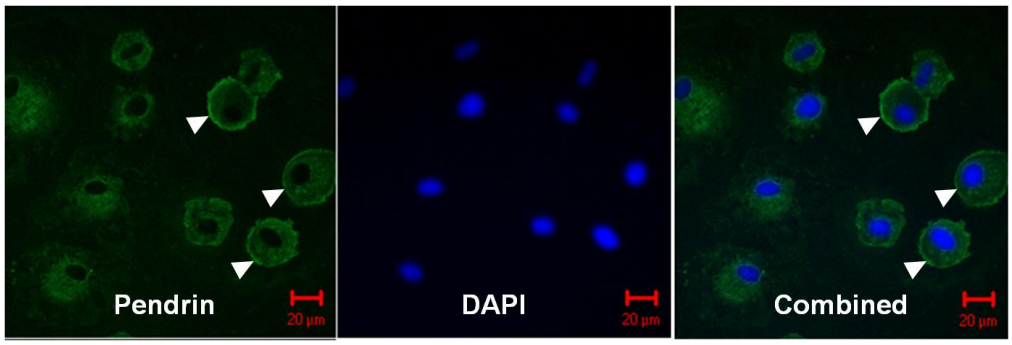

Figure 6. Immunostaining of pendrin(green) and DAPI(blue) in cultured HESE cells. Some cells were positive for pendrin antibody in their plasma membrane (arrowheads).

IV. DISCUSSION

Because of the importance of ES epithelial cells in maintaining inner ear homeostasis, ES epithelia culture has been performed by several researchers. Agrup et al. successfully measured Ca^{2+} influx in the cultured cells using ES tissue from the guinea pig[21]. Linder et al. first reported a serial culture method

for human ES epithelial cells and demonstrated that cultured cells have the morphological and biochemical characteristics of epithelial cells[22]. Nevertheless, they failed to obtain confluent and differentiated cells that would be more appropriate for various biochemical and electrophysiological studies. We modified their methods in several ways. Instead of using keratinocyte growth media, we used bronchial epithelial basal media for cellular proliferation because we gained knowledge from previous experiments that this media is more efficient than keratinocyte growth media for both epithelial proliferation and inhibition of fibroblast contamination. For proliferation, we used culture media containing high EGF (25 ng/ml) concentrations, with which we can increase the cell number 20- to 30-fold in every passage. However, low EGF media was used for cellular diffentiation since the morphology of the cultures maintained in low EGF concentrations (0.5 ng/ml) revealed a much more uniform pattern lacking the signs of epithelial cell necrosis and cyst formation. Because the ES tissue specimens harvested during neuro-otological surgery inevitably contains abundant connective tissue, fibroblast contamination is the main obstacle for establishment of an efficient HESE cell culture system. In this study, fibroblast contamination was prevented by removing connective tissue from the tissue specimens by careful microdissection and by incubating the separated cells in a culture dish for 30 minutes, after which only the supernatant was collected for subsequent cultures.

The ES is composed of a heterogeneous collection of epithelial cells[22]. In rats, the ES epithelial cells can be classified as mitochondria-rich cells or ribosome-rich cells. Peters et al. further classified mitochodria-rich cells into subclasses according to specific configurations analogous to renal intercalated cells[11]. They suggested that mitochondria-rich cells were involved in anion exchange, and ribosome-rich cells, which are a major cell type, might contribute to endolymph homeostasis by secretion and/or absorption of osmotically active protein components. Although there is no clear distinction between mitochondria-rich cells and ribosome-rich cells, 5 different types of

epithelia can be observed in human ES[22-23]. We can differentiate the cultured cells into 3 types of epithelial cells (mitochondria-rich cells, ribosome-rich cells and intermediate cells). This discrepancy might stem from either incomplete differentiation of cultured cells in our system or artifact during the process of cell lysis for cytospin slide preparation. Although the exact role of these intermediate cells is not yet fully understood, the intermediate cells have many mitochondria and long microvilli, indicating that these cells may be involved in ion and fluid transport across the cell membrane. Interestingly, most of the intermediate cells have filopodia on their apical surface and we have observed active endocytotic and phagocytotic activity around the filopodia. These findings suggest that intermediate cells in ES epithelia may play an active role in phagocytosis. Previous studies reported that ES epithelia showed phagocytotic activity, which is one of the main functions of ES[12,24-25]. However, the nature of intermediated cells in our culture system is still unclear. We cannot exclude the possibility that this type of cell represents simply incompletely differentiated cells in our culture condition. Some of the HESE cells expressed not just cytokeratin, but also vimentin, which is an intermediate-sized filament made up of a single peptide of about 58 kD that is present in all mesenchymal cells. The expression of more than one type of intermediate filament in the same cell is not a common phenomenon, but the co-expression of two different intermediate filaments in some normal cells and in a number of malignant neoplastic cells has been previously described[26]. Furthermore, previous studies on human and guinea pig endolymphatic sacs have demonstrated co-expression of cytokeratin and vimentin in epithelial cells[10,27-29].

pH homeostasis in the inner ear fluid is essential for maintaining the function of cochleovestibular organs. Various ion transporters are involved in the pH regulation process. Anion exchangers regulated luminal fluid pH in various systems by translocating HCO_3^- with monovalant anions. Anion exchangers were expressed in outer hair cells and supporting cells of the organ of Corti. Furthermore, they may play a role in maintaining motility and the integrity of

the organ[4,30]. Pendrin, which is an anion exchanger that is encoded by the *SLC26A4* gene, is expressed in the cochlea and vestibule and is essential for the development of normal hearing in mice. Mutations of *SLC26A4* are among the most prevalent causes of hereditary deafness in Asians[30]. Pendrin is known to function primarily as a Cl^-/HCO_3^- exchanger in spiral prominence epithelial cells. Loss of pendrin-mediated HCO_3^- secretion leads to luminal acidification and subsequent Ca^{2+} overload in the endolymph[31]. The role of pendrin in ES also appears to be essential in the development of normal hearing. Lack of fluid absorption in the ES causes hearing loss in mice lacking pendrin expression[7]. *Foxi1*$^{-/-}$ mice, which exhibit pendrin expression in the cochleovestibular labyrinth but not in the endolymphatic sac, are deaf[8].

In this study, we demonstrated that two unrelated multigene families of anion exchangers(SLC4 and SLC26 transporters) are expressed in cultured HESE cells with Cl^-/anion exchange activity, which support the involvement of anion exchangers in pH regulation by mediating Cl^-/HCO_3^- exchange. Although we demonstrated pendrin expression in the plasma membrane of cultured HESE cells by immunohistochemical staining, the DIDS-sensitive anion exchanger activity is not definite for pendrin activity and our result of the formate experiment did not detect a functional contribution to the measured flux, therefore, further studies are needed to elucidate the exact role of pendrin in cultured HESE cells.

Because we measured the anion exchanger activity in heterogenous cell type, the exact role of each cell type in the regulation of pH could not be determined. We are now investigating the role of each cell types in pH and fluid regulation by collecting each type of cells using flow cytomety system. Another limitation of our study is in regard to the tissue used for the cell culture. We used only the extraosseus part of endolymphtic sac which could be easily harvested during surgery. Because the intraosseus portion of ES is known to be most active portion of the entire ES, further evaluation of cultured cells from this area need to be performed in the future.

V. CONCLUSION

We demonstrated that cultured HESE cells exhibit anion exchanger activity and express pendrin. It is possible to further investigate the role of ES in inner ear homeostasis using this culture system.

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ABSTRACT(KOREAN)

배양된 사람 내임파낭 상피세포에서의 음이온 교환체의 발현

<지도교수 : 최 재 영>

연세대학교 대학원 의학과

양 원 선

내임파낭은 내이의 막성 구조물로 측두골과 후두와에 위치하여 내임파액의 양과 pH 변화등 항상성 유지에 중요한 역할을 할 것으로 생각된다. 내임파액의 조성 변화와 함께 선천성 난청 또는 메니에르 질환 발병과 관련하여 여러가지 가설이 제기되어 왔으나 아직 완전히 밝혀지지 않았으며 접근 및 채취의 어려움으로 인해 인체에서의 연구는 매우 제한적으로 이루어져 왔다. 본 연구에서는 사람에서 내임파낭 조직을 채취하고 내임파낭의 상피세포 배양 방법을 정립하여 배양된 세포의 특성 및 나아가 내임파낭의 기능을 알아보고자 하였다.

저자들은 본원에서 경미로 접근법으로 청신경 종양 제거술을 받는 24명의 환자에서 내임파낭 상피세포를 채취하여 높은 상피세포 증식 인자를 포함시킨 배양액에서 계대배양한 후 다른 조성을 갖는 배양액에서 분화시키고 세포 특성을 확인하였다. 또한 배양된 상피세포에서 pendrin을 포함한 음이온 교환체의 기능 및 발현을 확인하는 실험을 시행하였다.

전체 세포 중 50.2 ± 15.1% 에서 리보솜-풍부 세포로 분화하였으며 상대적으로 적은 7.3 ± 6.7% 에서 미토콘드리아-풍

부 세포로 분화하였다. 이들 세포의 내강면에서 음이온 교환체들이 발현하는 것을 기능적 및 조직학적 실험을 통하여 확인하였다.

결론적으로 내임파낭 상피세포에서 pendrin을 포함한 음이온 교환체가 내임파액의 항상성 유지에 관여함을 추론할 수 있었으며 내임파낭 상피세포의 계대배양방법 정립을 통해 앞으로의 추가 연구범위를 확대할 수 있었다.

핵심되는 말 : 내임파낭; pendrin; pH; 내이세포배양