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The development of human nasal epithelial organoid

사람 코 상피 오가노이드 제작

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서울대학교 대학원

의학과 중개의학

한 승 훈

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지도교수 김현직

이 논문을 의학석사 학위논문으로 제출함

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한 승 훈

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위 원 장   임 재 준   (인)

부위원장   김 현 직   (인)

위   원   권 성 근   (인)

Abstract

The development of human nasal epithelial organoid

Seung Hoon Han

Department of Translational Medicine

The Graduate School

Seoul National University

Introduction: Nasal epithelial cells (NECs) are a suitable model for studying the properties of the nasal epithelium and the pathophysiology of pathogens. However, since it is difficult to obtain sufficient cells that can be cultured for a long period, there are many limitations in using it for repeated in vitro experiments. This study aimed to describe the cell differentiation of human nasal epithelial (HNE) organoid derived from nasal samples and compare the characteristics of the normal nasal mucosa and cultured primary NECs

Methods: HNE organoids derived from human nasal mucosa were cultured. Then air-liquid interface (ALI) cultures from the HNE organoids were cultured until passages 3 and 5. Histologic analysis, electron microscopy, and real-time polymerase chain reaction of the cultured cells were performed.

Results: The histological findings showed that a well differentiated nasal epithelium was established 14 days after confluency in the ALI culture systems. Ciliated and secretory cells were observed through H&E and PAS staining. Scanning electron microscopy and transmission electron microscopy showed secreted mucus and well-differentiated cilia, and tight cellular junctions. Our HNE organoid models showed a significant expression of TP63, KRT5,

MUC5AC, and FOXJ1 genes until passage 5.

Conclusion: Findings suggest that our HNE organoid model will be suitable for long-term, repetitive in vitro experiments as it can accurately recapitulate the morphological and physiological characteristics of the nasal epithelium.

Keywords: Primary nasal epithelial cell culture, Nasal epithelial organoid, Airway epithelium

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I. Introduction

Airway epithelial cells serve as a natural wall to inhaled potential pathogens and other environmental insults and are the initial line of immunological defense against invading microbes [1,2]. Airway epithelial cells cultured in vitro can recapitulate in vivo physiological processes and morphology. These cultures are good surrogate models for studying respiratory diseases like cystic fibrosis and testing new therapies [3].

Human primary airway epithelial cell culture has several limitations, including the limited number of cells available from a donor, donor-to-donor heterogeneity, and contamination with pathogens [4]. Therefore many studies have been conducted on animal airway epithelial cells or tumor cells, which expand easily. However, human primary airway epithelial cell culture more appropriately represents the characteristics of the in vivo conditions and cellular response of normal human airway epithelial cells than immortalized cell lines or animal cell models. Attempts have been made to overcome this limitation of obtaining sufficient cells by subculturing bronchial epithelial cells and nasal epithelial cells [5, 6]. In 1996, Gray et al. reported that normal human lower airway epithelial cells were subcultured and that passage-2 airway epithelial cells were suitable for studying secretion mechanisms or differentiation. Currently, many research institutes are conducting various studies according to this method [5]. However, as the number of passages increases, cell division slows after passage 4 due to cellular senescence, and the ability to produce mucus or form cilia is gradually lost [6, 7]. Organoid models that culture cells in three dimensions can proliferate while maintaining differentiation capacity for a long time [8].

Organoids are three-dimensional cell aggregates formed through self-renewal and self-organization of stem cells. Organoids can overcome the limitations of two-dimensional cell culture methods while easily utilizing existing efficient two dimensional culture-based biochemical and cell biological analysis techniques. The airway organoid (AO) was first reported by Benail et al. using a collagen grid to obtain a three-dimensional culture of airway epithelial cells from nasal polyps [9]. Rossant first described the production of lung organoids from cystic fibrosis transmembrane conductance regulator (CFTR)-mutant induced pluripotent stem cells for the purpose of cystic fibrosis modeling [10].
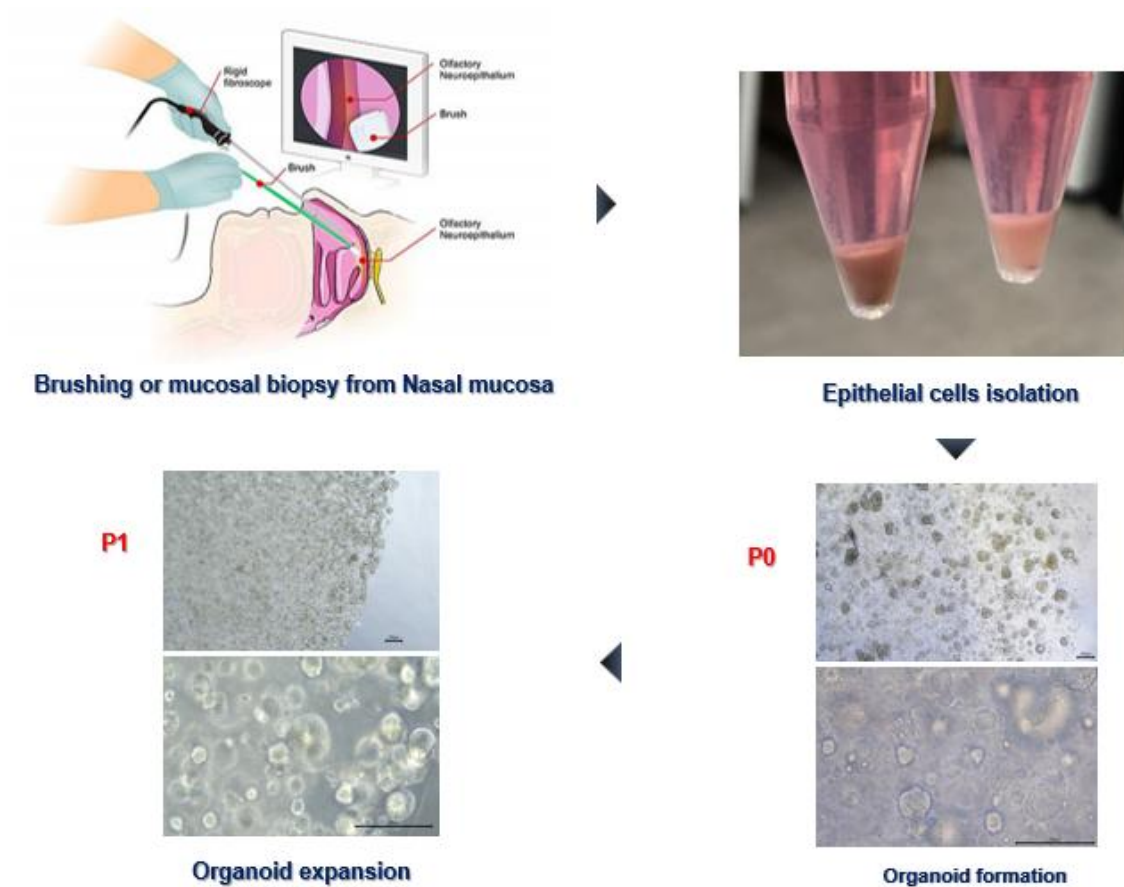
This study aimed to describe the cell differentiation of HNE organoids derived from human nasal samples and compare the characteristics of the normal nasal mucosa and cultured primary nasal epithelial cells.

II. Methods

1. Establishing human nasal epithelial (HNE) organoids

HNE organoids derived from human nasal samples were established according to a published protocol [10]. The nasal mucosa of the middle turbinate was harvested from the nasal cavity of four patients undergoing surgery for a deviated septum. The nasal tissue was washed with transfer medium (Dulbecco's modified Eagle's medium [DMEM]/HAM's F12K medium [F12], 1% penicillin and streptomycin). Digestion medium (AO medium, collagenase, and amphotericin B) was added, and the nasal tissues were digested on an orbital shaker for 60–120 min at 37°C. After digestion, the nasal tissue was removed, and collagenase was inactivated by adding fetal bovine serum. The solution was sheared, filtered through a strainer (100 µm), and spun at 1200 rpm for 5 min at 4°C. After removing the supernatant, the cell pellet was washed using washing medium (DMEM/F12, Glutamax 100x, HEPES 1M, penicillin, streptomycin and amphotericin B) and centrifuged at 4°C at 1200 rpm for 5 min. Then, the wash medium was taken away, the cell pellet was placed in Matrigel®, plated in a 24-well plate, and cultured at 37°C for 10 min. After the Matrigel® had hardened, 500 μL of AO medium was added with penicillin, streptomycin, amphotericin to each well, and the plates were kept in a 37°C incubator. Every four days, the AO medium was replaced, and every other week, subculture was carried out in a 1:2 ratio for expansion.

Figure 1. Schematic of the protocol to generate HNE organoids.

Brushing or mucosal biopsy from Nasal mucosa

Epithelial cells isolation

P1

Organoid expansion

P0

Organoid formation

2. HNE organoid-Air Liquid Interface (ALI) cultures

ALI cultures were carried out according to a previously published protocol [6]. Briefly, passage-2 and passage-4 HNE organoids (1×10^5) were seeded on Transwell clear culture inserts containing culture medium. The culture medium was composed of a plasma-free culture solution containing DMEM and basal epithelial growth medium with several supplements [11]. For the first 9 days, the cells were immersed in the culture medium. The culture medium was then replaced at the upper and lower parts 1 day after the start of the culture and every other day afterward. After culturing in the culture medium submerged for 9 days, the upper culture medium was removed to form an ALI. The upper part of the cells was exposed to air, and the lower culture medium was replaced daily. Cultivation was performed at 37°C, and 5% CO_2. The culture period was two weeks after the creation of ALI.

Figure 2. Schematic protocol of HNE organoid-ALI cultures for HNECs expansion and differentiation.

HNE, Human nasal epithelium; ALI, Air liquid interface; HNEC, Human nasal epithelial cell
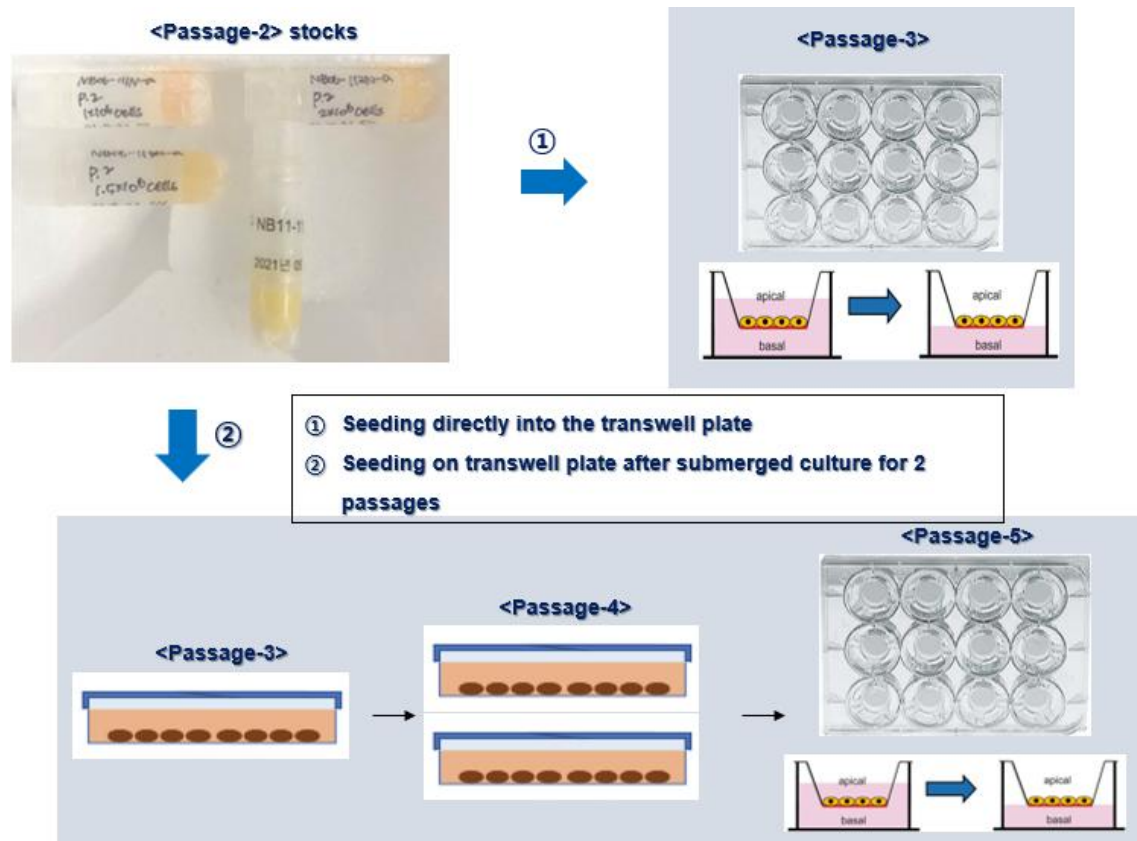
3. Histologic analysis and electron microscopy

For morphological observations, the culture was fixed in 10% formalin, and then embedded in paraffin and cut to a thickness of 5μm. Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining were performed on the cut tissues. The stained specimens were observed using an Olympus UTV0.63XC microscope (Olympus, Tokyo, Japan). For scanning electron microscopy (SEM), the cultured cells were fixed with 2.5% glutaraldehyde for 3hours and then washed with 0.1M PBS. After re-fixation in 1% osmium tetroxide(OsO_4) for 90 min, and dehydration using 100% alcohol, the tissue was dried at the critical point, and gold sputter-coating was performed. The specimen was visualized

using a Hitachi S-800 microscope (Hitachi, Tokyo, Japan). For transmission electron microscopy (TEM), the culture was washed with PBS, then pre-fixed in 2.5% glutaraldehyde for 5 minutes and washed. After being fixed in 1% OsO_4 for 30 min and dehydrated. It was replaced with isopropyl alcohol, embedded in epoxy resin, and polymerized in an incubator at 60°C for 48 hours. The fully polymerized resin was isolated from the plate, and trimmed and cut into 50-70nm sections using a diamond knife. The sections were double-stained in uranyl acetate and lead citrate for 10min each and observed under a JEM1011 microscope (JEOL, Tokyo, Japan).

4. Real-time PCR

Real-time PCR was performed as previously described [12]. Briefly, Trizol® (Molecular Research Center, Cincinnati, OH, USA) was added to the culture. The culture was left at room temperature for 5 min, chloroform was added, and mixed for 15 seconds, and centrifuged at 12,000g for 15 min. For RNA precipitation, the supernatant was transferred to a new tube, and isopropyl alcohol was added, followed by 75% ethanol for washing. The RNA precipitate was dissolved in DEPC solution and stored at -70oC. cDNA was synthesized using 1 μg of RNA, RNase inhibitor, oligo-(dT) 15 primer, 2.5mM dNTP, 50mM KCl, 10mM Tris-HCl, 5mM $MgCl_2$, and reverse transcriptase at 25°C for 15 min, 42°C for 60 min, 99°C for 5 min. The TaqMan method using the ABI Prism® 7000 Sequence Detection System was used to quantify the mRNA expression level. Human GAPDH was used as an endogenous control gene. The PCR procedure was performed for 2 min at 50°C, AmpliTaq Gold polymerase was activated for 10 min at 95°C, followed by 15 seconds at 95°C and 1 min at 60°C, this was repeated 40 times. Each reaction was repeated three times for all samples, and the standard curve method recommended by the manufacturer was used for comparative analysis of the expression results of each sample.

III. Results

1. The establishment of HNE organoid and the confirmation of cellular types in organoid.

Optimal cell viability of HNE organoids derived from human nasal tissue was demontrated in light microscope findings (Figure 3). There was no significant difference in the growth rate of live cells even when the number of passages increased . In HNE organoids established from human nasal samples, basal cell markers KRT5 and TP63 and ciliated cell marker FOXJ1 mRNA were expressed at similar levels compared to normal nasal mucosa(Figure 4).

2. Morphologic characterization and electronic microscopic findings of HNE organoid-ALI cultures

ALI culture was performed by seeding passage 2 cell stock of organoids into Transwell clear culture inserts at a cellular density of 1×10^5 cells/well. Confluency was achieved after 10 days of ALI culture. The finding of a high-magnification light microscope (40X) showed polygonal small tightly bound cells and ciliary beating at 14 days after confluence in ALI culture. Histological findings, including H&E and PAS staining, showed well-differentiated multi-layered columnar epithelium, including strongly stained secretory cells and ciliated cells (Figure 5). Fourteen days after confluency in the ALI, cells which adhere tightly to each other in a polygonal shape and secreted mucus and differentiated cilia were visualized under SEM. The finding of TEM also showed differentiated secretary cells and ciliated cells (Figure 6).

To compare the histological findings of the normal human nasal mucosa with the histological findings of HNE oragnoid-ALI culture, H&E and PAS staining was performed and observed on the normal human nasal mucosa. Light microscopy findings showed a multi-layered nasal epithelium containing well-stained ciliated and secretory cells and SEM finding also showed well-differentiated ciliated and secretory cells (Figure 7).

Figure 3. HNE organoids growth characteristics according to subculture. (A) passage 0, (B) passage 1, (C) passage 2, (D) passage 3, Scale = 100 μm (upper

panel), Scale = 50 μm (lower panel), (E) There is no difference in the growth rate of live cells according to the number of passages.

HNE, human nasal epithelium.
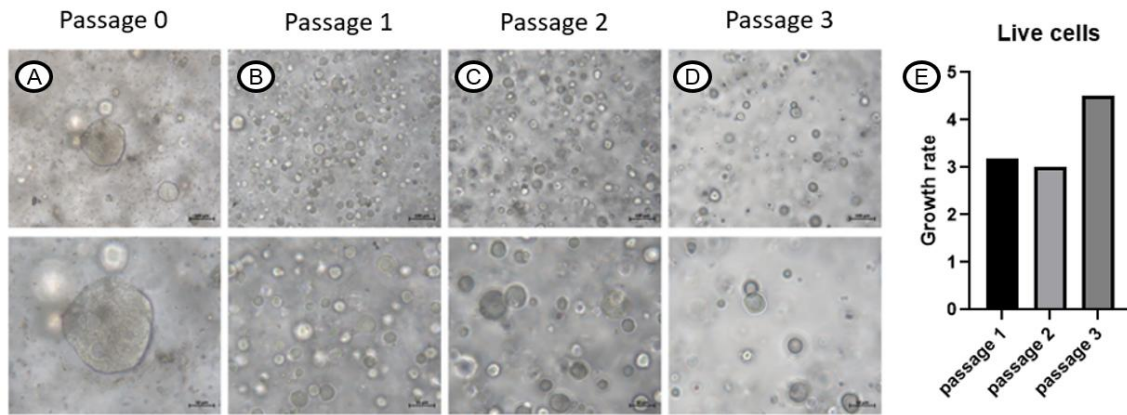
Figure 4. The mRNA levels of (A) TP63, (B) KRT5, (C) Foxj1 in nasal mucosa and HNE organoids. HNEO, human nasal epithelial organoid.
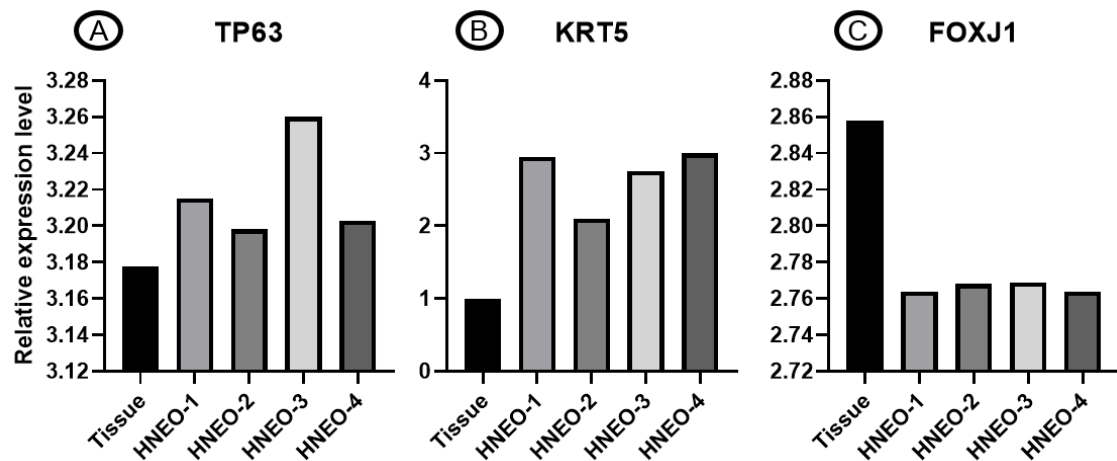
Figure 5. Histologic appearance of ALI cultures from HNE organoids and primary cultured nasal epithelial cells under light microscope. (A) Primary NECs (left panel) and NECs derived from HNE organoid (right panel) growing on ALI

culture periods. Scale = 200 μm. (B) Cross section (5 μm) of primary NECs (upper panel) and NECs derived from HNE organoid (lower panel) with H&E and (C) PAS at ALI 14days. Scale = 50 μm.

H&E, hematoxylin and eosin; PAS, periodic acid Schiff; ALI, air-liquid interface; NEC, nasal epithelial cell.
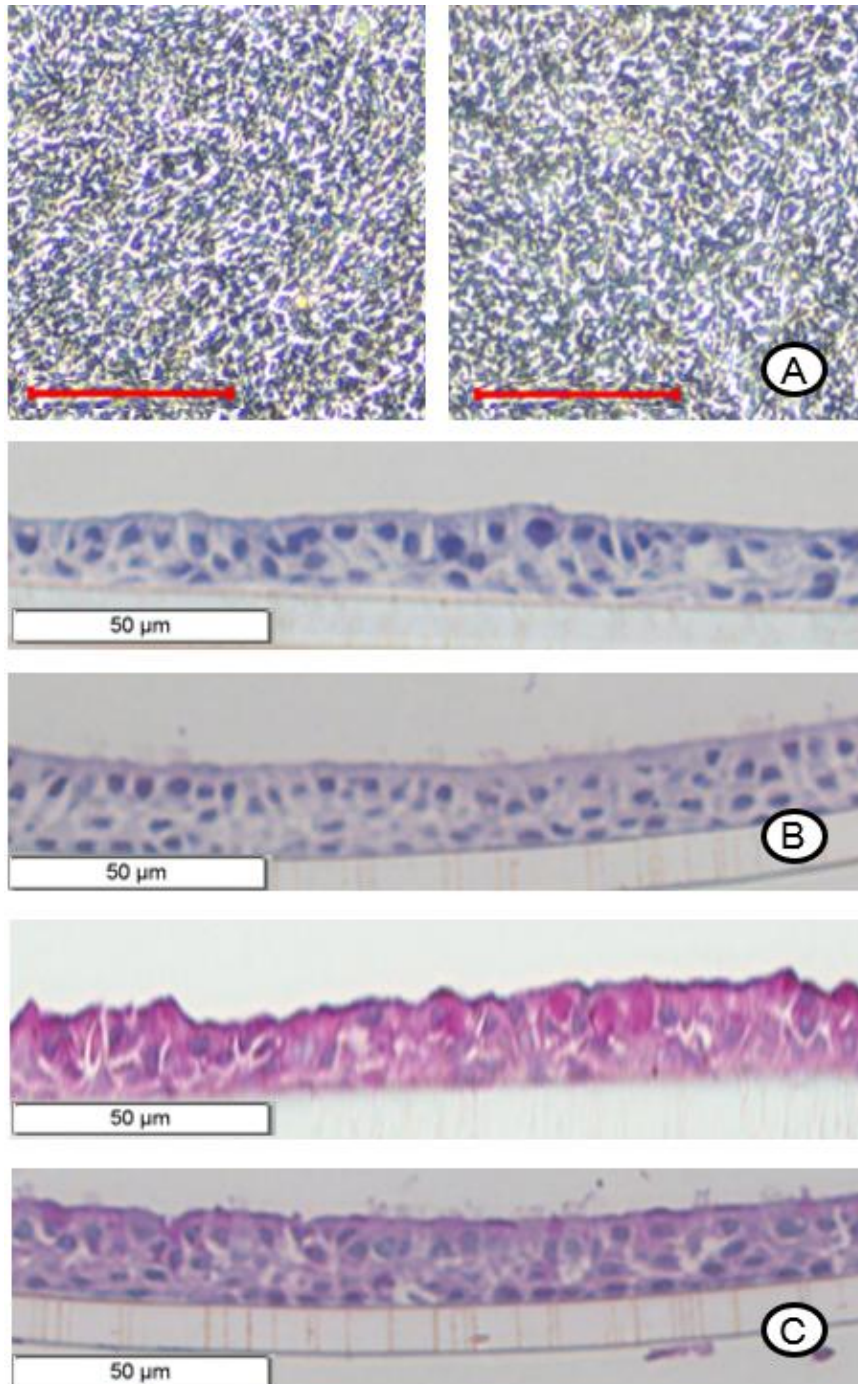
Figure 6. SEM and TEM findings of HNE organoid-ALI cultures and primary NEC-ALI cultures at 14 days after confluency. (A) SEM shows well-differentiated secretory and ciliated cells in both HNE organoid-ALI cultures (upper panel) and primary NEC-ALI cultures (lower panel). Scale = 10 μm, (B) TEM shows columnar secretory cells in both HNE organoid-ALI cultures (upper panel) and primary NEC-ALI cultures (lower panel). Scale = 1000 nm and (C) TEM shows well-differentiated ciliated cells in HNE organoid-ALI cultures. Scale = 500 nm.

SEM, scanning electron microscopy; TEM, transmission electron microscopy; HNE, human nasal epithelium; ALI, air-liquid interface.
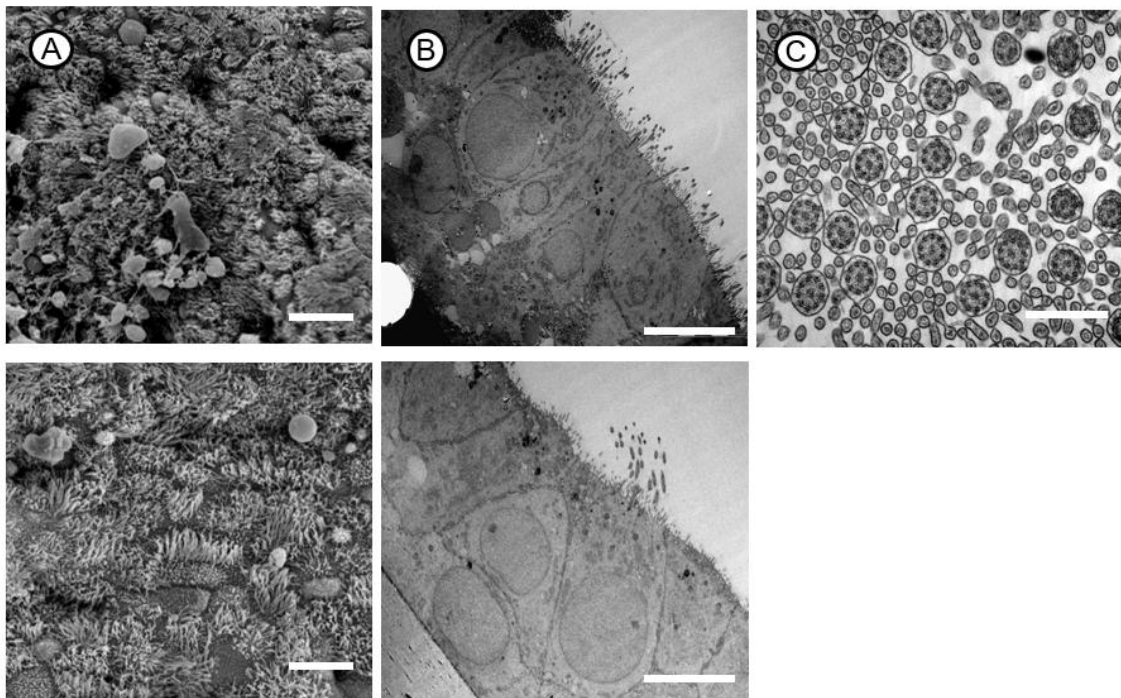
Figure 7. Histologic appearance of normal human nasal mucosa with H&E, PAS staining and SEM finding. (A) The structure of human nasal epithelium, including ciliated and secretory cells and basement membrane is observed in H&E. Scale = 50 μm. (B) PAS staining shows stained secretory cells in HNE. Scale = 50 μm. (C) SEM findings of HNE cells showing well-differentiated ciliated cells. Scale = 10nm.

H&E, hematoxylin and eosin; PAS, periodic acid Schiff; HNE, human nasal

epitelium.
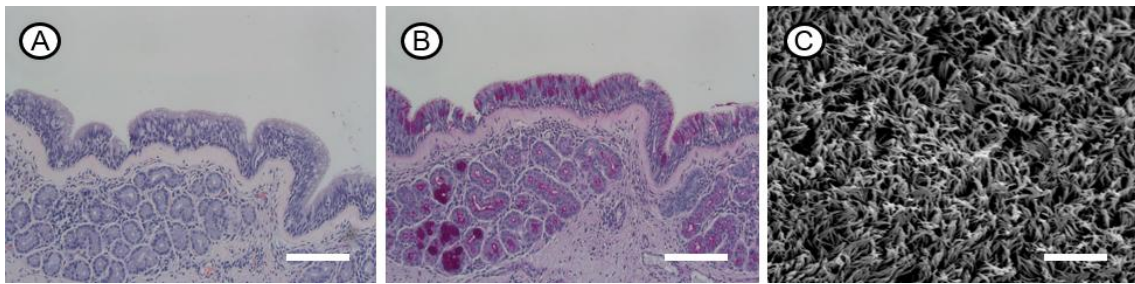
3. Cell type-specific mRNA expression in HNE organoid-ALI cultures

In nasal epithelial organoid-ALI cultures, KRT5 and TP63 mRNA, known as cell markers of basal cells, were expressed at a similar expression level compared to primary normal nasal epithelial cells and nasal mucosa. In addition, FOXJ1, a ciliated cell marker, and MUC5AC, a secretory cell marker, also demonstrated a similar expression level compared to primary normal nasal epithelial cells and nasal mucosa. KRT5, TP63, FOXJ1, and MUC5AC expression levels were similar in passage 3 and passage 5 cells of HNE organoid-ALI culture (Figure 4).

Figure 8. The mRNA levels of TP63, KRT5, Foxj1, and MUC5AC in (A) HNE organoid-ALI cultures (passage-3), (B) HNE organoid-ALI cultures (passage-5), (C) Primary nasal epithelial cell culture (passage-2) and (D) Normal human nasal mucosa. (1/2)^(Gene-GAPDH) of MUC5AC : passage-3 organoid = 0.0021, passage-5 organoid = 0.00064, primary cell culture = 0.0017, nasal mucosa = 0.014.

ALI, air-liquid interface.
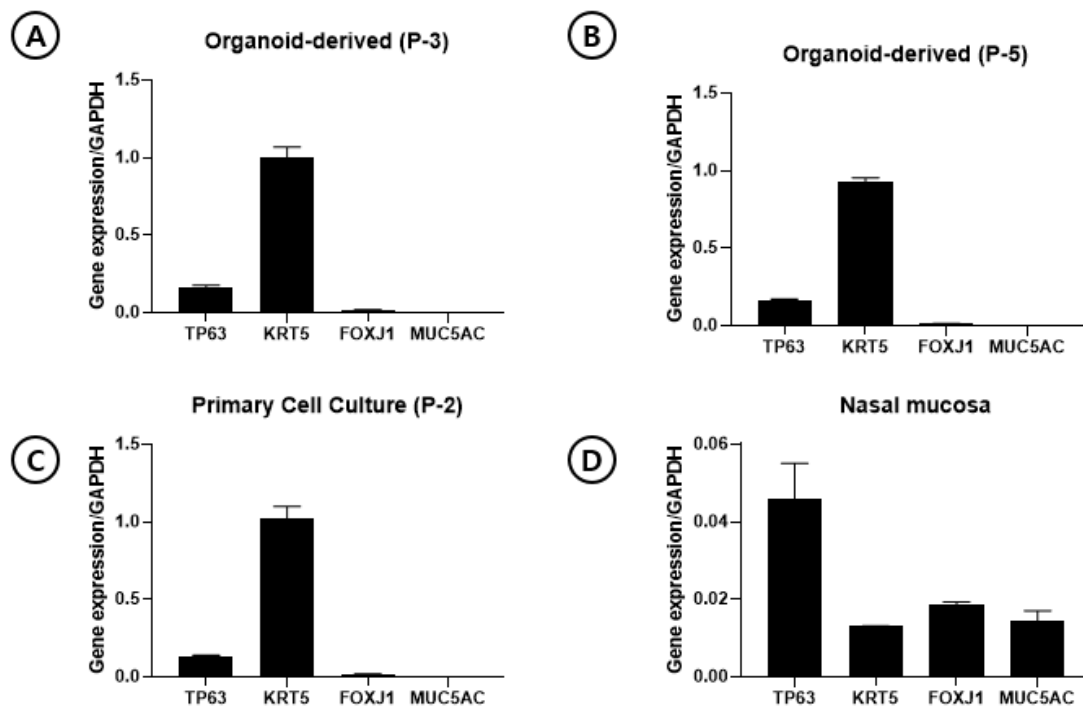
IV. Discussion

With the development of cell culture methods, it is possible to induce human airway epithelial cell differentiation with similar morphological, biochemical, and physiological characteristics to in vivo airways [11, 13]. Primary nasal epithelial cells have been utilized to diagnose and study nasal pathophysiology, including inflammation, allergy, viral and bacterial infections, and the function of airway epithelial cells [4]. However, for a primary cell culture, an invasive biopsy of human tissue is required for each experiment. Therefore, obtaining a sufficient number of cells is challenging, and complete differentiation takes a long time to achieve. A sufficient number of cells cannot be obtained as the number of passages is limited. As the number of passages increases, cell division slows down due to cellular aging and the ability to produce mucus or form cilia is lost [5, 7]. This study describes a well-differentiated HNE organoid model derived from human nasal tissues that accurately recapitulates the airway epithelium according to multiple outcome measurements. Passage-5 cells cultured from this organoid also showed optimal cell growth and differentiation, allowing sufficient cells to be obtained.

In various airway diseases, the production and secretion of mucus by airway epithelial cells is a common phenomenon. The airway is composed of basal cells and pseudo stratified ciliated epithelial cells composed of various types of secretory and ciliary cells. Understanding the pathophysiology and fundamental biology of airway cells and tissues is necessary for the accurate diagnosis and effective treatment of respiratory diseases. The lack of existing efficient models of differentiated respiratory epithelium makes basic studies of pathogenic processes associated with respiratory diseases difficult and time-consuming. Respiratory epithelial models must be able to efficiently and adequately recapitulate the human phenotypic and genotypic diversity of the respiratory system [14, 15].

Initially, airway animal models using guinea pigs or rats as a source of differentiated respiratory epithelium have been frequently used for research on respiratory diseases [16, 17]. However, these animal models have limitations in that only a small number of cells can be obtained, and they do not fully reflect the morphology and physiology of the human airway epithelium [18]. In mice, the composition of airway epithelium in certain parts of the airway tract different from that in humans [15]. In the mouse model of PCD resulting from a deficit of DNAH5 protein, mice without DNAH5 developed hydrocephalus, which does not occur in humans with DNAH5 mutations [19]. Because of the differences between animal and human disease models, reproducing human manifestations in animal disease models is difficult. Results in animal studies may appear different than those in human clinical trials [18]. Therefore, caution should be exercised when interpreting the results of animal studies. Additionally, tumor cells, which are relatively easy to culture, or immortalized airway cell lines were used for research. However, since the cell line cannot reproduce mucociliary differentiation, it cannot be used for studies on ciliary movement or mucus-related diseases [20, 21]. For these reasons, culturing primary human respiratory epithelial cells appears to be the most suitable method for studying host responses to external stimuli, such as respiratory pathogens and allergens, and testing personalized therapeutics [22, 23].

In the early 1980s, primary human respiratory epithelial cell culture was first reported [3]. Primary human respiratory epithelial cell culture became more popular as more information was discovered about the factors stimulating cell

proliferation or differentiation [24, 25]. Since the cell expansion and differentiation steps are separated in vitro, media containing stage-specific supplements such as retinoic acid, calcium ion, and culture platforms, including ALI culture, as mechanical conditions significantly impact the growth and differentiation of cells in primary cell culture [26, 27].

However, primary human respiratory epithelial cell culture has several limitations, such as difficulty obtaining a sufficient number of cells and their limited potential to expand and differentiate over a longer period. Primary human respiratory epithelial cells can only divide optimally for three to four passages [6, 28]. After this passage, the cells proliferate more slowly and lose their ability to differentiate into cilia and secrete mucus [5,7]. Furthermore, due to the limited amount of tissue available from the donor, acquiring sufficient cells in culture is difficult. The storage of donor tissues is also limited because the differentiation capacity of cells decreases after cryopreservation [29].

Organoid culture is a recently established method for growing human respiratory epithelial cells in a three-dimensional structure [30]. Primary human respiratory epithelial cells are embedded in an extracellular matrix with media-containing supplements that promote tissue self-renewal. Therefore, organoid cultures can be efficiently expanded for a long period [8]. In the early stage of organoid formation, expansion of basal cells is promoted by the composition of the culture medium and the cells proliferate rapidly and form three-dimensional spheroids. After the three-dimensional structure of organoids is created, some stem cells differentiate into functional cells constituting tissues due to changes in the physical environmental conditions. In this way, the self-renewal and differentiation of basal cells in the organoid is properly maintained, and expansion is possible even after many passages. In this study, to maintain the stem cells in a pre-mitotic state, a culture medium containing TGF-beta inhibitor and WNT signal agonist was used to prevent the differentiation of epithelial stem cells and promote self-renewal [31]. Although the exact mechanism remains unclear, it is known that the ability to expand is maintained because cell aggregation through three-dimensional culture creates structural characteristics similar to the stem cell niche in the actual tissue.

The nasal epithelium consists of basal cells, ciliated cells, non-ciliated columnar cells, and goblet cells [32]. We made HNE organoid-ALI cultures

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derived from human nasal samples and described the cell differentiation and characteristics of organoids. After 14 days of confluence in the ALI culture system, histological findings showed fully differentiated nasal epithelium. Secreted mucus was observed in HNE organoid-ALI culture, and cilia and secretory cells were also identified through H&E and PAS staining. Transmission electron micrographs also showed secreted mucus and well-differentiated cilia, and SEM revealed well-differentiated cilia, mucin granules, and tight cellular junctions. Previous studies of submerged monolayer cultures taken from the nasal and bronchus have described the functions of basal epithelial cells [33, 34]. However, the submerged monolayer cultures cannot observe mucociliary differentiation. In the ALI culture system, the apical part of epithelial cells is exposed to air, and cilia movement and mucus-secreting secondary cells can be observed because optimal airway epithelial differentiation is possible [35, 36]. Therefore, many studies have investigated the physiological function of the airway epithelium using the ALI culture system [37-39].

Basal cells are abundantly distributed in human airways. In basal cells, TP63 and CD44 are highly expressed, and the expression of desmosomal proteins such as KRT5 is also increased [40]. The differentiation of progenitors into ciliated cells consists of the expression of the MCIDAS-CCNO-MYB-FOXJ1 gene cascade [41]. Foxj1 regulates cilia differentiation as well as motile cilia formation processes such as cilia elongation and centrosome proliferation [42]. Additionally, progenitors that differentiate into goblet cells show high expression of MUC5AC [41]. In our HNE organoid model, we observed the significant expression of TP63, KRT5, MUC5AC, and FOXJ1 genes, which are specific cell markers of basal progenitor cells, secretory cells, and ciliated cells, until passage 5.

Several studies have evaluated the CFTR function and analyzed disease features of respiratory syncytial virus infection using organoids derived from distal airways, such as bronchial or tracheobronchial epithelial cells [8, 10]. There have been attempts to establish organoids from human nasal samples because nasal epithelial cells are much less invasive than distal airways [43, 44]. Liu et al. established an HNE organoid model using the patient's nasal tissue. Forskolin-induced swelling that can be used to evaluate CFTR activity could be measured using HNE organoid model [43]. And Sett et al. also generated HNE organoid model derived from the nasal sample of CF patients and used for

CFTR pharmacological testing [44]. In this study, development a HNE organoid was succeeded in a similar methods, it was described that proliferation and differentiation ability were maintained even when the number of passages of the organoids increased. The nasal cavity is the first barrier to immunological defense and a less invasive tissue source. Therefore, it is considered that organoid models derived from human nasal tissues would be useful for studying pathogens, including respiratory viruses, testing therapeutics, or *in vitro* studies of the immunological mechanisms of allergic rhinitis.

In the primary nasal epithelial cell culture, it takes about 6 weeks until the cells are completely differentiated from the donor [12]. However, the HNE organoid model can shorten the time required. Moreover, since the organoid model can be frozen and thawed, a sufficient number of cells can be obtained, and the results of multiple studies can be interpreted because it is possible to proliferate from the same donor [45]. Additionally, the organoid model produced from the patient reflects the patient's genotype and phenotype, and can be used as a preclinical model to evaluate the therapeutic effect of drugs, which will be of great help in establishing a patient-specific treatment plan.

V. Conclusion

This study demonstrates that the HNE organoid model adequately recapitulates the histological morphology and physiological features of the human nasal epithelium. Organoids derived from human nasal tissue can be effective models for repeated studies because sufficient cells can be obtained over a long period. Moreover, this organoid model can be used to recapitulate the complex interactions between pathogens, drugs, and airway cells and to develop therapeutic agents targeting the airway mucosa..

VI. References

1.   Ganesan S, Comstock AT, Sajjan US. Barrier function of airway tract epithelium. Tissue Barriers. 2013 Oct 1;1(4):e24997.

2.   Eisele NA, Anderson DM. Host Defense and the Airway Epithelium: Frontline Responses That Protect against Bacterial Invasion and Pneumonia. J Pathog. 2011;2011:249802.

3.   Randell SH, Fulcher ML, O'Neal W, Olsen JC. Primary epithelial cell models for cystic fibrosis research. Methods Mol Biol. 2011;742:285-310.

4.   Bukowy-Bieryłło Z. Long-term differentiating primary human airway epithelial cell cultures: how far are we? Cell Commun Signal. 2021 May 27;19(1):63.

5.   Gray TE, Guzman K, Davis CW, Abdullah LH, Nettesheim P. Mucociliary differentiation of serially passaged normal human tracheobronchial epithelial cells. Am J Respir Cell Mol Biol. 1996 Jan;14(1):104-12.

6.   Yoon JH, Kim KS, Kim SS, Lee JG, Park IY. Secretory differentiation of serially passaged normal human nasal epithelial cells by retinoic acid: expression of mucin and lysozyme. Ann Otol Rhinol Laryngol. 2000 Jun;109(6):594-601.

7.   Stewart CE, Torr EE, Mohd Jamili NH, Bosquillon C, Sayers I. Evaluation of differentiated human bronchial epithelial cell culture systems for asthma research. J Allergy (Cairo). 2012;2012:943982.

8.   Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, Heo I, Böttinger L, Klay D, et al. Long-term expanding human airway organoids for disease modeling. EMBO J. 2019 Feb 15;38(4).

9.   Benali R, Tournier JM, Chevillard M, Zahm JM, Klossek JM, Hinnrasky J, et al. Tubule formation by human surface respiratory epithelial cells cultured in a three-dimensional collagen lattice. Am J Physiol. 1993 Feb;264(2 Pt 1):L183-92.

10.   Wong AP, Bear CE, Chin S, Pasceri P, Thompson TO, Huan LJ, et al. Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. Nat Biotechnol. 2012 Sep;30(9):876-82.

11.   Yoon JH, Gray T, Guzman K, Koo JS, Nettesheim P. Regulation of the

secretory phenotype of human airway epithelium by retinoic acid, triiodothyronine, and extracellular matrix. Am J Respir Cell Mol Biol. 1997 Jun;16(6):724-31.

12. Park DY, Kim S, Kim CH, Yoon JH, Kim HJ. Alternative Method for Primary Nasal Epithelial Cell Culture Using Intranasal Brushing and Feasibility for the Study of Epithelial Functions in Allergic Rhinitis. Allergy Asthma Immunol Res. 2016 Jan;8(1):69-78.

13. Ostrowski LE, Nettesheim P. Inhibition of ciliated cell differentiation by fluid submersion. Exp Lung Res. 1995 Nov-Dec;21(6):957-70.

14. Mou H, Brazauskas K, Rajagopal J. Personalized medicine for cystic fibrosis: establishing human model systems. Pediatr Pulmonol. 2015 Oct;50 Suppl 40:S14-23.

15. Basil MC, Katzen J, Engler AE, Guo M, Herriges MJ, Kathiriya JJ, et al. The Cellular and Physiological Basis for Lung Repair and Regeneration: Past, Present, and Future. Cell Stem Cell. 2020 Apr 2;26(4):482-502.

16. Adler KB, Cheng PW, Kim KC. Characterization of guinea pig tracheal epithelial cells maintained in biphasic organotypic culture: cellular composition and biochemical analysis of released glycoconjugates. Am J Respir Cell Mol Biol. 1990 Feb;2(2):145-54.

17. Kaartinen L, Nettesheim P, Adler KB, Randell SH. Rat tracheal epithelial cell differentiation in vitro. In Vitro Cell Dev Biol Anim. 1993 Jun;29a(6):481-92.

18. Calvert BA, Ryan Firth AL. Application of iPSC to Modelling of Respiratory Diseases. Adv Exp Med Biol. 2020;1237:1-16.

19. Ibañez-Tallon I, Gorokhova S, Heintz N. Loss of function of axonemal dynein Mdnah5 causes primary ciliary dyskinesia and hydrocephalus. Hum Mol Genet. 2002 Mar 15;11(6):715-21.

20. Hiemstra PS, Grootaers G, van der Does AM, Krul CAM, Kooter IM. Human lung epithelial cell cultures for analysis of inhaled toxicants: Lessons learned and future directions. Toxicol In Vitro. 2018 Mar;47:137-46.

21. Xia S, Liu J, Yang Y, Wu M, Ye L, Chen S, et al. Coupled CRC 2D and ALI 3D Cultures Express Receptors of Emerging Viruses and Are More Suitable for the Study of Viral Infections Compared to Conventional Cell Lines. Stem Cells Int. 2020;2020:2421689.

22.   Broadbent L, Manzoor S, Zarcone MC, Barabas J, Shields MD, Saglani S, et al. Comparative primary paediatric nasal epithelial cell culture differentiation and RSV-induced cytopathogenesis following culture in two commercial media. PLoS One. 2020;15(3):e0228229.

23.   Berg A, Hallowell S, Tibbetts M, Beasley C, Brown-Phillips T, Healy A, et al. High-Throughput Surface Liquid Absorption and Secretion Assays to Identify F508del CFTR Correctors Using Patient Primary Airway Epithelial Cultures. SLAS Discov. 2019 Aug;24(7):724-37.

24.   Randell SH, Walstad L, Schwab UE, Grubb BR, Yankaskas JR. Isolation and culture of airway epithelial cells from chronically infected human lungs. In Vitro Cell Dev Biol Anim. 2001 Sep;37(8):480-9.

25.   Fulcher ML, Gabriel S, Burns KA, Yankaskas JR, Randell SH. Well-differentiated human airway epithelial cell cultures. Methods Mol Med. 2005;107:183-206.

26.   Bukowy Z, Ziętkiewicz E, Witt M. In vitro culturing of ciliary respiratory cells--a model for studies of genetic diseases. J Appl Genet. 2011 Feb;52(1):39-51.

27.   Gilpin SE, Wagner DE. Acellular human lung scaffolds to model lung disease and tissue regeneration. Eur Respir Rev. 2018 Jun 30;27(148).

28.   Martinovich KM, Iosifidis T, Buckley AG, Looi K, Ling KM, Sutanto EN, et al. Conditionally reprogrammed primary airway epithelial cells maintain morphology, lineage and disease specific functional characteristics. Sci Rep. 2017 Dec 21;7(1):17971.

29.   Mao H, Wang Y, Yuan W, Wong LB. Ciliogenesis in cryopreserved mammalian tracheal epithelial cells cultured at the air-liquid interface. Cryobiology. 2009 Dec;59(3):250-7.

30.   Hild M, Jaffe AB. Production of 3-D Airway Organoids From Primary Human Airway Basal Cells and Their Use in High-Throughput Screening. Curr Protoc Stem Cell Biol. 2016 May 12;37:Ie.9.1-ie.9.15.

31.   Kong J, Wen S, Cao W, Yue P, Xu X, Zhang Y, et al. Lung organoids, useful tools for investigating epithelial repair after lung injury. Stem Cell Res Ther. 2021 Jan 30;12(1):95.

32.   Watelet JB, Van Zele T, Gjomarkaj M, Canonica GW, Dahlen SE, Fokkens W, et al. Tissue remodelling in upper airways: where is the link with lower airway remodelling? Allergy. 2006 Nov;61(11):1249-58.

33.   Wesley UV, Bove PF, Hristova M, McCarthy S, van der Vliet A. Airway epithelial cell migration and wound repair by ATP-mediated activation of dual oxidase 1. J Biol Chem. 2007 Feb 2;282(5):3213-20.

34.   Perotin JM, Adam D, Vella-Boucaud J, Delepine G, Sandu S, Jonvel AC, et al. Delay of airway epithelial wound repair in COPD is associated with airflow obstruction severity. Respir Res. 2014 Nov 27;15(1):151.

35.   Ashraf S, Brockman-Schneider R, Bochkov YA, Pasic TR, Gern JE. Biological characteristics and propagation of human rhinovirus-C in differentiated sinus epithelial cells. Virology. 2013 Feb 5;436(1):143-9.

36.   Ashraf S, Brockman-Schneider R, Gern JE. Propagation of rhinovirus-C strains in human airway epithelial cells differentiated at air-liquid interface. Methods Mol Biol. 2015;1221:63-70.

37.   Krunkosky TM, Jordan JL, Chambers E, Krause DC. Mycoplasma pneumoniae host-pathogen studies in an air-liquid culture of differentiated human airway epithelial cells. Microb Pathog. 2007 Feb-Mar;42(2-3):98-103.

38.   Thaikoottathil JV, Martin RJ, Zdunek J, Weinberger A, Rino JG, Chu HW. Cigarette smoke extract reduces VEGF in primary human airway epithelial cells. Eur Respir J. 2009 Apr;33(4):835-43.

39.   Bérubé K, Prytherch Z, Job C, Hughes T. Human primary bronchial lung cell constructs: the new respiratory models. Toxicology. 2010 Dec 30;278(3):311-8.

40.   Reynolds SD, Rios C, Wesolowska-Andersen A, Zhuang Y, Pinter M, Happoldt C, et al. Airway Progenitor Clone Formation Is Enhanced by Y-27632-Dependent Changes in the Transcriptome. Am J Respir Cell Mol Biol. 2016 Sep;55(3):323-36.

41.   Ruiz García S, Deprez M, Lebrigand K, Cavard A, Paquet A, Arguel MJ, et al. Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. Development. 2019 Oct 23;146(20).

42.   Yu X, Ng CP, Habacher H, Roy S. Foxj1 transcription factors are master regulators of the motile ciliogenic program. Nat Genet. 2008 Dec;40(12):1445-53.

43.   Liu Z, Anderson JD, Deng L, Mackay S, Bailey J, Kersh L, et al. Human Nasal Epithelial Organoids for Therapeutic Development in Cystic

Fibrosis. Genes (Basel). 2020 May 29;11(6).

44.   Sette G, Lo Cicero S, Blaconà G, Pierandrei S, Bruno SM, Salvati V, et al. Theratyping cystic fibrosis in vitro in ALI culture and organoid models generated from patient-derived nasal epithelial conditionally reprogrammed stem cells. Eur Respir J. 2021 Dec;58(6).

45.   Mahnaz R, Harrison B, Hon K, Gohar S, Hadi R, Aaron FK, et al. Characterization of human nasal organoids from chronic rhinosinusitis patients. Biol Open. 2022 Apr 22.

초 록

서론: 인간 비강 상피 세포는 비강 상피의 특성과 병원체에 대한 면역반응을 연구하는데 유용한 모델이다. 그러나 인간 비강상피세포는 체외에서 장기간 배양할 수 없고 조직으로부터 충분한 세포를 얻기 어렵기 때문에 반복적인 시험관내 실험에 사용하는데 많은 한계가 있다. 본 연구는 인간 정상 비점막에서 유래한 비강 오가노이드 모델의 세포 분화를 살펴보고 기존 체외 배양 비강 상피세포와 비교하여 시험관내 실험에서 비강 오가노이드 모델의 유용성을 평가하고자 한다.

방법: 인간 정상 비점막을 채취하여 비강상피 오가노이드 모델을 만들고자 하였다. 제작된 비강상피 오가노이드를 이용하여 air-liquid interface법으로 배양하여 비강상피세포들의 분화를 유도하였으며 배양된 세포의 형태학적 관찰을 시행하였고 비강상피를 구성하는 특정 세포들의 mRNA을 세포에서 추출하여 발현정도를 RT-PCR을 이용하여 비교하였다.

결과: 비강상피 오가노이드는 ALI 배양 시스템을 통하여 완전히 분화된 비강 상피를 형성하였다. H&E 및 PAS 염색을 통해 분화된 섬모 세포와 분비 세포가 관찰할 수 있었다. 투과 전자현미경으로 분화된 섬모와 분비된 점액이 관찰되었으며 주사전자현미경으로 점액과립과 분화된 섬모, 세포간 밀착연접을 관찰 되었다. Passage-3세포 뿐만 아니라 Passage-5 세포에서도 basal celll의 마커인 TP63, KRT5, secretary cell의 마커인 MUC5AC 및 ciliated cell의 마커인 FOXJ1 유전자의 유의한 발현을 확인 할 수 있었다.

결론: 인간 비점막으로부터 만들어진 비강 오가노이드모델이 비강 상피의 형태 및 생리학적 특성을 유지하고 분화할 수 있음을 보여주었고 반복되는 시험관 내 연구에 유용할 것임을 시사한다.

주요어: 오가노이드, 비강상피세포, 일차상피세포배양, 기도 상피 조직

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