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From Cell Biology to Tissue Engineering

A subpopulation of airway epithelial cells that express hepatocyte nuclear factor 4α - its implication in the development of non-terminal respiratory unit-type lung adenocarcinoma

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Summary. A normal counterpart and precancerous lesion that non-terminal respiratory unit (TRU) lung adenocarcinomas (LADCs) develop from have not been clarified. Non-TRU LADCs specifically express hepatocyte nuclear factor 4α (HNF4α). Thus, we have been interested in airway epithelial cells that express HNF4α as the potential precursor of non-TRU LADC. The purposes of the present study are to report the frequency and distribution of HNF4α-expressing cells at the different airway levels, and to investigate the potential significance of the expression of HNF4α in the histogenesis of non-TRU LADC with a special reference to the relationship to bronchiolar metaplasia in idiopathic interstitial pneumonia. We herein identified a minor subpopulation of epithelial cells that express HNF4α in a physiological state. This subpopulation was mainly located in the terminal bronchioles and had the appearance of ciliated cells, which were mutually exclusive from Clara cells and others that strongly expressed thyroid transcription factor 1. Furthermore, the expression of HNF4α was similar in bronchiolar metaplastic lesions and the terminal bronchioles, and some of the metaplastic lesions showed an unequivocally higher frequency and expression level of HNF4α, which was comparable to non-TRU LADC. In summary, this is the first study to describe a subpopulation of ciliated cells that express HNF4α as a potential normal counterpart for non-TRU LADCs and suggests that bronchiolar metaplastic lesions that strongly express HNF4α are a precancerous lesion for non-TRU LADCs.

Key words: HNF4α expression, Airway epithelia, Bronchiolar metaplasia, Non-TRU lung adenocarcinoma

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Introduction

Recent advances in molecular biology have resulted in the identification of essential molecules that maintain cellular differentiation phenotypes, such as thyroid transcription factor 1 (TTF1) in the lung (Kimura et al., 1996; Fujii et al., 2002), which has prompted us to reconsider normal anatomical architectures (Yatabe, 2010). TTF1 is specifically expressed in epithelial cells located in the peripheral airways of the gas-exchanging system, which includes Clara cells and type 2 pneumocytes (and potentially some ciliated cells and basal cells). The subpopulation of cells expressing TTF1 is now called "the terminal respiratory unit (TRU)" and is regarded as a precursor for most (~90%) lung

adenocarcinomas, which are referred to as TRU-type adenocarcinomas (Yatabe, 2010). Non-TRU-type adenocarcinomas account for ~10% of lung adenocarcinomas (Yatabe, 2010; Kunii et al., 2011; Sugano et al., 2013). Non-TRU lung adenocarcinomas show similar morphological features to bronchial surface epithelia and express hepatocyte nuclear factor 4α (HNF4α), but not TTF1 (Kunii et al., 2011; Sugano et al., 2013). Thus, airway epithelial cells that express HNF4α may be the potential precursor for non-TRU-type adenocarcinomas (Kunii et al., 2011; Okudela et al., 2018).

Recent studies reported that non-TRU lung adenocarcinomas developed at a significantly higher frequency in lungs with idiopathic interstitial pneumonia (Masai et al., 2016; Kojima et al., 2017; Okudela et al., 2018) and were often associated with bronchiolar metaplasia in honeycomb lesions (Kojima et al., 2017; Okudela et al., 2018). These findings implicate bronchiolar metaplasia in the histogenesis of non-TRU lung adenocarcinomas.

However, a normal counterpart that non-TRU lung adenocarcinomas originate from and a precancerous lesion that they develop through have not yet been identified. Furthermore, HNF4 expression in the normal airway epithelia and bronchiolar metaplasia has not yet been examined in detail.

The two main purposes of the present study are to report the frequency and distribution of HNF4α-expressing cells at the different airway levels in a physiological state, and to investigate the potential significance of the expression of HNF4α in the histogenesis of non-TRU lung adenocarcinoma with special reference to the relationship to bronchiolar metaplasia in idiopathic interstitial pneumonia.

Materials and methods

Patients and lung tissues

Ninety-seven patients who underwent surgery at the Kanagawa Prefectural Cardiovascular and Respiratory Center (Yokohama, Japan) were enrolled. The surgical procedures performed were 55 surgical lung biopsies for idiopathic interstitial pneumonia and 60 lobectomies for lung tumors (39 idiopathic interstitial pneumonia associated cases and 21 unrelated cases). Informed consent for the research use of resected materials was obtained from all subjects.

Conventional histopathological examination

Lung tissues were fixed with buffered 10% formaldehyde solution for approximately 24 hours and embedded in paraffin wax. Tissues were cut into 4-μm-thick sections and stained with hematoxylin and eosin. The cytological subtypes of lung adenocarcinomas were identified according to previously described criteria (Kojima et al., 2017). Briefly, non-TRU adenocarcinoma was defined as adenocarcinomas consisting of tall columnar epithelial cells that are similar to well-differentiated bronchial surface epithelial cells. All the non-TRU adenocarcinomas examined here (39/39) were immunohistochemically positive for HNF4α, and some tumors (3/39) were also positive for TTF1 in small proportion (less than 10% of tumor cells). TRU adenocarcinoma was defined as tumors consisting of low columnar/cuboidal epithelial cells that show similar features to club cells and/or type 2 alveolar epithelial cells. All the TRU adenocarcinomas examined (21/21) were positive for TTF1, but were negative for HNF4α. Interstitial pneumonia was diagnosed using a multidisciplinary discussion according to the ATS/ERS/JRS/ALAT 2011 classification. Of 39 interstitial pneumonia lesions, these included 27 idiopathic pulmonary fibrosis, and 8 respiratory bronchiolitis interstitial lung disease, and 4 unclassifiable interstitial pneumonia.

Immunohistochemistry

Immunohistochemistry was performed using the Histostainer system (Nichirei, Tokyo, Japan). Briefly, tissue sections were deparaffinized, rehydrated, and incubated with blocking solution to block endogenous peroxidase activities and non-immunospecific protein binding. Sections were boiled in antigen retrieval buffer (heat processor solution pH 9.0) to retrieve masked epitopes and then incubated with the primary antibody against HNF4α (mouse monoclonal antibody clone H6939, Perseus Proteomics Inc., Tokyo, Japan) or TTF1 (mouse monoclonal antibody clone 8G7G3/1, DAKO, Ely, UK). Sections were then incubated with a horseradish peroxidase-labeled anti-mouse immunoglobulin antibody. Immunoreactivity was visualized using diaminobenzidine as a substrate, and nuclei were lightly counterstained with hematoxylin. Alternatively, dual immunohistochemistry was performed manually. Sections were incubated with antibodies against HNF4α (mouse monoclonal antibody clone H6939, Perseus Proteomics Inc.) and TTF1 (rabbit monoclonal antibody clone EP1584Y, Abcam, Cambridge, MA) after antigen retrieval. Sections were then incubated with a horseradish peroxidase-labeled anti-mouse immunoglobulin antibody (DAKO) and alkaline phosphatase-labeled anti-rabbit immunoglobulin antibody (DAKO). Immunoreactivity was visualized using NBT (DAKO) and ACE (Vector Laboratories, Burlingame, CA) as substrates.

Morphometry

Glass slides were scanned with a virtual slide system (Nanozoomer slide scanner, Hamamatsu Phonics, Hamamatsu, Japan). Morphometric analyses, such as the diameters of the bronchus/bronchioles, alveolar areas, and frequencies of HNF4α-expressing cells, were performed using analyzing software (NDP view version

2, Hamamatsu Phonics). The frequency of HNF4α-expressing cells was calculated as the rate of positive nuclei per up to 1000 or more epithelial cells in bronchi, bronchioles, alveoli, metaplastic lesions, and adenocarcinomas.

Quantitative real-time RT-PCR

Total RNA was extracted using the RNeasy mini kit (Qiagen, Valencia, CA) from snap frozen tissues. First-strand cDNA was synthesized from total RNA using the Superscript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA). The cDNA generated was used as a template in real-time PCR with SYBR Premix EXTaq (Takara, Kyoto, Japan) and run on a Thermal Cycler DICE real-time PCR system (Takara). The primer sets used were 5'-AGGCAAGATGCTGGCATAGCT (forward) and 5'-AGGCAAGATGCTGGCATAGCT (reverse) for HNF4α [NM_002046.5], and 5'-AGGCAAGATGCTGGCATAGCT (forward) and 5'-AGGCAAGATGCTGGCATAGCT (reverse) for KRT7 [NM_002205.2]. HNF4 expression levels were normalized to KRT7 levels.

Statistical analysis

Differences in the frequency of HNF4α-expressing cells and HNF4α mRNA level among bronchi, bronchioles, alveoli, metaplastic lesions, and adenocarcinomas were analyzed by the Wilcoxon/Kruskal-Wallis test. P values less than 0.05 were considered significant.

Results

HNF4α-expressing cells in a physiological state of the lung

Twelve bronchi, 20 interlobular bronchioles, 40 terminal bronchioles, 50 respiratory bronchioles, and 50 fields of alveolar areas were examined in the surgically resected lung tissues of 10 non-smoker patients with lung adenocarcinomas, that did not exhibit any histopathological changes. Here, we dared to examine the non-smokers' lung tissues to any confounding effects of smoking-related tissue damage on the expression of HNF4α. The frequencies and distributions of HNF4α-expressing cells were investigated at different airway levels. A representative photograph showing a morphometric analysis is shown in (Fig. 1). Overall, the expression of HNF4α was not frequently detected and was restricted to epithelial cells. In the bronchus, the expression of HNF4α was rarely detected in ciliated cells (Fig. 2), and was mostly absent in the goblet cells, basal cells, and epithelial cells constituting the bronchial glands (not shown). In lobular and terminal bronchioles, a small fraction of epithelial cells, mainly ciliated cells and occasionally basal cells, weakly expressed HNF4α (Fig. 2). Ciliated cells that expressed HNF4α tended to gather and form clusters consisting of 5 to 10 (or more) cells. In respiratory bronchioles, the expression of HNF4α was also mainly detected in ciliated cells and occasionally in basal cells (Fig. 2.), but at a lower frequency than in terminal bronchioles. The expression of HNF4α was extremely infrequent in the alveoli and was only rarely detected in flat pneumocytes (type 1 pneumocytes) (Fig. 2). The results are summarized in Table 1.

Immunostaining for HNF4α and TTF1 on serial sections revealed that HNF4α-expressing ciliated cells weakly expressed TTF1, but they were mutually exclusive from Clara cells and others that strongly expressed TTF1 (Fig. 3).

HNF4α-expressing cells in pathological states of the lung

Bronchiolar metaplasia (225 lesions from 55 patients who underwent surgical lung biopsy and 39 patients who

Table 1. Frequencies of HNF4α-expressing cells in physiological and pathological states.

Subjects	Diameter (μm)/*Area (mm^2)	Cell counts	Frequencies (%)
Physiological states			
Bronchus (12)	1234±550 (518-2280)	1099±429 (339-2005)	0.16±0.12 (0.00-0.36)
Interlobular bronchiole (20)	481±191 (224-981)	587±322 (249-1493)	2.52±3.51 (0.00-9.98)
Terminal bronchiole (40)	187±97 (89-258)	388±259 (71-1078)	6.99±5.39 (0.00-21.10
Repiratory bronchiole (50)	96±31 (38-168)	191±113 (66-665)	2.14±2.15 (0.00- 9.05)
Alevelolus (50)	*1.6±0.7 (0.8-4.9)	489±251 (211-1446)	0.07±0.15 (0.00-0.83)
Pathological states			
Bronchiolar metaplasia (255)	*0.2±0.2 (0.2-2.0)	327±259 (55-1962)	10.87±11.39 (0.00-78.34)
Non-TRU adenocarcinoma (39)	*0.7±0.7 (0.2-1.2)	523±310 (300-1200)	94.58±5.61 (79.00-100.00)
TRU adenocarcinoma (21)	*1.0±0.8 (0.3-1.8)	550±463 (100-1100)	#0.00±0.00 (0.00-0.00)

( ), number of samples; values of mean ± standard deviation (range) are shown. Differences in frequencies of HNF4α-expressing cells between every pair of subjects are analayzed with Wilcoxon test. The those are significantly higher; interlobular bronchiole than bronchus (P=0.0002) and alveolus (P<0.0001); terminal bronchiole than interlobular bronchiole (P=0.0004), respiratory bronchiole (P<0.0001), and alveolus (P<0.0001); non-TRU adenocarcinoma than brochiolar metaplasia (P<0.0001), and TRU adenocarcinoma (P<0.0001). The difference between brochiolar metaplasia and terminal bronchiole is not significant (P=0.1963). # is out of the range, actually, that is 0.0007± 0.001 (0-0.003).

underwent lobectomy for non-TRU lung adenocarcinomas) were examined. Generally, metaplastic lesions often appear from terminal bronchiole to alveolar ducts, and are called peribronchiolar metaplasia. Metaplastic lesions also appear on lumens of honeycomb lesions (but not only in

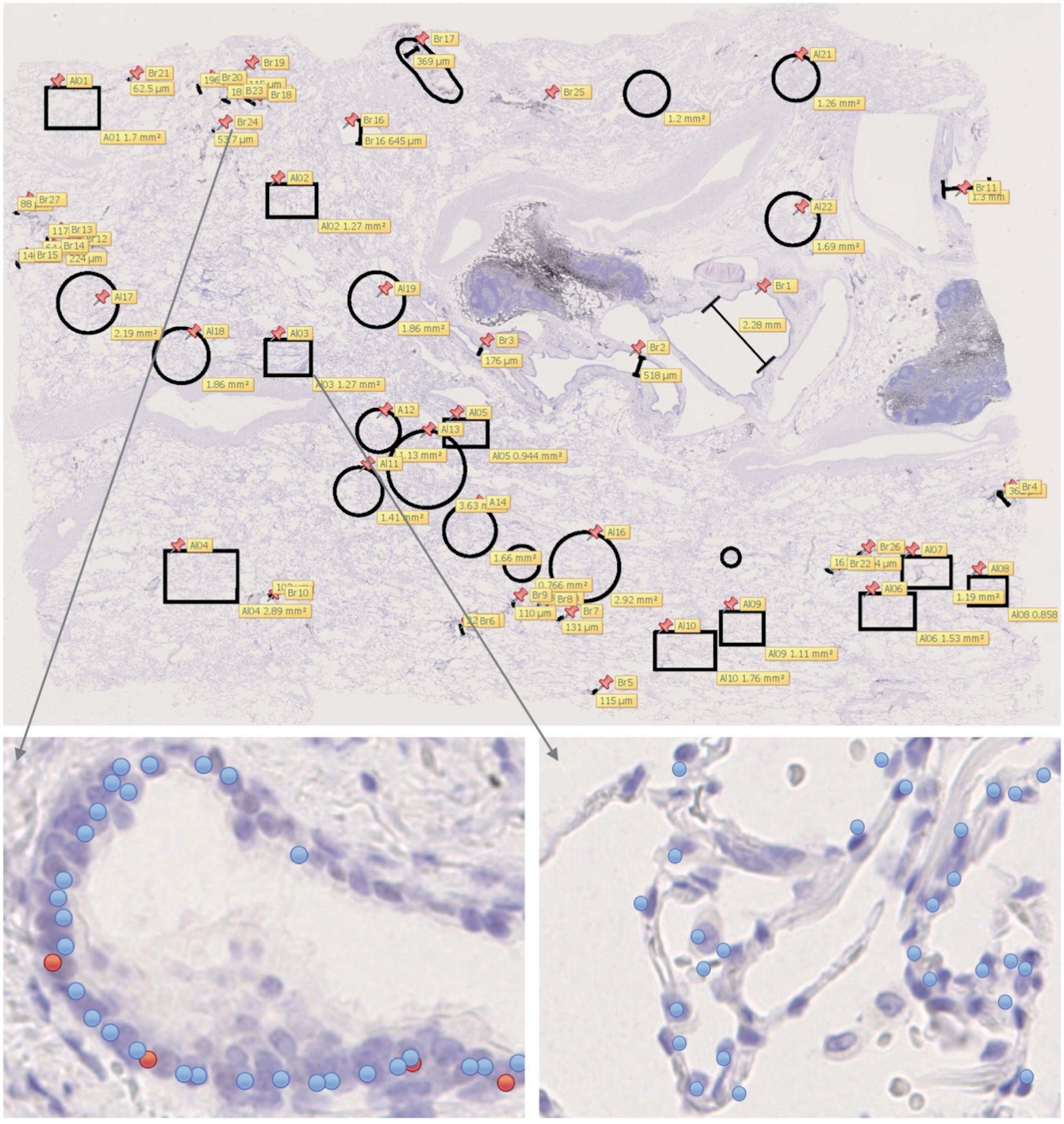

Fig. 1. Morphometric values, such as the diameters of the bronchus/bronchioles (µm), and alveolar areas (mm^2), and frequencies of HNF4α-expressing cells, were measured using analyzing software (NDP view version 2, Hamamatsu Phonics). HNF4α-positive and negative cells are counted by manually marking them red and blue dots, respectively. Br: conducing airway including bronchus and different levels of bronchiole. Al: alveolus.

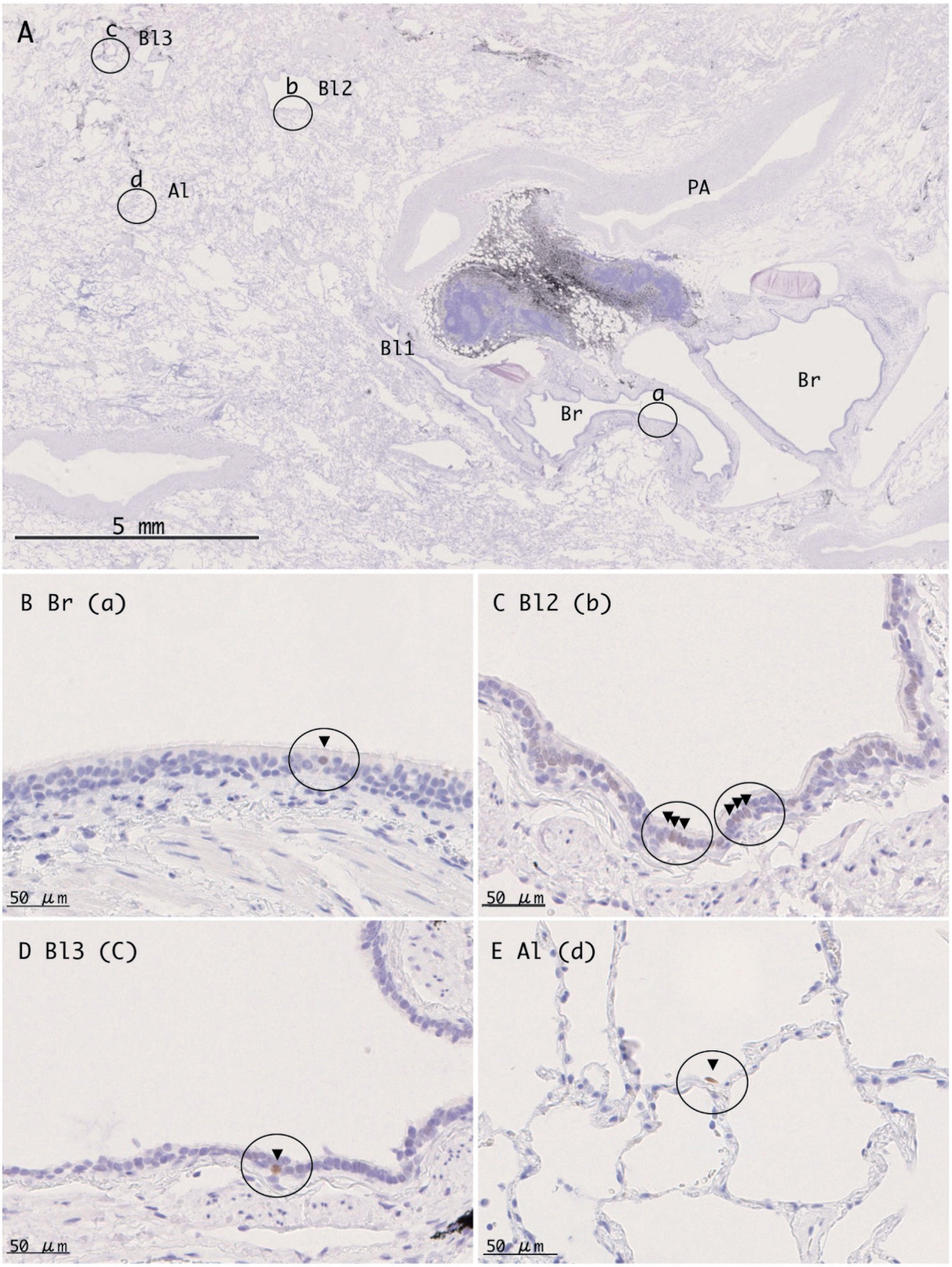

Fig. 2. Immunohistochemical expression of HNF4α at different levels of airway epithelia: A scanning view is shown **(A)**. Bronchus (Br), lobular bronchiole (Bl1), terminal bronchiole (Bl2), respiratory bronchiole (Bl3), alveolus (Al), and PA (pulmonary artery) are indicated. The circled areas a, b, and c are magnified and shown in panels **(B), (C), and (D),** respectively. HNF4α-expressing cells are circled in the panels. In the bronchus, HNF4α expression was rarely detected in columnar ciliated cells (**B**). In terminal bronchioles, a small fraction of cuboidal ciliated cells expressed HNF4α **(C).** In respiratory bronchioles, HNF4α expression was mainly detected in cuboidal ciliated cells and occasionally in basal cells **(D)**. In the alveolus, HNF4α expression was extremely infrequent, and only rarely detected in flat pneumocytes (type 1 pneumocytes) **(E)**.

peribronchiolar areas). Thus, we here preferentially used a term "bronchiolar metaplasia" to mean both. Non-TRU lung adenocarcinomas (39 tumors from 39 patients) that developed in lungs with idiopathic interstitial pneumonia, and TRU lung adenocarcinomas that developed in patients unrelated to idiopathic interstitial pneumonia (21 tumors for 21 patients), were also examined. The TRU lung adenocarcinomas served as a reference group. The majority of bronchiolar metaplastic lesions mainly consisted of cuboidal ciliated cells, similar to the terminal bronchioles (Fig. 4). The frequencies of HNF4α-expressing cells and their immunohistochemical intensities considerably varied among the lesions. Typically, the metaplastic lesions expressed HNF4α as frequently and strongly as the terminal bronchioles (Fig. 4). Some of the lesions expressed HNF4α more frequently (Fig. 4), while others sometimes expressed it more strongly than the terminal bronchioles (Fig. 4). In all non-TRU lung adenocarcinomas, the majority of neoplastic cells strongly expressed HNF4α (Fig. 4). In contrast, in TRU lung adenocarcinomas, almost no neoplastic cells expressed HNF4α (not shown). The results are summarized in Table 1.

The levels of HNF4α expressions appeared to be increasing during the transition from metaplasia to non-TRU adenocarcinoma (Fig. 5). In contrast, the levels of TTF1 expressions appeared to be decreasing (Fig. 5). Intermediate lesion consisted of (a mixture of) cells expressing either HNF4α, TTF1, or both, and showed atypical morphological changes that were less remarkable than overt non-TRU adenocarcinoma (Fig. 5).

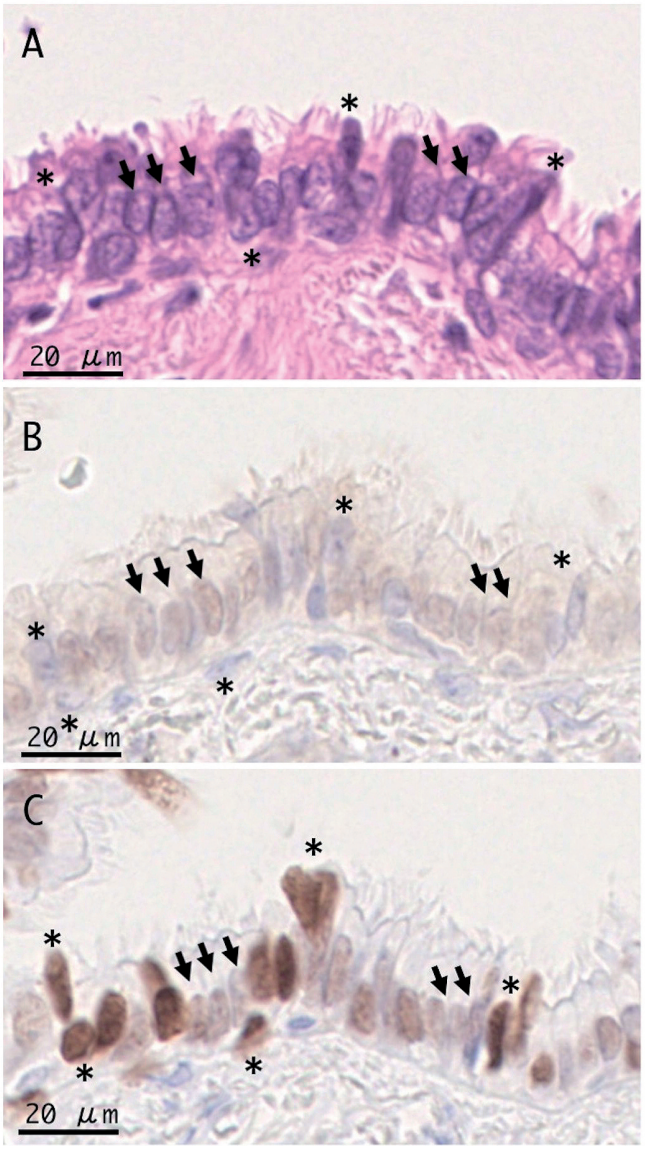

Fig. 3. Immunohistochemical expression of HNF4α and TTF1 in ciliated cells in terminal bronchioles: Serial sections are examined. Hematoxylin and eosin staining **(A)**, immunohistochemistry for HNF4α **(B)**, and for TTF1 **(C)** on serial sections are shown. In all panels, asterisks indicate HNF4α-positive cells, and arrows indicate TTF1-positive cells.

Difference in HNF4α levels between physiological and pathological states

The frequencies of HNF4α-expressing cells at different airway levels, in bronchiolar metaplastic lesions, in non-TRU adenocarcinomas, and in TRU adenocarcinomas were plotted on a graph (Fig. 6), and differences among groups were analyzed. In a physiological state, the frequency of HNF4α-expressing cells was the highest in the terminal bronchioles, and a significant difference was observed between the terminal bronchioles and others, including the bronchi, respiratory bronchioles, and alveoli (Table 1; Wilcoxon/Kruskal-Wallis test, $P<0.05$). The frequencies of HNF4α-expressing cells were similar between the terminal bronchioles and the bronchiolar metaplastic lesions (Table 1). The frequency of HNF4α-expressing cells in non-TRU adenocarcinoma was significantly higher than those in the others (Table 1; Wilcoxon/Kruskal-Wallis test, $P<0.05$). As described above, some metaplastic lesions expressed HNF4α at a markedly higher frequency. Particularly, two out of 255 bronchiolar metaplastic lesions (from 2 out of 87 patients) showed more frequent and stronger HNF4α expression (Fig. 6 dots circled), and those showed atypical morphological changes that looked less remarkable than overt non-TRU adenocarcinoma (Fig. 4 third panel).

To further confirm this relationship, HNF4α mRNA levels were quantified in normal lungs (10 tissues), idiopathic interstitial pneumonia with bronchiolar metaplasia (11 lesions), idiopathic interstitial pneumonia without bronchiolar metaplasia (12 lesions), non-TRU adenocarcinoma (20 tumors) and TRU adenocarcinoma (16 tumors), and differences in these levels between the groups were analyzed. HNF4α mRNA levels were significantly higher in idiopathic interstitial pneumonia

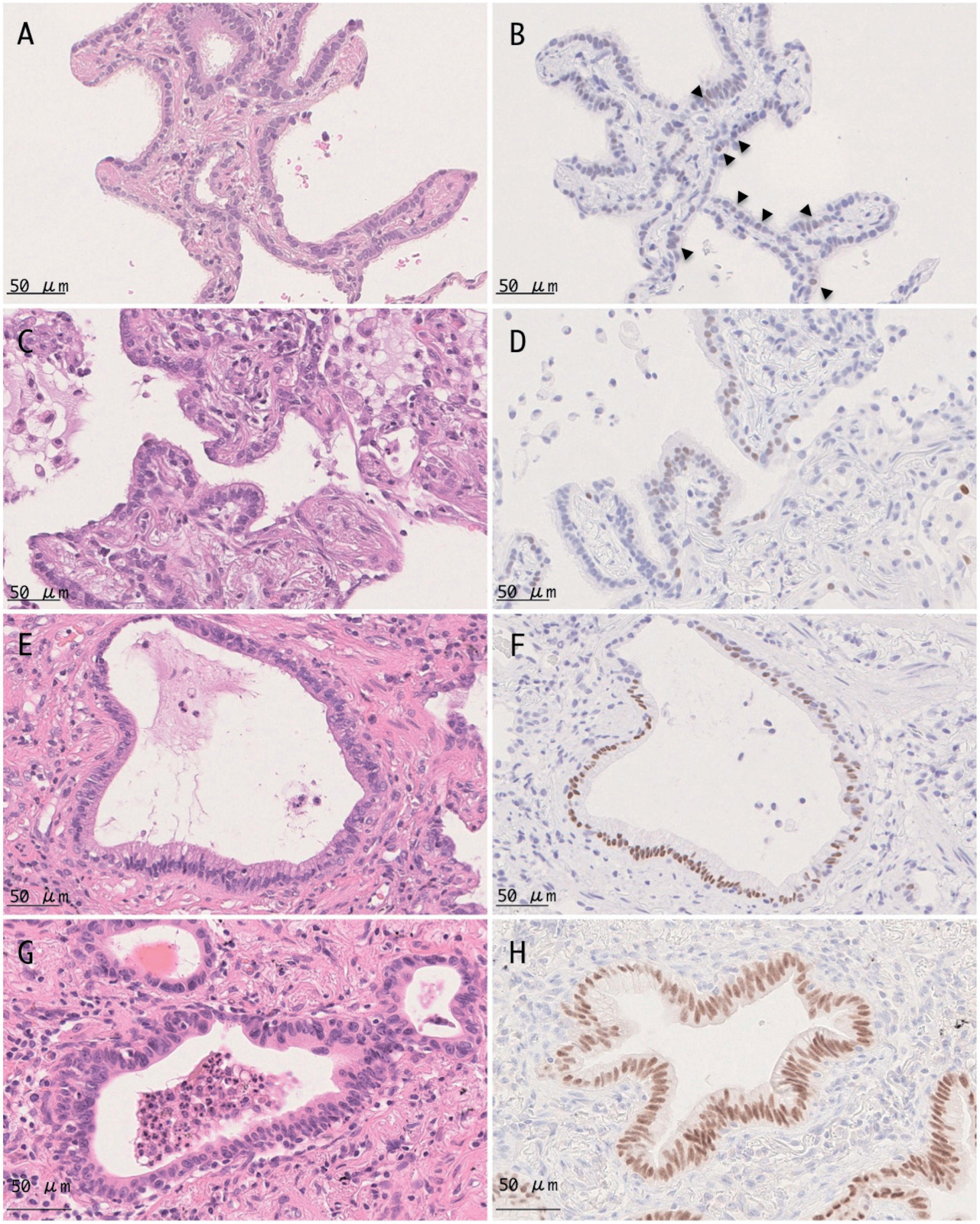

Fig. 4. Immunohistochemical expression of HNF4α in bronchiolar metaplasia and non-TRU lung adenocarcinoma that develop in lungs with idiopathic interstitial pneumonia: Typical bronchiolar metaplastic lesions mainly consist of ciliated cells and occasionally and weakly express HNF4α (**A, B**). Some lesions express HNF4α more frequently **(C, D**), while others express it more strongly than the terminal bronchioles **(E, F)**. Non-TRU lung adenocarcinoma expresses HNF4α more frequently and strongly **(G, H).** A, C, E, and G, hematoxylin and eosin staining. B, D, F, and H, immunohistochemistry for HNF4α.

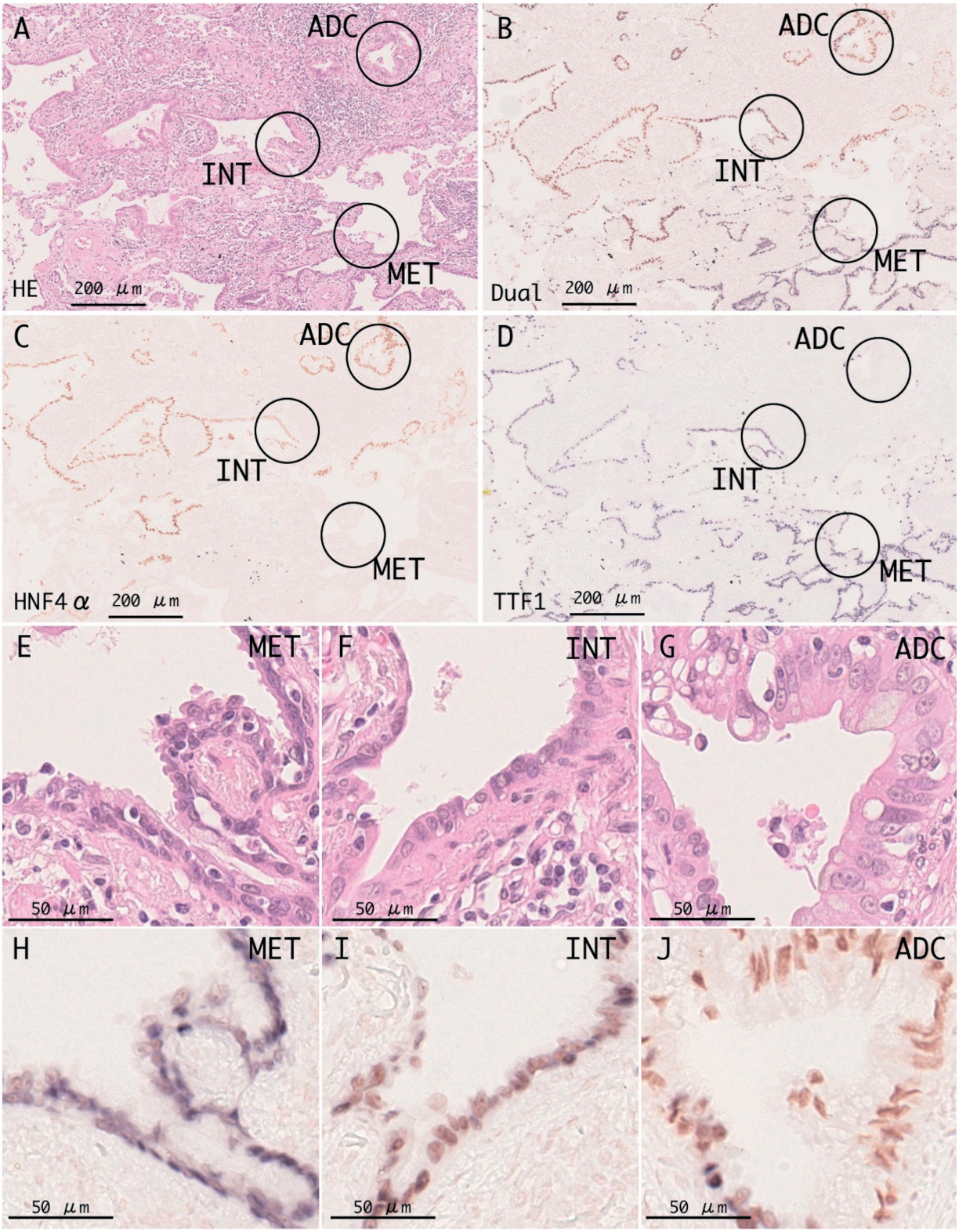

Fig. 5. Immunohistochemical expressions of HNF4α and TTF1 during the transition from metaplasia to non-TRU adenocarcinomas: A representative case with dual immunohistochemistry for HNF4α (brown signal from ACE as a substrate) and TTF1 (black signal from NBT as a substrate) are shown. **A** (HE), **B** (dual for both HNF4α and TTF1), **C** (single for HNF4α), and **D** (single for TTF1), show scanning views. The circles mark focused parts of bronchiolar metaplasia (MET), intermediated lesion (INT), and non-TRU adenocarcinoma (ADC). The focused parts were shown with close-up views; metaplasia **E** (HE) and **H** (dual for both HNF4α and TTF1), intermediate **F** (HE) and **I** (dual for both HNF4α and TTF1), non-TRU adenocarcinoma **G** (HE) and **J** (dual for both HNF4α and TTF1).

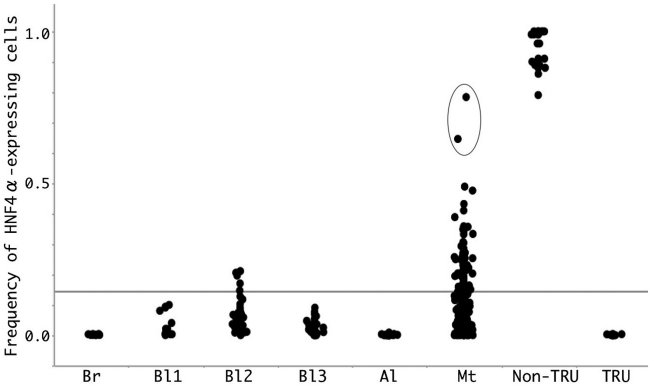

Fig. 6. Frequencies of HNF4α-expressing cells at different airway levels, in bronchiolar metaplasia, in non-TRU adenocarcinomas (non-TRU), and in TRU adenocarcinomas. The frequencies of HNF4α-expressing cells in the bronchus (Br), lobular bronchiole (Bl1), terminal bronchioles (Bl2), respiratory bronchioles (Bl3), alveoli (Al), bronchiolar metaplasia (Mt), non-TRU adenocarcinomas (non-TRU), and TRU adenocarcinomas (TRU), are plotted. The frequency of HNF4α-expressing cells was significantly higher in Bl2 than in Br, Bl3, and Al (Wilcoxon/Kruskal-Wallis test, $P<0.05$). The frequency of HNF4α-expressing cells was significantly higher in ADC than in the others. The frequencies of HNF4α-expressing cells were similar between Bl2 and Mt. Notably, two metaplastic lesions express HNF4α with an unequivocally higher frequency and stronger level (circle).

with marked metaplasia and non-TRU adenocarcinomas than in normal lungs and idiopathic interstitial pneumonia without metaplasia (Wilcoxon/Kruskal-

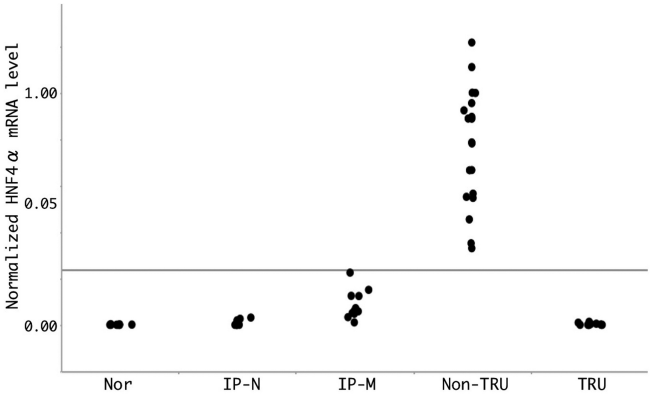

Fig. 7. HNF4α mRNA expression levels in normal lungs (Nor), lungs with interstitial pneumonia without bronchiolar metaplasia (IP-N), lungs with prominent bronchiolar metaplasia (IP-M), non-TRU adenocarcinomas (non-TRU), and TRU adenocarcinomas, are plotted. HNF4α mRNA expression levels were significantly higher in IP-M and non-TRU than in either Nor, IP-N, or TRU (Wilcoxon/Kruskal-Wallis test, $P<0.05$).

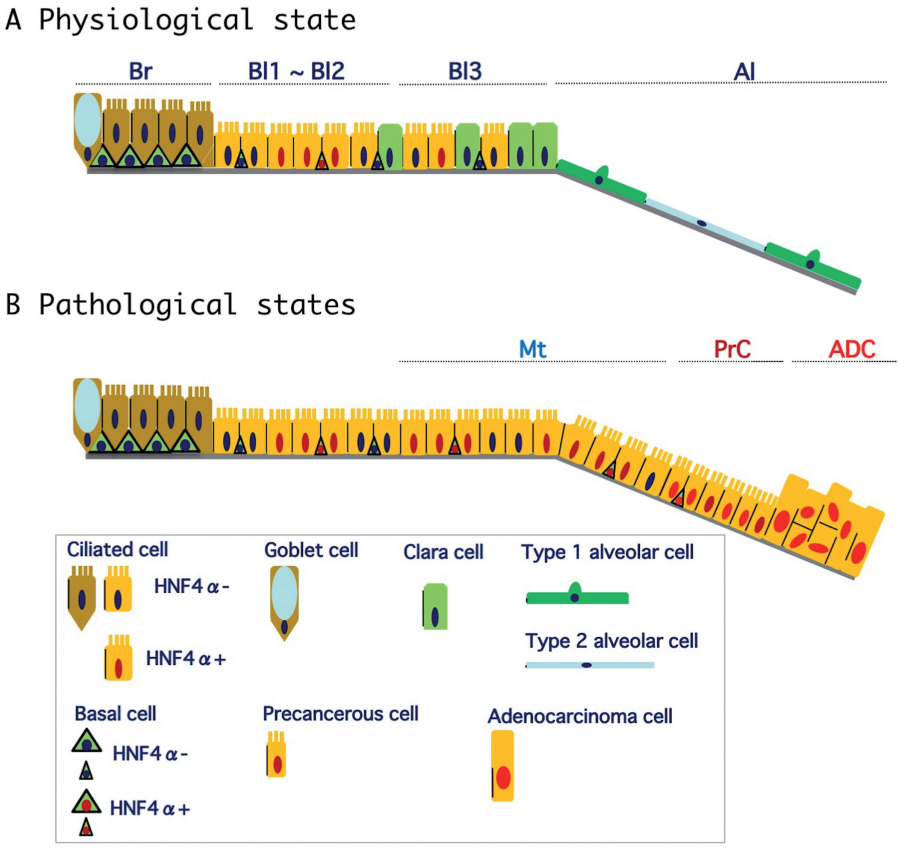

Fig. 8. Schema showing the distribution of HNF4α-expressing cells at different airway levels (Br, bronchus; Bl1, lobular bronchiole; Bl2, terminal bronchiole; Bl3, respiratory bronchiole; Al, alveolus) in a physiological state (upper panel) and changes in pathological states, including bronchiolar metaplasia and non-TRU adenocarcinoma (lower panel): HNF4α-expressing cells are mainly located in the terminal bronchioles (Bl2) and have the appearance of ciliated cells in a physiological state (upper panel). Typical bronchiolar metaplasia (Mt) mainly consists of ciliated cells, similar to the terminal bronchioles, and express HNF4α as frequently as the terminal bronchioles (lower panel). Metaplastic lesion occasionally expresses HNF4αα with an unequivocally higher frequency and stronger level, and this may be a precancerous lesion (PrC) (lower panel). Non-TRU lung adenocarcinoma (ADC) is speculated to originate from ciliated cells and develop through this precancerous (PrC) lesion (lower panel).

Wallis test, P<0.001) (Fig. 7). HNF4α mRNA levels increased in the order of idiopathic interstitial pneumonia without bronchiolar metaplasia, idiopathic interstitial pneumonia with marked bronchiolar metaplasia, and non-TRU adenocarcinomas (Fig. 7).

Discussion

The concept of TRU and non-TRU subpopulations has contributed to our understanding of the histogenesis of lung adenocarcinomas (Yatabe, 2010). We have been interested in airway epithelial cells that express HNF4α as the potential precursor for non-TRU lung adenocarcinoma. We herein immunohistochemically examined normal lungs for the expression of HNF4α and described its frequency and distribution at different airway levels. HNF4α expression was also examined in bronchiolar metaplasia and non-TRU lung adenocarcinomas that developed in lungs with idiopathic interstitial pneumonias to confirm their sequential association.

The present results revealed a minor subpopulation of epithelial cells that expressed HNF4α, even in a physiological state. This subpopulation was mainly located in the terminal bronchioles and had the appearance of ciliated cells, which are mutually exclusive from Clara cells and others that strongly express TTF1. This result suggests that these ciliated cells are a non-TRU subpopulation. HNF4α is well known as an essential transcriptional factor maintaining differentiation of digestive tract epithelia, hepatocytes, and pancreatic cells (Taraviras et al., 1994; Colleypriest et al., 2017). A non-TRU subpopulation may have the potential to differentiate to these cell types. It seems consistent with the fact that non-TRU lung adenocarcinomas often showed the gastric/intestinal phenotypes such as some mucins expression (Kunii et al., 2011; Sugano et al., 2013). Moreover, columnar ciliated cells located in bronchi and lobular bronchioles rarely expressed HNF4α. Thus, ciliated cells in the distal and proximal airways may essentially be distinct.

We also demonstrated that the majority of bronchiolar metaplastic lesions mainly consisted of ciliated cells, similar to the terminal bronchioles, and expressed HNF4α as frequently as the terminal bronchioles. This result suggests that bronchiolar metaplasia is a heterotopic expansion of distal airway epithelia, particularly of the terminal bronchioles, but not proximal airway epithelia (Fig. 8). We recently proposed that bronchiolar metaplasia is the potential field in which non-TRU lung adenocarcinomas develop (Kojima et al., 2017; Okudela et al., 2018). Ciliated cells were previously speculated to be a normal counterpart that non-TRU lung adenocarcinomas originate from (Park et al., 2012; Kim et al., 2016) since Kimula et al. originally named this group of adenocarcinomas the bronchial surface epithelial type (Kimula, 1978). Therefore, non-TRU lung adenocarcinomas appear to originate from ciliated cells that express HNF4α in either bronchiolar metaplasia or possibly normal distal airways (Fig. 8).

Metaplasia is a precancerous condition in certain types of cancers, e.g., gastric adenocarcinoma develops from gastric intestinal metaplasia and squamous cell carcinoma from squamous cell metaplasia in different organs, such as the bronchus, esophagus, and uterine cervix (Meyer and Liebow, 1965). A recent molecular biological study suggested that metaplasia is a re-programming process in which tissue stem cells accumulate, and, thus, results in increased susceptibility to neoplastic transformation (Fujii et al., 2002). Furthermore, metaplasia is not simply heterotopic tissue regeneration, and may be a pathological state caused by some molecular alterations (Nielsen et al., 2004; Coon et al., 2006). Our results showing that some metaplastic lesions exhibited unequivocally higher frequencies and stronger levels of HNF4α expression are consistent with this notion. These may be precancerous lesions (Fig. 8). We are now interested in investigating metaplastic lesions that strongly express HNF4α in more detail in order to elucidate the histogenesis of non-TRU lung adenocarcinomas. A comprehensive analysis of genetic alterations and comparison of mutation profiles between these metaplastic lesions and non-TRU adenocarcinomas are expected to further confirm their sequential association.

In summary, the present study is the first to describe the frequency and distribution of HNF4α-expressing cells in the different airway levels in a physiological state, and suggests that ciliated cells expressing HNF4α in bronchiolar metaplasia are the precursor for non-TRU lung adenocarcinomas particularly in idiopathic interstitial pneumonia.

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References

Colleypriest B.J., Burke Z.D., Griffiths L.P., Chen Y., Yu W.Y., Jover R., Bock M., Biddlestone L., Quinlan J.M., Ward S.G., Mark Farrant J., Slack J.M. and Tosh D. (2017). Hnf4alpha is a key gene that can generate columnar metaplasia in oesophageal epithelium. Differentiation 93, 39-49.

Coon D.R., Roberts D.J., Loscertales M. and Kradin R. (2006). Differential epithelial expression of shh and foxf1 in usual and nonspecific interstitial pneumonia. Exp. Mol. Pathol. 80, 119-123.

Fujii T., Dracheva T., Player A., Chacko S., Clifford R., Strausberg R.L., Buetow K., Azumi N., Travis W.D. and Jen J. (2002). A preliminary transcriptome map of non-small cell lung cancer. Cancer Res. 62, 3340-3346.

Kim M.H., Cho J.S., Kim Y., Lee C.H., Lee M.K. and Shin D.H. (2016). Discriminating between terminal- and non-terminal respiratory unit-type lung adenocarcinoma based on microrna profiles. PLoS One

11, e0160996.
Kimula Y. (1978). A histochemical and ultrastructural study of adenocarcinoma of the lung. Am. J. Surg. Pathol. 2, 253-264.
Kimura S., Hara Y., Pineau T., Fernandez-Salguero P., Fox C.H., Ward J.M. and Gonzalez F.J. (1996). The T/ebp null mouse: Thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes Dev. 10, 60-69.
Kojima Y., Okudela K., Matsumura M., Omori T., Baba T., Sekine A., Woo T., Umeda S., Takemura T., Mitsui H., Suzuki T., Tateishi Y., Iwasawa T., Arai H., Tajiri M., Ogura T., Kameda Y., Masuda M. and Ohashi K. (2017). The pathological features of idiopathic interstitial pneumonia-associated pulmonary adenocarcinomas. Histopathology 70, 568-578.
Kunii R., Jiang S., Hasegawa G., Yamamoto T., Umezu H., Watanabe T., Tsuchida M., Hashimoto T., Hamakubo T., Kodama T., Sasai K. and Naito M. (2011). The predominant expression of hepatocyte nuclear factor 4alpha (HNF4alpha) in thyroid transcription factor-1 (ttf-1)-negative pulmonary adenocarcinoma. Histopathology 58, 467-476.
Masai K., Tsuta K., Motoi N., Shiraishi K., Furuta K., Suzuki S., Asakura K., Nakagawa K., Sakurai H., Watanabe S.I., Hiraoka N. and Asamura H. (2016). Clinicopathological, immunohistochemical, and genetic features of primary lung adenocarcinoma occurring in the setting of usual interstitial pneumonia pattern. J. Thorac. Oncol. 11, 2141-2149.
Meyer E.C. and Liebow A.A. (1965). Relationship of interstitial pneumonia honeycombing and atypical epithelial proliferation to cancer of the lung. Cancer 18, 322-351.
Nielsen C.M., Williams J., van den Brink G.R., Lauwers G.Y. and Roberts D.J. (2004). Hh pathway expression in human gut tissues and in inflammatory gut diseases. Lab. Invest. 84, 1631-1642.
Okudela K., Kojima Y., Matsumura M., Arai H., Umeda S., Tateishi Y., Mitsui H., Suzuki T., Tajiri M., Ogura T. and Ohashi K. (2018). Relationship between non-tru lung adenocarcinomas and bronchiolar metaplasia - potential implication in their histogenesis. Histol. Histopathol. 33, 317-326.
Park W.Y., Kim M.H., Shin D.H., Lee J.H., Choi K.U., Kim J.Y., Park D.Y., Lee C.H. and Sol M.Y. (2012). Ciliated adenocarcinomas of the lung: A tumor of non-terminal respiratory unit origin. Mod. Pathol. 25, 1265-1274.
Sugano M., Nagasaka T., Sasaki E., Murakami Y., Hosoda W., Hida T., Mitsudomi T. and Yatabe Y. (2013). HNF4alpha as a marker for invasive mucinous adenocarcinoma of the lung. Am. J. Surg. Pathol. 37, 211-218.
Taraviras S., Monaghan A.P., Schutz G. and Kelsey G. (1994). Characterization of the mouse HNF-4 gene and its expression during mouse embryogenesis. Mech. Dev. 48, 67-79.
Yatabe Y. (2010). EGFR mutations and the terminal respiratory unit. Cancer Metastasis Rev. 29, 23-36.

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