DOCTORAL THESIS

Prognostic impact of HNF4α expression in interstitial lung disease

(間質性肺疾患における HNF4α 発現と予後指標としての有用性)

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Tomoe Sawazumi

澤住 知枝

Department of Pathology

Yokohama City University Graduate School of Medicine

横浜市立大学医学部 病態病理学

(Deputy Doctoral Supervisor: Satoshi Fujii)

代行指導教員：藤井 誠志 教授

(Supervisor: Koji Okudela, Associate Professor)

研究指導教員：奥寺 康司 准教授

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Prognostic impact of HNF4α expression in interstitial lung disease

Tomoe Sawazumi[1,2] | Tomohisa Baba[4] | Tae Iwasawa[5] | Hiromasa Arai[6] | Mai Matsumura[2] | Tamiko Takemura[7] | Misaki Sugiyama[7] | Motoki Sekiya[7] | Yusuke Saigusa[3] | Takashi Ogura[4] | Yoshiaki Inayama[1] | Kenichi Ohashi[2] | Koji Okudela[2]

[1]Division of Pathology, Yokohama City University Medical Center Hospital, Yokohama, Japan

[2]Department of Pathology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

[3]Department of Biostatistics, Yokohama City University Graduate School of Medicine, Yokohama, Japan

[4]Division of Respiratory Medicine, Kanagawa Cardiovascular and Respiratory Center Hospital, Yokohama, Japan

[5]Division of Radiology, Kanagawa Cardiovascular and Respiratory Center Hospital, Yokohama, Japan

[6]Division of General Thoracic Surgery, Kanagawa Cardiovascular and Respiratory Center Hospital, Yokohama, Japan

[7]Division of Pathology, Kanagawa Cardiovascular and Respiratory Center Hospital, Yokohama, Japan

Correspondence
Koji Okudela, MD, PhD, Department of Pathology, Yokohama City University Graduate School of Medicine, 3-9, Fukuura, Kanazawa-ku 236-0004, Yokohama, Japan.
Email: kojixok@yokohama-cu.ac.jp

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Abstract

Pneumocyte injury is a crucial factor influencing the severity of interstitial lung disease (ILD). In this study, we investigated the potential of hepatocyte nuclear factor α (HNF4α) as an immunohistochemical marker to detect pneumocyte injury and as a prognostic marker. Surgical lung biopsy specimens were collected from 309 patients with different types of ILDs (61 idiopathic pulmonary fibrosis (IPF), 173 non-IPF, and 75 unclassifiable ILD). HNF4α expression were examined and the frequency of positive cells (per mm^2) was calculated. HNF4α was strongly expressed in regenerating pneumocytes present on fibroblastic foci, Masson bodies/organizing alveoli. In the non-IPF and unclassifiable ILD groups, cases with high frequency expression showed significantly poorer outcome. Particularly, in the unclassifiable ILD group, the prognostic impact was more significant (death due to ILD, log-rank test, $p < 0.0001$), with a 10-year survival rate (hazard ratio 11.1, Wald test, $p = 0.0003$), as compared to the non-IPF group (log-rank test, *$p = 0.0269$; hazard ratio 2.7, Wald test, $p = 0.0334$).* Multivariable analysis focusing on the unclassifiable ILD group confirmed that the frequent HNF4α expression was an independent prognostic factor (hazard ratio 28.6; Wald test, $p = 0.0033$). Thus, HNF4α can be utilized as an immunohistochemical marker for pneumocyte injury and have prognostic impact particularly in unclassifiable ILD.

KEYWORDS
HNF4α, interstitial lung disease, prognosis

Abbreviations: ALAT, Latin American Thoracic Association; ATS, American Thoracic Society; DLco, diffusing capacity of carbon monoxide; ERS, European Respiratory Society; FVC, forced vital capacity; JRS, Japanese Respiratory Society; KL-6, Krebs von den Lungen-6; PaO_2, partial pressure of arterial oxygen; PFT, pulmonary function test; SP-D, surfactant protein-D.

INTRODUCTION

Interstitial lung disease (ILD) is an umbrella term for a large group of disorders that affect the alveolar septa, causing the airspaces to collapse, and they sometimes progress to honeycomb lesions. ILDs may be associated with other diseases or environmental exposures; for example, connective tissue disease-related ILD (CTD-ILD) and hypersensitivity pneumonia (HP). However, most ILDs are diseases of unknown causes (idiopathic interstitial pneumonia [IIP]).[1,2] The prognosis of patients depends on the disease type.[1–4] According to the current American Thoracic Society (ATS)/European Respiratory Society (ERS), IIPs are categorized as major IIPs, rare IIPs, and unclassifiable IIPs.[4] The major IIPs include three groups consisting of two disease types each; chronic fibrosing IIP (idiopathic pulmonary fibrosis [IPF], nonspecific interstitial pneumonia [NSIP]), smoking-related IIP (respiratory bronchiolitis-interstitial lung disease [RB-ILD]), desquamative interstitial pneumonia (DIP), and acute/subacute IIP (cryptogenic organizing pneumonia [COP]), and acute interstitial pneumonia (AIP). The rare IIPs include idiopathic lymphoid interstitial pneumonia (LIP) and idiopathic pleuroparenchymal fibroelastosis (PPFE).[4] The unclassifiable IIPs are ones that cannot be specifically classified even through multidisciplinary discussion (MDD) according to standard criteria.[5] A recent meta-analytical study indicated that there could be considerable heterogeneity in the unclassifiable IIPs because of the lack of diagnostic standardization.[6]

Surgical lung biopsy (SLB) is recommended for patients with atypical clinical features and radiological findings.[7,8] Histopathologically, disease subtypes are determined based on levels of inflammatory infiltrates and spatial/temporal patterns of active fibrosis, such as fibroblastic foci (young fibroblastic proliferation on collapsed alveoli at the periphery of the lobules) and Masson bodies/organizing alveoli (young fibroblastic proliferation into the alveolar space or on the intralobular alveolar septa). However, these pathological changes are nonspecific and often cause interobserver discrepancies.[9] Reliable immunohistochemical markers are necessary to improve the diagnostic reproducibility.

Hepatocyte nuclear factor 4α (HNF4α) was initially identified as a transcription factor that induces hepatocyte and enterocyte differentiation.[10,11] It is generally used as a molecular biological marker for mucinous and enteric subtypes of lung adenocarcinomas.[12] During a series of our recent studies on ILD-associated lung adenocarcinomas,[13,14] we noted that regenerating pneumocytes lying on either fibroblastic foci and Masson bodies/organizing alveoli in the background of non-tumorous lung strongly expressed HNF4α. HNF4α was also reported as weakly expressed in metaplastic epithelia in peri-bronchiolar areas and honeycomb lesions,[14] suggesting HNF4α-positive cells could proliferate in the damaged foci, and eventually produce bronchiolar metaplasia. HNF4α could play important roles in tissue remodeling, such as bronchiolization and honeycombing.

Thus, we focused on HNF4α and investigated its potential as an immunohistochemical marker to detect pneumocyte injury and as a prognostic marker.

MATERIALS AND METHODS

Patients and lung tissues

A total of 309 patients with ILD who underwent SLB at the Kanagawa Cardiovascular and Respiratory Center (Yokohama, Japan) between January 2008 and May 2019 were examined. The patients did not receive any treatment for ILD before undergoing a biopsy. The median follow-up period was 1650 days (range, 6–4422 days). During the follow-up period, 67 patients died of ILD-related respiratory failure (47), lung cancer (6), other cancers (2), infectious diseases (5), right ventricular failure (2), transplant rejection (1) and other unspecified causes (4). Informed consent for the use of the resected materials for research purposes was obtained from all patients. The Ethics Committees of Yokohama City University (A180726009) and Kanagawa Prefectural Cardiovascular Respiratory Center Hospital (KCRC-17-0015) approved this study.

Conventional histopathological examination

Lung tissues were fixed with 10% buffered formaldehyde solution for approximately 24 h and embedded in paraffin wax. Sections were cut from tissues and stained with hematoxylin and eosin, Elastica van Gieson, and Alcian blue periodic acid Schiff. We determined pathological categories: UIP-definite, UIP-probable, UIP-indeterminate, and alternative groups, according to the official ATS/ERS/Japanese Respiratory Society (JRS)/Latin American Thoracic Association (ALAT) 2018 clinical practice guidelines.[8]

Disease subtyping

Final diagnoses were reached through MDD based on clinical, radiological, and pathological findings, where three pathologists, two radiologists, and three respirologists participated, and included 61 cases of IPF, 173 of non-IPF, and 75 of unclassifiable ILDs (i.e. a meaning equal to unclassifiable IIPs). The proportion of disease type was comparable to that in a recently published study.[6,15–17] Furthermore, the non-IPF cases were classified into 48 cases of CTD-ILD, 60 of HP, 55

TABLE 1 Baseline characteristics of the patients

Subjects	IPF (61)	Non-IPF (173)	Unclass (75)	*p*-value
Age				
<65 y/o	34.4 (21)	51.5 (89)	38.7 (29)	0.0331
≥65 y/o	65.6 (40)	48.5 (84)	61.3 (46)	
Sex				
Male	78.7 (48)	51.5 (89)	60.0 (45)	0.0008
Female	21.3 (13)	48.5 (84)	40.0 (30)	
Smoking status				
Non/light (<40 PYI)	55.7 (34)	84.5 (142)	73.3 (55)	<0.0001
Heavy (≥40 PYI)	44.3 (27)	15.5 (26)	26.7 (20)	
Laboratory findings				
SP-D < 110 ng/ml	18.0 (11)	22.5 (39)	18.7 (24)	0.7386
SP-D ≥ 110 ng/ml	82.0 (50)	77.5 (134)	81.3 (61)	
KL-6 < 500 U/ml	18.0 (11)	10.0 (17)	13.3 (10)	0.2265
KL-6 ≥ 500 U/ml	82.0 (50)	90.0 (156)	86.7 (65)	
PFT				
%FVC ≥ 80	59.0 (36)	56.5 (96)	53.3 (40)	0.7951
%FVC < 80	41.0 (25)	43.5 (74)	46.7 (35)	
%DLco ≥80	50.8 (31)	41.8 (72)	41.3 (31)	0.4428
%DLco <80 or cannot perform	49.2 (30)	58.2 (100)	4 (44)	

Notes: % and number of cases () is indicated; the total number does not equal to 309, regarding the smoking status, PFT, and SP-D level, because there were some cases that could not be evaluated.

Abbreviations: DLco, diffusing capacity of carbon monoxide; FVC, forced vital capacity; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den Lungen-6; PFT, pulmonary function test; PYI, pack-year index; SP-D, surfactant protein-D; Unclass, unclassifiable ILD; y/o, years old at biopsy.

p values were calculated using Fisher's exact test or the Wilcoxon test among the three groups.

of idiopathic NSIP, five of idiopathic DIP, and five PPFE. The CTDs included eight cases of rheumatoid arthritis, 10 of systemic sclerosis, nine of Sjogren's syndrome, six of polymyositis/dermatomyositis, four of mixed connective tissue disease, and 11 of anti-neutrophil cytoplasmic antigen-related disease.[18–23]

Immunohistochemistry

Immunohistochemistry was performed using a Histostainer system (Nichirei). Briefly, tissue sections were incubated with the blocking solution to block endogenous peroxidase activity and non-immunospecific protein binding, and the blocked tissue sections were then boiled in antigen retrieval buffer (heat processor solution pH 9.0). The sections were incubated with primary antibodies against HNF4α (mouse monoclonal antibody clone H6939; Perseus Proteomics Inc.) or thyroid transcription factor-1 (TTF-1) (mouse monoclonal antibody clone 8G7G3/1; DAKO), followed by incubation with horseradish peroxidase-labeled anti-mouse immunoglobulin secondary antibody. Immunoreactivity was visualized using diaminobenzidine as the substrate, and the nuclei were lightly counterstained with hematoxylin.

Morphometry

Glass slides were scanned using a virtual slide system (NanoZoomer slide scanner; Hamamatsu Photonics). Morphometric analyses were performed using the freely published software Qu-Path (version 0.1.2, OS Windows 10).[24,25]

Statistical analysis

Significant differences in the frequency of HNF4α-positive cells (or relationships with HNF4α expression levels) among various disease categories and patients were analyzed using the Wilcoxon/Kruskal–Wallis test

(or the χ^2 test). Patient survival was analyzed using Kaplan–Meier curves and the log-rank test. A Cox proportional hazards model for survival was used to assess the utility of HNF4α. Predictive factors were selected based on Uno's concordance index. Hazard ratios and 95% confidence intervals were calculated using Cox's proportional hazards model, and the Wald test was also performed. Schoenfeld residuals were plotted to assess the proportional hazard assumption for HNF4α in the Cox model. Statistical significance was set at $p < 0.05$. Statistical analyses were conducted using JMP (version 15.0; SAS Institute Inc.) and R (version 3.5.1; R Foundation for Statistical Computing).

RESULTS

Baseline characteristics

The clinical characteristics and laboratory findings of patients with IPF, non-IPF, and unclassifiable ILD are shown in Table 1. Age, sex, and smoking status differed significantly among the three groups. Patients in the IPF and unclassifiable groups were older and predominantly male compared to those in the non-IPF group. The IPF group included more smokers than the other two groups. No significant differences were noted in the laboratory findings and respiratory function among the three groups.

Immunohistochemical expression of HNF4α in ILD lesions

HNF4α was strongly expressed in the cells present on fibroblastic foci (Figure 1) and Masson bodies/organizing alveoli (Figure S1). HNF4α-expressing cells typically co-expressed TTF-1 (Figures 1, S1, and S2), suggesting that they could originate from pneumocytes. There was a significant correlation between the HNF4α-positive cell frequency and young fibroblastic proliferation (Figure S3).

Precise measurement of HNF4α-positive cell frequency

HNF4α-positive cells were automatically counted using morphometric software (Qu-Path).[24,25] Regenerating pneumocytes lying on fibroblastic foci and Masson bodies/organizing alveoli strongly expressed HNF4α, while metaplastic epithelial cells also weakly expressed HNF4α. Here, we set a threshold to exclusively detect strong signals (Figure S4). The frequency of HNF4α-positive cells was determined as positive cells/mm^2 (Figure S5) and was separately calculated for the upper and lower lobes.

Relationship between HNF4α-positive cell frequency and disease category

We analyzed the relationships between the HNF4α-positive cell frequency and disease category. Among the pathological categories, the UIP-indeterminate and alternative groups showed a higher frequency of HNF4α-positive cells (Figure 2). While, among the disease subtypes determined through MDD (final diagnoses), the NSIP group, HP, and unclassifiable ILD groups showed higher frequencies (Figure 2). The CTD-ILD group showed an intermediate frequency (Figure 2). The IPF group showed lower frequencies of HNF4α-positive cells (Figure 2). The PPFE and DIP cases varied in frequency (Figure 2). Among the CTD subtypes, no significant differences were observed in frequency (Figure 2). The results are summarized in Table S1. The frequencies of HNF4α-positive cells were generally higher and varied in the upper lobes than the lower lobes (Figure S6)

Relationship between HNF4α levels and patient outcomes

We determined the cutoff values according to receiver operating characteristic curves describing the relationship between the frequency of HNF4α-positive cells and outcomes (death due to ILD-related respiratory failure). Differences in survival duration and 10-year survival rates from the time of SLB between the low and high expression groups were separately analyzed in the whole, IPF, non-IPF, and unclassifiable ILD groups. In addition, the analyses were performed separately for the upper and lower lobes. In the analyses of the upper lobes, the high expression group showed significantly shorter survival and lower 10-year survival rate in the whole, non-IPF, and unclassifiable-ILD groups but not in the IPF group (Figure 3; Table 2). In the analyses of the lower lobes, a significant difference was observed only in the unclassifiable ILD group (Figure S7). Thus, HNF4α-positive cell frequency from upper lobe suggested to be more prognostic. We thereafter focused on HNF4α-positive cell frequency from upper lobe to analyze its relationship to survival in different subtypes of the non-IPF group. The PPFE group only showed a slight difference in survival duration, although the number of cases examined may have been too small (Figure S8).

Furthermore, we also analyzed the relationship between HNF4α expression levels and acute exacerbation (AE). No significant correlations were observed (Figure S9).

The survival durations in the IPF, non-IPF, and unclassifiable-ILD groups examined in the present study were similar to those generally reported,[26–31]

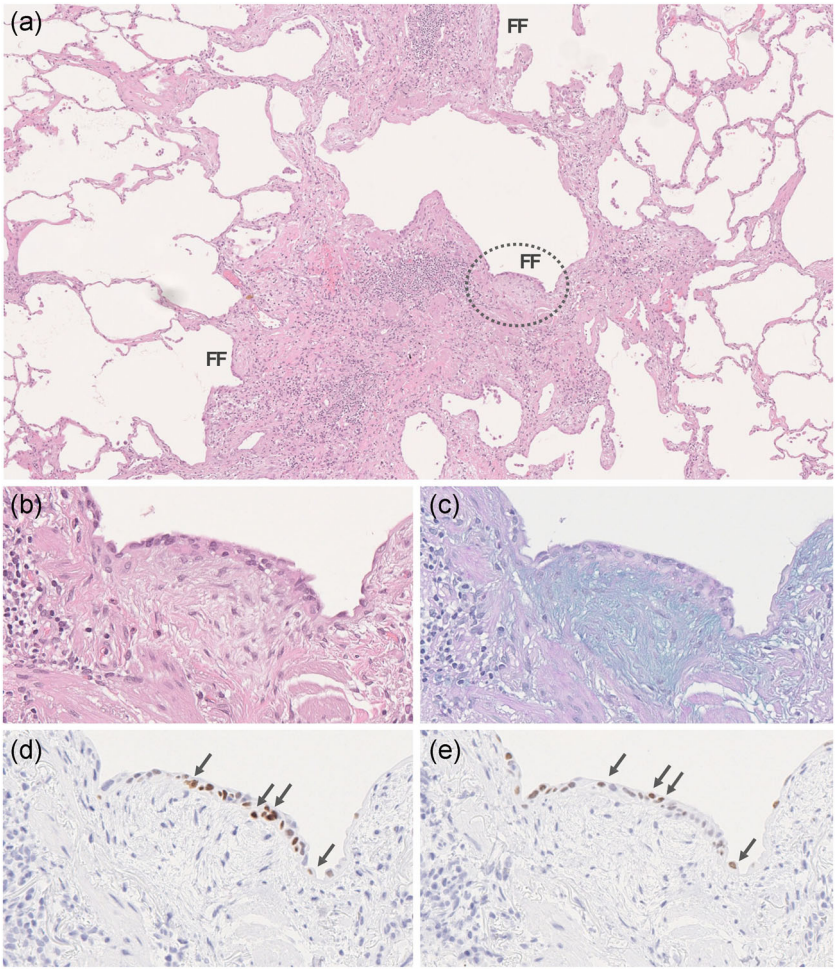

FIGURE 1 Histological appearance and immunohistochemical expression of hepatocyte nuclear factor α (HNF4α) in a usual interstitial pneumonia (UIP) lesion are shown. (a) Scanning view of a hematoxylin and eosin (HE)-stained section (×40). Collapsed lesions are seen at the lobular periphery, where fibroblastic foci (FF; one of them is circled with a dotted line) are scattered. (b) A closer view of the FF circled with the dotted line in panel A (HE, ×400). Proliferation of young fibroblastic cells to form a focus, on which regenerating epithelial cells are spreading. (c) A closer view of the FF marked with the dotted line circle in panel (a) (Alcian blue and PAS, x400). Young fibroblastic cells were stained light blue and are well visualized. (d) HNF4α is strongly expressed in some of the regenerating epithelial cells (arrows) in the FF (immunohistochemistry, ×400). (e) TTF-1 is weakly expressed in some of the regenerating epithelial cells (arrows) in the FF (immunohistochemistry, ×400)

which confirmed that our series of cases was appropriate for survival analysis (Figure S10).

Independent prognostic value of HNF4α level in unclassifiable ILD

We also analyzed the generally known prognostic factors of ILD in relation to patient outcomes in the unclassifiable ILD group.[8,32] Univariate analysis showed that in addition to a high HNF4α, low partial pressure of arterial oxygen (PaO_2) and low forced vital capacity (FVC) % predicted were negative prognostic factors, as patients with these factors had significantly lower 10-year survival rates (Table S2).

We then selected FVC % predicted from the putative prognostic factors (PaO_2, FVC % predicted, diffusing capacity of carbon monoxide (DLco) % predicted, and Krebs von den Lungen-6 [KL-6])[1,5,8] according to the concordance index (the details are not shown). Schoenfeld residuals suggested that the proportional hazard assumption for HNF4α was appropriate (not shown). Multivariable analysis confirmed that a high HNF4α level (≥4.0) was an independent prognostic factor with an estimated hazard ratio of 28.6 (95% confidence interval, 3.1–268.5) (Table 3).

Histopathological features in unclassifiable ILDs with a high HNF4α level

We were interested in the histopathological appearance of the unclassifiable ILDs with high HNF4α levels. Most patients showed frequent alveolar collapse and numerous fibroblastic foci, where fibrin exudate or

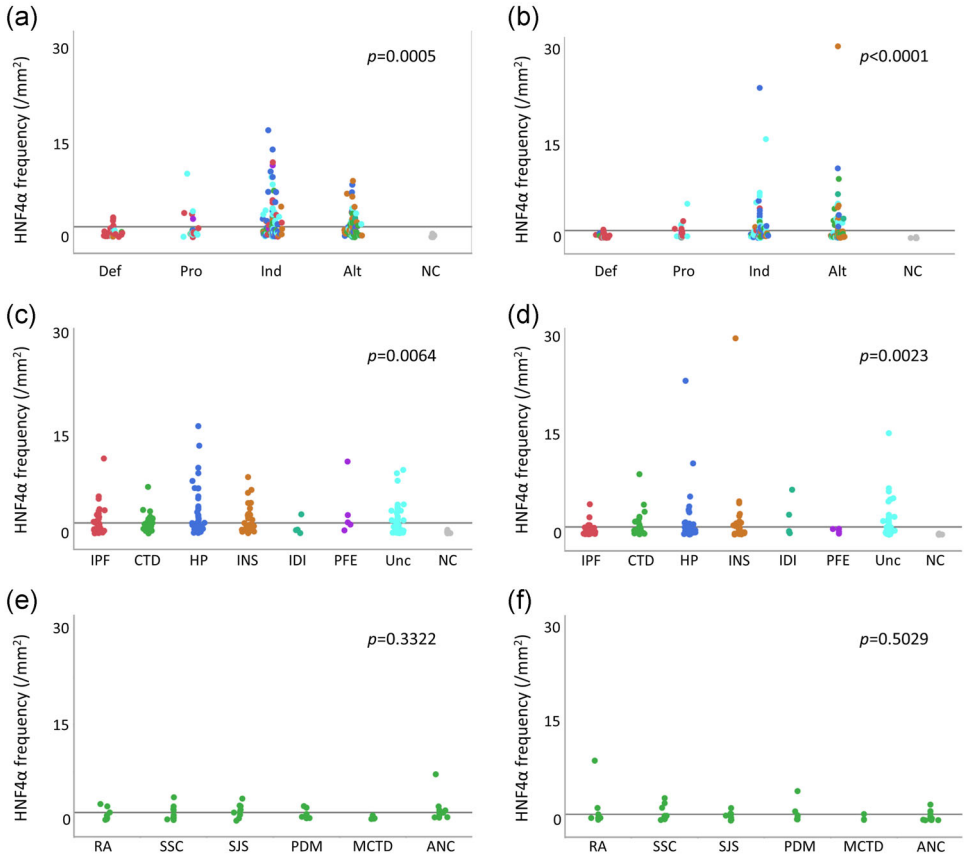

FIGURE 2 Relationships between frequency of HNF4α-positive cells and disease subtypes in different levels (a and b; pathological category; c and d, final diagnosis through MDD; e and f, types of CTD) are shown separately in each lobe (a, c, e, upper lobe; b, d, f, lower lobe). HNF4α-positive cell frequencies from individual cases were plotted. The colors of the data dots were compatible among the different levels of disease classifications. The colors to discriminate the disease types in the final diagnosis are a standard set. The horizontal lines indicate mean values. Differences were analyzed using the Wilcoxon/Kruskal–Wallis rank-sum test. *p* values are indicated. ANC, antineutrophil cytoplasmic antibody (ANCA)-related disease; Alt, alternative lesion; CTD, connective tissue disease-related interstitial pneumonia; Def, UIP-definite; HNF4α, hepatocyte nuclear factor α HP, Hypersensitivity pneumonitis; IDI, idiopathic desquamative IP; ILD, interstitial lung disease; INS, idiopathic NSIP; Ind, UIP-indeterminate; MCTD, mixed connective tissue disease; MDD, multidisciplinary discussion; NC, normal control (lung tissues without any pathological changes from lung cancer patients who under underwent lobectomy); PDM, polymyositis/dermatomyositis (PM/DM); PFE, pleuroparenchymal fibroelastosis (PPFE); pro, UIP-probable; RA, rheumatoid arthritis; SJS, Sjogren syndrome; SSC, systemic sclerosis; Unc, unclassifiable ILD

hemosiderin deposition was almost never seen, or quite focal if there was. The temporal phase of the lesions seemed almost uniform, and the spatial distribution was diffuse or random (Figure 4). Thus, these patients were suggested to have severe pneumocyte injury and progressive fibrosis. In contrast, the unclassifiable ILDs with low HNF4α levels significantly varied in histopathological appearance, as they showed combined features of a couple or more subtypes where fibroblastic foci and Masson bodies/organizing alveoli were seen only scantly (Figure S11). Unclassifiable ILD remains miscellaneous. We divided the unclassifiable ILD cases into two groups, according to the pathological criteria:[8] UIP-definite and UIP-probable versus UIP-indeterminate and alternative. HNF4α expression levels were not significantly different between the two groups. Conversely, in both groups, the cases with high expression levels of HNF4α showed poorer survival (Figure S12).

DISCUSSION

We demonstrated that HNF4α was strongly expressed in regenerating pneumocytes lying on fibroblastic foci and Masson bodies/organizing alveoli, where HNF4α and TTF-1 were co-expressed. TTF-1 is a master regulator of pneumocyte differentiation and has been reported to negatively regulate HNF4α expression and to further suppress differentiation of gastric and intestinal cell lineages.[33,34] Thus, the observed HNF4α/TTF-1-co-expression might indicate a unique/specific dedifferentiation status and the double positive pneumocytes could play a distinctive role in tissue repair and remodeling in ILD. Therefore, it is of great interest to investigate their biological characteristics.

We also showed the potential prognostic utility of HNF4α levels, particularly in non-IPF and unclassifiable ILD groups. Strong HNF4α expression was specific to regenerating pneumocytes (epithelial cells) and was

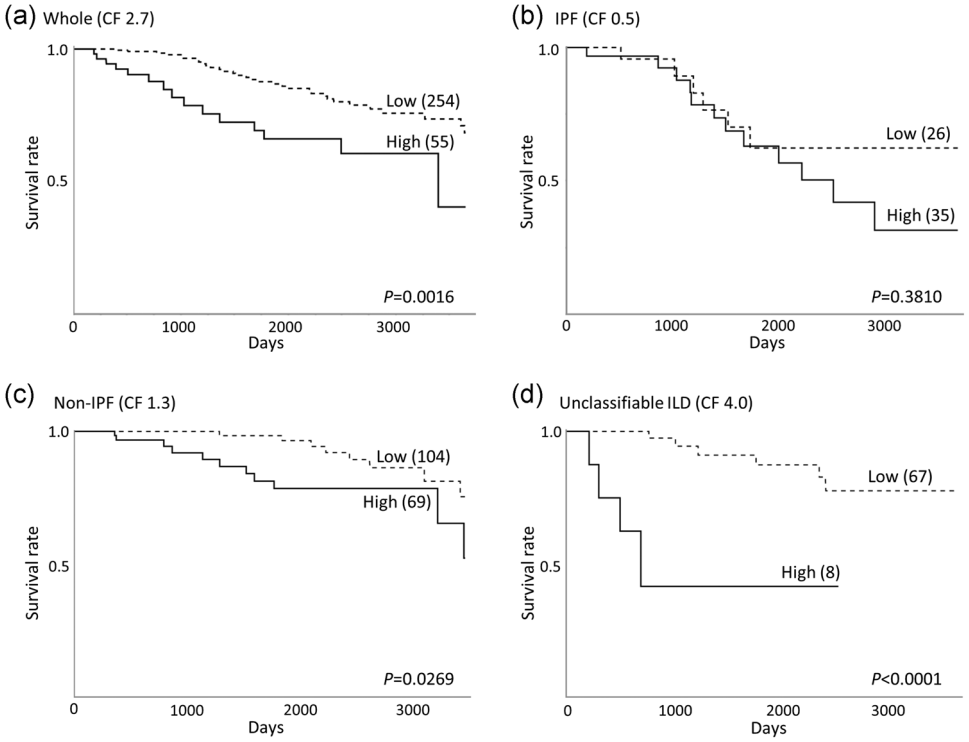

FIGURE 3 Relationship between the hepatocyte nuclear factor α (HNF4α) level in the upper lobe and patient outcome. Kaplan–Meier survival curves for the high and low expression groups in the whole (a), idiopathic pulmonary fibrosis (IPF) (b), non-IPF (c), and unclassifiable interstitial lung disease (ILD) groups (d) are shown. Cutoff values (CF) are determined based on HNF4α-positive cell frequencies from the upper lobe: 2.7/mm^2 in the whole group, 0.5/mm^2 in the IPF group, 1.3/mm^2 in the non-IPF group, and 4.0/mm^2 in the unclassifiable ILD group. The differences were analyzed using the log-rank test

TABLE 2 Relationships between the HNF4α level and patient outcomes among the different disease categories

Groups	10-year SR (%)	Hazard ratio	95% CI	*p*-value
Whole—CF 2.7–				
Low (254)	68.4	Ref.	Ref.	0.0023
High (55)	40.4	2.6	1.4–4.8	
IPF—CF 0.5–				
Low (26)	62.4	Ref.	Ref.	0.3848
High (35)	31.5	1.5	0.6–4.1	
Non-IPF—CF 1.3–				
Low (104)	75.5	Ref.	Ref.	0.0334
High (69)	52.4	2.7	1.1–6.7	
Unclass—CF 4.0–				
Low (67)	77.7	Ref.	Ref.	0.0003
High (8)	0.0	11.1	3.0–41.2	

Abbreviations: CF, cutoff value; CI, confidence interval; HNF4α, hepatocyte nuclear factor α; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; Ref., reference; SR, survival rate (estimated survival rate calculated from Kaplan–Meier survival curves); Unclass, unclassifiable ILD.
p, significant level from the Wald test.

TABLE 3 Relationships between the HNF4α level and patient outcomes in the unclassifiable ILDs: a multivariable analysis with putative prognostic factors

Subjects	Hazard ratio	95% CI	*p*-value
HNF4α level			
<4.0 (67)	Ref.	Ref.	0.0033
≥4.0 (8)	28.6	3.1–268.5	
FVC, %predicted			
≥80 (40)	Ref.	Ref.	0.0082
<80 (35)	29.7	2.4–366.5	

Note: *p*, significance level from the Wald test.
Abbreviations: CI, confidence interval; FVC, forced vital capacity; HNF4α, hepatocyte nuclear factor α; ILD, ILD, interstitial lung disease; Ref., reference.

never detected in mesenchymal cells. Because pneumocyte injury is a critical factor for assessing the severity of ILD,[34,35] this is an important benefit. To our knowledge, fibroblastic foci have been the only prognostic marker for ILD in SLB examinations.[36] A previous study manually measured the number and area of fibroblastic foci using morphometric software and demonstrated a correlation with the

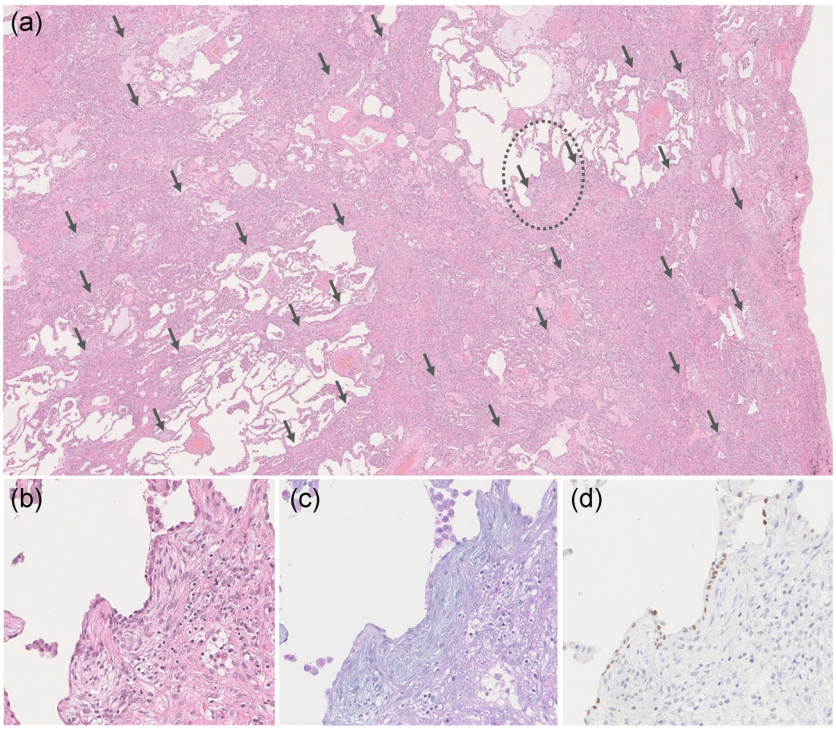

FIGURE 4 Histological appearance in a representative case of high-expression unclassifiable interstitial lung disease (ILD) is shown. (a) A scanning view of a hematoxylin and eosin (HE)-stained section (×40). Severe alveolar collapse with a larger number of fibroblastic foci (arrows) is seen. (b) A closer view of the area circled with the dotted line in panel (a) (HE, ×200). (c) Young fibroblastic cells proliferate to form a focus, on which regenerating epithelial cells are spreading (Alcian blue and PAS, ×200). (d) A high number of hepatocyte nuclear factor α (HNF4α)-positive regenerating epithelial cells can be seen on the fibroblastic foci (immunohistochemistry, ×200)

outcomes of patients with IPF.[37,38] Performing accurate measurements is often difficult as fibroblastic foci sometimes have ill-defined borders and have different appearances depending on the stage of scarring.[39] Immunohistochemical signals are more objective and quantitative and could be used to overcome conventional limitations. Moreover, positive nuclear signals are easily countable and may be automatically counted in the entire desired sections on virtual side glasses using public morphometric software.[24,25] This is sufficiently easy and quick for routine clinical use. Thus, we expect that the HNF4α immunohistochemical level could be a biological marker, not only for predicting outcomes but also for determining the adaptation of antifibrotic molecular targeting agents, such as pirfenidone and nintedanib.[3]

In the present study, another notable finding was that the unclassifiable ILDs could include the potential subgroup showing significantly poorer outcome (that can be separated by using HNF4α level). We considered that an essential pathobiological event in the worst subgroup of unclassifiable ILD could be severe pneumocyte injury and progressive fibrosis. Future studies focusing on this subgroup may lead to the proposal of a novel disease category.

On the other hand, unexpectedly, the IPF group showed lower frequencies of HNF4α-positive cells. The severity of pneumocyte injury is a crucial factor influencing the outcomes of ILD, but its spatial distribution may also be important. Actually, fibroblastic foci in IPF appear in the periphery of lobules, in contrast to Masson bodies/organizing alveoli in the other subtypes, which scatter intralobularly. In IPF, pneumocyte injury may be concentrated at the periphery of lobules, followed by wound healing responses leading to alveolar collapse and dense collagen deposition, which could result in more severe restrictive respiratory impairment than other subtypes.[40] Thus, the potential causes and pathogenesis of IPF may differ from those of other ILDs.[41] Moreover, IPF is temporally or spatially heterogeneous. Pneumocyte injury observed in a one-time/one-site biopsy may be focal. This could be a possible explanation for the discrepancy that the HNF4α level in the IPF group was rather lower.

Recent advances in molecular biology have uncovered precise diagnostic approaches and prognostic biological markers for ILD, such as signatures of exosome miRNAs[42] and serum antigens.[43] However, they are currently not available for routine use because of their associated costs and technical difficulties. We still need to obtain as much information as possible from biopsy specimens, and thus, reliable immunohistochemical markers are required. Here, we demonstrated that HNF4α has prognostic impact.

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CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

The contribution of each author was as follows: Tomoe Sawazumi wrote most parts of the manuscript; Tomohisa Baba, Tae Iwasawa, and Hiromasa Arai collected the information of the patients and compiled the clinical database; Koji Okudela, Yoshiaki Inayama, Kenichi Ohashi, and Takashi Ogura designed this study and suggested the content of the manuscript; Koji Okudela, Mai Matsumura, and Tamiko Takemura contributed to the review for pathological diagnosis; Yusuke Saigusa conducted the statistical analysis; Misaki Sugiyama and Motoki Sekiya carried out the immunohistochemical analysis.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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論文目録

Ⅰ主論文

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