Expression Pattern of the Hippo Pathway Effector TAZ in Cellular and Fibrotic Nonspecific Interstitial Pneumonia

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Interstitial lung disease (ILD) is a comprehensive term referring to a group of lung diseases affecting the interstitium of the lung. Idiopathic pulmonary fibrosis (IPF) is the most common idiopathic ILD, and nonspecific interstitial pneumonia (NSIP) is the second most common. As the name suggests, NSIP is diagnosed after many other diseases are excluded. The main pathological finding in NSIP is homogeneous interstitial inflammation with or without fibrosis.[1] NSIP can be categorized by cellular type or fibrotic type, according to the grade of inflammation and fibrosis. The cellular type has mostly inflammatory lesions with good responses to steroid, but the fibrotic type has a large proportion of fibrosis mixed with inflammatory lesions and a relatively poor response to steroid treatment.[1] So far, the exact mechanism underlying idiopathic ILD has not been clarified. Determining key regulators of these ILDs will be helpful in the diagnosis and development of novel drugs for ILD.

The Hippo signaling pathway regulates organ size through control of cell proliferation and apoptosis. It is also deeply involved in cell differentiation and cancer development.[2] Recent studies have revealed that the Hippo pathway has an important role in lung development and alveolar differentiation.[2,3] The Hippo signaling pathway consists primarily of LATS1/2, MST1/2, WW45, and the transcriptional effector Yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ).[4] TAZ has been shown to sense the matrix stiffness and control the cell differentiation fate.[5] TAZ can be activated in lung fibroblasts when grown on a stiff matrix, and TAZ regulates the functions of fibroblasts including matrix synthesis, contraction, and proliferation.[5] These data suggest that TAZ is involved in mechanotransduction in the lung and may have an important role in the pathophysiology of pulmonary disease. In this study, we analyzed the expression pattern of TAZ in both cellular and fibrotic NSIP.

This study was approved by the institutional review board of our hospital (No. CNUH 2016-11-007). Adult patients who were admitted to the pulmonology department and underwent video-assisted thoracoscopic surgery -guided lung biopsies were screened from January 2009 to December 2015. In total, 32 patients with NSIP were selected according to the pathology with compatible clinical and radiological findings. Clinical data, including modified Medical Research Council (mMRC) dyspnea scale, and treatment response based on chest computed tomography (CT) findings were reviewed. Patients were divided into good treatment response group and nonresponse group. NSIP was subdivided into cellular NSIP showing interstitial chronic inflammatory cell infiltration with little fibrosis and fibrotic NSIP showing interstitial fibrotic thickening with varying amounts of cellular inflammation, following the 2008 American Thoracic Society project.

The sections were incubated at room temperature for 1 h with a primary polyclonal rabbit anti-TAZ antibody (diluted 1:50; Cell Signaling, Danvers, MA, USA). Nuclear TAZ expression was the sum of the percentage of area stained multiplied by

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the intensity (range: 0–3; 0 is no staining, 1 is weak staining, 2 is moderate staining, and 3 is strong staining).

Probability values were obtained from two-sided tests, with statistical significance set at $P < 0.05$. We used SPSS software for the analyses (version 21.0; SPSS Inc., Chicago, IL, USA).

The mean age of the 32 patients was 62.9 ± 9.4 years (range: 44–80 years), and 19/32 (59.4%) were women. There were 24/32 (75%) nonsmokers, 3/32 (9.3%) ex-smokers, and 5/32 (15.6%) current smokers. There were 15 cellular-type and 17 fibrotic-type patients. The two groups were similar in terms of clinical features and laboratory findings. Patients who had an improvement on follow-up CT were categorized as a "good response", while patients who had no change and aggravation were categorized as "nonresponse". We also compared the mMRC dyspnea scale to check clinical improvement [Table 1].

To elucidate the function of TAZ in NSIP by analyzing the differences in TAZ expression between the two types of NSIP, we compared TAZ expression levels in fibroblasts, bronchiolar cells, and alveolar cells between the NSIP cellular and fibrotic groups. A previous study showed that IPF tissues show high expression of TAZ versus normal lung tissue.

Hence, we predicted that TAZ expression is higher in fibrotic type than cellular type. However, unexpectedly, the results showed that TAZ expression in fibroblasts and bronchiolar cells was significantly lower in fibrotic type versus cellular type [Figure 1a and 1b]. TAZ expression levels in the alveolar cells of NSIP cellular-type patients tended to be higher than those of the fibrotic-type patients, but the difference was not statistically significant [Figure 1c]. These results suggest that the expression of TAZ changed during progression from inflammatory to fibrotic NSIP and that TAZ may play different roles in each phase.

To validate the relevance between TAZ expression and treatment response in NSIP, we also compared TAZ expression in fibroblasts, bronchiolar cells, and alveolar cells between the good response and nonresponse groups. The results indicated that TAZ expression in fibroblasts, bronchiolar cells, and alveolar cells was significantly higher in the good response group than in the nonresponse group [Figure 1d-1f].

Because previous studies of TAZ were also focused on fibrosis, it is difficult to evaluate the function of TAZ in early inflammatory lesions. Our result that TAZ expression levels were higher in cellular-type than fibrotic-type NSIP suggests an important role of TAZ in the more dynamic inflammatory phase of ILD in addition to irreversible fibrotic phase. We also evaluated the relationship between TAZ expression and steroid response. This is the first study to identify the role of TAZ as predictive factor for prognosis of ILD.

We concluded that it may be possible that TAZ expression is high in fibrotic lung including IPF and fibrotic NSIP, but that is much higher during the inflammatory state such as cellular NSIP [Figure 1g]. This study is the first study to compare the TAZ expression between inflammatory state and fibrotic state. The expression of TAZ may be useful as a predictive biomarker of the response to steroid treatment in NSIP, and targeting TAZ may offer novel therapeutic options for NSIP by inhibiting inflammation and fibrosis.

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Conflicts of interest

There are no conflicts of interest.

Table 1: Clinical features and laboratory finding of patients

Characteristics	Cellular NSIP (n = 15)	Fibrotic NSIP (n = 17)
Age, years, mean ± SD (range)	63.2 ± 7.8 (52.0–80.0)	62.7 ± 10.8 (44.0–80.0)
Gender (male/female), *n*	7/8	6/11
Smoking habit (current/former/never), *n*	2/1/12	3/2/12
Symptoms and signs		
Dyspnea scale (mMRC 0/1/2/3/4), *n*	0/2/9/3/1	1/1/8/7/0
Cough, *n* (%)	8 (53.3)	9 (52.9)
Dry eyes and dry mouth, *n* (%)	1 (6.6)	1 (5.8)
Fever, *n* (%)	1 (6.6)	0 (0)
Arthralgia, *n* (%)	1 (6.6)	1 (5.8)
Rash, *n* (%)	0 (0)	1 (5.8)
CT findings at the time of biopsy, *n* (%)		
Ground-glass opacity	15 (100.0)	17 (100.0)
Patchy lesions	3 (20.0)	15 (88.2)
Irregular reticular opacity, *n* (%)	6 (18.7)	12 (70.5)
Traction bronchiectasis	2 (40.0)	3 (17.6)
Response of treatment, *n*		
CT finding (improve/no change/aggravation)	12/2/1	12/3/2
Change of mMRC dyspnea scale (improve/no change/aggravation)	8/6/1	9/6/2

CT: Computed tomography; mMRC: modified Medical Research Council; NSIP: Nonspecific interstitial pneumonia; SD: Standard deviation.

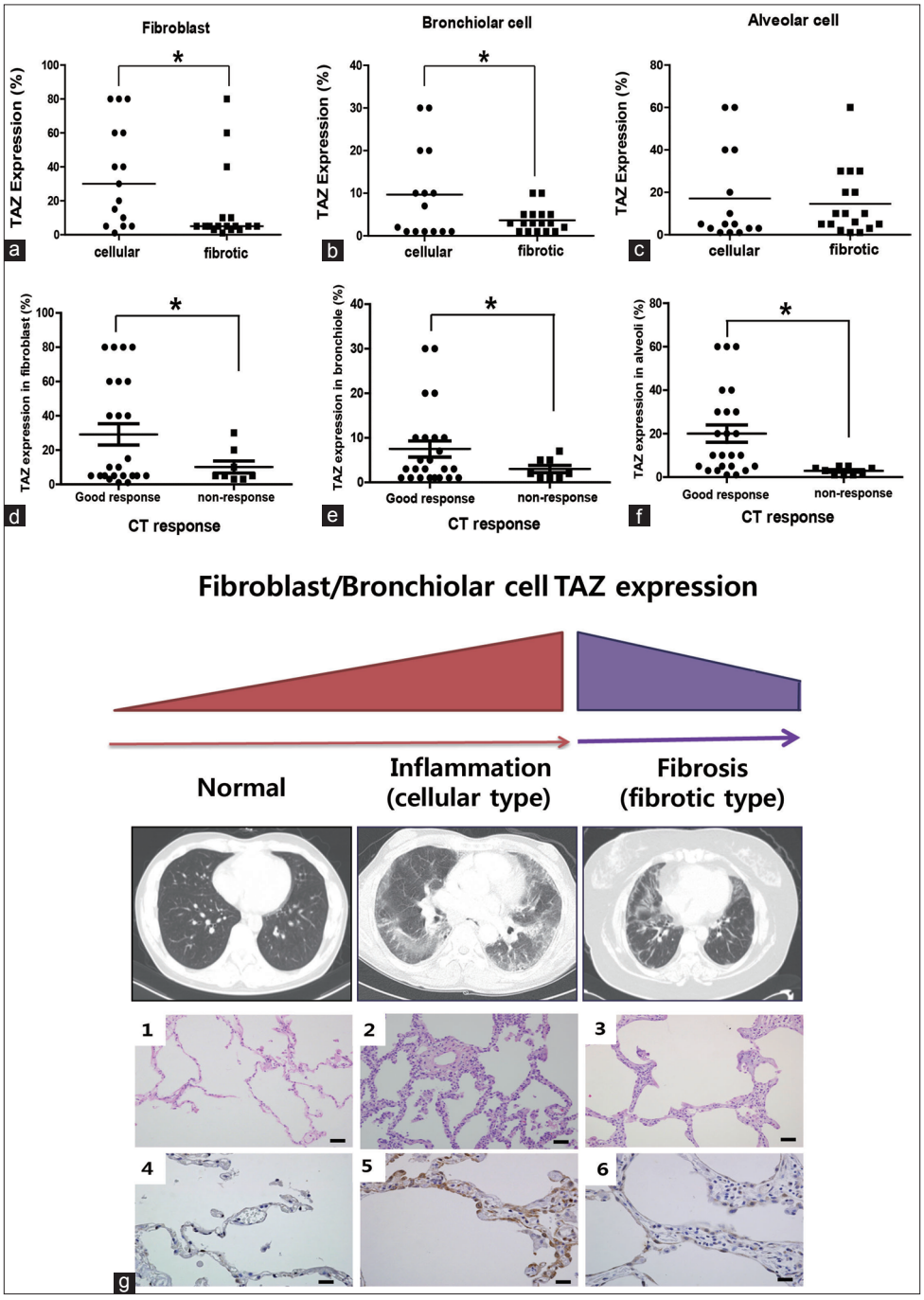

Figure 1: Expression pattern of TAZ in fibroblast, bronchial cell, and alveolar cell of NSIP. Comparison of TAZ expressions of fibroblast (a), bronchiolar cell (b), and alveolar cell (c) between NSIP cellular type and fibrotic type. Comparison of TAZ expressions of fibroblast (d), bronchiolar cell (e), and alveolar cell (f) between good response group and nonresponse group. *$P < 0.05$. (g) Schematic model of TAZ expression pattern in fibroblast and bronchiolar cell of NSIP progression. The representative images of chest computed tomography, hematoxylin and eosin staining and TAZ expression of each normal lung (1 and 2), cellular NSIP (3 and 4), and fibrotic NSIP (5 and 6). NSIP: Nonspecific interstitial pneumonia.

References

1. Vogelmeier C. IPF or NSIP? That is the question. Eur Respir J 2003;22:191-2. doi: 10.1183/09031936.03.00037003.
2. Chung C, Kim T, Kim M, Kim M, Song H, Kim TS, *et al.* Hippo-Foxa2 signaling pathway plays a role in peripheral lung maturation and surfactant homeostasis. Proc Natl Acad Sci U S A 2013;110:7732-7. doi: 10.1073/pnas.1220603110.
3. Lee JE, Park HS, Lee D, Yoo G, Kim T, Jeon H, *et al.* Hippo pathway effector YAP inhibition restores the sensitivity of EGFR-TKI in lung adenocarcinoma having primary or acquired EGFR-TKI resistance. Biochem Biophys Res Commun 2016;474:154-60. doi: 10.1016/j.bbrc.2016.04.089.
4. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, *et al.* Role of YAP/TAZ in mechanotransduction. Nature 2011;474:179-83. doi: 10.1038/nature10137.
5. Liu F, Lagares D, Choi KM, Stopfer L, Marinković A, Vrbanac V, *et al.* Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. Am J Physiol Lung Cell Mol Physiol 2015;308:L344-57. doi: 10.1152/ajplung.00300.2014.