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Clinical implication of protease-activated receptor-2 in idiopathic pulmonary fibrosis

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Summary
Idiopathic pulmonary fibrosis (IPF) is a lethal pulmonary disease which is characterized by progressive fibrosis. In general, the exaggerated activation of the coagulation cascade has been observed during initiation or maintenance of the fibrotic disease. In a recent study, protease-activated receptor (PAR)-2, which plays a key role in coagulation cascade, was up-regulated in IPF patients, however, its clinical implications have not been understood. The objective of the present study was to evaluate the clinical significance of PAR-2 expression in the alveolar epithelial cells of IPF patients. PAR-2 expression was evaluated by immunohistochemical method in formalin-fixed and paraffin-embedded tissues of surgical lung biopsies from patients with IPF. Fibrosis scores from hematoxylin and eosin-stained lung sections and honeycombing scores in chest CT were calculated. Medical records were retrospectively reviewed and the correlation between the expression of PAR-2 and clinical profiles were assessed. Among thirty-three IPF patients, PAR-2 expression was observed in 25 (75.8%). The lymphocyte counts in peripheral blood (2317 vs. 1753, $p = 0.044$) and honeycombing scores in chest CT (4.0 vs. 3.0 $p = 0.046$) were higher in PAR-2 positive group compared with PAR-2 negative group. During a follow-up duration of median 40.3 months, 7 (21.2%) patients died and they were all included in the PAR-2 positive group ($p = 0.113$). We conclude that PAR-2 is expressed in the alveolar epithelial cells of a substantial number of IPF patients, and the expression of PAR-2 significantly correlates with the extent of honeycombing shown in chest CT.

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a lethal fibrotic lung disease of unknown etiology. Unfortunately, it is a progressive and irreversible disorder. In general, median survival of IPF patients is 2.5—5 years with or without treatment.[1] Even to this day, pathogenesis of IPF is poorly understood and prognostic factor is not clearly defined.[2] Various therapeutic approaches, such as anti-inflammatory, immunomodulatory, and anti-fibrotic agents have been tried, but effective treatment has not been established. Thus, new insights into pathogenesis of IPF are essential for the development of effective treatments.

One of the major advances in the pathogenesis of IPF is the shift in current paradigms from inflammation to abnormal wound healing.[3,4] In the wound healing process, the coagulation cascade is activated locally from the tissue factor dependent extrinsic pathway.[5] Recent studies implicated that protease-activated receptor (PAR)-2 may play a significant role in the pathogenesis of fibrosis. Factor Xa-dependent PAR-2 activation showed a profibrotic response in fibroblasts and caused lung fibrosis in an experimental acute lung injury model.[6] Furthermore, PAR-2 induced myofibroblast differentiation in a bleomycin-induced pulmonary fibrosis model.[7]

PAR-2 is a G-coupled 7-transmembrane receptor that is activated by tethered peptide ligand, which is exposed after enzymatic cleavage of the specific site in the extracellular N-terminal.[8] PAR-2 is expressed in various tissues and is found more in kidney, pancreas, and gastrointestinal tissue than in heart and lungs.[9] PAR-2 expression is mainly located in endothelial and epithelial cells.[10] In lungs, PAR-2 is highly expressed in ciliated and non-ciliated epithelial cells.[11] Recent studies suggested the association of PAR-2 activation with airway inflammation[11—13] and pulmonary fibrosis.[7] In addition, up-regulation of PAR-2 was observed in lung tissue of IPF patients and PAR-2 could be a significant contributor in fibrotic process.[14] These findings suggest that PAR-2 expression may be associated with the development of fibrosis in IPF. However, to our knowledge, there are no previous studies reporting the relationship between PAR-2 expression and the clinical parameters implicating fibrosis. In this study, we investigated the correlations between PAR-2 expression and the clinical parameters for fibrosis, such as fibrosis scores in surgical specimens and honeycombing scores in chest computed tomography (CT), and all-cause mortality.

Methods

Study population

Surgical lung biopsy-proven IPF patients in one tertiary referral hospital in Seoul, Korea between January 2005 and May 2010 were included in this study. The surgical biopsy was performed at the time of initial diagnosis of IPF. Pathologic diagnosis was based on the established criteria and clinical diagnosis was made according to the international guidelines.[15] Medical records were reviewed retrospectively. The study protocol (H-1007-137-324) was approved by institutional review board of Seoul National University Hospital. The board waived informed consent from participants. This study was conducted in accordance with the Declaration of Helsinki.

Fibrosis score

The slides of hematoxylin and eosin-stained lung sections were reviewed by two independent board certified pathologists to score fibrosis. In brief, the whole lung section of each slide was observed to assess the area of fibrosis using light microscopy (Olympus Optical Co. Ltd., Tokyo, Japan). The extent of fibrotic lesions was scored as 0 (0—10%), 1 (10—25%), 2 (25—50%), 3 (50—75%), or 4 (75—100%). The severity of fibrosis was scored from 0 (normal lung) to 8 (total fibrosis) according to Ashcroft scoring system[16] at a magnification of $\times$200 in average of 10 different fields. Finally, fibrosis scores were calculated by multiplying the extent of fibrotic lesion to Ashcroft scores. A mean value of the scores from two pathologists was considered as the final fibrosis score.

Honeycombing score

Honeycombing on chest CT image was defined as thick-walled air-filled cysts between the sizes of several millimeters to several centimeters in diameter. Previous method shown by Akira et al.[17] was used to assess the honeycombing score by two independent board certified pulmonary radiologists. In brief, the whole lung was divided into six lobes (right upper, right middle, right lower, left upper, lingular division, and left lower lobe) and the extent of honeycombing lesion was assessed as 0 (absent), 1 (0—25%), 2 (25—50%), 3 (50—75%), or 4 (>75%) in each lobe. Honeycombing score was the final sum of all six lobes. A mean value of the scores from two radiologists was considered as the final honeycombing score.

Immunohistochemical staining

Formalin-fixed and paraffin-embedded surgical lung biopsies of IPF patients were used for immunohistochemical staining. After deparaffinization in xylene and rehydration through graded alcohol to water, these slides were soaked in Target Retrieval Solution (Dako, Carpenteria, CA, USA), then placed under 60 °C water bath for 20 min for antigen retrieval. We used 1% H_2O_2 in methanol to quench endogenous peroxidase activity. The 1:50 goat polyclonal anti-PAR-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was added at 4 °C overnight. The secondary antibody, 1:200 biotinylated anti-goat antibody (Vector, Burlingame, CA, USA), was placed on the slides for 1 h at room temperature. After brief washing in Tris-buffered saline, Vectastain avidin—biotin complex elite kit (Vector, Burlingame, CA, USA) was applied for 1 h. Reaction products were visualized using 3,3-diaminobenzidinetetrahydrochloride. After washing with distilled water, counter staining was performed with hematoxylin. Human pancreas tissue was used for positive and negative control.[18] Negative control was performed with the same protocol but without the primary antibody. Positive immunohistochemical staining of