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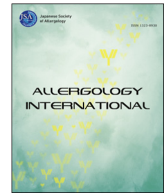

Original article

Fragmented gelsolins are increased in rheumatoid arthritis-associated interstitial lung disease with usual interstitial pneumonia pattern

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Abbreviations:
ABPs, actin binding proteins; BAL, bronchoalveolar lavage; CRP, C-reactive protein; ELISA, Enzyme-linked immunosorbent assay; GM, gel matching; GSN−/−, gelsolin knockout; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; IPG, immobilized pH gradient; LDH, lactate dehydrogenase; LC-nESI-MS/MS, liquid chromatography nano electron spray ionization tandem mass spectrometry; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; MMPs, matrix metalloproteinases; NSIP, non-specific interstitial pneumonia; OP, organizing pneumonia; PVDF, polyvinylidene difluoride; RA, rheumatoid arthritis; RA-ILD, rheumatoid arthritis-associated interstitial lung disease; SP-D, surfactant protein D; TBS-T, tris buffered saline with Tween 20; 2-DE, two-dimensional gel electrophoresis; UIP, usual interstitial pneumonia; %DLco, percentage predicted

A B S T R A C T

Background: Rheumatoid arthritis-associated interstitial lung disease (RA-ILD) occurs in 10%–30% of patients with RA, and interstitial lung disease (ILD) is associated with increased mortality in up to 10% of patients with RA. The pathogenesis of RA-ILD is virtually unknown. The aim of this study is to investigate the proteins related to UIP pattern by comparing to OP pattern in RA-ILD using proteome analysis of bronchoalveolar lavage fluid (BALF).
Methods: Proteomic differences in BALF were compared between the UIP pattern and OP pattern by examining BALF from 5 patients with the UIP pattern and 7 patients with the OP pattern by two-dimensional gel electrophoresis and mass spectrometry.
Results: In individual comparisons of BALF samples, the levels of the protein gelsolin and Ig kappa chain C region were significantly higher in the UIP pattern than in the OP pattern. In contrast, the levels of α-1 antitrypsin, CRP, haptoglobin β, and surfactant protein A (isoform number 5) were all significantly higher in the OP pattern than in the UIP pattern. Gelsolin was cleaved into two fragments, a C-terminal half and N-terminal half, and the levels of both were significantly higher in the UIP pattern than in the OP pattern.
Conclusions: Fragmented gelsolins may be associated with the pathogenesis of fibrosis in RA-ILD.
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diffusing lung capacity for carbon monoxide; %FEV1.0, percentage of forced expiratory volume in one second; % VC, percentage predicted vital capacity

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology that affects 0.5–1.0% of the adult population worldwide.[1] Rheumatoid arthritis-associated interstitial lung disease (RA-ILD) occurs in 10%–30% of patients with RA, and interstitial lung disease (ILD) is associated with increased mortality in up to 10% of patients with RA. The most common pathological findings in RA-ILD are usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP). Organizing pneumonia (OP) and lymphocytic interstitial pneumonia are less common, but not rare.[2] RA-ILD patients with UIP have a worse prognosis than those with OP.[3,4] While genetic, clinical, serological, and environmental factors are thought to contribute to the development of RA-ILD, little is known about the pathogenesis of the disease.

The actin cytoskeleton plays a central role in many fundamental cellular processes involving the generation of force and facilitation of movement, processes enabled by the assembly of actin monomers into filaments and cooperation with a wide variety of actin binding proteins (ABPs).[5] One particularly abundant ABP is gelsolin, an actin filament capping/severing protein cleaved by caspases or matrix metalloproteinases (MMPs).[6,7] Caspase-3, an important effector of apoptosis, cleaves gelsolin into two fragments.[8] The C-terminal gelsolin fragment (C-gelsolin) is generally anti-apoptotic, while the N-terminal fragment (N-gelsolin) is pro-apoptotic.[5]

Increased gelsolin expression correlates with reduced pulmonary function. Gelsolin upregulation has recently been found in idiopathic pulmonary fibrosis (IPF) and fibrotic NSIP, but not in other forms of interstitial lung disease. Gelsolin affected both neutrophil infiltration and epithelial apoptosis in a bleomycin- or lipopolysaccharide-challenged model of lung inflammation.[5,9] Gelsolin knockout ($GSN^{-/-}$) mice were protected from lung inflammation and fibrosis, as well as ventilator-induced lung injury.[9,10] Gelsolin is likely to play a role in RA. Gelsolin expression leads to severe alterations in cytoskeletal organization and induction of RA in $GSN^{-/-}$ mice, thereby resulting in the exacerbation of signs of disease.[5,11] The combination of reduced plasma gelsolin and presence of actin and gelsolin–actin complexes in synovial fluids suggest a local consumption of this potentially anti-inflammatory protein in the inflamed joint.[5,12] While the relationships of gelsolin with pulmonary fibrosis and RA have been reported, there have been no reports on the relationship between gelsolin and RA-ILD.

The aim of this study is to investigate the proteins related to the UIP pattern in RA-ILD by comparing them with the proteins related to the OP pattern by performing a proteomic analysis of bronchoalveolar lavage fluid (BALF). We also focused on fragmented gelsolins, which were observed at higher levels in the UIP pattern, and studied the relationship between gelsolin and RA-ILD.

Methods

Patients

The study conformed to the Declaration of Helsinki and was approved by the internal review boards of our institution (No. 1270). The requirement for informed consent was waived. BALF samples were obtained from 12 patients with RA-ILD in our hospital between 2001 and 2011. The diagnosis of RA was based on the 1987 American College of Rheumatology classification criteria. The patients were divided into two groups, UIP pattern and OP pattern, based on the findings of high-resolution computed tomography (HRCT). Five patients with the UIP pattern and 7 patients with the OP pattern were examined. We diagnosed the UIP pattern by referring from IPF guideline.[13] All patients were definite UIP pattern and none of them were inconsistent with UIP pattern in HRCT. One patient underwent surgical lung biopsy. On the other hand, we diagnosed the OP pattern by clinical, HRCT, BALF, and transbronchial lung biopsy (TBLB) findings. Five patients underwent TBLB.

Clinical data, pulmonary function data on the predicted vital capacity percentage (%VC) and percentage of forced expiratory volume in one second (%$FEV_{1.0}$), and laboratory data on serum levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), Krebs von den Lungen-6 (KL-6), and surfactant protein D (SP-D) were collected. The patients gave their informed consent to undergo bronchoscopy, and none of them were receiving prednisolone or other immunosuppressive agents at the time of enrollment.

Bronchoalveolar lavage (BAL)

BAL was performing using three 50-ml aliquots of sterile 0.9% saline, as previously described.[14] The cellular composition of the BALF was determined by counting 200 cells in a cytospun smear with Wright's stain. The lymphocyte phenotypes were analyzed by flow cytometry using monoclonal antibodies for CD4 and CD8.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2-DE) was performed as previously described.[15] BALF was concentrated by acetone precipitation and diluted in lysis buffer (8 M urea, 4% CHAPS, 65 mM dithioerythritol, 0.1 M acetic acid, pharmalyte [pH 3 to 10 for isoelectrofocusing; GE healthcare], and a trace of bromophenol blue) to a concentration at which 54 μl of samples contained 108 μg of proteins. Samples of the same volume (54 μl) were loaded on immobilized pH gradient (IPG) strips (pH 4–7, 18 cm; GEhealthcare, Uppsala, Sweden) in each analytical experiment. The samples were rehydrated in the strip holder of the IPG-IEF Cool-PhoreStar system (Anatech, Tokyo, Japan) at 20 °C, and isoelectrofocusing was terminated at 47 kV. The IPG strips were equilibrated first in the urea/SDS/Tris buffer for 30 min and then in the same buffer containing 2.5% iodoacetamide. The second dimensional run was performed on 10% polyacrylamide linear gradient gels at a constant current of 20–30 mA/gel at 20 °C until the dye front reached the bottom of the gels. The gels were stained with SYPRO-Ruby Protein Gel Stain (Molecular Probes, Carlsbad, CA, USA).

Evaluation and identification of proteins

The gels were scanned using FluoroPhoreStar 3000 (Anatech, Tokyo, Japan) and analyzed using Progenesis PG220 Software (Nonlinear Dynamics, Newcastle upon Tyne, UK). The proteins were analyzed automatically using the spot detection feature of the software, with automatic warping and matching. Spot volumes were corrected for background using the “mode of non-spot”

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