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Elevated protein arginine methyltransferase 1 expression regulates fibroblast motility in pulmonary fibrosis

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ABSTRACT

Objective: Idiopathic pulmonary fibrosis (IPF) is a devastating disease characterized by epithelial cell injury, fibroblast activation and excessive extracellular matrix deposition. Although protein arginine methyltransferase 1 (PRMT1) was found to regulate cell proliferation, differentiation and migration, its role in the development/progression of IPF has not yet been described.

Results: Expression of PRMT1 was elevated in lung homogenates from IPF patients. Significant upregulation of PRMT1 expression was also observed in the lungs of bleomycin-treated mice. Immunohistochemical analysis revealed PRMT1-positive staining in fibroblasts/myofibroblasts and alveolar type II cells of IPF lungs and in fibrotic lesions of bleomycin-injured lungs. Fibroblasts isolated from IPF lungs demonstrated increased PRMT1 expression. Interleukin-4 (IL-4), a profibrotic cytokine, enhanced the expression of PRMT1 and the migration of donor and IPF fibroblasts. Interference with the expression or the activity of PRMT1 diminished the migration of the cells in response to IL-4. Strikingly, even though the incubation of donor and IPF fibroblasts with IL-4 did not affect their proliferation, depletion, but not blockage of PRMT1 activity suppressed cell growth.

Conclusions: PRMT1 can contribute to the development of pulmonary fibrosis by regulating fibroblast activities. Thus, interference with its expression and/or activity may provide a novel therapeutic option for patients with IPF.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease with a median survival time of less than three years following diagnosis [1]. It is characterized by a histological pattern of usual interstitial pneumonia (UIP) with fibroblast foci consisting of activated, collagen producing myofibroblasts [2]. Although the fundamental mechanisms that initiate and propagate IPF have not yet been fully defined, it is believed that abnormal alveolar epithelial cell activation leads to the impairment of epithelial–mesenchymal crosstalk resulting in the accumulation of fibroblasts and extracellular matrix (ECM) proteins in the lung [2,3].

A growing body of evidence suggests that inflammatory cells recruited to the site of an injury might contribute to the fibrotic process through the production of profibrotic cytokines such as interleukin (IL)-4, IL-13 and transforming growth factor (TGF)-β [4]. In line with this notion, elevated levels of IL-4 were measured in IPF bronchial alveolar lavage fluid (BALF) and an increased expression of the IL-4 and IL-13 receptors was detected in IPF lung fibroblasts [5,6,7]. In addition, IL-4 deficient mice or mice with targeted disruption of the key components of the IL-4 signaling pathway were found to be protected against pulmonary fibrosis [8,9]. However, the anti-inflammatory therapies based on broad immunosuppression have been unsuccessful, and newly discovered pharmacotherapeutic options for IPF still remain limited [10,11].

Protein arginine methylation is a posttranslational modification which is catalyzed by a family of intracellular enzymes termed protein arginine methyltransferases (PRMT) [12]. In humans PRMTs have been classified into type I (PRMT1, 2, 3, 4/CARM1, 6 and 8) and type II (PRMT5, 7 and FBXO11) enzymes depending on their specific catalytic

Abbreviations: ADMA, asymmetric dimethylarginine; BALF, bronchoalveolar lavage fluid; Col, collagen; DAPI, 4′, 6-diamidino-2-phenylindole; DMEM, Dulbecco's Modified Eagle's Medium; ECM, extracellular matrix; HIF, hypoxia-inducible factor; HLF, human lung fibroblast; IPF, idiopathic pulmonary fibrosis; IL, interleukin; LDH, lactate dehydrogenase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; PBGD, porphobilinogen deaminase; PRMT, protein arginine methyltransferase; SDMA, symmetric dimethylarginine; proSP-C, prosurfactant protein C; SD, standard deviation; SEM, standard error of the mean; TBS, tris-buffered saline; UIP, usual interstitial pneumonia.

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activity [12]. Type I PRMTs catalyze the formation of asymmetric dimethylarginine (ADMA), whereas type II PRMTs form symmetric dimethylarginine (SDMA) [12,13]. PRMT1 is a predominant type I PRMT in mammalian cells that is responsible for approximately 85% of total protein arginine methylation [14]. Thus, PRMT1 is engaged in various cellular processes including signal transduction, gene transcription, DNA repair, and mRNA splicing [12]. Hence, dysregulated PRMT1 expression was found to contribute to the pathogenesis of a variety of diseases including cardiovascular and pulmonary disorders [13,15]. In our study, we sought to investigate whether dysregulated PRMT1

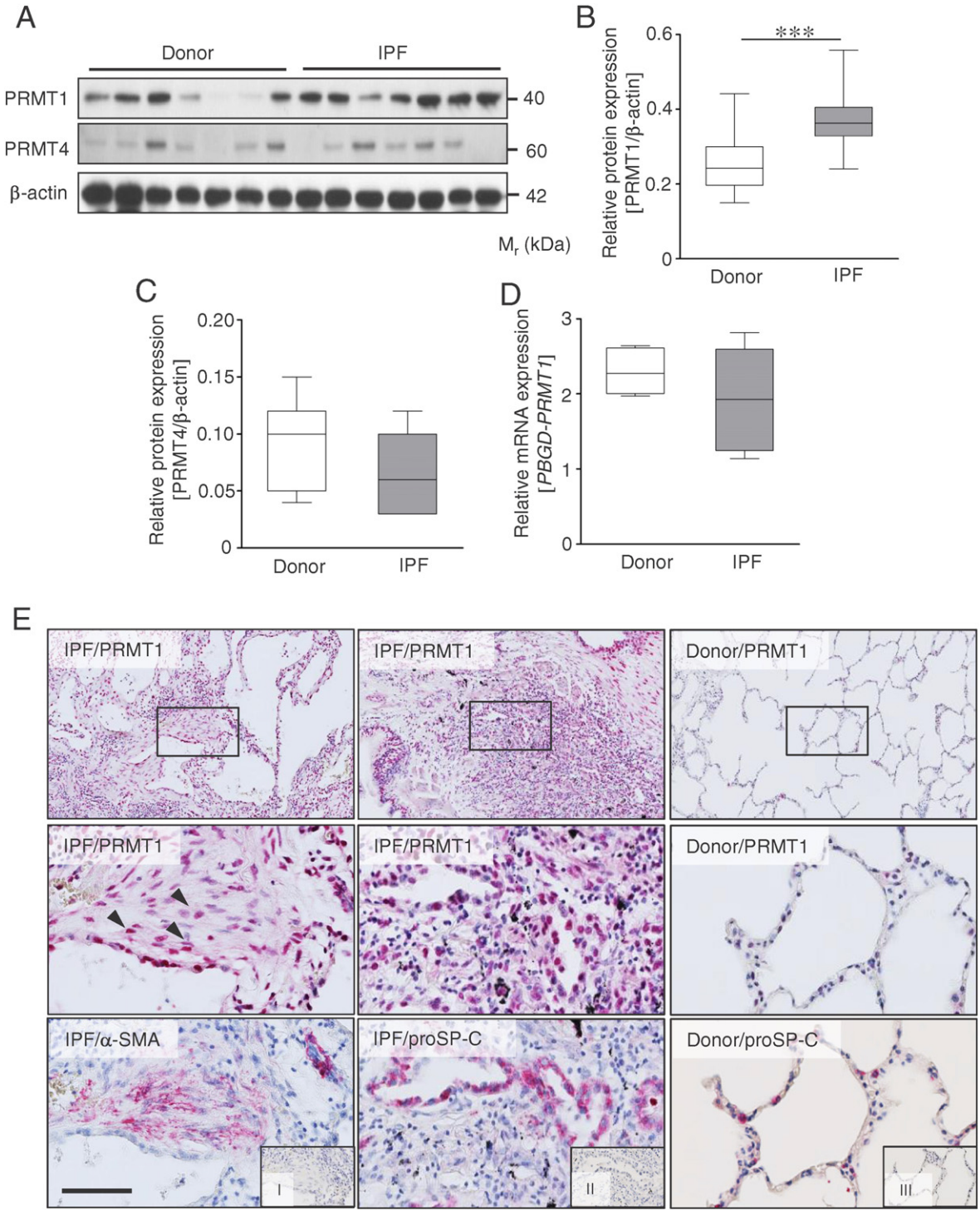

Fig. 1. Expression of PRMT1 is upregulated in the lungs of IPF patients. (A) Lung homogenates obtained from IPF patients and donors were subjected to Western blotting and the protein expression of PRMT1 and PRMT4 was analyzed. Representative donors (7/15) and patients (7/25) are shown. β-actin served as a loading control. (B, C) Densitometry analysis of (A); n = 15 (donors), n = 25 (IPF patients); ***, p ≤ 0.001. (D) PRMT1 mRNA expression in lung tissue of donors (n = 5) and IPF patients (n = 6) was assessed by RT-qPCR. RT-qPCR results are expressed as ΔCt using *PBGD* as the reference gene. (E) IPF and donor lung tissue sections were stained for PRMT1, α-SMA and proSP-C. Staining was performed on at least three independent sections obtained from at least three different patients or donors. Selected areas were magnified (boxed areas). Arrowheads indicate PRMT1-positive fibroblasts. Negative control was performed by omitting a primary antibody (I, II, III). Bar size 50 μm.