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Article

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EMBO reports

miR-9-5p suppresses pro-fibrogenic transformation of fibroblasts and prevents organ fibrosis by targeting NOX4 and TGFBR2

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Abstract

Uncontrolled extracellular matrix (ECM) production by fibroblasts in response to injury contributes to fibrotic diseases, including idiopathic pulmonary fibrosis (IPF). Reactive oxygen species (ROS) generation is involved in the pathogenesis of IPF. Transforming growth factor-β1 (TGF-β1) stimulates the production of NADPH oxidase 4 (NOX4)-dependent ROS, promoting lung fibrosis (LF). Dysregulation of microRNAs (miRNAs) has been shown to contribute to LF. To identify miRNAs involved in redox regulation relevant for IPF, we performed arrays in human lung fibroblasts exposed to ROS. miR-9-5p was selected as the best candidate and we demonstrate its inhibitory effect on TGF-β receptor type II (TGFBR2) and NOX4 expression. Increased expression of miR-9-5p abrogates TGF-β1-dependent myofibroblast phenotypic transformation. In the mouse model of bleomycin-induced LF, miR-9-5p dramatically reduces fibrogenesis and inhibition of miR-9-5p and prevents its anti-fibrotic effect both *in vitro* and *in vivo*. In lung specimens from patients with IPF, high levels of miR-9-5p are found. In omentum-derived mesothelial cells (MCs) from patients subjected to peritoneal dialysis (PD), miR-9-5p also inhibits mesothelial to myofibroblast transformation. We propose that TGF-β1 induces miR-9-5p expression as a self-limiting homeostatic response.

Keywords fibrosis; miRNAs; myofibroblast; oxidative stress; TGF-β signaling
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Introduction

The problem of organ fibrosis constitutes a major biomedical challenge. Fibrosis can be defined as an excessive accumulation of ECM components leading to the irreversible replacement of cellular compartments by ECM, ultimately leading to stiffness, scarring and devitalized tissue [1]. While the past 25 years have witnessed a very significant increment in our understanding of fibrogenesis from the molecular standpoint, it is evident that very little progress has been made toward the possibility of preventing, deferring, or curing organ fibrosis. The fact that major organs such as the liver, heart, kidney, or lung can be affected by fibrosis, in many cases with lethal outcomes, adds an important and social burden to these clinical conditions. Fibrosis of these organs underlies the development of diseases with significant prevalence and scarce therapeutic margin including liver cirrhosis, myocardial sclerosis, renal diabetic disease, or IPF [2]. IPF is a chronic progressive and lethal fibrotic lung disease of unknown etiology that is currently untreatable. The majority of IPF patients die from respiratory failure within 2–5 years of diagnosis [3,4]. The annual incidence of IPF appears to be rising, the disease is more common in men and the prevalence rises significantly with age [5,6].

IPF is one of the conditions where the established interaction between TGF-β and disturbance of redox homeostasis is sustained by a well-defined molecular mechanism [7]. TGF-β enhances the expression of NOX4 in numerous cell types, leading to increased oxidative stress, which in itself is capable of amplifying the powerful fibrogenic program induced by TGF-β [8–10], thus leading to a vicious cycle and self-perpetuating fibrotic response [11].

An important pathological scenario where the appearance of fibrosis has a devastating effect is the peritoneum of patients subjected to continuous PD. Peritoneal fibrosis (PF) leads to peritoneal membrane failure and ultrafiltration dysfunction [12–14]. It has been shown that peritoneal mesothelial cells (PMCs) play a crucial role in the development and progression of PF through the acquisition of a myofibroblast-like phenotype by mesothelial-to-mesenchymal transition (MMT) [15,16].

miRNAs are short single-stranded RNAs that regulate post-trancriptional mRNA expression by binding to complementary

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