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ARTICLE

Melatonin ameliorates bleomycin-induced pulmonary fibrosis via activating NRF2 and inhibiting galectin-3 expression

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Pulmonary fibrosis (PF) is a chronic interstitial lung disease with no effective therapies. Galectin-3 (Gal-3), a marker of oxidative stress, plays a key role in the pathogenesis of PF. Fibroblast-myofibroblast differentiation (FMD) is an important source of fibrotic cells in PF. Previous studies showed that melatonin (MT) exerted anti-fibrotic effect in many diseases including PF through its antioxidant activity. In the present study we investigated the relationships among Gal-3, NRF2, ROS in FMD and their regulation by MT. We established an in vitro model of FMD in TGF-β1-treated human fetal lung fibroblast1 (HFL1) cells and a PF mouse model *via* bleomycin (BLM) intratracheal instillation. We found that Gal-3 expression was significantly increased both in vitro and in vivo. Knockdown of Gal-3 in HFL1 cells markedly attenuated TGF-β1-induced FMD process and ROS accumulation. In TGF-β1-treated HFL1 cells, pretreatment with NRF2-specific inhibitor ML385 (5 μM) significantly increased the levels of Gal-3, α-SMA and ROS, suggesting that the expression of Gal-3 was regulated by NRF2. Treatment with NRF2-activator MT (250 μM) blocked α-SMA and ROS accumulation accompanied by reduced Gal-3 expression. In BLM-induced PF model, administration of MT (5 $mg \cdot kg^{-1} \cdot d^{-1}$, ip for 14 or 28 days) significantly attenuated the progression of lung fibrosis through up-regulating NRF2 and down-regulating Gal-3 expression in lung tissues. These results suggest that Gal-3 regulates TGF-β1-induced pro-fibrogenic responses and ROS production in FMD, and MT activates NRF2 to block FMD process by down-regulating Gal-3 expression. This study provides a useful clue for a clinical strategy to prevent PF.

Keywords: pulmonary fibrosis; fibroblast-myofibroblast differentiation; galectin-3; NRF2; ROS; melatonin

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INTRODUCTION

The formation of pulmonary scarring induced by autoimmune diseases, environmental and occupational exposures, pneumonia, and side effects of certain drugs is known as pulmonary fibrosis (PF), and its irreversible pathogenesis and severe impairment of lung function make it one of the most lethal respiratory diseases [1]. PF with unknown etiology is termed idiopathic pulmonary fibrosis (IPF), whose median survival time from diagnosis is 3~4 years and the incidence continues to rise [2]. Currently, lung transplantation is the only intervention shown to extend the life expectation of patients with IPF [3]. Notably, one of the major complications with infection of the novel coronavirus severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is PF, which leads to increased chronic dyspnea and impaired quality of life in patients with COVID-19 [4]. Viral-induced acute respiratory distress syndrome (ARDS) and PF share many risk factors and biological processes, including gender, aging, hypertension and diabetes [5], and lung epithelial cells and fibroblasts also participated in COVID-19 induced PF [6]. Therefore, investigating the pathogenesis of PF and searching for new therapeutic targets is a novel strategy to treat severe COVID-19 and prevent the possible long-term fibrotic consequences after current pandemic.

Accumulating evidences suggest that the pathological myofibroblast cells in PF mainly originate from epithelial cells and fibroblasts, and these processes are termed as EMT (epithelial-mesenchymal transition) and FMD (fibroblast-myofibroblast differentiation) respectively. Galectin-3 (Gal-3), the only chimera-type of the β-galactoside-binding lectin family, has been found to play pivotal role in TGF-β1 induced EMT in lung fibrosis and Gal-3 inhibitor TD139 has been proved to be effective in lung fibrosis model and IPF patients [7, 8]. However, engagement of Gal-3 in FMD process and the related mechanism remain poorly understood.

As a marker of oxidative stress, Gal-3 has been shown to be potentially associated with perturbations in mitochondrial homeostasis and the subsequent formation of reactive oxygen species (ROS) and glutathione (GSH) depletion [9–12]. Recombinant Gal-3 could stimulate ROS production in neutrophils [13] and monocytes [14] in a dose-dependent manner and increased Gal-3 contributed to superoxide production [15]. NRF2 is known as the major negative regulator of ROS and oxidative stress [16]. Our previous studies demonstrated that NRF2 attenuated the EMT process in PF through inhibition of the Numb or high-mobility group box 1 (HMGB1) pathway [17, 18]. Melatonin (MT, N-acetyl-5-methoxytryptamine), a hormone mainly secreted by the pineal

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