
# Hyaluronan synthase 2 regulates fibroblast senescence in pulmonary fibrosis

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## Abstract

Dysregulated repair of lung injury often results in lung fibrosis characterized by unremitting deposition of matrix components including glycosaminoglycan hyaluronan (HA). HA is mainly produced by hyaluronan synthases (HAS) in mesenchymal cells. We previously demonstrated that over-expression of HAS2 in mesenchymal cells in mice regulates the invasiveness of fibroblasts and promotes severe lung fibrosis. The mechanisms that control the resolution of lung fibrosis are unknown. We propose that a critical step in resolving fibrosis is the induction of senescence in fibrotic fibroblasts and hyaluronan synthase 2 may regulate this process. We found that fibrotic fibroblasts developed the characteristics of replicative senescence in culture and that HAS2 expression was dramatically down-regulated. Furthermore, down-regulation of HAS2 initiated and regulated fibroblast senescence through a p27-CDK2-SKP2 pathway. Deletion of HAS2 in mouse mesenchymal cells increased the cellular senescence of fibroblasts in bleomycin-induced mouse lung fibrosis in vivo. These data suggest that HAS2 may be a critical regulator of the fate of pulmonary fibrosis and we propose a model where over-expression of HAS2 promotes an invasive phenotype resulting in severe fibrosis and down-regulation of HAS2 promotes resolution. Targeting HAS2 to induce fibroblast senescence could be an attractive approach to resolve tissue fibrosis.



## Introduction

Dysregulated tissue injury and repair often result in tissue fibrosis. Fibroblasts in fibrotic diseases are heterogeneous in morphological, secretory, and behavioral properties, possibly because of their cell origin, activation state, and local environment. Resolution of tissue fibrosis has not been adequately addressed. The fate of fibrotic fibroblasts is largely unknown. Liver fibrosis is often reversible [1], while idiopathic pulmonary fibrosis (IPF), a severe disease with high mortality rate has proved irreversible to date [2]. The hypothesis has been put forth that fibroblasts from IPF patients are resistant to apoptosis leading to failure of resolution. For example, fibroblasts from IPF patients are resistant to Fas-L-induced apoptosis compared with normal fibroblasts [3]. A variety of extracellular matrix regulators such as CD44 [4], soluble fibronectin peptides [5], and PAI-1 [6] have also been shown to regulate fibroblast apoptosis. Collectively, these studies suggest there may be both intrinsic components of fibrotic fibroblasts as well as interactions with the surrounding matrix that may regulate apoptosis.

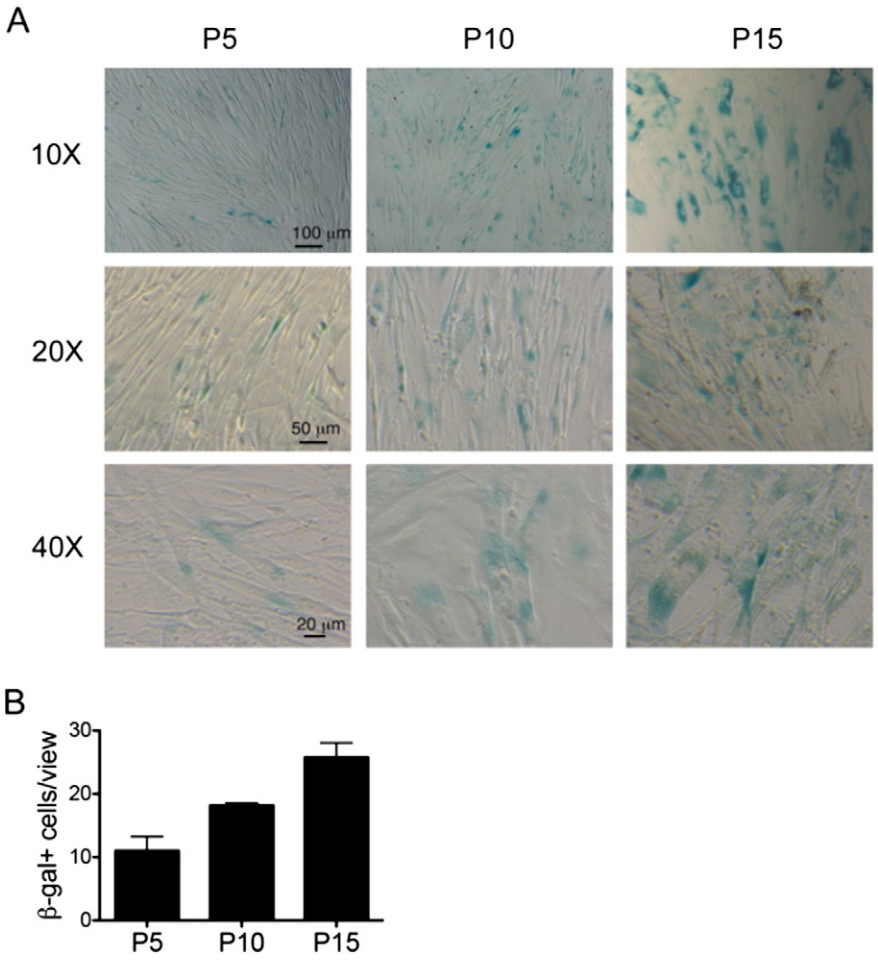


**Fig. 1.** (A). Replicative senescence in fibrotic fibroblasts. Fibrotic fibroblasts at passages (p) 5, 10 and 15 cultured in 15% FBS-DMEM were stained for SA-β-gal. $n = 5$ patients. Scale bars are shown. (B). Number of SA-β-gal positive cells per view with 20× magnification. 4–6 random views were counted for each passage of cells.

Senescence is a complex phenomenon related to cellular stress where cells lose their ability to proliferate [7]. Senescent cells irreversibly arrest in the G1 phase of cell cycle, develop a flattened, enlarged morphology, and demonstrate increased activity of senescence associated β-galactosidase (SA-β-gal) [7]. Various mechanisms including telomere erosion, chromatin disruption, DNA damage, oncogene activation, and oxidative stress are reported to trigger replicative or premature senescence [7]. Several pathways including p53, p16, and p27 are involved in initiating and regulating senescence [7]. Senescence is implicated in development [8], cancer [9], and tissue fibrosis [10]. The chronic inflammation caused by cellular senescence may be related to the pathogenesis of various chronic diseases [9,11]. A few studies of senescent epithelial cells suggest a role in IPF [12,13] and in bleomycin-induced lung fibrosis [14]. Insufficient autophagy may be responsible for accelerated cellular senescence [13]. In liver fibrosis models, hepatic myofibroblasts undergo senescence when fibrosis resolves [10]. The role of fibroblast senescence in lung fibrosis has not been investigated.

The matrix in which a cell resides has profound effects on cellular functions. Hyaluronan (HA) is one of the major components of extracellular matrix, which is mainly produced by mesenchymal cells [15]. All three hyaluronan synthase isoforms, designated as HAS1, HAS2 and HAS3, synthesize HA at the inner face of the cytoplasmic membrane in vertebrates [16]. HAS2 is the major isoform responsible for hyaluronan production in mesenchymal cells. HAS2 deficiency leads to embryonic lethality [17], and the vast majority of mice with targeted deletion of HAS2 in collagen-expressing mesenchymal cells died in utero [18]. Accumulation of HA is a characteristic of disorders that are associated with lung diseases [19] and progressive tissue fibrosis [20]. HA is able to modulate TGF-β induction of lung myofibroblasts and to control collagen deposition [21]. HAS2 has been implicated in cellular senescence since suppression of HAS2 in tumor cells