

Effect of purine-rich box1 on proliferation of fibroblasts

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Abstract

• Fibroblasts are pleomorphic cells that have a multi-directional effect on organ morphogenesis, tissue homeostasis, and immune response. In fibrotic diseases, fibroblasts synthesize large amounts of extracellular matrix (ECM), leading to scarring and organ failure. Purine-rich box1 (PU.1) is a specific transcription factor of hematopoietic cell and belongs to the E26 transformation specificity (ETS) family. Recently, it was found that the transcription factor PU.1 is an important regulatory factor of the profibrotic gene expression program. TGF- β had been proved to play an important role in many ocular tissue fibrosis diseases, and up-regulated the expression of PU.1 in fibroblasts producing ECM in a Smad-3 dependent manner. We explore the effect of PU.1 on fibrosis of different ocular tissues from this perspective. This article reviews the role of PU.1 and its effects on fibrosis of ocular tissue and other tissues.

• **KEYWORDS:** purine-rich box1; TGF- β ; extracellular matrix; fibroblasts; fibrosis; ocular tissue

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INTRODUCTION

Fibrosis is a non-physiological scar formation process associated with excessive deposition of extracellular matrix (ECM) and leads to damage to organ function^[1]. Fibrosis has involved in several cell populations, including

circulating fiber cells derived from bone marrow, endothelial cells, epithelial cells, and perivascular cells which are recently called pericytes^[2]. A research has shown that fibroblasts are the main source of ECM in fibrosis^[3]. Therefore, fibroblasts are the focus in fibrosis research, and the inhibition of ECM production mediated by fibroblast is a major goal of anti-fibrotic therapy. Fibrosis is a complex process involving many cell factors, including growth factors [transforming growth factor- β (TGF- β), connective tissue growth factor (CTGF) and platelet derived growth factor (PDGF)], Th1/Th2 interleukins (IL), pro-inflammatory cytokines [IL-1 and tumor necrosis factor- α (TNF- α)], IL-6 cytokine family [oncostatin-M (OSM), IL-6 and IL-11] and hematopoietic growth factor. The key role of TGF- β in tissue fibrosis has not been controversial in any organ, making TGF- β a major target for potential anti-fibrotic therapy^[4]. Studies have reported that TGF- β is the typical profibrotic growth factor that contributes to the development of fibrosis in all organs^[1].

Purine-rich box1 (PU.1) is a member of the E26 transformation specificity (ETS) family and is a major regulator of hematopoietic stem cell proliferation and differentiation, it maintains the cellular homeostasis and plays an important role in inflammation and immunity. Studies have shown that TGF- β further upregulates the basal level of PU.1 in a Smad3-dependent manner in fibroblasts, and PU.1 is highly expressed in fibrotic fibroblasts that produce ECM. Inhibiting the expression of PU.1 may be an effective method for treating a variety of fibrous diseases. Studies have shown that the expression of PU.1 is up-regulated in fibroblasts of various fibrotic diseases. Wohlfahrt *et al*^[5] used the CRISPR-Cas9 technology to knock out the PU.1 gene (human, SPI 1; mouse, Spi1) from human fibroblasts from fibrous tissue. Fibroblastic fibroblasts with SPI 1 knockout showed that the release of collagen reduced, and expression levels of α -smooth muscle actin (α -SMA) and F-actin were similar to those of resting fibroblasts. In contrast, overexpression of PU.1 in resting fibroblasts induces a shift in resting fibroblasts from healthy donors to highly activated profibrotic phenotypes, the release of collagen and the expression of α -SMA and F-actin are upregulated. PU.1 is expressed in fibroblasts of the mouse fibrosis model, which is similar to humans, and fibroblast-specific gene knockout SPI1 improves fibrosis in the above model. Although PU.1 is highly expressed in fibroblasts

producing ECM, it is silenced by epigenetic mechanisms in resting fibroblasts and inflammatory fibroblasts which degrade the ECM. Thus, the activity of PU.1 is a genetic switch that promotes the production of fibrotic fibroblasts that produce ECM. In various fibrotic diseases, the interaction between the transcriptional and post-transcriptional mechanisms that normally control the expression of PU.1 is disrupted, leading to up-regulation of PU.1, induction of fibrosis-related genes, and phenotypic transformation of fibrotic fibroblasts producing ECM. In contrast, the inactivation of PU.1 effectively restored the fibrotic phenotype of fibroblasts to a resting state and induced the regression of tissue fibrosis^[5].

Mechanism, Expression and Function of PU.1 PU.1 was isolated from the gene product of SPI1 in the proto-oncogene in 1988 by Moreau-Gachelin *et al*^[6]. PU.1 is a hematopoietic cell-specific transcription factor belonging to the ETS family. It is named for its ability to recognize and bind to core DNA elements which are rich in GGAA/T (PU box) motifs and its expression has obvious tissue specificity. The difference in expressing levels determines the differentiation of many kinds of immune cells (determining that hematopoietic stem cells eventually differentiate into different functional cell types)^[7]. PU.1 is a major regulatory factor of hematopoietic stem cell proliferation and differentiation. When PU.1 is expressed at a high level, it is beneficial to the differentiation of macrophage, neutrophil and monocyte, while at a low level, it is beneficial to the differentiation of B lymphocyte; it isn't expressed in mature megakaryocytes, erythrocytes and T lymphocytes. In recent years, studies have reported that PU.1 is also expressed in fibroblasts, osteoclasts, and epithelial cells^[8]. As a transcription factor, PU.1 has identified more than 110 direct target genes, and it can regulate downstream gene networks through multiple levels. It has several main functions: 1) regulating the expression of antibody, receptor and complement; 2) regulating the proliferation and differentiation of immune cells; 3) regulating the expression of inflammation-associated cytokines and effector enzymes^[9].

PU.1 and Ocular Tissue Fibrosis

Effect of PU.1 on trabecular meshwork fibrosis Glaucoma refers to a group of optic neuropathies characterized by degeneration of retinal ganglion cells (RGC) and visual field defects. The most common glaucoma is chronic open angle glaucoma (OAG), in which the iris cornea angle remains open and the increase in intraocular pressure (IOP) is mainly due to the hardening of the trabecular meshwork (TM) and related ciliary muscles (CM)^[10]. Furthermore, elevated IOP cause RGC dysfunction, which in some cases can occur in an IOP-dependent manner^[11]. The pathological high resistance to aqueous outflow may be due to the conversion of TM cells to myofibroblast (MFB)-like cells^[12]. TGF- β 1 and TGF- β 2

induce the contraction of TM and CM and the deposition of ECM in OAG patients, and this study indicates that sustained high TGF- β levels lead to a sustained increase in TM fibrosis and IOP. Similarly, since TGF- β in fibrotic fibroblasts can up-regulate PU.1 level, the expression of PU.1 may affect the fibrosis of TM.

Effect of PU.1 on proliferative vitreoretinopathy Proliferative vitreoretinopathy (PVR) is a blinding disease characterized by the formation of a preretinal membrane by a defective wound repair process^[13]. The development of PVR involves several cell types, including glial cells, retinal pigment epithelial (RPE) cells, inflammatory cells and fibroblasts (primarily derived from RPE cells)^[14]. Among these cell types, RPE cells are thought to play a major role in the pathogenesis of PVR, because it is the main cellular component of the preretinal membrane of PVR patients^[15]. When the retina is damaged, the blood-retinal barrier is destroyed, and the RPE cells are separated from the bruch membrane and migrate to the vitreous cavity or subretinal space. The vitreous is rich in cytokines that are released from the serum due to impaired blood-retinal barrier or secreted by activated cells during the progressive stage of PVR^[16]. Under the stimulation of these factors, RPE cells undergo epithelial-mesenchymal transition (EMT), gain the abilities of proliferation, migration and invasion, resistance to apoptosis, and transit from epithelial cells to mesenchymal phenotype to form fibroblasts and MFB which produce matrix, and involve in the formation of retinal fibrous tissue^[17]. TGF- β belongs to a superfamily molecule that regulates the differentiation and growth of cells. EMT can be triggered by different signaling molecules, such as epidermal growth factor and fibroblast growth factor, but TGF- β 1 is considered to be the main regulator of EMT^[18]. TGF- β in fibroblastic fibroblasts can up-regulate the level of PU.1, therefore, whether PU.1 can promote the occurrence and development of PVR needs to be further studied.

Effect of PU.1 on posterior cataract Posterior cataract (PCO) is the most common complication after extracapsular cataract extraction^[19]. Years after surgery, lens epithelial cells may continue to undergo aberrant proliferation, migration and fibrosis. The lens cells then lose epithelial characteristics and become mesenchymal cells, which is the process of EMT^[20]. EMT is a cellular phenotypic change in which epithelial cells lose their established cell-cell basement membrane contact, its structure becomes fusiform, and is morphologically similar to interstitial cells/myofibroblasts^[21], and LEC migrates to posterior capsule after proliferation, and transforms into MFB, producing α -SMA, which has a contractile function that causes the lens capsule surface to shrink.

Studies have found that TGF- β plays an important role in inhibiting the proliferation of lens epithelial cell (LEC) and

promoting the synthesis of EMT and ECM^[22]. Moreover, TGF- β is highly expressed in the human lens and forms a TGF- β signal transduction pathway together with the corresponding receptor and signal transduction molecule (mainly Smad protein). Among them, TGF- β /Smad signaling pathway plays an important role in regulating the development of PCO. The EMT and the ability of migration of Smad2 overexpressing cells are enhanced, and the ECM synthesis of Smad3 overexpressing cells is increased^[23]. In fibrotic fibroblasts, TGF- β further upregulates the basal level of PU.1 in a Smad 3-dependent manner, and thus whether the transcription factor PU.1 can promote the processes of EMT and the synthesis of ECM, and whether it promotes the formation of PCO remain unknown, these could be our new direction of the research .

Effect of PU.1 on Tenon's capsule fibroblasts Glaucoma is the leading cause of irreversible blindness throughout the world and is characterized by irreversible damage to the optic nerve and defects in the visual field^[24]. Till now, the most effective treatment for glaucoma is glaucoma filtration surgery; however, excessive scarring of filtration blebs is the most important cause of surgical failure^[25]. fibroblasts of Tenon's capsule can be activated by cytokines and growth factors to promote the proliferation and migration of cells and the synthesis of ECM, and TGF- β is known to regulate cell activity, including contraction, differentiation, proliferation, and ECM production^[26-27], and the TGF- β family is the main stimulating factor for scar formation after glaucoma filtration surgery. At present, we have three subtypes of TGF- β found in human cells, namely TGF- β 1, TGF- β 2 and TGF- β 3, of which TGF- β 2 was found to be the main cause of eye scar formation after glaucoma filtration surgery^[28]. Studies have found that TGF- β 2 promotes cell proliferation by inhibiting the expression of Nrf2 and miR-29b mRNA in human Tenon's fibroblasts (HTFs). In fibrotic fibroblasts, TGF- β further upregulates the basal level of PU.1 in a Smad3-dependent manner, and thus the proliferation of Tenon's capsule fibroblasts may be related to the level of PU.1. Studies have shown that PU.1 and IL-9 are significantly reduced after blocking TGF- β signaling *in vitro*^[29], and PU.1 may promote the proliferation of Tenon's capsule fibroblasts.

PU.1 and Other Tissue Fibrosis

Effect of PU.1 on pulmonary fibrosis Pulmonary fibrosis (PF) is the most common type of interstitial pneumonia with a poor prognosis. It is a terminal stage of lung disease characterized by the proliferation of fibroblast and the aggregation of a large number of ECM with inflammatory injury and tissue destruction. The lesions are confined to the lungs, causing diffuse PF, leading to symptoms such as impaired lung function and difficulty breathing. PU.1 specifically binds to a site in the

IL-9 promoter in Th9 cells, promotes the development of Th9 cells, and plays an very important role in the development of allergic and autoimmune diseases^[30]. Jiang *et al*^[31] found that the abnormal expression of IL-9 in connective tissue disease with interstitial lung disease (CTD-ILD) is associated with the severity of PF. Studies have shown that the overexpression of IL-9 in mice is associated with the development of PF and the reduction of IL-9 may suppress the PF^[32-33]. The results of immunohistochemistry of IL-9 and PU.1 in lung tissue of rats with PF showed that there was a positive correlation between IL-9 protein expression and PU.1 protein expression in lung tissue^[34]. Yang *et al*^[35] detected the content of PU.1 in rat alveolar lavage fluid by fluorescence PCR, and found that PU.1, as a specific transcription factor of Th9 cells, increased gradually in the process of fibrosis. Bao *et al*^[36] found that PU.1 can promote the formation of PF in rats.

Effect of PU.1 on liver fibrosis Liver fibrosis is a pathological process and result of excessive deposition of ECM and inflammation caused by various chronic pathogenic factors during liver tissue repairs after its injury^[37]. ECM is produced by MFB and is mainly derived from activated hepatic stellate cells (HSCs). HSCs are pericytes located in the space surrounding the hepatic sinus, which are the major cell types involved in healing and scar formation associated with liver injury. Stellate cells are in rest state under normal conditions. When the liver is damaged, the quiescent HSCs transform into activated MFB, which results in the upregulation of the α -SMA and the deposition of ECM, activated HSCs were considered to be main mechanism for liver fibrosis^[38-39]. The imbalance between synthesis and degradation of ECM, increased production of ECM or reduced degradation are the most fundamental cause of liver fibrosis. PU.1 showed lower expression in hepatocellular carcinoma and was identified as a tumor suppressor, suggesting that PU.1 may play a role in the cell fate or function of hepatocytes^[40]. PU.1 is a key transcription factor involved in many pathological processes. However, its exact role in HSCs and liver fibrosis activation is rarely reported. Activation and proliferation of HSC are involved in a variety of cellular signaling pathways. Among them, TGF- β blocks the growth of hepatocytes through the TGF- β /Smad signaling pathway, thereby inducing and promoting the interstitial transformation of precursor cells, leading to high expression and excessive deposition of ECM, and then leading to liver fibrosis^[41]. However, PU.1 is involved in TEAD-Hippo, the classical TGF- β /Smad and the profibrotic factor network of the AP1 signaling pathway. In fibroblastic fibroblasts, TGF- β further upregulates the basal level of PU.1 in a Smad3-dependent manner. Therefore, PU.1 may be involved in the process of liver fibrosis, and elevated levels of PU.1 may be associated with high expression of

ECM. In addition, Sirt1 acts as an inducer of apoptosis and inhibits proliferation and activation of various cells. The overexpression of PU.1 promotes proliferation, migration, activation, oxidative stress and inflammatory responses of HSCs by inhibiting Sirt1 protein. Thus, PU.1 positively affects the activation of HSC and liver fibrosis by negatively regulating the expression of Sirt1 protein^[42]. Therefore, PU.1 may have a certain promoting effect on liver fibrosis.

Effect of PU.1 on systemic sclerosis Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by localized or diffuse skin thickening and fibrosis, in which an abnormal immune response leads to the activation and expansion of immune cells by self-reactive effects, resulting in multiple organ fibers^[43]. Studies have shown that Th9 cells and IL-9 are involved in the pathogenesis of SSc. Increased expression of IL-4, thymic stromal lymphopoietin (TSLP) and TGF- β was detected in skin tissue of SSc patients, particularly in patients with diffuse disease. According to the expansion of Th9 cells, TGF- β , TSLP and IL-4 are significantly up-regulated in SSc skin and directly related to the expression of IL-9 and the number of Th9 cells^[44]. The differentiation of Th9 cells requires the balance of signaling molecules, including IL-4 and TGF- β , as well as the epithelial cytokine TSLP, which leads to the induction of transcription factor PU.1. PU.1 directly binds to the IL-9 promoter and activates gene expression in Th9 cells, which is consistent with interferon regulatory factor 4 (IRF4)^[45-46]. In addition, the differentiation of Th9 and the expression of IL-9 were enhanced by IL-4, TGF- β and TSLP, and activated by induction of ETS family transcription factor PU.1^[47]. Therefore, PU.1 is able to induce Th9 differentiation and IL-9 expression and has a positive effect on SSc. Moreover, studies have confirmed an increase in the expression of PU.1 in the skin of patients with SSc^[5].

Effect of PU.1 on cystic fibrosis Cystic fibrosis (CF) is a hereditary exocrine gland disease that primarily affects the gastrointestinal tract and respiratory system and is often characterized by exocrine pancreatic dysfunction, chronic obstructive pulmonary disease, and abnormally elevated sweat electrolytes. CF is an autosomal recessive disorder caused by mutation of the CF gene on chromosome 7. The pathogenesis of CF exocrine gland dysfunction is unclear. Studies have shown that it is caused by an abnormality in the gene encoding the chloride channel. The regulation of chloride channel in epithelial cells of the patient is defective. The transport of water and electrolytes across the membrane of respiratory mucosa is hindered, and the content of acid glycoprotein in mucus gland secretions increases, changing the characteristics of mucus rheology, which may be the cause of thickening of secretions. Peripheral blood mononuclear cells isolated from CF patients were found to be in an endotoxin-tolerant state.

In patients with CF, down-regulation of Trem-1 (myeloid cell-triggering receptor), at least to some extent, is one of the causes of endotoxin tolerance in which monocytes are locked, transcription factor PU.1 plays an important role in the regulation of Trem-1 expression^[48]. Studies have found that high levels of PU.1 are detected in the nuclei isolated from CF monocytes, and transcription factor PU.1 inhibits upregulation of Trem-1^[49]. Therefore, PU.1 may promote CF.

Conclusion and Future Trends At present, studies have confirmed that PU.1 is expressed in lung, liver, kidney, skin and other related fibrotic diseases, but its specific molecular mechanism is still unclear, and the relationship between PU.1 and eye-related fibrotic diseases is rarely reported. The role of PU.1 in tissue and its effect on related fibrosis diseases need to be further studied. With the continuous improvement of molecular biology technology, the research of PU.1 in various fibrotic diseases may become one of the hotspots for the treatment of fibrotic diseases in the future, providing a new method for the treatment of fibrotic diseases. It is believed that in the near future, the functions of PU.1 will be continuously researched and discovered. The treatment of fibrosis diseases based on PU.1 can certainly be developed in clinic, which will open up broader prospects for the diagnosis and treatment of fibrotic diseases.

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PU.1 on proliferation

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