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Journal of Cancer Epidemiology & Treatment

ETS-Related Gene (ERG) and Friend leukemia integration – 1 (FLI-1) Transcription Factors in the Precision Treatment of Pulmonary Arterial Hypertension and Pulmonary Fibrosis

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Abstract

Pulmonary arterial hypertension (PAH) is a chronic debilitating cardiopulmonary disease characterized by abnormal remodeling of peripheral lung vasculature resulting in progressive vasoconstriction. Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and irreversible disease that is often associated with significant morbidity and poor quality of life. The prognosis of PAH and IPF is poor and currently available medications focus on relieving symptoms and slowing down progression. Hence, there is a clear necessity to develop new therapies. ETS-related genes and Friend leukemia integration–1 (FLI–1) are transcription factors involved in angiogenesis, cellular homeostasis, vascular remodeling, and the genetic regulation of inflammation, apoptosis, and fibrosis seen in PAH and IPF. Simultaneous small-interfering-RNA (siRNA) knockdown of ERG and FLI-1 in human pulmonary artery endothelial cells (HPAEC) and human pulmonary microvasculature endothelial cells (HPMEC) has been associated with up-regulation of pro-inflammatory genes and interferon (IFN) pathway-related genes. Notably, the endothelium in normal lungs has also been shown to have high levels of nuclear ERG and Fli-1 compared to significantly lower levels in diseased lungs. Recently, ERG upregulation was found to promote liver homoeostasis by regulating canonical TGFβ1-SMAD signaling and promoting the SMAD1 pathway while repressing SMAD3 activity. Improvement in pulmonary fibrosis through medications that suppress the TGF-β1/Smad3 pathway has also been a subject of study. In this review, we hypothesize that targeting the regulation of ERG, FLI-1 and ERG-mediation of TGF-β1/Smad3 signaling may be a promising therapeutic strategy in PAH and IPF.

Keyword: Pulmonary Diseases, ETS genes, Signaling pathways, Targeted therapy, small molecules, Smad3 inhibitors, ERG/Fli-1 inducers.

Introduction

Pulmonary arterial hypertension (PAH) is a chronic debilitating cardiopulmonary disease characterized by abnormal remodeling of peripheral lung vasculature resulting in progressive vasoconstriction [1]. PAH is associated with an elevated pulmonary arterial pressure (> 25 mm Hg at rest to > 30 mm Hg with exercise) and if left untreated leads to right-heart hypertrophy, and consequently heart failure [2]. PAH could be classified as idiopathic (formerly known as primary pulmonary hypertension) or associated with autoimmune, infectious, vascular, metabolic, and congenital disorders [1]. The underlying pathology of PAH involves the recruitment of inflammatory cells with release of cytokines which enhance cell proliferation and elastin fibers degradation [3]. Histologically, PAH is characterized by intimal fibrosis, increased medial thickness, predominance of complex plexiform lesions resulting in vasoconstriction and pulmonary arteriolar occlusion [4].

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and irreversible disease that is often associated with significant morbidity and poor quality of life [5]. Till date, the cause of IPF is unknown and the pathogenesis is not completely clear [6]. However, studies have shown that the fibrosis is driven by the

abnormal activation of alveolar epithelial cells (AECs) by TGF-β1, which enhance the formation of fibroblasts and myofibroblasts, through the proliferation of resident mesenchymal cells and stimulation of the epithelial to mesenchymal transition (EMT) [6].

The disease mechanism in PAH and IPF has been the target of numerous therapeutic interventions [7]. The current mainstay of PAH treatment essentially involves promoting vasodilation, inhibiting prothrombotic and inflammatory processes. Similarly, in IPF, treatment is mainly geared towards relieving symptoms as much as possible and slowing down the progression. The prognosis ofPAH and IPF is poor [1,6], hence, there is a clear necessity to develop new therapies.

Recent evidence suggests that there are families of transcription factors (ETS-related gene (ERG) and Friend leukemia integration–1(FLI1) involved in the angiogenesis, cellular homeostasis, vascular remodeling, and the genetic regulation of inflammation, apoptosis, and fibrosis seen in PAH and IPF[8-10]. Improved understanding of the genetic modulation of these processes has revealed novel therapeutic strategies.In this review, we will discuss the role of the transcription factors in the pathogenesis of PAH and IPF, and they can hypothetically contribute to precision and targeted therapy.

FLI1 and ERG in the Modulation of Vascular Homeostasis, Inflammation and Tissue Fibrosis

ERG and human FLI1 both belong to the ETS family of transcriptional factors and were discovered by Drs. Reddy and Rao [11,12].. ETS transcription factors share an 85 amino acid DNA binding domain (ETS domain) that binds to a DNA core consensus motif, 5'GGA(A/T)3' [13-17].Both FLI1 and ERG proteins were shown to inhibit apoptosis [18]. Depending on the cellular environment, these factors can act as transcriptional activators, repressors, or both [19]. Of all the ETS factors, the ERG and FLI1 are the most closely related in terms of protein structure and have been extensively studied in the context of vascular homeostasis and remodeling [20,21].

Though ERG and FLI1 have similar structures, they have different cellular regulatory roles. ERG is mainly expressed in endothelial cells, megakaryocyte erythroid progenitor cells, T and B early lineage-committed cells, and myocardium [22].On the other hand, FLI1 expression is mostly found in endothelial cells, hematopoietic progenitors and their mature derivatives (megakaryocytes, monocytes, erythroblasts, and natural killer cells), neural cells, and dermal fibroblasts [23].

ERG and FLI-1 in PAH Pathogenesis

Several in-vivo studies have shed more light on the biological roles of the ERG and FLI1 transcription factors especially in embryogenic development of cardiac structures and hematopoietic stem cells[24,25].Notably, Looney and collaborators showed that the simultaneous small-interfering-

RNA (siRNA) knockdown ofERG and FLI1 in human pulmonary artery endothelial cells (HPAEC) and human pulmonary microvasculature endothelial cells (HPMEC) was associated with up-regulation of pro-inflammatory genes and interferon (IFN) pathway-related genes [26].

Additionally, dual knockdown of these factors had a significantly higher pro-inflammatory effect than obtained from knockdown of ERG or FLI1 alone. The study collaborators further compared the levels of endothelial ERG in the pulmonary vasculature of lung sections from patients with idiopathic PAH and scleroderma associated PAH to that of non-diseased lungs (healthy controls). The endothelium in normal lungs was found to have high levels of nuclear ERG compared to significantly lower levels to near absence of ERG in the endothelium of patients with diseased lungs. This suggests that down-regulation or loss of ERG in the HPAEC and HPMEC of humans is associated with systemic and pulmonary vasculopathy seen in PAH.

ETS Related Gene (ERG) and TGF-β1/Smad3 pathway in the Pathogenesis of IPF

Smad3 has been shown to be a critical mediator in the pathogenesis of tissue fibrosis.In animal models, Smad3 signaling through phosphorylation and cytosol-nucleus translocation upregulated TGF-β1-induced EMT processes and subsequent lung fibrosis [27,28]. Targeted suppression of the TGF-β1/Smad3 pathway improved pulmonary fibrosis in rats[10.] Significantly elevated levels of TGF-β1 has also been demonstrated in patients hitherto diagnosed with idiopathic pulmonary fibrosis [29].

In another study by Webb *et al*. [8], upregulation of ETS-related gene (ERG) promoted liver homoeostasis by regulating canonical TGFβ1-SMAD signaling and promoting the SMAD1 pathway while repressing SMAD3 activity.Molecular ablation of ERG expression in this study further resulted in a SMAD3-dependent endothelial-to-mesenchymal transition (EMT) and spontaneous liver fibrogenesis. Decreased ERG expression was also shown to correlate with the degree of EndMT end-stage liver fibrosis. This study highlights ERG mediated fibrogenesis through the TGFB1-SMAD pathway and the potential of ERG as a reliable biomarker in assessing EMT in tissue fibrosis.

Targeted Tumor necrosis factor-alpha (TNF-α) inhibition in PAH and IPF Treatment

Tumor necrosis factor-alpha (TNF-α) is a cell signaling protein (cytokine) produced by lymphocytes and macrophages. TNF-α mediates the immune response by attracting white blood cells to sites of injury and regulating inflammatory processes [30]. Dysregulation of TNF production has been implicated in a variety of autoimmune diseases. PAH and IPF patients demonstrate a high circulating levels of inflammatory cytokines especially TNF α, which also correlate with poor prognosis and survival [31,32].

Etanercept is a fusion protein produced by recombinant DNA that fuses the TNF receptor to the constant end of the immunoglobulin

(Ig) G1 antibody thereby reducing the inflammatory effects of TNF-α [33].It is the most studied TN-α inhibitor in PAH treatment research. Studies have demonstrated that Etanercept administration prevented and reversed Monocrotaline (MCT) - induced PAH in experimental rats through the suppression of TNF-α expression and NF-κB pathway [34-36].Similar effects were also noted when Piguet and Vesin prevented Bleomycin and Silica induced lung fibrosis with the infusion of a TNF – alpha inhibitor in mice [37].

Etanercept as a Potential Treatment of PAH and IPF through ETS Expression and ERG mediation of TGF B1 / Smad3 Pathway

TNF-α inhibition may not be the only mechanism through which etanercept could be beneficial in PAH treatment. As already described, reduced expression of ERG and FLI1 transcription factors is associated with dysregulation of vascular homeostasis in HPAEC and HMPEC [26].Likewise, upregulation of the TGF-β1/Smad3 activate the cascade of lung fibroblasts proliferation and formation of aberrant epithelial cells leading to pulmonary fibrosis [38].

Notably, Neil *et al.* induced liver injury and fibrosis through administration of tetrachloric acid (CCL4) which caused a significant hyperplasia in smooth muscle actin (SMA), reduction of ERG expression and enhanced SMAD 3 phosphorylation [8]. The co-administration of etanercept prevented these changes and normalized endothelial ERG expression. Interestingly, etanercept was not able to prevent or correct the CCL4- induced injury in $Erg^{cEC-het}$ mice which suggests that it potentially exerts its therapeutic effects through the endothelial ERG pathway in the prevention of fibrosis and restoration of vascular homeostasis.

Conclusion

Targeting the regulation of ERG, FLI1 and ERG-mediation of TGF-β1/Smad3 signaling may be a promising therapeutic strategy in PAH and IPF. Etanercept appears to reduce endothelial inflammation and tissue fibrosis through increased expression of ERG and FLI1, promotion of ERG-mediated reduction of smad3 phosphorylation and suppression of TGFβ1/Smad3 signaling pathway. Tyrosine kinase (Ponatinib) has been shown to improve pulmonary fibrosis by inhibiting the TGF-β1/Smad3 pathway invitro[10]. It is also tempting to speculate that FDA approved Smad3 inhibitors can be used as therapeutic agents through modulation of the functional roles of ERG and Fli-1 in PAH and IPF. ERG and Fli-1 protein levels may serve as early biomarkers for monitoring the health of blood vessels connected to the lung tissue in PAH and IPF patients. Similarly, serum levels of ERG and Fli-1 can be useful in monitoring treatment of patients with PAH and IPF as restoration to normal levels tend to correlate with positive response.

Conflict of Interest

We acknowledge that we have no conflict of interest or any financial interest that could influence our work.

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