Vascular Endothelial Growth Factor Signaling in Fibrosis: An Ophthalmic Perspective

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Abstract

Fibrosis is a common condition, transcending all areas of medicine. Its pathophysiology has eluded researchers for decades with no universal cure. The cellular processes which underpin these conditions begin with cellular proliferation, followed by migration, epithelial to mesenchymal transformation, and extracellular matrix contraction. This short review highlights the complexities in the signaling cascade of the key event in fibrosis: epithelial to mesenchymal transition with particular emphasis on the role of vascular endothelial growth factor (VEGF). Other growth factors, mitogens, and markers of fibrosis included in this review are transforming growth factor β (beta), platelet-derived growth factor, cadherins, Smads, Slug, Snail, α-SMA, and the Rho-kinease group, among others. The ocular lens capsule serves as an ideal model to study this pathophysiology due to its isolation from other organs, avascular environment, and experimental models available. Anti-VEGF treatments are well established for neovascular conditions such as age-related macular degeneration and may therefore form a component of anti-fibrotic treatment in the future. Evidence for reduction of fibrotic markers following VEGF inhibition is revealed last.

Keywords: VEGF, Vascular endothelial growth factor, Fibrosis, Lens capsule, Posterior capsule Opacification

Fibrosis

Fibrosis is a pathological process in which a tissue’s architecture is disturbed by excessive fibro connective tissue, typically in response to a reparative or reactive response to a cellular insult [1,2]. From metabolic malfunction, ischemia, degeneration, or autoimmune inflammatory processes, fibrosis results from proliferation and Trans differentiation of a cellular phenotype (e.g. epithelial cells) to activated fibroblasts known as my fibroblasts. These cells contribute to ‘excessive’ production of extra-cellular matrix (ECM) components, resulting in contraction and disruption of tissue architecture [1]. In an effort to resolve the damaged tissue, this complex proliferative process often wreaks havoc, leading to worse survival rates than cancer in the most severe cases (i.e. idiopathic pulmonary fibrosis) [3]. In ophthalmology, this results in loss of vision.

Vascular Endothelial Growth Factor

Vascular Endothelial Growth Factor (VEGF), also known as Vascular Permeability Factor (VPF), is a family of potent mitogens originally thought to solely stimulate endothelial cells in angiogenesis. Multiple genes translate the five known VEGF subtypes (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor), leading to differences in molecular structure, biological properties, and regulation [4,5]. Appreciating the gross variability in both the VEGF ligand and its receptor, it is the subtleties in this interaction leading to its differential effects. Its functions range from normal physiological regulation of wound healing, angiogenesis (capillary tube formation of endothelial cells), bone formation, hematopoiesis, to embryological development and morphogenesis. In the eye, VEGF is required for visual function through playing a survival role on Muller cells and photoreceptors, indicating an important role in the maintenance and functioning of the adult retina [6]. VEGF will also drive proliferation, migration, cell survival, and even epithelial to mesenchymal transition [7,8].

Epithelial to Mesenchymal Transition

In epithelial to mesenchymal transition (EMT), which all fibrotic processes share, epithelial cells experience a multitude of biochemical and morphological changes, disengaging from their...
TGF-β, a superfamily of βα, βα, and βα, known as “master regulators” of fibrogenic response and angiogenesis, generally suppress cell proliferation, induce apoptosis, promote EMT, and cell motility. These isoforms also regulate VEGF and VEGF has even been reported to induce TGF-β, depending on a direct interaction between its signaling proteins, Smads, and members of the Wnt/B-catenin signaling group [22,23]. T cell factor (TCF) provides two binding sites on the VEGF promoter region to which TGF-β binds for transcription, illustrated through absent levels of (TGF-β-induced) VEGF promoter activity after blocking these binding sites. Smad4, a common isoform in TGF-β/Smad signaling, will translocate to the nucleus of lens epithelium as soon as 2-12hrs post-surgical trauma. α-SMA is subsequently produced during EMT response. Rho-kinase, PI3-kinase, and Src-family kinases (SKF) also regulate EMT [24-26]. VEGFR-2(KDR/Flk-1) has proven to recruit Src homology 2 domain-containing molecules that lead to activation of MAP kinase signaling. This is significant because p85, a regulatory subunit of PI3-kinase, is strongly associated with Flk-1/KDR; treatment of human umbilical vascular endothelial cells (HUVECs) with VEGF caused tyrosine auto phosphorylation of VEGFR2 which, in turn, caused phosphorylation of p85, showing an increase in PI 3-kinase activity and implicating a larger role in EMT regulation [27]. It is when the PI-3 kinase/Akt pathway is down-regulated that bone morphogenetic protein 2 (BMP2), a member of the TGF-β super-family induces EMT and migration through loss of E-cadherin [28]. This hypothesis is consistent with reduced α-SMA in sub conjunctival layers of the eye post-anti-VEGF treatment after trabeculectomy (a form of glaucoma surgery) compared to control [22].

The Lens Capsule as a Model for VEGF’s Role in Fibrosis

Further studies investigated the role of VEGF in the lens, an avascular environment, after an active VEGF-A signaling system was revealed [29]. Assessment of medium from human lens capsular bag cultures demonstrated high levels of VEGF relative to other growth factors. In fact, levels were >10 fold more than detected for FGF [30]. It has also been proven that treatment of cells with TGF-β, EGF, PDGF, and IL-6 induce VEGF mRNA production, suggesting that paracrine or autocrine release of these compounds works with hypoxic conditions in regulating VEGF. Inflammatory cytokines such as IL-1 and IL-6 can even induce VEGF in synovial fibroblasts, hinting at an enormous array of compounds mediating its effects, particularly in inflammatory conditions [31,32]. This may therefore serve as a novel therapeutic target.

VEGF inhibition has been shown to reduce key markers of fibrosis and EMT (α-SMA, FN1, Collagen 1, SNAIL1/2, and MMP-2) [11]. Using cell-based contraction assays, VEGF inhibition prevents TGF-β mediated contraction. Immunocytochemical analysis shows that anti-VEGF therapy works independent of Smad signaling, which suggests contraction is not entirely due to trans differentiation, but perhaps through interaction of actin with myosin in intracellular stress fiber formation. TGF-β2 has proven to induce the Rho/Rho kinase pathway, which also relies on myosin light chain phosphatase (MLCP;Pase) and myosin light chain kinase (MLCK) in regulating contraction. Likewise, myosin activity is a key regulator for TGF-β induced contraction in lens cells [33]. In addition to Rho kinase, ERK signaling (regulated by VEGF) can also
induce matrix contraction via MLCK [34], JNK, and p38 pathways, all of which are induced by TGF-β2 as well [35]. Conversely, TGF-β2 induced collagen I transcription has been shown to decrease with VEGFR inhibition [11]. There is evidence that Smad3 is key for collagen I synthesis, but also the ras/MEK/ERK MAP kinase cascades [18]. The anti-fibrotic effects of VEGFR inhibition is likely dependent on the regulation of Smad signaling and instead through inhibition of ras/MEK/ERK MAP kinase.

Evidence suggests that VEGF induces SNAIL expression by inhibiting GSK3β (glycogen synthase kinase-3), which normally cooperates with AKT to stabilize SNAIL activity [36]. Blocking this pathway via VEGF’s receptor tyrosine kinases clearly exerts downstream effects on these compounds and prevents regulation of EMT.

Given VEGF’s role in an array of signaling pathways and physiology, there is clear evidence of an active biochemical pathway in fibrosis, even in an avascular environment such as the human lens. Anti-VEGF treatments are well established for neovascular ocular diseases (e.g., neovascular age-related macular degeneration) so may serve as a readily available treatment for fibrotic conditions. Future directions in fibrosis research, both in ocular and extraocular environments, should focus on targeted therapy on more than one signaling pathway due to the interdependencies illustrated above.

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Conflict of Interest

None to declare

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