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Molecular mechanisms of subretinal fibrosis in age-related macular degeneration

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ABSTRACT

Subretinal fibrosis is a result of a wound healing response that follows choroidal neovascularization in neovascular age-related macular degeneration (nAMD). Although anti-vascular endothelial growth factor therapy has become a standard treatment that improves visual acuity in many nAMD patients, unsuccessful treatment outcomes have often been attributed to the progression of subretinal fibrosis. In this review, we summarize the cellular and extracellular components of subretinal fibrous membranes and also discuss the possible molecular mechanisms including the functional involvement of growth factors and the inflammatory response in the process. Moreover, we present an murine animal model of subretinal fibrosis that might facilitate greater understanding of the pathophysiology and the development of novel therapeutic strategies for the inhibition of subretinal fibrosis in nAMD.

1. Introduction

Neovascular age-related macular degeneration (nAMD) is a leading cause of blindness resulting from the development of choroidal neovascularization (CNV), which may progress to an end stage fibrous plaque/disciform scar (Lim et al., 2012; Ryan, 1979). Currently, anti-vascular endothelial growth factor (VEGF) therapy has been the first choice for the primary treatment for CNV (Holz et al., 2014). Although anti-VEGF therapy generally stabilizes or improves visual function, subretinal scarring (fibrosis) can develop in approximately half of all treated eyes within two years after anti-VEGF treatment and has been identified as one cause of unsuccessful outcomes (Daniel et al., 2014). Subretinal fibrosis formation can cause local destruction of photoreceptors, retinal pigment epithelium (RPE), and choroidal vessels leading to permanent dysfunction of the macular visual system. Histopathologic studies of human AMD eyes suggest that the progression of photoreceptor degeneration is proportional to the diameter and thickness of the subretinal fibrosis (Green and Enger, 1993).

Fibrosis is considered an excessive wound healing response to tissue damage (Wynn, 2007). In the wound healing process, angiogenesis is initiated to aid in the repair of damaged tissue, to increase the oxygen supply, and to recruit inflammatory cells to the wounded tissue (Greaves et al., 2013). In nAMD, CNV develops in the subretinal and/or sub-pigment epithelial space, leading to hemorrhage and exudative change and culminating in subretinal fibrosis. This process is characterized by proliferation and/or infiltration of various types of cells: RPE, glial cells, fibroblasts,

Abbreviations: α-SMA, alpha-smooth muscle actin; BM, bone marrow; CNV, choroidal neovascularization; CNVMs, choroidal neovascular membranes; CTGF, connective tissue growth factor; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial–mesenchymal transition; FAK, focal adhesion kinase; FGF, fibroblast growth factor; FN, fibronectin; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinase; nAMD, neovascular age-related macular degeneration; PDGF, platelet derived growth factor; PDR, proliferative diabetic retinopathy; PI3K, phosphatidylinositol-3 kinase; POSTN, periostin; PVR, proliferative vitreoretinopathy; RPE, retinal pigment epithelium; S1P, sphingosine-1-phosphate; SPARC, osteonectin; TGF-β, transforming growth factor-β; TNC, tenascin; TNF-α, tumor necrosis factor-α; TSP1, thrombospondin 1; VEGF, vascular endothelial growth factor; ZEB, zinc finger E-box-binding homeobox; ZO-1, zonula occludens protein-1.

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myofibroblast-like cells and macrophages, interacting with inflammatory cytokines and growth factors and resulting in substantial remodeling of the extracellular matrix (ECM) (Kent and Sheridan, 2003). Because of the complexity of the cellular interactions and the numerous mediators, effective therapeutic intervention for fibrosis has yet to be developed. We will review the pathophysiology associated with subretinal fibrosis and discuss the potential animal models to study this process, as well as possible therapeutic strategies for preventing fibrotic scar formation in nAMD.

2. Clinical implications

Although anti-VEGF therapy has become a standard treatment that improves visual acuity or at least prevents severe vision loss in nAMD patients with CNV, some patients have a poor response to the therapy, resulting in visual impairment despite frequent intravitreal injections of the drugs (Cohen et al., 2012). These unsuccessful outcomes have often been attributed to progression of the underlying AMD, such as the development of subretinal fibrosis, the most common natural history pattern of subfoveal CNV (Pauleikhoff, 2005). To identify the risk factors for subretinal scars, a prospective cohort study was performed in eyes of nAMD patients receiving anti-VEGF therapy (Daniel et al., 2014). It was noted that eyes with “classic” CNV lesions, which penetrate the RPE monolayer and grow in the subretinal space, are more likely to develop scar formation than those with “occult” CNV lesions, which are usually confined to the space beneath the RPE. This suggests that a subretinal lesion containing extensively damaged and scattered RPE would be more likely to progress to fibrosis; and this increased likelihood could be related to the presence of transdifferentiated RPE cells in surgically removed CNV fibrous membranes, as discussed in Section 4. Furthermore, it has been reported that fibrosis may develop after treatment with anti-VEGF drugs in nAMD and proliferative diabetic retinopathy (PDR) (Arevalo et al., 2008; Hwang et al., 2011; Van Geest et al., 2012; Barikian et al., 2015) and development of subretinal fibrosis may be associated with a bi-weekly treatment regimen in nAMD (Barikian et al., 2015). However, prompt initiation of therapy may also be beneficial for the prevention of fibrosis since development of subretinal fibrosis is associated with a longer interval between diagnosis of nAMD and treatment with anti-VEGF drugs (Bloch et al., 2013).

In proliferative diabetic retinopathy (PDR), it has been reported that a balance between the levels of the growth factors pro-angiogenic VEGF and pro-fibrotic connective tissue growth factor (CTGF) regulates the angiogenesis to fibrosis conversion, the so-called “angio-fibrotic switch” (Kuiper et al., 2008). An increased ratio of CTGF to VEGF levels in the vitreous after anti-VEGF therapy has been suggested to be a trigger for fibrosis progression in PDR (Sohn et al., 2012; Van Geest et al., 2012). The molecular mechanism for the effect of VEGF inhibition on pro-fibrotic factors associated with subretinal fibrosis remains unclear.

3. Basic mechanisms of subretinal fibrosis

Subretinal fibrosis in nAMD shares common molecular mechanisms with fibrosis in organs such as lung, liver, kidney, heart and skin (Friedlander, 2007). Generally, soon after tissue injury, epithelial cells release mediators that recruit and activate inflammatory cells, endothelial cells, and fibroblasts. In addition, the cells undergo epithelial–mesenchymal transition (EMT), which enables transdifferentiation, resulting in the conversion of epithelial cells to myofibroblasts (Kalluri and Weinberg, 2009; Wynn, 2007). The induction of neovascularization can result in the recruitment of more inflammatory cells and fibroblasts, which can be a direct or indirect source of additional myofibroblasts. Those cells produce ECM, and proliferate and migrate over the basal layers to cover and regenerate the damaged tissue; however, in the presence of repeated injury and/or chronic inflammation, the fibrotic scar will persist.

4. Cellular components of subretinal fibrous membranes

Previously, surgical removal of choroidal neovascular membranes (CNVMs) was a common treatment option for patients with nAMD. According to the histological studies of the tissues excised from those patients, CNVMs consist of connective tissues such as ECM and cellular components such as vascular endothelial cells, RPE, macrophages, myofibroblasts, pericytes and fibroblast-like cells (Grossniklaus and Green, 1998; Grossniklaus et al., 1994; Hinton et al., 1998; Lopez et al., 1996). CNVM-scars have been defined as those with an increased proportion of fibrous tissue to neovascular channels (Macular Photocoagulation Study Group, 1991). Histologic studies of human CNVMs has also demonstrated that development of fibrous scar is accompanied by an increase in apoptosis and a decrease in cellularity, suggesting that subretinal fibrosis may evolve along with regression of CNV in nAMD (Hinton et al., 1998).

In surgically excised CNV, many of the stromal cells are immunoreactive for both alpha-smooth muscle actin (α-SMA) and cytokeratin (Lopez et al., 1996). Additionally, it was observed that there was a gradient change of RPE from cytokeratin-positive, mildly α-SMA-positive cells adjacent to the normal RPE monolayer, to non-pigmented cytokeratin-positive, α-SMA-positive stromal cells, and finally to cytokeratin-negative, α-SMA-positive cells in the stroma (Lopez et al., 1996). Thus, RPE could be the origin of myofibroblastic cells through development of EMT (Grisanti and Guidry, 1995).

An additional source of α-SMA-positive cells in experimental CNV is bone marrow-derived cells. When CNV was induced in irradiated mice that had been engrafted with green fluorescent protein (GFP) positive bone marrow, many of the α-SMA-positive cells were also GFP-positive (Espinosa-Heidmann et al., 2005). Thus there is evidence to support both a local (RPE) and systemic (Bone marrow-derived cells) source for α-SMA-positive cells in CNV lesions.

4.1. Epithelial–mesenchymal transition of the RPE

In the normal eye, the RPE is a highly polarized monolayer of pigmented cells located between the neural retina and the choroid that plays a critical role in the maintenance of visual function (Strauss, 2005). Normally, the RPE retains a mature epithelial phenotype and is mitotically quiescent with cell–cell contact inhibition mediated by the homotypic adhesion of cadherins on adjacent cells (Binder et al., 2007). Once these contacts are disrupted, RPE cells lose their epithelial phenotype with decreasing expression of epithelial markers such as E-cadherin and ZO-1 and gain mesenchymal properties with increasing expression of mesenchymal markers such as N-cadherin, vimentin and α-SMA (Kalluri and Weinberg, 2009). In nAMD, RPE detachment and dissociation can occur as part of the CNV process (Ambati and Fowler, 2012). In vitro, RPE that are dissociated into single cells gain the ability to proliferate and undergo EMT (Grisanti and Guidry, 1995). It has been observed that the loss of RPE cell–cell contact induces EMT and that the initiation of proliferation coincides with a switch in cadherin isoform expression, from P- to N-cadherin. Moreover, transforming growth factor (TGF)-β, a well-established EMT-inducer in RPE cell suspensions, does not initiate EMT in differentiated RPE with well-established cell–cell contacts, suggesting that disruption of cell–cell contact is a crucial step in