Signal transducer and activator of transcription 3 (STAT3) signaling in retinal pigment epithelium cells

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Abbreviations: STAT3, signal transducer and activator of transcription 3; RPE, retinal pigmented epithelium; JAK, Janus kinase; TLR, toll-like receptor; VEGF, vascular endothelial growth factor; AMD, age-related macular degeneration; UVB, medial wave ultraviolet light; CFB, complement factor B; IL6, interleukin 6; LIF, leukemia inhibitory factor; CNTF, ciliary neurotrophic factor; OsM, oncostatin M; CNV, choroidal neovascularization; NAC, N-acetyl cysteine; EGF, epidermal growth factor; GlcN, glucosamine

The retinal pigmented epithelium (RPE) is a monolayer of specialized epithelial cells located between the photoreceptors of the retina and the choroidal blood supply. The RPE is essential for maintaining retinal health and vision. Recent findings identified STAT3 as a newly recognized regulator of RPE survival, inflammatory response, visual cycle maintenance, and cytokine release. Additionally, STAT3 is implicated in retinal diseases that affect the RPE, including the common blinding disease age-related macular degeneration. Determining how STAT3 influences RPE functions ultimately may lead to novel therapeutics for retinal disease. In this review, we summarize the roles of JAK-STAT3 signaling in the RPE, and its potential contribution to retinal degenerations.

Introduction

The retina is a thin neural–epithelial tissue that lines the posterior region of the eye. The retina is primarily responsible for vision, and its cellular layers are specialized for converting photons of light to electrical signals that are perceived by the brain as color, contrast, depth and movement. Two major cell types in the retina contribute to vision: neurons detect and process light, and the adjacent RPE cells provide essential supportive functions. Dysfunction of either the neural layers or the RPE results in visual deficits of varying severity, and often leads to complete loss of vision. A major effort in the retina field is to characterize molecular and cellular mechanisms that regulate the function and survival of the RPE, and to understand processes that influence retina metabolism in its normal and pathological states. Recent evidence indicates that the JAK-STAT pathway plays important roles in the RPE during normal and diseased conditions. Accumulating data demonstrates that STAT3 integrates signals from the external environment, such as light, and the internal environment, such as inflammation, in order to regulate survival and biological activities of the RPE. Furthermore, several exciting studies demonstrate that STAT3 cross-talks with other signaling pathways in the RPE, such as Wnt signaling, toll-like receptor (TLR) signaling, and vascular endothelial growth factor (VEGF) signaling. Therefore, JAK-STAT signaling may be a central pathway in maintaining the health and function of the RPE, and consequently, of the retina, suggesting that STAT3 could be a potential therapeutic target for treating retinal degenerations.

The Retinal Pigmented Epithelium (RPE) is Essential to the Function of the Retina

The RPE is a monolayer of tightly packed, interconnected epithelial cells that lies between the photoreceptor layer of the retina and the choroid blood supply. RPE cells are essential for vision by providing crucial support and maintenance functions for photoreceptors. The RPE transports nutrients from the blood to the photoreceptors and the retina, including oxygen, fatty acids and retinol, and takes up metabolic end products from the retina and delivers them back into the circulation. The RPE is also critical for maintaining the visual cycle by recycling components of phagocytosed photoreceptor outer segments. Additional functions of the RPE include regulating pH balance, fluid uptake, growth factor secretion, and removing reactive oxygen species (ROS). As a result of the close interaction between RPE and photoreceptors, RPE dystrophy directly leads to photoreceptor death in humans and in animal models of retinal disease. Indeed, RPE dysfunction is a primary cause of many retinal degenerative diseases, such as age-related macular degeneration (AMD).
Major Functions of STAT3

JAK-STAT3 signaling is activated by specific ligands, including growth factors and cytokines, which bind to JAK receptors (Fig. 1). Upon initiation of STAT3 signaling, the STAT3 transcription factor is phosphorylated, leading to dimerization and translocation to the nucleus, where it binds to specific consensus sites within promoters of its target genes and activates major functions.

Figure 1. Functions of STAT3 in the RPE. Ligands bind to the gp130 receptor complex and activate JAK phosphorylation, leading to STAT3 phosphorylation and dimerization. The phosphorylated STAT3 dimer translocates to the nucleus and binds to the promoter of STAT3 target genes. In the RPE, STAT3 target genes can modulate RPE survival and proliferation, immune response, cytokine release, and visual cycle activity. Abnormal regulation of these pathways may be underlying causes of retinal diseases and degenerations.
transcription. STAT3 activity is critical for a wide range of functions in numerous cell types, including cellular differentiation, cell-cycle progression, proliferation, and survival. STAT3 also plays roles in regulating the immune response and inflammation. Based on the various functions of STAT3 and its upstream ligands in other cell types, several candidate roles for STAT3 have been investigated in RPE to determine whether STAT3 activation regulates and integrates biological processes that are essential to the health of the retina. The roles of STAT3 in the RPE are described in detail below.

**STAT3 Expression in the RPE**

Within the eye, STAT3 plays an important role in the development of many ocular structures, including the lens and retina. Zhang et al. examined the expression of different STATs throughout the developing mouse eye. Expression of STAT3 protein was noted first in the lens during early development, with onset beginning at embryonic day 11.5. STAT3 was observed in neurons in the differentiated inner retina starting at embryonic day 14.5, and then appeared in the outer retina (photoreceptors) and RPE beginning at postnatal day 1, and continuing to day 5, which corresponds to the phase of rapid differentiation of retinal progenitors. The expression of STAT3 in the outer retina is consistent with its critical role in regulating photoreceptor differentiation. STAT3 acts as a negative regulator of rod photoreceptor differentiation and mediates the effects of ciliary neurotrophic factor (CNTF) on the retina. Inhibition of STAT3 was shown to dramatically alter the development of the outer retina. STAT3 was detected in both the nucleus and cytoplasm of RPE cells during retinal development, unlike other STAT family members, which show only cytoplasmic expression. In the adult retina, STAT3 is constitutively expressed in the RPE, as well as neurons in the ganglion and inner nuclear layers and Muller glia. Phosphorylated STAT3 was also observed in the nucleus of the RPE cells, indicating that there is a basal level of STAT3 signaling in the adult retina. Interestingly, STAT3 is often upregulated and highly activated during retinal disease, suggesting that STAT3 may play a role in disease progression or the intrinsic tissue survival response, as described in detail below.

**Activities of STAT3 in the RPE**

**Survival.** STAT3 is known to upregulate many pro-survival genes, such as survivin and Bcl-xl, in various cell types. Consistent with a pro-survival activity of STAT3, upregulation of STAT3 was shown to protect RPE cells from injury. We recently demonstrated that STAT3 mediated the Wnt-dependent protection of RPE cells during oxidative stress injury. Furthermore, Wnt signaling increased total STAT3 and nuclear phosphorylated STAT3 levels. The importance of STAT3 to RPE survival was shown in experiments that used siRNA to silence STAT3, which eliminated the protective effect of Wnt signaling. In another recent study, STAT3 was also shown to mediate the protective effect of innate immune signaling in the RPE. Activation of the innate immunity receptor TLR3 led to time-dependent increases in total STAT3 and phosphorylated STAT3. Furthermore, the protective effect of TLR3 activation against oxidative stress in RPE was also mediated through STAT3, suggesting that STAT3 serves to integrate immunity pathways with cell survival signaling. These results indicate that STAT3 is an important downstream mediator of RPE survival following injury, and can be activated through multiple pathways.

**Immune regulatory role.** An important function of the RPE is to secrete immunosuppressive factors and to absorb excess light and neutralize light-induced formation of free radicals. Exposure of the retina to UV light is an established activator of various immune and inflammatory responses, and recent findings implicate STAT3 in this process. Chou et al. showed that medial wave UV light (UVB) upregulated IL6 RNA and protein levels and STAT3 phosphorylation in a time-dependent manner in the RPE. Furthermore, induction of STAT3 and IL6 by UVB led to an increase in complement factor B (CFB), which is a major component of the complement branch of the innate immune system. Inhibition of STAT3, using the JAK-STAT blocker AG490 or STAT3-specific shRNA, attenuated CFB elevation and RPE inflammatory signaling, indicating that STAT3 is a necessary component of the inflammatory response of the RPE. Therefore, these data are the first to link STAT3 with light-induced regulation of inflammation in the RPE. Although IL6 is an upstream activator of STAT3, it remains to be determined whether the regulation of STAT3 by UVB required IL6.

**Regulation of the visual cycle.** The RPE plays a critical role in the maintenance of vision by recycling retinal chromophores. The rate of chromophore production is important for retinal function because too little or too much chromophore leads to retinal degeneration. A key enzyme in the reconversion of all-trans retinal back to its x-retinal form is RPE65. Interestingly, STAT3 activation was recently associated with RPE65 levels, suggesting a role for STAT3 in regulating the visual cycle. Chuchair-Elliott et al. showed that intraretinally injected leuke mia inhibitory factor (LIF) activated STAT3 in the mouse RPE, which led to significantly reduced RPE65 expression. The regulation of RPE65 was shown to be STAT3 dependent through the use of conditional knockout mice that lack STAT3 specifically in the RPE. Because the maintenance of the visual cycle and proper RPE function are essential for vision, these findings indicate an important activity for STAT3 in vision.

**Cytokine response.** The RPE is responsive to multiple cytokines and growth factors, and many of these signal through STAT3. Activation of STAT3 by cytokines leads to various effects on the RPE, including proliferation, fluid transport, cytokine and growth factor secretion, and visual cycle maintenance. CNTF, LIF, and oncostatin M (Osm) are all members of the IL6 family and bind to several types of receptors that initiate JAK-STAT3 signaling in RPE. Interestingly, the activation of STAT3 by these cytokines shows specific cellular polarization properties. For example, Osm increased STAT3 activation differentially on the apical (photoreceptor) side of the RPE, in contrast to CNTF, which increased STAT3 phosphorylation in both the apical and basal (blood) sides. Although it is not yet understood why these cytokines cause directional activation of STAT3,
we speculate that one possible reason is that differential localization of STAT3 phosphorylation may contribute to activating or altering RPE functions that are polarized, such as ion and fluid transport and visual molecule recycling. An additional point of interest is that the downstream activities of these cytokines differ, despite their common activation of STAT3. While CNTF increases RPE cell survival, it had no effect on cellular proliferation, while OsM decreased both cell survival and proliferation. CNTF also increased transepithelial fluid transport across the RPE, which was shown to be mediated by the JAK-STAT3 pathway through the use of specific inhibitors.

**Potential Roles of STAT3 in RPE Pathology during Retinal Disease**

Age-related macular degeneration (AMD) is a progressive, irreversible degenerative disease of the macula that causes severe central visual loss. It occurs frequently in adults over the age of 60 and is the most common cause of blindness in developed countries. Early stage AMD is characterized by RPE abnormalities leading to photoreceptor dysfunction. Although the exact pathogenesis of AMD is unknown, molecular and cellular damage in RPE cells that is caused by oxidative stress is believed to be a major contributor to pathology. In neovascular AMD, new blood vessel formation within the choroid occurs. These new vessels are leaky, which causes subretinal hemorrhage and subsequent fibrovascular membrane formation, resulting in progressive and irreversible visual loss. Investigating the mechanism of choroidal neovascular (CNV) membrane formation and RPE abnormalities will enable understanding of the molecular pathogenesis involved in AMD.

Several lines of evidence have associated STAT3 with CNV in AMD. STAT3 is a critical transcriptional mediator of the angiogenic factor VEGF. VEGF is an inducer of CNV in AMD eyes, and blocking VEGF activity is the primary therapeutic strategy for treating neovascular AMD. Additionally, activated STAT3 was localized to RPE cells overlying areas of CNV in eyes of patients with AMD. STAT3 was mainly identified in areas of developing scar tissue that were rich in RPE cells, fibroblasts, macrophages, and lymphocytes, and was absent from avascular scar tissue and control eyes without AMD. Therefore, the activation of STAT3 in RPE cells adjacent to developing CNV scar tissue is compelling associative evidence for a role of STAT3 in the formation of CNV membrane and/or the subsequent reactive tissue response. Although it is unclear whether irregular STAT3 activation induces CNV or if CNV induces increased STAT3 signaling, the following studies suggest the former, rather than latter, possibility.

The presence of macrophages and lymphocytes indicates a role of inflammation in the formation of CNV, and STAT3 has been implicated as a regulator of the inflammatory response in the RPE. Indeed, the pro-inflammatory cytokine IL6, a ligand activator of STAT3, was elevated in serum of AMD patients. Also, using a murine laser-induced CNV model, Izumi-Nagai et al. demonstrated that CNV induction stimulated IL6 expression and activation of STAT3 in the RPE-choroid complex, as measured by increased phosphorylated STAT3 protein. Furthermore, inhibiting IL6 receptor signaling using the IL6 receptor monoclonal antibody MR16-1 significantly suppressed STAT3 phosphorylation and was associated with suppression of CNV formation. Pharmacological blockage of STAT3 via the JAK-STAT pathway using the JAK inhibitor AG490 also significantly and consistently suppressed CNV. In summary, this important study confirmed that IL6 receptor mediated activation of STAT3 plays a significant role in the formation of CNV in the mouse.

Another line of evidence for the involvement of STAT3 in CNV is the association between STAT3, VEGF, and hyperglycemia. Li et al. demonstrated that induction of VEGF in a cellular model of hyperglycemia was correlated with STAT3 activation in cultured RPE cells. Phosphorylated STAT3 protein significantly increased in a time-dependent manner, reaching maximum levels after 3 h of exposure to high-glucose medium. VEGF mRNA was also upregulated during high-glucose exposure and the time-course of this upregulation was similar to that of STAT3. The increase in STAT3 in RPE by hyperglycemia may potentially be a cellular protective response, which would be consistent with findings by our group and others that demonstrated that STAT3 protects cells from injury.

The Li et al. study also investigated the relationship between oxidative stress and STAT3 activation. Oxidative stress has been implicated as a contributor of CNV in AMD. It has also been previously demonstrated that an increase in superoxide production in the retina caused by hyperglycemia contributes to the pathogenesis of diabetic retinopathy. N-acetyl cysteine (NAC) is a thiol-containing compound that has been used as an antioxidant to counteract oxidative stress in several diseases. Li et al. demonstrated that treating an RPE cell line with NAC while in a high-glucose medium reduced phosphorylated STAT3 protein levels and VEGF expression. Moreover, treatment with the JAKSTAT3 pathway inhibitor AG490 also reduced VEGF levels. Therefore, anti-oxidant therapy reduced VEGF/STAT3 signaling, which suggests an upstream role of oxidative stress in the STAT3 pathway. Because STAT3 protects RPE from oxidative stress, the Li study suggests a complex signaling pathway by which oxidative stress and STAT3 are co-regulated.

Another retinal disease that involves the RPE is proliferative vitreoretinopathy, which occurs when the RPE has accelerated proliferation and migration, and which leads to retinal detachments and blindness. RPE proliferation is related to epidermal growth factor (EGF) signaling, which has been linked to STAT3 activation. EGF signaling increased STAT3, ERK1/2, and AKT phosphorylation in a dose-dependent manner in RPE cells, leading to increased proliferation in vitro. Furthermore, the carbohydrate glucosamine (GlcN) inhibited STAT3 phosphorylation and reduced RPE proliferation.

**Future Directions**

Although analysis of the functional role of STAT3 in RPE biology is a relatively new field, there are already several major findings, including the regulation of RPE survival, proliferation,
inflammation, and visual cycle function by STAT3. These functions of STAT3 may have important clinical implications in various retinal degenerations, such as AMD, diabetic retinopathy, and proliferative vitreoretinopathy. However, despite recent progress, there are several notable challenges that still need to be solved. Identifying target genes of STAT3 that mediate its effects will be essential for properly understanding the mechanism of action of STAT3 in the RPE and retina. Also, the activators of STAT3 in the RPE are mostly unknown, with the notable exceptions of IL6, Wnt, and VEGF. Finally, STAT3 cross-talk with other major signaling pathways in RPE is only just beginning to be recognized (for example, its interactions with TLR3 and TLR7), and further examination of STAT3 interactions with other pathways will reveal a great deal about its regulation. Because STAT3 regulates the survival of other cell types, particularly cancer cells, there are already major efforts focused on finding specific targets to interfere with STAT3 signaling. Therefore, potential future therapeutic strategies may involve delivering highly specific STAT3 regulators to diseased RPE cells, in order to delay or halt the progression of retinal degenerations.

Disclosure of Potential Conflicts of Interest No potential conflicts of interest were disclosed.

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