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Article

NLRP3 Inflammasome Blockade Inhibits VEGF-A-Induced Age-Related Macular Degeneration

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SUMMARY

The NLRP3 inflammasome is activated in age-related macular degeneration (AMD), but it remains unknown whether its activation contributes to AMD pathologies. VEGF-A is increased in neovascular ("wet") AMD, but it is not known whether it plays a role in inflammasome activation, whether an increase of VEGF-A by itself is sufficient to cause neovascular AMD and whether it can contribute to nonexudative ("dry") AMD that often co-occurs with the neovascular form. Here, it is shown that an increase in VEGF-A results in NLRP3 inflammasome activation and is sufficient to cause both forms of AMD pathologies. Targeting NLRP3 or the inflammasome effector cytokine IL-1β inhibits but does not prevent VEGF-A-induced AMD pathologies, whereas targeting IL-18 promotes AMD. Thus, increased VEGF-A provides a unifying pathomechanism for both forms of AMD; combining therapeutic inhibition of both VEGF-A and IL-1β or the NLRP3 inflammasome is therefore likely to suppress both forms of AMD.

INTRODUCTION

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in the elderly (Friedman et al., 2004; van Leeuwen et al., 2003). AMD manifests with the cardinal features of progressive atrophic degeneration of the retinal pigment epithelium (RPE) with sub-RPE deposits that affect metabolic exchanges between the RPE and choroidal vessels in nonexudative ("dry") AMD or with choroidal neovascularization (CNV) in neovascular ("wet") AMD (Bird et al., 1995; Kliffen et al., 1997). Importantly, patients with nonexudative AMD can progress to develop neovascular AMD, and both forms of AMD can occur simultaneously, suggesting a common pathomechanism that is currently unknown, in part due to the lack of a mouse model with features of both forms of AMD (Sunness et al., 1999).

Increased VEGF-A levels have been observed in neovascular AMD, but it remains to be shown whether an increase in VEGF-A alone is sufficient to cause neovascular AMD and through which pathomechanisms it may promote the disease process (Funk et al., 2009). Increased hypoxia and oxidative damage to the RPE have been regarded as critical factors in AMD pathogenesis, which induce VEGF-A expression in the RPE (Klettner and Roider, 2009). Consistent with the observed increased VEGF-A levels in neovascular AMD, anti-VEGF-A treatments have shown significant clinical benefit in patients with neovascular AMD (Martin et al., 2011). In contrast, nonexudative AMD is more common than neovascular AMD and causes loss of vision in millions of individuals, but no established treatments exist for nonexudative AMD. Thus, identifying a common pathogenetic step for both forms of AMD would provide the opportunity for a targeted broad therapeutic approach for neovascular and nonexudative AMD.

Genetic association data have provided evidence for linkage of both forms of advanced AMD with the VEGF-A gene locus (Fritsche et al., 2013; Yu et al., 2011), suggesting that increased VEGF-A levels may promote not only neovascular AMD but also nonexudative AMD. Thus, both forms of AMD may arise as distinct manifestations of a common underlying process of VEGF-A dysregulation.

Recently, NLRP3 inflammasome activation has been reported in both nonexudative and neovascular AMD, but it is not known whether VEGF-A promotes its activation (Kaneko et al., 2011; Tarallo et al., 2012; Tseng et al., 2013). Activation of the NLRP3 inflammasome results in autocatalytic cleavage of caspase-1 precursor (with the generation of the active p10 and p20 subunits), which leads to proteolytic activation of the potent proinflammatory cytokines IL-1β and IL-18 (Latz et al., 2013). Importantly, inflammasome activation has been suggested to influence various metabolic and aging diseases, including atherosclerosis, diabetes, gout, or obesity (Wen et al., 2012). However, it is not known whether NLRP3 inflammasome activation has a pathogenic role in the development of AMD, due to the lack of a mouse model that manifests chorioretinal pathologies as seen in both forms of AMD with progressive age, in which the role of the inflammasome could be tested. Recent studies have proposed either an inhibitory or a promoting role of the NLRP3 inflammasome for AMD (Doyle et al., 2012; Tarallo et al., 2012). Inhibition of the NLRP3 inflammasome prevented RPE degeneration, induced by DICER1 loss or *Alu* RNA exposure, whereas it increased neovascular lesions in an acute laser wound-healing model (Doyle et al., 2012; Tarallo et al., 2012).

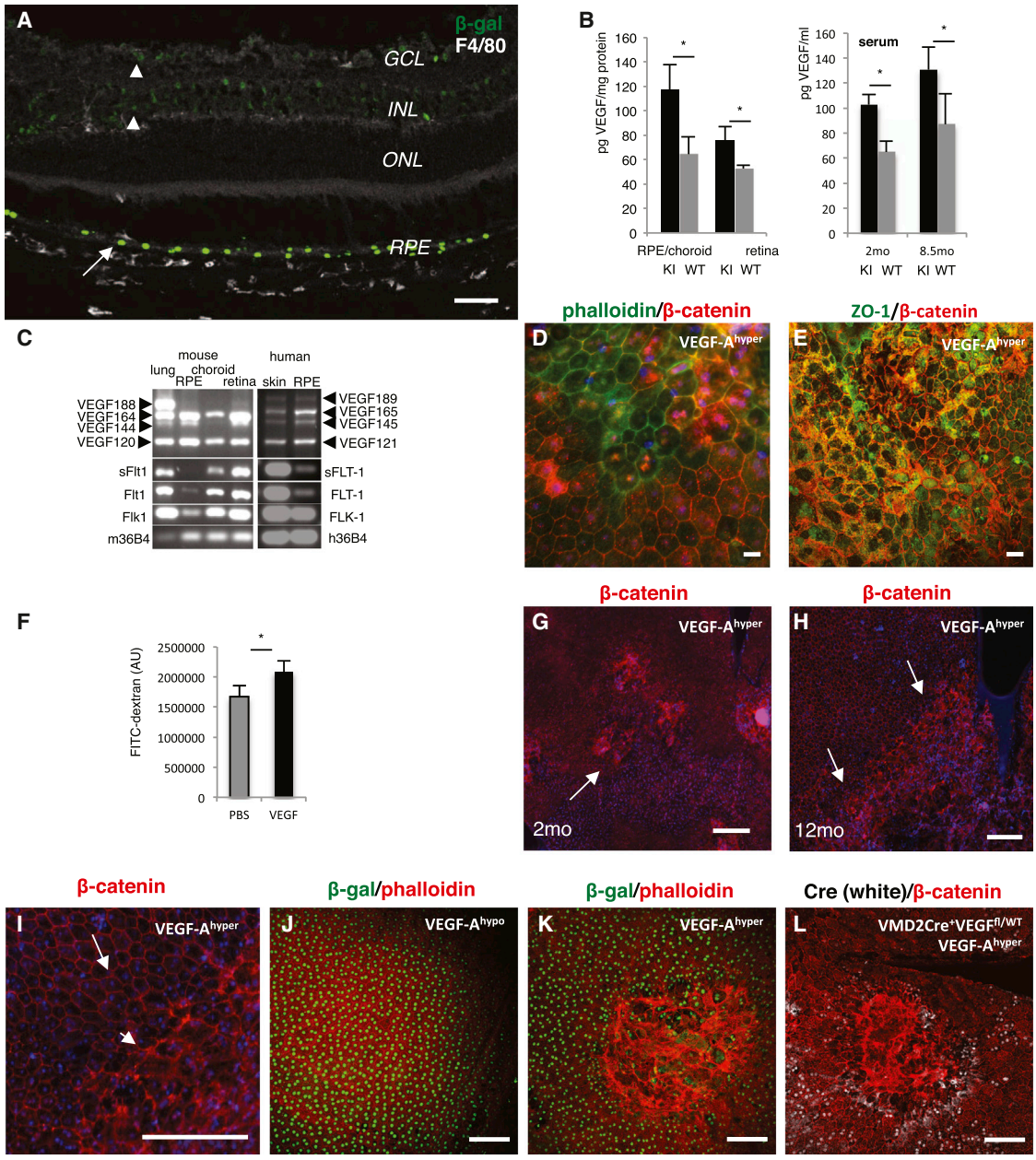

Figure 1. Increased VEGF-A Levels Result in a Progressive RPE Barrier Breakdown

(A) The RPE is the main cell type of VEGF-A expression in the adult eye. Immunolabeling of an adult posterior eye of a VEGF-A^{hyper} mouse (15 months old) for β-gal reflects cellular expression of VEGF-A (green). Strong VEGF-A expression is observed in RPE cells (arrow), whereas low-level VEGF-A expression is seen in retinal cells of the ganglion cell layer (GCL) and inner nuclear layer (INL) (arrowheads), but not the photoreceptor layer (ONL). Scale bar, 50 μm.

(B) VEGF-A ELISA of RPE/choroid and retinal tissue in adult VEGF-A^{hyper} mice (KI) and control littermate (WT) mice (n = 7/group) shows a significant increase of VEGF-A levels in the RPE/choroid tissues in VEGF-A^{hyper} mice. Serum levels of VEGF-A are elevated in mutant mice independently of age as well (n = 3/group). Error bars, mean ± SD. *$p < 0.05$.

(C) Mouse and human RPE cells express the VEGF-A receptors Flt1 and Flk1 and all major VEGF-A isoforms, particularly VEGF-A^{164} and VEGF-A^{120} (VEGF-A^{165} and VEGF-A^{121} in human).

(D) Focal RPE barrier breakdown is observed in VEGF-A^{hyper} mice with cytoplasmic and nuclear accumulation of β-catenin. Colabeling with Alexa 488-conjugated phalloidin (green) shows that in normal RPE cells, phalloidin and β-catenin (red) labeling is strongest along cell membranes, whereas in RPE cells with RPE barrier breakdown, β-catenin labeling along the cell membranes is attenuated and increased in the nuclei or cytoplasm. A 7-month-old VEGF-A^{hyper} mouse is shown. Scale bar, 20 μm.

(E) Colabeling of cell junction proteins β-catenin (red) and ZO-1 (green) shows loss of membrane-bound ZO-1 and β-catenin with nuclear and cytoplasmic accumulation. A 7-month-old VEGF-A^{hyper} mouse is shown. Scale bar, 50 μm.

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