ImmunoTools *special* Award 2014

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Regulation of HIF-1α activity and hypoxia-induced VEGF up regulation in retinal pigment epithelium cells and angiogenesis of choroidal vascular cells

Choroidal neovascularization is the third leading cause of blindness in the western world. Its etiology remains unknown. However, it has been established that both up-regulation of VEGF and down-regulation of PEDF at the outermost layer of the retina, in the so-called retinal pigment epithelium – choroid complex, is the milestone of this pathological process. The regulatory mechanisms that ultimately fail to inhibit this pro-angiogenic environment are unknown. Nevertheless, the transcriptional factor named hypoxia-induced factor -1 or HIF-1 has been implicated in the pathogenesis of choroidal neovascularization. The action of HIF-1, upon hypoxia-mediated stabilization, is to activate the transcription of specific hypoxia adaptive genes, including VEGF.

Retinal pigment epithelium (RPE) is the main source of VEGF, which activates choroidal vascular endothelial (CVE) cells and generates neo-vessels into the retina, leading to irreversible blindness. Different natural existing molecules from the family of phenols, display anti-oxidant activity, and in some cases, HIF-1 inhibitory activity has been reported in tumor cells. However, their effect on retinal cells exposed to hypoxia has not been previously reported. Hence, we aimed to study the effects of various anti-oxidant phenols on the expression of VEGF by RPE cells.

We have discovered that anti-oxidant molecules display a novel inhibitory effect in hypoxia-driven VEGF up regulation in RPE cells. Vascular cells are also sensible to this regulation, leading to decreased biological activity and angiogenesis. Thus, hypoxia-mediated increase of VEGF-A gene expression in RPE cells, as well as angiogenesis induced by secreted factors from RPE cells, may be modulated by natural anti-oxidant compounds. Further studies with this family of compounds on retinal cells are needed to establish its potential usefulness as a novel ocular anti-angiogenic molecule.

In order to model this pathogenic pathway *in vitro*, RPE cells are challenged with hypoxia (1% O2) and HIF-1α, VEGF-A165 and VEGF-A189 gene expression is studied. The response of vascular cells to stimulus from RPE cells are studied using migration and vasculogenic assays. Human recombinant VEGF isoforms (rh VEGF-A/VEGF-165) from Immunotools will be used to emulate what has been described in patients with this retinal condition, where VEGF-A isoform has been implicated as

primary disease trigger and mediator of the angiogenic response. In vascular cells, the pro-mitotic and migratory effect of VEGF isoforms has been mainly mediated through VEGF receptor type 2 activation. Hence, Immunotools Recombinant Human Vascular Endothelial Growth Factor Receptor-2 (rh VEGFR2 / CD309) will be used to block VEGF induced activity in vascular cells. Also, dysregulated complement is thought to play a central role in age-related macular degeneration pathogenesis. Hence Immunotools CD 46 and CD 59 flow cytometry antibodies will be used to study these complement regulatory proteins in RPE cells.

ImmunoTools *special* AWARD for

Dario Vasquez Zuloaga includes 25 reagents

FITC - conjugated Annexin V, control-IgG1, anti-human CD36, CD40, CD44, CD46, CD59, CD69,

recombinant human cytokines rh VEGF A, VEGF 165, VEGF 121, rh MCP1 / CCL2, rh MCP2 / CCL8, rh PDGF-AA, rh PDGF-BB

human IL-4 ELISA-set, human IL-6 ELISA-set, human IL-8 ELISA-set, human IL-12p40-set, human TNF alpha ELISA-set

DETAILS