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PROGRAM ABSTRACTS

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Abstract ID: 381

Study of the participation of surface proteins on inflammatory cellular component during choroidal neovascularization (CNV)

Section: RPE-Choroid

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In the last years, increased attention has been put in understanding the modulatory role of inflammation in the development and progression of the age-related macular degeneration (AMD). Epidemiological evidence suggests that the activation of inflammatory cellular component, composed by resident microglia and infiltration of monocyte derived macrophages participates on the choroidal neovascularization (CNV). This represent a pathological angiogenesis of the choroidal plexus of the retina which is key feature of the wet form of AMD. On the other hand, evidence of reduced of vascular leakage through the suppression of retinal immune cells reactivity supports the involvement of this cells activation in CNV progression. However, the underlying mechanisms involved in the recruited and activation of the inflammatory cellular component are not clear yet. We hypothesize that these could be modulated by surface proteins in inflammatory cells that promote CNV pathology.

Our objective is to describe and understand histological, biochemical and molecular consequences associated to functional retinal changes in response of inflammatory component modulation during CNV progress.

We employed genetic and pharmacological tools in a mouse model of laser-induced CNV, where C57BL/6 mice 2- months old 4 injuries were performed in the retina using a photocoagulation laser with a 532nm wavelength slit lamp. We studied the roles of surface proteins involved in inflammatory events such as p75 neurotrophin receptor ($p75^{NTR}$) and low density lipoprotein receptor-related protein 1 (LRP1) by western blot and immunofluorescence. Activation and recruitment of microglia and macrophage analysed by Flow cytometry. Expression of this proteins surface were evaluated on microglia, macrophage, cell lines exposed to supernatant from RPE cells.

After laser injury, expression of $p75^{NTR}$ and LRP1 increased in microglia near the CNV area in the retina and macrophage on the retinal pigmented epithelium (RPE)-choroid. In $p75^{NTR}$ knockout mice ($p75^{NTR}$ KO) with CNV, there is significantly reduced glia activation and a minor recruitment of macrophages, with a consequent reduction of CNV area. Also, retinal function is preserved in $p75^{NTR}$ KO CNV mice. Notably, a pharmacological $p75^{NTR}$ antagonist on wild type mice with CNV showed similar effect of the $p75^{NTR}$ KO mice. Moreover, using an in vitro model of macrophage or microglia exposure to RPE supernatant we found higher level of LRP1. Although endogen level of LRP1 ligand (α_2M) showed no changes in CNV, the exogen administration of it by intravitreal injection reduced the recruitment of microglia and macrophage significantly and reduced the CNV area.

Our results suggest that two different surface protein such as $p75^{NTR}$ and LRP1 expressed on the glial and macrophage cells has a role on the reactivity of this effector immune cells and could represent potential therapeutic targets.

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Classical Phospholipase D isoforms in retinal pigment epithelium cells response to stress

Section: RPE-Choroid

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Inflammation and oxidative stress (OS) are key factors in the pathogenesis of many ocular and retinal neurodegenerative diseases that eventually end in vision loss and blindness, such as age-related macular degeneration, diabetic retinopathy and uveitis,

among others. For all these retinal inflammatory conditions, pharmacological treatment options are limited. Therefore, to provide new insights for the treatment of ocular inflammatory diseases, it is important to elucidate the molecular mechanisms involved in these disorders.

Findings from our laboratory described for the first time the participation of classical Phospholipase D isoforms (PLD1 and PLD2) in the inflammatory responses of human retinal pigment epithelium (RPE) cells exposed to lipopolysaccharide (LPS) and also to high glucose levels (HG). Classical PLD isoforms catalyze phosphatidylcholine (PC) hydrolysis to generate phosphatidic acid (PA) which can be further dephosphorylated by lipid phosphate phosphatases (LPPs) to diacylglycerol (DAG). Thus, the PLD pathway modulates the activities of PA-responding proteins, as well as DAG-responding proteins. In recent years, we have reported the participation of classical PLDs in the LPS-induced inflammatory response of human RPE cells (ARPE-19 and D407 cells) through extracellular regulated kinase 1/2 (ERK1/2) activation and cyclooxygenase-2 (COX-2) induction. We also showed that even though LPS treatment promoted an inflammatory response in human RPE cells, it also triggered autophagy which served as a cell protective mechanism, and that the PLD pathway modulates this autophagic process. Moreover, we described that PLD1 and PLD2 mediate the inflammatory response generated by exposing RPE cells to HG concentration. This response involved concatenated PLDs and ERK1/2 activation, NFκB (nuclear factor kappa B) nuclear translocation, expression of pro-inflammatory interleukins (IL-6 and IL-8) and COX-2, and reduced cell viability. Specific PLD1 and PLD2 pharmacological inhibitors were able to prevent the LPS-induced inflammatory responses in RPE cells and also the HG-induced inflammatory responses, OS and phagocytic function loss in RPE cells.

Our findings postulate that PLD inhibition presents a novel pharmacological tool to prevent at the same time oxidative stress and the inflammatory responses, the two hallmarks of several retinal diseases that are usually addressed by different therapeutic strategies.

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Retinal Pigment Epithelial cells, a culture of resilience.

Section: RPE-Choroid

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Introduction
Retinal pigment epithelial (RPE) cells sustain photoreceptor integrity, and when this function is disrupted, retinal degenerations ensue. To fulfill their function, RPE must endure oxidative, proteostatic and metabolic stress, showing responsiveness and resiliency.

Objectives
We aim to characterize the principal pathways involved in the response of RPE to these three type of stress and relate them to the protective effects of lipid mediators derived from omega 3 fatty acid, DHA.

Methods
For this purpose, we developed cultures of human RPE cells that keep desirable features to use them as tool for the study of the Retinal pigmen epithelium in vitro. We use healthy human 19-year-old donor eye cup from the human eye bank NDRI. ABC cells were characterized by comparing them with cultured RPE from elderly human donors and ARPE-19 human cell line using RNAseq and immunocytochemistry. The phagocytosis was determined using photoreceptor outer segments (POS) or polystyrene microspheres (beads) to compare ABC cells to ARPE-19. Limited proteolysis coupled to mass spectrometry (LiP-MS) to define protein targets of the lipid messengers and poly(Q)-induced proteotoxic stress to investigate mechanisms to escape from apoptosis and cell death.

Results
The newly characterized cell line from human RPE that we termed ABC remarkably recapitulates human eye native cells. They formed microvilli, tight junctions, and honeycomb packing and expressed distinctive markers. Outer segment phagocytosis, phagolysosome fate, phospholipid metabolism, and lipid mediator release were tested and showed resemblance with the human