ARPE-19-DERIVED VEGF-CONTAINING EXOSOMES PROMOTE NEOVASCULARIZATION IN HUVEC: THE ROLE OF THE MELANOCORTIN RECEPTOR 5

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Introduction: The melanocortins α-, β-, and γ-melanocyte-stimulating hormone (α-, β-, γ-MSH) and adrenocorticotropic hormone (ACTH) are a family of peptides that exert several biological activities, including pigmentation, steroidogenesis, energy homeostasis, exocrine secretion, sexual function, analgesia and the promotion of the resolution phase of inflammation through five distinct receptors, termed MCR_1, MCR_2, MCR_3, MCR_4, and MCR_5 . These receptors are 7-transmembrane G protein-coupled receptors that are ubiquitously expressed in several organs, including the eye. MCR_3 and MCR_4 are localized in the layer of retinal ganglion cells, whilst the retinal pigment epithelial cells abundantly express MCR_5. The aim of this study was to investigate the role of the melanocortin receptor (MCR) 5in the high glucose induced release of VEGF-containing exosomes from human retinal pigment epithelial cells ARPE-19and in the resulting neovascularization.

Materials and methods: The exposure of these cells for 9days to 10^{-10}M of PG-901, a melanocortin receptor (MCR) 5agonist, reduces this pro-oxidant challenge by reducing the ROS generation, the number of exosomes released and their VEGF content. ARPE-19derived VEGF-containing exosomes promoted neovascularization in HUVEC cells, an effect that was markedly reduced by 10^{-10}M PG-901but not by the MCR3/4agonist MTII (0.30 nmol) or the MCR1agonist BMS-470539(10^{-5}M).

Results: Exposing these cells to the melanocortin 5receptor agonist (MCR_5) PG-901(10^{-10}M), for 9 d reduced ROS generation, the number of exosomes released and their VEGF content. In contrast, incubating the cells with the melanocortin receptor MCR_1 agonist BMS-470539(10^{-5} M) or with the mixed $MCR_{3/4}$agonist MTII (0.30 nmol) did not produce any significant decrease in ROS levels. ARPE-19-derived VEGF-containing exosomes promoted neovascularization in human umbilical vein endothelial cells (HUVEC), an effect that was markedly reduced by PG-901(10^{-10}M) but not by the $MCR_{3/4}$ agonist MTII (0.30 nmol) or the MCR_1 agonist BMS-470539(10^{-5} M). Here we show that retinal pigment epithelial cells ARPE-19cultured in a medium containing 35mM of D-glucose have augmented ROS formation and augmented release of vascular endothelial factor (VEGF)-containing exosomes, compared to ARPE-19cells cultured in a medium containing 5mM of D-glucose (standard medium).

Discussion: The MCR_5 receptor plays a role in glucose metabolism- and inflammation-based pathologies, and is expressed in retinal pigment epithelial cells. We demonstrated that MCR_5 activation reverse the angiogenic potential of high glucose-induced exosomes, released by ARPE-19. In fact, the exosomal release from these cells and the consequent exosomes-induced node and tube formation in the HUVEC were significantly reduced by ARPE-19treatment with PG-901, a melanocortin receptor MCR_5 agonist. The MCR5-related action in ARPE-19cells was accompanied by an increased expression of two coupled factors, the cytochrome p4502E1(CYP2E1) and the nuclear factor kappa b (Nf- kB). These are both involved in the high glucose signaling, in the ROS generation and interestingly, reduced by the MCR5agonist in ARPE-19cells.

Conclusion: The anti-angiogenic activity of MCR_5 agonists is related to modulation of exosomal release of VEGF by RPE cells, indicating that MCR_5 agonists can be developed as indirect inhibitors of angiogenesis. On the basis of evidences herein presented, other melanocortin receptors do not influence the exosome angiogenic potentials. Another interesting direction in further works would be showing the mechanism by which HG-induced OS causes the overexpression of MCR_5.