UPARANT mitigates human iris angiogenesis through uPAR/LRP-1 in an organotypic ex vivo model

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Conclusions

Our findings broaden the understanding of the mechanisms of action of UPARANT in inhibiting iris angiogenesis, which could benefit the treatment of patients afflicted with multifactorial ocular neovascular diseases.

Introduction

Rubeosis Iridis (RI) is characterized by an increase in neovascularization and inflammation factors in the iris. During angiogenesis, the urokinase plasminogen activator (uPA) and its receptor (uPAR) play a pivotal role in the regulation of endothelial cell migration and proliferation through transmembrane receptors (FPRs), which are involved in the vascular endothelial growth factor (VEGF) regulation. It has been shown that a non-canonical pathway involving uPAR and low-density lipoprotein receptor-related protein 1 (LRP1) interaction upregulates angiogenesis. The tetra-peptide UPARANT, is a FPRs binding antagonist which has been demonstrated to reduce ocular angiogenesis in *in vitro* and *in vivo* models. In the present study, in the context of hypoxia-induced angiogenic stimulus, UPARANT effects were investigated in an ex vivo human iris angiogenesis assay, and compared to the clinically used anti-VEGF, Aflibercept.

Aim

To assess the efficacy of UPARANT in a novel *ex vivo* model of human iris neovascularization, induced by hypoxia stimulus, through an interference of uPAR/LRP1 interaction.

Results

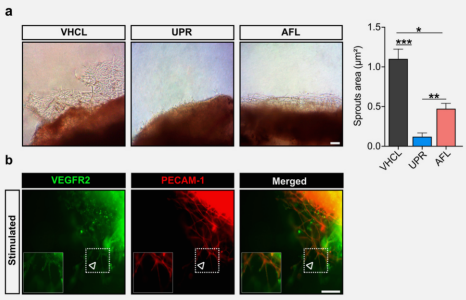


FIGURE 1. UPARANT mitigates neoangiogenesis in human *ex vivo* iris. (**a**) Hypoxia-stimulated iris neoangiogenesis was significantly mitigated by UPARANT and Aflibercept as shown by sprouts area analysis; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (**b**) Endothelial sprouts were characterized by positive staining of VEGFR2 and PECAM-1, as endothelial cell markers (arrow indicates a sprouting tip-cell). Scale bars = 100µm.

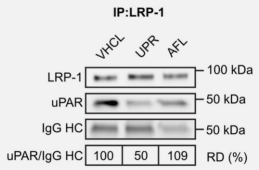


FIGURE 2. Effect of UPARANT on uPAR/LPR1 interactome. Co-immunoprecipitation shows an uPAR/LRP-1 interaction in hypoxia-stimulated iris, displaced by UPARANT.

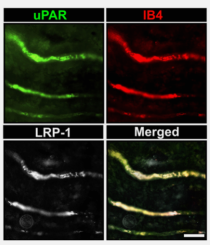


FIGURE 3. Human irises immunofluorescence micrograph. uPAR (green), isolectin B4 (red) and LRP-1 (white) colocalization in human iris blood vessels. Scale bar =200 µm.

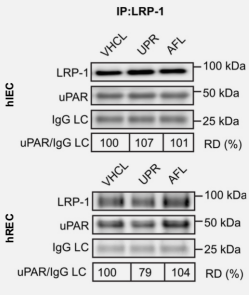


Figure 4. Effect of UPARANT on uPAR/LRP-1 complex in hIEC and hREC. Co-immunoprecipitation shows a uPAR/LRP-1 interaction in iris epithelial and retinal endothelial cells, displaced by UPARANT only in retinal endothelial cells.

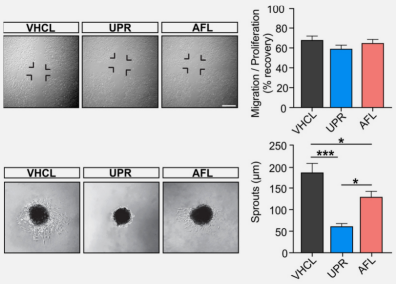


Figure 5. Effect of UPARANT on uPAR/LRP-1 complex in hIEC and hREC. UPARANT and Aflibercept reduce the average sprout length in hypoxia-stimulated retinal endothelial cells spheroids. No effects is observed in wound recovery of iris epithelial cells; * $P < .05$, *** $P < .001$. Scale bar = 100 µm.

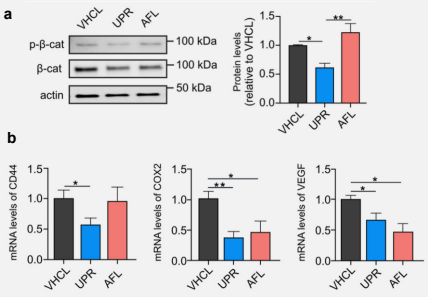


Figure 6 . Effect of UPARANT on phospho-β-catenin levels in hREC. a) UPARANT reduces phosphorylation of β-catenin in hypoxia-stimulated retinal endothelial cells. **b)** Transcript levels of β-catenin target genes CD44, COX-2 and VEGF are significantly reduced by UPARANT; a significant decrease was observed in both COX-2 and VEGF transcript levels when treated with Aflibercept; ** $P < .01$, *** $P < .001$, * $P < .05$.

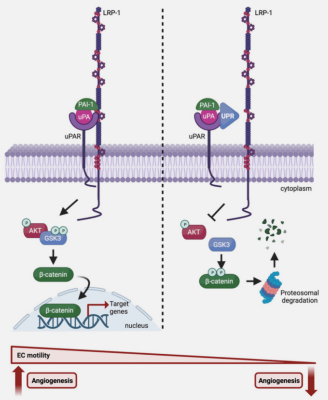
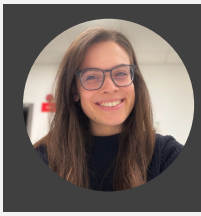


Figure 7 . Schematic view of UPARANT antagonism on uPAR/LRP-1 interactome. In the presence of PAI-1, uPAR interacts with LRP-1 and activates β-catenin to mediate endothelial cell (EC) motility in association with pro-angiogenic stimuli. In the presence of UPR the protein complex uPAR/LRP-1 is displaced, thus leading to an increased degradation of phospho-β-catenin.



Characterizing non-canonical pathways involved in hypoxia-induced angiogenesis opens the opportunity for multi-target treatements of ocular neovascular diseases.

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