Ocular angiogenesis:

A new era from multitarget inhibition to gene therapy

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On September 13, 2024, Flavia Plastino defended her thesis "Angiogenesis in ocular neovascular diseases: from multitarget inhibition to gene therapy tools" at the Dept. of Clinical Neuroscience, Division of Eye and Vision, Karolinska Institutet (KI). The supervisor(s) were Prof. Anders Kvanta, MD, PhD, Dept. of Clinical Neuroscience, Division of Eye and Vision, KI; Dr. Helder André, PhD, Dept. of Clinical Neuroscience, Division of Eye and Vision, KI; Dr. Filippo Locri, PhD, Dept. of Clinical Neuroscience, Division of Eye and Vision, KI; and Prof. Fredrik Lanner, PhD, Dept. of CLINTEC, KI.

Key points:

- The uPAR/LRP-1 axis as a potential anti-angiogenic target distinct from VEGF inhibition
- Targeting HIF-1α upstream of neovascular events is a promising strategy for treating ocular neovascular diseases
- The establishment of time and cost-effective ARPE-19 cells redifferentiation protocol offers a valid model for studying cell-specific anti-angiogenic gene therapy strategies with potential for in vivo applications.

Ocular neovascular diseases such as neovascular age-related macular degeneration (nAMD) and proliferative retinopathies (PRs) remain leading causes of vision loss worldwide. These conditions are characterized by the growth of abnormal, fragile blood vessels, triggered by hypoxia-mediated responses. The hypoxia-inducible factor-1α (HIF-1α) and its downstream target genes orchestrate multiple aspects of the angiogenic cascade, including the induction of vascular endothelial growth factor (VEGF), a central mediator of blood vessel growth. The urokinase plasminogen activator receptor (uPAR) also plays a critical role by remodeling the extracellular matrix (ECM), facilitating endothelial cell migration and vessel formation.

Current treatments focus on intravitreal anti-VEGF agents, which, while effective, require repeated injections and fail to benefit all patients. The multifactorial nature of ocular angiogenesis highlights the need for complementary therapeutic strategies targeting alternative molecular pathways. This thesis explored alternative strategies to inhibit ocular angiogenesis using a multitarget inhibition approach that combined the modulation of the uPAR system, the inhibition of HIF-1α, with the evaluation of specific tools for the targeted delivery of gene therapy constructs in the eye, by developing an optimized model for retinal pigment epithelium (RPE)-specific gene therapy.

Using a novel *ex vivo* human iris organotypic culture, the peptide UPARANT was shown to antagonize the uPA/uPAR system by disrupting the interaction between uPAR and its co-receptor LRP-1. This led to the inhibition of β-catenin–mediated angiogenesis specifically in endothelial cells, independent of VEGF signaling, highlighting a previously unidentified non-canonical mechanism for controlling pathological ocular neovascularization. Complementing this, the HIF-1α inhibitor, Echinomycin, was evaluated in retinal cell cultures and a mouse model of laser-induced choroidal neovascularization. The inhibition of HIF-1α decreased the hypoxic response of retinal cells and reduced vascular lesion area in vivo. These findings demonstrate that blocking HIF-1α can simultaneously suppress several pro-angiogenic pathways, providing a powerful upstream approach to control pathological angiogenesis.

The use of ocular-specific gene therapy opened the possibility for gene therapy as a potential treatment of other ocular disease. To facilitate future gene therapy strategies, commonly used ARPE-19 cells were redifferentiated into a more mature RPE phenotype using a combination of nicotinamide and laminin-521. This protocol promoted the expression of RPE-specific genes and proteins, improved cellular organization, and enhanced transduction by adeno-associated viral (AAV) vectors at low multiplicity of infection, mimicking in vivo tropism. The resulting platform provided a physiologically relevant system to evaluate novel RPE-targeted gene therapies efficiently.

Together, these studies offer new insights into the mechanisms of ocular angiogenesis and propose complementary strategies to existing anti-VEGF therapies. By combining molecular pathway inhibition with advances in gene delivery, this work contributes to the development of more durable, targeted, and patient-friendly treatments for ocular neovascular diseases.

Remaining questions:

- Develop localized, long-lasting anti-HIF-1α gene therapies to achieve sustained suppression of ocular neovascularization.
- Advance uPAR-targeting agents, such as UPARANT, toward preclinical and clinical evaluation as complementary or alternative treatments to anti-VEGF therapy.
- Refine RPE-specific viral vectors and promoters to improve safety, precision, and durability of gene delivery in the eye.

References

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