3D Printing . OPA1 . Autosomal Dominant Optic Atrophy . Artificial Transcription Factor
Fuchs' Dystrophy . Corneal Implant

Ocular Pharmacology and Physiology

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Translational research to treat blinding diseases

Visual impairment and the associated socio-economic impact affects an estimated 290 million people worldwide. Our research is aimed at understanding the causes of vision loss and to develop novel therapies to prevent or treat blinding diseases.

Treating autosomal dominant optic atrophy
The optic nerve formed by the axons of retinal ganglion cells (RGCs) relays visual information form the light-sensing retina to the vision centers in the brain. Thus, loss of RGCs results in visual impairment and ultimately blindness.
Autosomal dominant optic atrophy (ADOA) is the most common inherited form of optic nerve degeneration. Mainly caused by mutations in the OPA1 gene, visual impairment develops in the first decade of life and can progress to complete blindness. The OPA1 gene codes for a mitochondrial protein involved in maintaining mitochondrial morphology by modulating mitochondrial fusion. In addition, OPA1 is essential for mitochondrial cristae organization and, thus, electron transport chain function. Loss of OPA1 is known to diminish mitochondrial fidelity and to increase sensitivity to apoptotic stimuli. Critically, OPA1 mRNA is subject to extensive alternative splicing giving rise to at least eight different OPA1 isoforms with a balanced isoform expression important for OPA1 function.
About 70 % of ADOA cases are caused by OPA1 haploinsufficiency. Using artificial transcription factors (ATFs) capable of upregulating OPA1 expression, we aim to alleviate OPA1 haploinsufficiency while maintaining OPA1 isoform balance, thus providing a functional cure for ADOA. Based on TAL effectors, we generated ATFs capable of upregulating mouse or human OPA1 (Fig. 1). To test efficacy of such ATFs, we generated a novel *in vivo* model of pharmacologically accelerated RGC degeneration in ADOA mice. This allowed us to synchronize RGC death and shortened the time it takes to detect treatment efficacy from 12-15 months to 14 days. Currently, we are in the process of optimizing viral delivery to allow for efficient delivery of OPA1-upregulating ATFs to RGCs by simple intravitreal injection.

Towards a treatment for Fuchs' dystrophy
Vision starts with light entering the eye through a transparent cornea. The cornea is a five-layered tissue with the outermost corneal epithelium together with Bowman's layer and the innermost corneal endothelium with Descemet's membrane sandwiching the almost cell-free corneal stroma. To keep the corneal stroma transparent, corneal endothelial cells (CECs) actively remove water from this tissue. During Fuchs' dystrophy and due to CEC loss, water accumulates inside the stroma causing a disorganization of the carefully arranged stromal matrix leading to severe visual impairment. Interestingly, about 4 % of the population over 40 years of age suffers from Fuchs' dystrophy to a varying degree. Severe Fuchs' dystrophy is treated by replacing the damaged CEC layer with human donor material in a procedure called Descemet Membrane Endothelial Keratoplasty (DMEK). However, the demand for corneal transplants outweighs availability about 70:1.
By combining additive manufacturing and stem-cell technology, we want to address this unmet medical need by generating a drop-in replacement for human donor material for DMEK. We employ melt electrowriting to fabricate a suitable scaffold for culturing human CECs (Fig. 2). Furthermore, a differentiation protocol for CECs from induced pluripotent stem cells (iPSCs) was developed and adapted to be compatible for culturing these cells on such scaffolds. To assess whether these implants are compatible with the established transplantation procedure, cell-free scaffolds were implanted into porcine eyes *ex vivo*. Imaging revealed that the fabricated structural support is well suited as drop-in replacement during DMEK. Taken together, with developing a donor tissue replacement for DMEK we hope to address the unmet medical need of patients suffering from Fuchs' dystrophy.

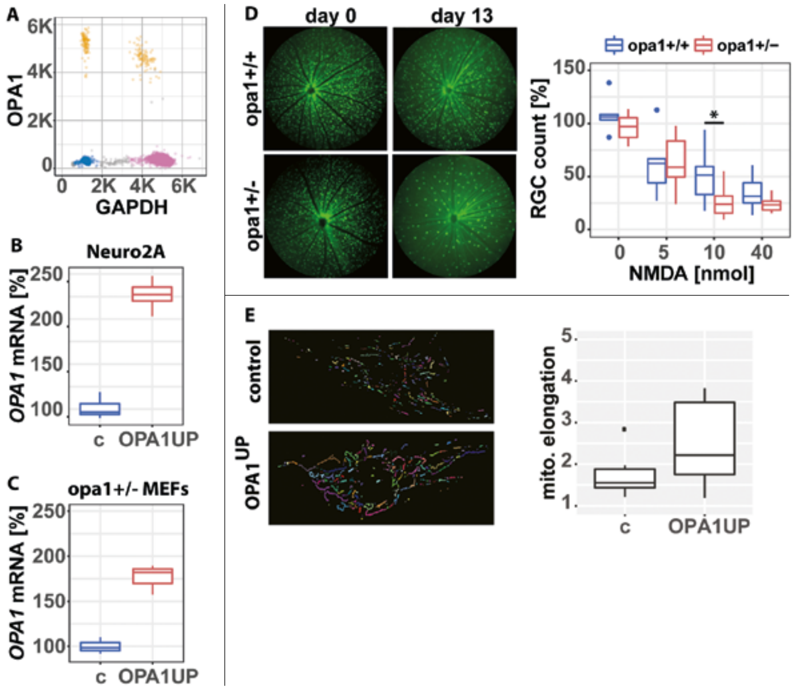

Fig. 1: *Xanthomonas* transcriptional activator like (TAL)-based artificial transcription factors (OPA1^{UP}) capable of upregulating *OPA1* in mouse or human cells was generated. **(A)** To accurately measure *OPA1* mRNA levels, a digital droplet PCR (ddPCR) assay was established. Following expression of OPA1^{UP} fused to T2A-GFP, GFP$^+$ cells were sorted and multiplexed measurement of *OPA1* and *GAPDH* mRNA levels was possible in as little as 100 cells. **(B)** Mouse neuron-like Neuro2A and **(C)** opa1$^{+/-}$ mouse embryonic fibroblasts (MEFs) were transfected with expression plasmid for OPA1^{UP}-T2A-GFP, GFP$^+$ cells were isolated by fluorescence activated cell sorting and *OPA1* mRNA levels were quantified relative to *GAPDH*. Please note, opa1$^{+/-}$ MEFs are haploinsufficient for *OPA1*. **(D)** OPA1$^{+/-}$ mice with GFP-expressing retinal ganglion cells (RGCs) and their wildtype littermates were treated with increasing amounts of NMDA by intravitreal injection. Fluorescent fundoscopy was performed before and 13 days after NMDA application. Please note the significantly lower amount of surviving RGCs in opa1$^{+/-}$ animal compared to wildtype controls following application of 10 nmol of NMDA (n>15 animal/condition). (E) Patient-derived, primary human fibroblasts transfected with OPA1^{UP} or inactive control were stained for the mitochondrial marker cytochrome c and mitochondrial morphology was analyzed. Please note the increase in mitochondrial length following OPA1^{UP} expression compared to controls indicative for restored OPA1 function.

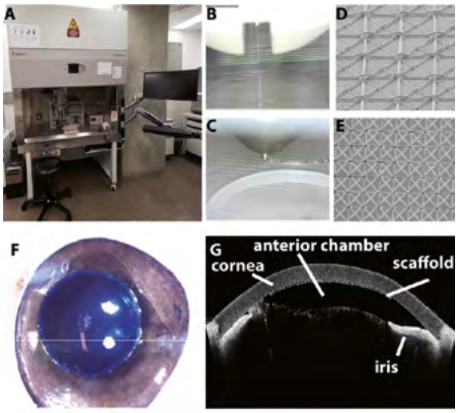

Fig. 2: (A) Precision 3D printer (regenHU, 3D Discovery) equipped with four printheads including a melt electrowriting head capable of depositing molten polymer with a lower diameter of 600 nm in a highly reproducible fashion. **(B)** Microscopic image of molten polycaprolactone (PCL) deposited onto a glass collector plate in a 6000 KV electric field to form 10 µm fibers. **(C)** Deposition of PCL support structures onto melt electrowritten scaffold using a fused deposition printhead. **(D+E)** Electron microscopic images of melt electrowritten PCL fibers. **(F)** A PCL scaffold embedded into artificial extracellular matrix (stained blue for better visualization) was implanted *ex vivo* into a porcine eye using the standard surgical procedure to perform Descemet Membrane Endothelial Keratoplasty (DMEK). **(G)** Anterior chamber optical coherence tomography was used to assess placement and attachment of the implanted scaffold.

Connection to Clinical Practice

Prof. Hendrik Scholl
Prof. Dr. Zisis Gkatzioufas
University Eye Hospital

Visual impairment greatly affects a patient's quality of life. Thus, the focus is on halting or even reversing loss of vision. Understanding degenerative mechanisms affecting ocular tissues such as the optic nerve, the retina or the cornea is of great importance to develop novel treatment modalities. Such modalities include gene therapeutic approaches as well as stem cell-based treatments for retinitis pigmentosa, Stargardt disease, age-related macular degeneration, diabetic retinopathy, autosomal dominant optic atrophy, but also corneal dysfunction as encountered in Fuchs' dystrophy. The clinical research center of the Institute of Molecular and Clinical Ophthalmology (IOB) in collaboration with the University Eye Hospital offers the opportunity for clinical research into causes of vision loss as well as the natural history of ocular diseases. Together with the evaluation of clinical outcome measures and the possibility to conduct clinical trials, the clinical research center provides an integrated environment for successful transition from bench-to-bedsite by bridging the "valley of death" often encountered by translational research. Only careful studies into the natural history of diseases as well as thoughtful selection of outcome parameters - especially in the case of slowly progressing eye diseases – minimize the inherent risk of translational research projects and development of new therapies. The close connection between the ocular pharmacology and physiology lab and the IOB clinical research center, both headed by Prof. Hendrik Scholl, is instrumental to leverage our translational research for the benefit of the patients.

Selected Publications

Bento AC, Bippes CC, Kohler C, Hemion C, Frank S and Neutzner A (2018). UBXD1 is a mitochondrial recruitment factor for p97/VCP and promotes mitophagy. Sci Rep 8, 12415.

Kaeslin MA, Killer HE, Fuhrer CA, Zeleny N, Huber AR and Neutzner A (2016). Changes to the Aqueous Humor Proteome during Glaucoma. PLoS One 11, e0165314.

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Zeleny TNC, Kohler C, Neutzner A, Killer HE and Meyer P (2017). Cell-Cell Interaction Proteins (Gap Junctions, Tight Junctions, and Desmosomes) and Water Transporter Aquaporin 4 in Meningothelial Cells of the Human Optic Nerve. Front Neurol 8, 308.