Ocular Pharmacology and Physiology

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The optic nerve: Why does it fail and what to do about it?

Loss of retinal ganglion cells (RGCs), responsible for relaying visual information from the retina to the visual centers of the brain, causes vision deficits in about 100 million patients worldwide. Reasons for RGC degeneration are manifold and include genetic mutation and predisposition as well as environmental factors. Recently, the role of the optic nerve microenvironment shaped by meningothelial cells (MECs) became evident with optic nerve compartmentalization connected to optic nerve damage and visual impairment. In addition, mitochondrial dysfunction is also central to RGC failure and death.
Our work focused on characterizing the cellular component of the optic nerve microenvironment and the role of mitochondrial maintenance on neuronal survival. Furthermore, to translate insight into mitochondrial dysfunction towards treatment options for patients suffering from optic nerve degeneration, we are developing artificial transcription factors targeting OPA1, a key regulator of mitochondrial morphology, to treat autosomal dominant optic atrophy.

The optic nerve microenvironment

As part of the central nervous system, the optic nerve is ensheathed by meninges and bathed in cerebrospinal fluid forming a specific microenvironment. Meningothelial cells covering the meninges constitute the cellular component of this microenvironment. Previous work point to an involvement of MECs in pathophysiological processes leading to optic nerve damage as observed during glaucoma. Our recent work revealed, MECs are highly active facultative phagocytes capable of ingesting bacteria and also apoptotic cells, thereby acting immune modulatory by secreting pro- or anti-inflammatory cytokines and chemokines, respectively. Furthermore, MECs are capable of ingesting large amounts of neurotoxic substances, e.g. the Alzheimer's disease-related amyloid-beta peptide, and display a remarkable resistance to such substances. Our work strongly suggests a neuroprotective function for MECs through cerebrospinal fluid conditioning and clearance.

Mitochondrial maintenance and degeneration of neuronal cells

Mitochondrial dysfunction is at the heart of neurodegeneration with specific quality control mechanisms responsible for maintaining mitochondrial and therefore neuronal function. Previously, we studied the role of mitochondrial ubiquitin ligases, now we focused on the AAA-ATPase p97 and its role in mitochondrial maintenacne. We found that p97 is responsible for the removal of oxidatively and nitrosatively damaged mitochondrial proteins and that blockage of this process is deleterious to neuronal cells. We also recently identified three novel co-factors of p97 involved likely involved in maintaining mitochondrial function through targeted protein degradation and targeted autophagic destruction of mitochondria.

Treating autosomal dominant optic atrophy

Dominant optic atrophy (DOA) is a neuro-ophthalmic condition characterized by the degeneration of the optic nerve resulting in blindness. DOA is inherited in an autosomal dominant fashion and is caused by mutations in *OPA1*. OPA1 is a mitochondrial protein and is involved in mitochondrial dynamics and maintenance of mitochondrial cristae structure. Thus, interfering with OPA1 function leads to disturbed mitochondrial dynamics and higher susceptibility to apoptotic stimuli. In DOA, cellular OPA1 levels are reduced due to various haploinsufficient mutations. While this reduction of OPA1 levels to about 50 % does not impact the function of most cells, neurons and especially RGCs depend on full OPA1 function. Thus, restoring wildtype levels of OPA1 in RGCs would constitute a functional cure for DOA.

To this end, we generated a CRISPR-Cas9-based artificial transactivator capable of upregulating *OPA1* in mouse neuronal cells *in vitro* and are in the process of developing viral vectors for delivery of such therapeutics to retinal ganglion cells *in vivo*. In addition, we established two mouse models of DOA in the lab to assess a therapeutic benefit of such artificial CRISPR-Cas9 transactivators for the treatment of this blinding disease.

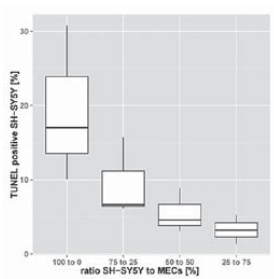

Fig. 1: MECs act neuroprotective by scavenging Aβ. Neuron-like SH-SY5Y cells were co-cultured at identical total density with CellTrace Violet-labeled MECs (Ben-Men-I) at ratios of 100:0, 75:25, 50:50, 25:75 SH-SY5Y:MECs and treated for 24 hours with 25 µM Aβ. TUNEL staining as measure for apoptotic cell death was determined flow cytometrically for CellTrace Violet-negative cells. Shown is the median of three independent experiments as boxplot. Please note the reduction of SH-SY5Y death in the presence of Aβ-scavenging MECs.

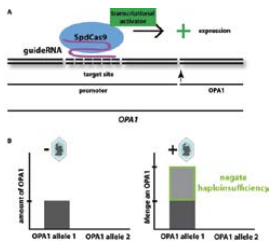

Fig. 2: A novel therapeutic approach for the treatment of dominant optic atrophy. (A) By combining a catalytically inactive form of the RNA-guided *Staphylococcus aureus* endonuclease Cas9 (SpdCas9) with a protein domain capable of driving gene expression (e.g. VP64), a RNA-guided transactivator can be generated. By selecting appropriate guideRNAs, virtually every gene promoter of interest can be targeted. While not all guideRNAs will direct SpdCas9 to a site suitable for inducing gene activation, the relative ease of guideRNA generation allows probing multiple sites inside a gene promoter greatly accelerating development times compared to other artificial transcription factor approaches. **(B)** AAV2-mediated delivery of SpdCas9 to retinal ganglion cells together with a suitable guideRNA targeting the *OPA1*-promoter is envisioned to increase *OPA1* transcript levels, thereby restoring wildtype levels of OPA1 protein, negating haploinsufficiency, and preventing RGC death.

Connection to Clinical Practice

Prof. Hendrik Scholl
Professor and Chairman,
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The ocular pharmacology and physiology lab is closely connected to the University Hospital Eye Clinic under the chairmanship of Prof. Dr. Hendrik Scholl. The main focus is to not only understand mechanisms of (neuro)-degenerative processes of the retina and the optic nerve, but also to modulate these processes to provide better outcomes for patients. In particular, new treatment approaches, including gene therapy and pharmacotherapy, for retinal diseases such as retinitis pigmentosa (RP), Stargardt disease, age-related macular degeneration and diabetic retinopathy are being developed or applied in clinical trials to patients. Also, a close and fruitful collaboration with the Institute of Pathology enables access to valuable tissue samples to study human optic nerves to better understand and validate findings from *in vitro* studies.

Selected Publications

Fang L, Hemion C, Pinho Ferreira Bento AC, Bippes CC, Flammer J, Neutzner A. (2015) Mitochondrial function in neuronal cells depends on p97/VCP/Cdc48-mediated quality control. Front Cell Neurosci. 9, 16

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Li J, Fang L, Meyer P, Killer HE, Flammer J, Neutzner A. Anti-inflammatory response following uptake of apoptotic bodies by meningothelial cells. J Neuroin- ammation. 11, 35

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