Mitochondrial DNA at the interface between mitochondrial dysfunction and neuroinflammation in glaucoma

Author: Yasmin Alameldin

Background: Glaucoma is a leading cause of irreversible vision loss characterized by retinal ganglion cell (RGC) degeneration. Mitochondrial DNA (MtDNA) mutations are known to accumulate with age and are linked to neurodegenerative disorders. The retina is known to be a metabolically active tissue and is prone to mitochondrial dysfunction. The role of mtDNA instability in retinal neuroinflammation ,structural changes, and the role of the CGAS- STING pathway in these changes remains poorly understood.

Method: Proteomics and bulk RNA seq were performed on retinal tissue from 35 week old WT, polg mutator (Mut), sting knockout (KO), and a double knockout (DKO; polg mut+sting ko) mice. Retinal structure was evaluated by immunolabeling retinal flat-mounts for Iba1 (microglia), Brn3a (RGC) and IB4 (vasculature morphology), along with cryosectioning for H&E staining and tunel staining assessed cell death.

Results: Proteomics showed downregulation of oxidative phosphorylation (complex I) alongside upregulation of iron sulfur (fe-S) cluster assembly and heme biosynthesis proteins (Isca2,Nfu1, Fdx2, Fech ,Abcb7) in Mut retinas indicating mitochondrial iron loading and compensatory biogenesis. Metabolic rewiring toward fatty acid beta-oxidation, amino acid catabolism as well as antioxidants, indicated mitochondrial stress. Anti-apoptotic protein down- regulation was accompanied by up-regulation of proapoptotic protein signatures in Mut retinas. This was further supported by tunel positive apoptotic cell death in Mut retinas as well as in DKO mic indicating sting independent apoptotic dysregulation. Low grade innate immune activation including Rigi pathway components was present in both Mut and DKO retinas. DKO vs Mut omics comparison revealed no significant differential regulation of innate immune signaling pathways implicating cGAS–STING independent rna sensing mechanisms. Morphologically Mut retinas displayed dystrophic and amoeboid microglia, vascular rarefaction and reduced retinal layer.

Conclusion: These findings suggets that mtDNA instability can drive a glaucoma phenotype encompassing metabolic and iron dysregulation, apoptotic cell death and microglial dystrophy through a cGAS–STING independent pathway. Rigi mediated rna sensing could be a candidate neuroinflammatory mechanism for understanding and potentially targeting IOP independent glaucoma progression.

Modulation of Autophagy by High Glucose and Metformin in Mouse Retinal Explants

Presenter: Elisabetta Benfatto

BACKGROUND: Diabetic retinopathy (DR) is a major complication of diabetes mellitus and the leading preventable cause of blindness worldwide (Seewoodhary, 2021). This condition is driven by chronic hyperglycemia which induces oxidative stress and inflammation, leading to microvascular and neuroretinal alterations (Al-Kharashi, 2018). Despite being considered a microvascular disease, increasing evidence suggests that neurodegeneration and neuroinflammation are key contributors to DR pathogenesis (Che et al., 2026). Autophagy, the main intracellular pathway responsible for degrading organelles and macromolecules (Palmer et al., 2025), is a known player in retinal diseases (Adornetto et al., 2020), yet its role in DR remains unclear. Nevertheless, several studies have shown that pharmacological manipulation of autophagy exerts neuroprotective effects in the retina (Satriano et al., 2025). In this study, we investigated autophagy changes in mouse retinal explants exposed to normal and high-glucose conditions and evaluated the effects of the direct exposure of retinal explants to metformin, a glucose-lowering agent known for its autophagy-modulating and neuroprotective properties.

METHODS: Organotypic retinal cultures from C57BL/6J mice (8-10 weeks) were exposed to normal (15mM) (NG) or high (50mM) glucose (HG) for 7 days or maintained for 4 days in vitro (DIV) in the presence or absence of metformin (1 M or 10 M). Neuronal survival and glial activation were analyzed by immunofluorescence. Protein and mRNA levels of autophagy-related proteins (LC3, p62, p-ULK1, LAMP1, p- AMPK) and inflammatory markers (IL-1b, GS, GFAP) were analyzed by western blotting or qPCR.

RESULTS: Retina exposed to HG for 7 day exhibited loss of neuronal components and increased glial reactivity compared to the ones cultured under NG conditions. HG treatment was associated with the upregulation of inflammation-related proteins and significant changes in autophagic flux. Metformin modulated autophagy-related markers in retinal explants maintained for 4DIV and counteracted the increased glial reactivity markers compared to baseline (DIV0).

CONCLUSIONS: Our preliminary data suggest that autophagy is significantly modulated in the retina under HG conditions and can be directly targeted by metformin. These finding provides a rational for future studies aimed at pharmacologically modulating HG-induced autophagy to clarify its role in the pathogenesis of diabetic retinopathy

Exploring the potential of retinal organoids for modeling the XLRS-associated RS1 variants

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Variants in the RS1 gene are known to cause X-linked retinoschisis (XLRS), characterized by the splitting of the inner retinal layers. This schisis is a structural defect that leads to loss of retinal organization and eventually disrupted communication between photoreceptors and bipolar cells. Although animal models have proved valuable for studying disease pathomechanisms and have shown the potential of gene therapy for XLRS, no treatment is currently available for these patients. Human- relevant models to understand the underlying biology of XLRS may be necessary for that. In this context, retinal organoids have emerged as a valuable model for studying inherited retinal dystrophies. In this study, we characterize two pathogenic variants in the RS1 gene: a missense variant c.214G>A (p.E72K) in exon 4 and a deletion in exon 3, c.78+365_c.184+607del (del.ex3). These variants were modeled in retinal organoids generated using a 3D-embedding system. Our findings demonstrate that patient-derived retinal organoids exhibited reduced RS1 protein presence compared to control organoids, while lamination remains unaffected. Furthermore, reduced RS1 protein levels appeared to be linked to functional dysregulation (as measured by microelectrode arrays), as well as disruptions of the photoreceptor developmental timeline and ultrastructure. Nevertheless, although the simplified architecture of organoids can be beneficial for modeling certain phenotypic characteristics, this may not be the case for schisis formation, since the variants modeled in this study did not result in the formation of this characteristic XLRS hallmark. In this study, we further assess the current limitations of retinal organoids and the critical components needed to advance in vitro XLRS modeling.

Studying the role of potassium channels in mouse model of Kcnv2 retinopathy.

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KCNV2 retinopathy, caused by mutations within the KCVN2 gene, is a slow progressing inherited condition that leads to complete blindness. The KCVN2 gene, which encodes for the voltage-gated potassium (Kv) subunit Kv8.2. Kv8.2 pairs with the Kv2.1 subunit to form a functional heterotetrameric potassium channel. The diagnosis of KCNV2 retinopathy is confirmed through an electroretinogram (ERG), which measures the retina's electrical activity in response to light, as patients exhibit a unique ERG phenotype called a supernormal b-wave response. This study utilised wildtype (WT), Kv8.2 knockout (KO) and Kv2.1 KO mouse models to probe the origins of this supernormal b-wave. Three different pharmacological agents, L-AP4, BaCl2, and Guangxitoxin-1E, were used to temporarily modulate the scotopic ERG response. The pharmacological agents were administered directly into the eye via an intravitreal injection to mice aged between 6-8 weeks. This was followed by ERG recordings 16-24h later. LAP-4 and BaCl2 decreased b-wave amplitude in Kv2.1 KO and WT (only at lower light intensities). The application of guangxitoxin-1E did not affect WT and Kv2.1 KO retinas but lead to a decrease in ERG amplitude of Kv8.2 KO in both a- and b-wave suggesting that a negative voltage shift of the Kv2.1 homomeric channels lead to the supernormal b-wave. The results show that both bipolar cells and Muller glia contribute to the b-wave and the supernormal b-wave. Further exploration is needed into the contribution of homomeric Kv2.1 channels to this unique phenotype.

Developing a cone-specific promoter using the PDE6C gene

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Background: Targeting cone photoreceptors for gene therapy requires promoters that drive specific expression within the limited packaging capacity of adeno-associated viral (AAV) vectors. However, commonly used cone-specific promoters such as the PR1.7 promoter are relatively large (~1700bp), which can restrict vector design when combined with large therapeutic genes of interest. In this study, we designed and evaluated novel promoters derived from the human PDE6C gene to identify shorter sequences capable of driving cone-specific expression.

Methods: Promoter sizes of 400bp, 700bp, and 1500bp were packaged into AAV7m8 vectors with an mCherry reporter gene. Constructs were administered to C57BL/6J mice and expression patterns were compared to established PR1.7 and ProA1 cone-specific promoters. Intravitreal injections were investigated as a less invasive approach for gene therapy, whilst subretinal injections were investigated as the gold-standard method for photoreceptor targeting.

Results: Following intravitreal administration, the PR1.7 promoter demonstrated the highest cone specificity, the PDE6C promoters transduce cones but also had some transduction of cells within the inner retina, whereas the ProA1 exhibited the least specificity and had broad expression across the retina. The PDE6C promoters showed a size-dependent increase in mCherry RNA expression, with the 1500bp construct having a higher expression than the PR1.7 promoter. When delivered subretinally, all PDE6C-derived promoters were capable of transducing cone photoreceptors to a high efficiency.

Conclusions: These findings demonstrate that human PDE6C-derived promoter sequences can drive transgene expression in the mouse retina. Although intravitreal delivery had lower specificity than the PR1.7 standard, all PDE6C promoter sizes transduced cone photoreceptors efficiently when delivered subretinally. These novel promoters provide a promising alternative for AAV-based gene therapies where current established promoters may exceed packaging capacity limits for large therapeutic genes.

Human Retinal Organoids as a Platform for Evaluating Gene Delivery and Therapeutic Efficacy

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Gene-based therapies offer a promising strategy to restore cellular function in inherited retinal diseases (IRDs), particularly in patients who retain structural retinal integrity despite functional loss. However, clinical translation of gene therapy remains limited by the lack of delivery system capable of achieving efficient, cell type–specific targeting while minimising immune activation. Adeno-associated viral (AAV) vectors and lipid nanoparticles (LNPs) represent distinct gene delivery systems, with differing mechanisms of cargo delivery, expression profiles, and safety considerations; however, systematic and directly comparable evaluation in human-relevant models remains lacking.

To address this gap, we utilise human retinal organoids as a physiologically relevant platform for the systematic assessment of gene delivery strategies. We propose a stepwise pipeline: (i) optimisation and comparative screening of delivery systems in retinal organoids, incorporating clinically relevant administration routes (subretinal-like and intravitreal-like) to evaluate delivery efficiency, transgene expression, cell-type specificity, and vector distribution; (ii) application of optimised strategies in retinal disease models (e.g. X-linked juvenile retinoschisis (RS1) and retinitis pigmentosa (LRAT, RPGR)) to assess therapeutic efficacy and establish correlations between delivery performance and functional rescue; and (iii) incorporation of microglia into retinal organoids to model the retinal immune microenvironment and evaluate delivery-induced immune responses.

By integrating delivery optimisation, route modelling, disease-specific validation, and immune profiling within the human-based system, this platform provides a translational framework for the rational selection and development of gene therapies for retinal diseases.

Polymer Films for Retinal Prostheses: Material Properties and Cellular Response

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Retinal degeneration, associated with aging and metabolic disorders as diabetes, leads to photoreceptor loss and visual impairment, including blindness. Retinal prostheses offer a promising strategy to functionally replace damaged photoreceptors and partially restore vision. In this work, we investigated polymer-based semiconducting films as candidate materials for subretinal interfaces. Blends were composed of PEDOT:PSS combined with TQ1 or RP3HT to achieve high photosensitivity, efficient charge transport, and cytocompatibility.

Films with varying compositions were prepared by spin-casting. Chemical composition was characterized by Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and surface morphology by Atomic Force Microscopy (AFM). Photosensitivity and light- dependent polymer–cell interactions were studied under controlled irradiation cycles. Cytocompatibility of the blends with ARPE-19 cells was assessed through LDH assay, fluorescent staining, and reactive oxygen species (ROS) detection, both in darkness and under illumination.

ToF-SIMS and AFM measurements revealed uniform film structure with both constituent polymers present at the surface. Blends showed photosensitive properties, higher for HTL:TQ1. The films showed no cytotoxicity in darkness, however, lowered viability of the cells, together with ROS generation was observed after illumination for the cells cultured on HTL:TQ1 films. Therefore, we investigated blends with varying TQ1 abundance to identify compositions that exhibit light-induced electrical activity while remaining non-cytotoxic.

The obtained results demonstrate the potential of semiconducting polymer blend films as functional materials for subretinal prosthetic interfaces. They showed combined optoelectronic performance, stability under aqueous environment, and compatibility with ARPE-19 cells, which demonstrates their potential for the development of light- responsive retinal implants.

The study was funded by the “Research support module” as part of the “Excellence Initiative - Research University” program at the Jagiellonian University in Kraków the (A.C, RSM/80/CA).

Novel applications of Descemet's membrane for retinal diseases

Background: Descemet's membrane (DM) is the specialized extracellular matrix (ECM) located between the posterior corneal stroma and the endothelium. Thanks to its composition and mechanical properties, this basement membrane provides the biochemical cues that sustain endothelium viability and function, making it an optimal cell niche for the cell types that organize in polarized monolayers, like retinal pigment epithelium (RPE). Several decellularization techniques have been developed to isolate the ECM removing all cellular components from a variety of tissue, but it is important to select the optimal protocol that maintains ECM integrity. The present work describes the tailored-made decellularization protocol to obtain an acellular scaffold from DM, and its application for the differentiation of stem cells towards RPE lineage, with the final aim to obtain an implantable RPE graft for age- related macular degeneration treatment.

Methods: DMs were mechanically stripped from donor corneas. To induce cell lysis and eliminate all cytoplasmic and nuclear material, isolated DMs were subjected to osmotic (hypotonic) shock for two hours under constant agitation. Nuclear removal and ECM integrity after treatment were verified with DNA quantification and immunofluorescence analysis. RPE cells obtained from stem cell lines following a triphasic differentiation protocol were seeded on decellularized scaffolds, and the increase of transepithelial electrical resistance (TEER) and RPE marker expression was evaluated.

Results: DNA quantification showed a 99% reduction of total DNA in decellularized Dms compared to control ones, with no DAPI-positive nucleus still visible after decellularization. Moreover, ECM components staining maintained the same pattern and intensity as the fresh untreated control. The differentiated cells expressed typical markers of RPE phenotype, with apico-basal polarization and increased TEER reaching the threshold values corresponding to tight junction formation.

Conclusions: These results show that the decellularized DM is depleted of the donor DNA, avoiding the risk of unwanted immunogenicity when used as heterologous graft. Cells seeded on this scaffold complete their maturation towards RPE phenotype, creating a functional transplantable epithelium. DM is currently employed in intraocular surgery and prestripped by eye banks, therefore a decellularized DM could represent a valid scaffold for regenerative medicine purposes in ocular surgery.

Keywords: Age-related macular degeneration, retinal pigment epihelium, stem cells, Descemet's membrane, decellularization

Background. Beyond ATP production, mitochondria act as central signaling platforms regulating cell death and inflammatory pathways such as NF-κB and cGAS/STING, linking cellular bioenergetics to tissue-level inflammation. Alterations in mitochondrial respiration are increasingly recognized as upstream drivers of cytokine and chemokine production, particularly in immune cells whose functional state is tightly coupled to metabolic status in the central nervous system. The retina offers a uniquely sensitive system to study these processes: as a metabolically demanding neural tissue with a finely organized vascular network, it depends on tight regulation of inflammation and vascular growth. Disruption of this balance underlies blinding diseases such as diabetic retinopathy and age-related macular degeneration, in which inflammation is a key trigger of pathological angiogenesis. Myeloid cells are critical sensors of retinal stress and orchestrators of inflammatory and angiogenic responses, with distinct metabolic and mitochondrial features guiding their activation and functional states. How these metabolic features perpetuate inflammation in the central nervous system, however, remains unclear.

Methods: we employ a knockout of COX10 in CX3CR1^{+} cells to induce controlled Complex IV deficiency selectively in the immune compartment. Mitochondrial respiration is assessed by Seahorse extracellular flux analysis. The retinal phenotype is characterized i in vivo using OCT, BluePeak autofluorescence imaging, and optomotor response, complemented by histology, immunofluorescence, single-cell transcriptomics, and metabolomics.

Results. Deletion of COX10 in CX3CR1^{+} cells reduce oxygen consumption, confirming cell-intrinsic Complex IV deficiency. In vivo retinal imaging at the timepoints examined shows preserved retinal thickness, autofluorescence, and visual acuity, defining a pre-symptomatic window in which to dissect molecular and cellular pathology preceding functional decline. Ongoing analyses focus on microglial activation, transcriptional reprogramming, and immune-endothelial crosstalk within this window.

Conclusions. This project establishes a tractable in vivo system to dissect how mitochondrial dysfunction in CX3CR1^{+} immune cells reshape inflammatory signaling and contributes to pathological retinal vascular growth, positioning immune-cell mitochondrial metabolism as a candidate upstream regulator of retinal inflammation and a potential therapeutic node.

Introduction

Retinol-binding protein (RBP3) is a major component of the interphotoreceptor matrix (IPM) and supports retinoid transport between the retinal pigment epithelium (RPE) and the photoreceptor cells. Its presence within the interphotoreceptor matrix is light- and dark-dependent. Reduced RBP3 levels have been associated with the progression of diabetic retinopathy, making this protein an attractive candidate biomarker for early diabetic retinopathy detection.

We aim to develop a diagnostic approach in which we can measure the level of RBP3 using fluorescently labelled antibodies combined with two-photon excitation fluorescence (TPEF) imaging. The current diagnostic approach to diabetic retinopathy involves observing structural changes in the retina, which detects the disease at a late stage. Our innovative approach would potentially enable the diagnosis of diabetic retinopathy earlier, giving patients a better chance of maintaining their quality of vision for longer.

Methods

Microinjections of retinas at early and progressive diabetic retinopathy stages, with fluorescently labelled antibodies against RPB3, will be followed by in vivo two-photon imaging. Signal distribution and intensity will be validated ex vivo by immunofluorescence microscopy, Western blot, and mass spectrometry.

Results

Preliminary observation shows RBP3 displays a distinct light-dependent distribution, and is aligned with the changes in IPM volume due to hydration levels. Our preliminary data confirmed a strong concentration of RBP3 at the IPM’s borders in light conditions (the interface with the RPE and the junction of inner and outer segments of photoreceptors. Immunostainings will also show if there are any changes in the pattern of expression of RBP3 after forming complexes with fluorescent tags. The spotlight tool in our experiment is two-photon imaging – only minimally invasive while giving an early picture of starting the diabetic retinopathy.

Aims

Our project aims to develop a minimal invasive platform for tracking RBP3 abundance and distribution in retinal tissue. Additionally, RBP3 could be the therapeutic molecule alleviating the symptoms of diabetic retinopathy, and our proposed detection method can support follow-up checks on the treatments.

Immunostaining of fibrotic tissue isolated from the vitreoretinal interface

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Background: Fibrotic membrane can form at the vitreoretinal interface e.g. after the healing of a surgical wound or as a result of a disease such as rhegmatogenous retinal detachment, proliferative vitreoretinopathy, age-related macular degeneration, or various pediatric diseases 1–5. Fibrotic membrane consists of cells that have undergone epithelial-mesenchymal transition (EMT), proliferated, and migrated, e.g. RPE cells, glial cells, fibroblasts and myofibroblasts, as well as excessive extracellular matrix (ECM) 1,2. For example, vimentin and α-SMA are key indicators of the transition of mesenchymal cells into myofibroblast, which promotes the formation of fibrosis 6. Fibrosis leads to wrinkling, folding, contraction, and detachment of the retina 2.

Methods: Vitreoretinal interface scar tissue was placed in a well of 12-well plate and photographed using a light microscope (Zeiss Axio Vert). The membrane was fixed with 4% PFA, washed with DPBS, blocked with 1% BSA / 0.1%Triton X-100 in DPBS, and washed again with DPBS. The membrane was stained with primary antibodies to α-SMA and vimentin and then treated with Alexa Fluor secondary anti-mouse and anti-rabbit antibodies. Nuclei were stained with Hoechst 33342. Membrane was washed, mounted with Mowiol, and photographed using Olympus Apex View APX100.

Results: In the photograph, the membrane appeared thin, light, and almost translucent. Hoechst staining revealed that the membrane contained occasional cells and cell clusters, as well as abundant ECM. The stained scar tissue contained myofibroblast markers α-SMA and vimentin.

Conclusions: α-SMA and vimentin appeared to be at least partially localized to the nuclei staining in scar membrane. The role and inhibition of α-SMA and vimentin in membrane formation require further studies. Nuclei staining revealed occasional cells in the membrane, so the role and inhibition of cell migration and ECM production also require further studies.

Acknowledgement: Warm acknowledgement to the immuno-ophthalmology and ophthalmology groups. Scar tissue was collected at KYS, and the study was conducted in accordance with research the permit (256/13.00/2025) and ethical approval (297/13.00/2024).

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HIF-P4H-2 inhibition in experimental glaucoma

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Background: Oxygen plays a central role in the pathogenesis of retinal neurodegeneration. The high metabolic rate and O2 consumption make retina vulnerable to oxidative stress and hypoxia. Hypoxia-inducible factor (HIF) mediates an adaptive response to oxygen deficiency by activating compensatory pathways that both increase O2 delivery and decrease its consumption. HIF prolyl 4-hydroxylase-2 (HIF-P4H-2/PHD2/EGLN1) is the primary regulator of HIF. HIF-P4H-2 requires O2 for catalysis but has a low affinity for it, making it an O2-sensor. We hypothesize that moderate stabilization of HIF by HIF-P4H-2 inhibition protects from retinal ganglion cell (RGC) death and optic nerve damage seen in glaucoma.

Methods: Two different methods of HIF-P4H-2 inhibition were used: A mouse line with genetic repression of Hif-p4h-2 (Hif-p4h-2gt/gt) achieved by insertion of a beta-galactosidase reporter into truncated Hif-p4h-2, leading to HIF-P4H-2 hypomorphism, and second, systemically administered roxadustat, a pan-HIF-P4H inhibitor. X-Gal staining, qPCR and Western blot were used to determine HIF-P4H-2 and HIF levels at baseline. Experimental glaucoma was induced unilaterally by acute ocular hypertension (AOHT) or by optic nerve crush (ONC). Retinal neurodegeneration was quantified with Brn3a immunohistochemistry in retinal flat mounts and histology was evaluated from HE-stained cross-sections and optic nerves.

Results: Hif-p4h-2gt/gt mice had similar RGC survival with wild-type mice in the ONC model but showed significantly higher RGC survival compared to wild-type mice in the AOHT model. Hif-p4h-2gt/gt mice also showed preserved tissue integrity compared to wild type in the AOHT model. The mice treated with roxadustat had similar RGC survival compared to vehicle group in the AOHT model, however, induced hemoglobin levels in the roxadustat- treated mice correlated with higher RGC survival.

Conclusions: Genetic HIF-P4H-2 deficiency protects RGCs from ischemia-reperfusion injury in the AOHT model but does not protect RGCs from direct axonal injury in the ONC model. Systemically administered roxadustat does not offer similar level of protection, although induced hypoxia signaling correlates with higher RGC survival, suggesting that HIF stabilization must be local to effectively protect against glaucoma pathology. In conclusion, HIF-P4H-2 inhibition could be a treatment method in retinal neurodegeneration observed in high-pressure glaucoma.

Nicotinamide effect on the NAD+/NADH ratio in the diabetic rat retina

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Background: Diabetes mellitus is a chronic disease characterized by alterations in glucose metabolism that leads to complications in several organs, including the retina. Oxidative stress plays a key role in the progress of diabetic retinopathy and decrease in the NAD+/NADH ratio has been demonstrated in its pathogenesis. Therefore, NAD supplementation by nicotinamide (NAM) may be beneficial.

Methods: Four groups of Long-Evans rats were established: normal (control), normal supplemented with NAM, diabetic, and diabetic supplemented with nicotinamide (NAM). Diabetes was induced by a single intraperitoneal administration of streptozotocin (STZ). One day after STZ administration, rats received water containing 10 mM NAM for 5 h daily up to 20 or 45 days.

Results: Hyperglycemia induced increase in carbonylated proteins, as well as a progressive decrease in the NAD+/NADH ratio. Despite that, no significative changes in Nrf2 expression or GSH levels were found. NAM supplementation enhanced NAD+ levels in 20 days diabetic rat retina, reestablishing the NAD+/NADH ratio and carbonylated protein levels. However, NAM supplementation did not modify either NAD+/NADH ratio, nor levels of carbonylated protein at 45 days diabetes.

Conclusions: Alteration of the NAD+/NADH ratio induced protein oxidation independent of the Nrf2 function. Experiments aimed at clarifying the significance of these results are in progress.

The characterization of porcine retinal explants and their use as an ex vivo model for retinal gene therapy

Author: Jessica Hernandez

Purpose: Inherited retinal diseases (IRD) significantly contribute to vision loss worldwide, impacting the quality of life for affected individuals. Gene editing (GE) offers a promising future solution as an effective treatment through restoration of gene function, but translation into the clinic has proven difficult. Therefore, effective models to evaluate the specificity and efficacy of therapeutic GE are of particular importance.

Methods: Since findings from our preliminary in vitro experiments failed to translate to an in vivo pig model, porcine retinal explants (RE) were selected as an alternative tool for cellular systems. RE were analyzed at 5 different time points, days 0, 1, 3, 7, and 14, to assess their stability in culture. Characterization of RE was carried out by immunofluorescent staining, qPCR, and bulk RNAseq. Once established, RE were treated with Adeno-associated virus (AAV)9 in combination with a GFP reporter at different application sites and their transduction into retinal cells was assessed by flow cytometry and immunofluorescent staining.

Results: Histological analysis showed that RE retained their structure well with minimal signs of degeneration and were able to rapidly adapt and remain stable under culture conditions. However, at the molecular level, stress-related processes accompany adaptation to culture, highlighting the need for rapid culture after isolation and immediate treatment with vectors. By applying AAV9 with GFP at different application sites, we also found potential shifts in GFP expression in retinal cell populations dependent on REorientation. Finally, through flow cytometry analyses, we were further able to discriminate rod cells and assess GFP expression within a specific cellular population.

Conclusion: The conflicting results previously observed moving from an in vitro to in vivo model highlight the need for a better predictive tool which more closely replicates the post-mitotic tissues of the retina when assessing GE techniques. RE are an ideal candidate to bridge this gap since they preserve tissue architecture, physiological relevance, and have already demonstrated value as a model for GE vector testing.

Keywords: Gene therapy, Porcine retinal explants, Adeno-associated virus, Inherited retinal diseases

Delivering oxygen with nanobubbles to retinal cells

Author: Emma Ilonen

Background: Nanobubbles are small, < 1 µm, gaseous bubbles. Recent research has shown that they persist in solution for extended time periods, even for days, although theoretically, they should dissolve on the timescale of milliseconds. Here, we investigate whether nanobubbles participate in gas exchange in isolated tissue. We use the retina as a biosensor, since it is among the metabolically most active tissues. Without an additional oxygen supply, high metabolism results in hypoxia, which quickly reduces retinal signalling.

Methods: A custom-made microfluidics system was used to produce oxygen and carbogen nanobubbles. The nanobubble size distribution was determined with dynamic light scattering, and the oxygen concentration was measured with the Oxygraph+ system. The effect of nanobubbles on the retina was studied with transretinal ERG using WT C57BL/6J mice. The dark-adapted retina was stimulated with 1 ms 530 nm full-field flashes. Ames' medium was prepared from the water in which the nanobubbles had been produced the previous day. HEPES was used as a pH-buffer, as no additional bubbling was used in the solutions containing nanobubbles. 100 µM $BaCl_2$ was used to block the glial component, and in some experiments, 10 mM bicarbonate was added to the solution.

Results: The oxygen concentration in nanobubble dispersions was lower than in solutions constantly bubbled with traditional microbubblers but higher than in air- saturated solutions. The a-wave viability was sustained in nanobubble dispersions for several hours. The b-wave amplitude decreased in nanobubble dispersions but remained stable for several hours. The addition of bicarbonate increased both a-wave and b-wave viability.

Conclusions: The amount of oxygen achieved with this nanobubble production system maintains the a-wave, but the b-wave seems to require higher oxygen concentrations. The nanobubble production system should be developed further to achieve higher oxygen concentrations. Bicarbonate may have a role in cellular viability, not only by pH buffering but also with other mechanisms.

Keywords: ERG, transretinal ERG, nanobubble, retinal viability

A biologist’s guide to rapid and robust detection of GSK-J4

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BACKGROUND

Modulating epigenetic targets has shown therapeutic potential in various conditions, including inherited retinal disease. Of recent interest is the modulation of H3K27 trimethylation (H3K27me3). A seminal study by Miller et al. (2022) demonstrated that increasing H3K27me3 through continuous administration of GSK-J4 increases cone survival ex vivo in the Pde6ccpfl1 mouse model of achromatopsia. Although GSK-J4 is a useful probe for modulating H3K27me3, developing it as a treatment requires improved physicochemical and pharmacokinetic characterisation, as well as a simple method to detect and quantify the drug. Here, we present a fast, robust HPLC method for quantifying GSK-J4 that is accessible to non-chemists.

METHODS

Various mobile phases, flow rates, and elution conditions were evaluated to develop the high-performance liquid chromatography (HPLC) method. The optimised method was then validated by assessing precision, accuracy, linearity, and specificity. Importantly, the method’s compatibility with GSK-J4 extracted from treated 661W cone-like photoreceptor cells was also evaluated.

RESULTS

The optimised HPLC method met all acceptance criteria for precision, accuracy, linearity and specificity tests, highlighting its robustness. It employs simple mobile phases and isocratic conditions with rapid drug elution, enabling the detection of the drug in under 5 minutes. Notably, the method also allowed for successful quantification of GSK-J4 extracted from treated 661W cells.

CONCLUSIONWe developed and validated a novel HPLC method that provides a rapid, simple, and accessible way to quantify the drug. This may facilitate improved understanding of the pharmacokinetic properties of GSK-J4, and provides a practical platform for testing novel formulations. This extraction protocol can also be adapted for isolating the drug from other cell cultures, or retinal tissue obtained after ex vivo or in vivo treatment, which is the focus of ongoing studies.

Optoelectronic Biointerfaces for Ex Vivo Retina Stimulation

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Background: Degenerative retinal diseases such as age-related macular degeneration and retinitis pigmentosa result in photoreceptor loss with disruption in native phototransduction while largely preserving inner retinal circuitry. Artificial phototransduction strategies propose stimulation of surviving retinal neurons. Photovoltaic biointerfaces offer a wireless approach by converting incident light into electrical currents without external power sources.

Methods: We developed quantum dot integrated devices designed to generate pseudocapacitive currents under near infrared light illumination. The multilayer architecture was optimized for efficient photocurrent generation. Biocompatibility of the photovoltaic interface was first assessed in primary hippocampal neuron cultures through viability assays and neuronal marker analysis. Device functionality was evaluated using ex vivo retinal explants isolated from healthy and degenerated animals recorded on multi-electrode array (MEA) systems. Illumination (880 nm, 5Hz, 20ms ON, 180ms OFF cycles) was applied to the entire device surface, and neuronal responses were quantified through changes in active electrode number and firing dynamics.

Results: NIR illumination induced robust light-triggered modulation of retinal activity. Photovoltaic stimulation significantly increased the number of active electrodes compared to baseline recordings and altered firing dynamics across the network, indicating effective recruitment of retinal neurons.

Conclusion: Our findings demonstrate that a quantum dot-based photovoltaic interface can effectively modulate retinal network activity under NIR light illumination. This wireless, optoelectronic strategy represents a promising platform for next-generation subretinal prosthetic systems targeting photoreceptor degeneration.

Keywords: Retinal prosthesis; vision restoration; bioelectronic interface; NIR light stimulation

Pharmacological VCP inhibition confers neuroprotection in the Prph2Rd2 model of retinal degeneration.

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Objective: Inherited Retinal Degenerations are genetically heterogeneous disorders leading to progressive photoreceptor dysfunction and vision loss. Despite their genetic diversity, shared pathogenic mechanisms converge on disrupted proteostasis. Pharmacological inhibition of valosin-containing protein (VCP), a central regulator of protein homeostasis, has previously demonstrated neuroprotective eBects in rhodopsin- associated autosomal dominant models as well as in phosphodiesterase 6 (PDE6)- associated autosomal recessive models of retinal degeneration. Here, we evaluated the neuroprotective potential of UPCDC-30766, a recently developed VCP inhibitor, in the Prph2Rd2 mouse model, which exhibits structural outer segment defects and progressive degeneration aBecting both rod and cone photoreceptors.

Methods: Retinal explants from Prph2Rd2 mice were cultured using an interphase system from PN12 to PN18, corresponding to the peak of retinal degeneration in this model. This ex vivo system preserves retinal lamination and cellular architecture while allowing controlled pharmacological intervention during the degenerative phase. Cultures were maintained in a defined, serum-free medium to enhance reproducibility and minimize experimental variability. Explants were treated with UPCDC-30766 in escalating doses, and DMSO-treated samples served as vehicle controls. Neuroprotection was assessed by ONL thickness and photoreceptor cell rows, TUNEL-positive cell quantification within the ONL, OS length, and the ONL-to-OS ratio of rhodopsin fluorescence intensity to evaluate alterations in rhodopsin traBicking and localization.

Results: Treatment with UPCDC-30766 resulted in significant preservation of photoreceptor row numbers across all tested doses compared to vehicle controls. Although the percentage of TUNEL-positive cells per section did not reach statistical significance, a consistent reduction in apoptotic profiles was observed in treated explants, indicating a trend toward decreased photoreceptor cell death.

Conclusion: UPCDC-30766-mediated VCP inhibition confers structural neuroprotection in Prph2Rd2 retinal explants. These findings highlight the value of organotypic interphase cultures as a controlled platform for mechanistic studies and preclinical pharmacological testing in genetically diverse retinal degenerations.

Parallel Isolation and Selective Expansion of Primary Porcine Retinal Endothelial Cells and Pericytes from a Single Donor Source

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Background/intro:

Retinal endothelial cells and pericytes constitute the cellular core of the inner blood-retina barrier and are central to vascular stability and barrier function. Experimental investigation of their coordinated responses requires primary cultures that retain physiological characteristics. This study aimed to establish a cost-effective and reproducible method for the parallel isolation and selective expansion of primary porcine retinal endothelial cells (pECs) and pericytes (pPCs) from the same retinal preparation, thereby providing a translationally relevant in vitro platform for studies of retinal vasculature.

Methods: Fresh porcine eyes obtained as an excess product from the food industry were disinfected and dissected. The retinal tissue was collected and subjected to enzymatic digestion to generate a mixed microvascular cell suspension. Selective culture conditions were applied to favour either endothelial or pericyte expansion. Cell morphology was assessed microscopically, and phenotypic identity was validated using RT-qPCR and immunofluorescent staining for cell-type specific markers.

Results: The protocol enabled reproducible establishment of enriched pEC and pPC-like monocultures. pEC cultures formed cobblestone monolayers and expressed endothelial markers at both transcript (PECAM1, vWF, ERG) and protein (VE-Cadherin, ERG) levels. PPC cultures exhibited spindle-shaped morphology and expressed mural-associated markers at RNA (PDGFRB, DES, NG2, PRKG1, ACTA2) and protein (PDGFRα/ß, NG2, α-SMA) levels, indicative of a predominant pericyte phenotype. Markers of the alternate vascular cell type and glial cells were not detected, supporting selective enrichment of the intended populations.

Conclusions: The presented approach allows parallel isolation of pECs and pPCs from the same retinal source without reliance on advanced cell-sorting technologies. Cells isolated with the co-isolation protocol preserves key cellular characteristics of the retinal microvasculature and offers a practical platform for mechanistic studies of blood-retina barrier or retinal vascular diseases. To our knowledge, this is the first protocol presenting the successful isolation of porcine retinal microvascular cells.

Association between histone deacetylase compartmentalization and survival of Retinal Ganglion Cells and Primary Hippocampal Neuron

Author: Malwina Lisek

Class IIa histone deacetylases (HDAC4 and HDAC5) are critical transcriptional regulators whose function depends on intracellular localization. Whether class Iia HDACs are neuroprotective or proapoptotic is controversial. Class IIa HDACs when localized to the nucleus bind MEF2 on chromatin and mediate gene repression. In this study, it was tested if nuclear or cytoplasmic localization of HDAC4/5 and inhibition of MEF2A activity will promote neuronal survival and/or axon outgrowth in primary neurons. Neuronal survival and axon growth were studied using primary cultures of rat E18 hippocampal neurons. Cells were transfected with plasmids encoding wild-type (WT) forms and mutants of HDAC4 and HDAC5 with constitutive nuclear (3SA, S/A) or cytoplasmic (L175A, ANLS) localization. Surviving neurons spontaneously exhibit neurite outgrowth, and measurement of the longest neurite represents an assay for axon growth as a model. With the AAV2 intravitreal injections we want to confirm the neuroprotective effect of HDAC4 and 5 nuclear localization in RGC cells after optic nerve crush.

Keywords: retina ganglion cells, histone deacetylase

Photoswitchable mGluR6 agonist elicits light responses in human retinal organoids through selective recruitment of ON-pathway single units

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* Equivalent contribution

Background: Retinal degenerative diseases cause photoreceptor loss and vision impairment. Photopharmacology for vision restoration aims to bypass photoreceptors by directly activating downstream circuits. Prosthe6-20 (p6-20), a photoswitchable mGluR6 agonist, targets ON-bipolar cells to restore light-driven output independently of photoreceptors. P6-20 has demonstrated visual acuity restoration in blindness models both in zebrafish larvae and mice, supporting translation to human systems. Human iPSC-derived retinal organoids (Ros) provide a physiologically relevant ex vivo platform to evaluate such strategies. Current RO- multi-electrode array (MEA) systems face technical challenges including incomplete 9-cis retinal recycling and limited maturation, showing heterogeneous light responses.

Methods: Healthy iPSC derived ROs were plated on MEA for electrophysiological recordings (DIV 210+). Standardized 2-minute dark/light cycles were applied at baseline and after p6-20 (32 µM). Activity was recorded from 8 electrodes with consistent firing (5 organoids, >0.3 Hz). Light responses were quantified via normalized Light Response Index (LRI). Spike sorting resolved putative single units (≥180 spikes) to assess functional heterogeneity masked by population averaging.

Results: At baseline, ROs displayed spontaneous activity but minimal light responses (LRI 0.00±0.06), with no electrodes meeting ON-response criterion (LRI >0.3). With p6-20, electrode-level LRI increased (0.13±0.08), with 1/8 electrodes reaching ON-response threshold. Spike sorting resolved 13 active units at baseline and 19 post-treatment. Critically, single-unit analysis revealed ON-type responses in 3/19 units post-treatment (LRI 0.42-0.85) across 3 electrodes, compared to 1/13 units at baseline. Spike sorting unmasks functional heterogeneity invisible at population level, revealing selective recruitment of mGluR6- expressing neuronal subpopulations.

Conclusions: These findings provide proof-of-concept that p6-20 elicits selective ON- pathway responses in human ROs, in line with previous in vivo results, supporting mGluR6 agonism as a photoreceptor-bypass strategy. The sparse yet specific response pattern highlights both mGluR6 selectivity and need for technical refinement of RO-MEA systems. Single-unit analysis was essential for detecting responses. This establishes a foundation for systematic optimization of RO-MEA platforms with spike sorting for preclinical evaluation of vision restoration therapies.

The circadian modulation of melanin biosynthesis by targeting the ROR regulatory motif in the retinal pigment epithelium

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Introduction: Pigmentation deficits are implicated in blinding eye disorders, with albinism being the most prominent. Melanin is the main pigment in the eyes, skin and/or hair and is synthetized in complex biochemical pathways. The circadian clock is a molecular pacemaker comprised of transcriptional and translation feedback loops involving transcription factors such as BMAL1, CLOCK, PER1-2, CRY1-2. Our goal was to investigate the link between the circadian clock and melanogenesis. We also utilized this link to modulate pigmentation in stem cell-derived pigmented cells of the eye, called the retinal pigment epithelium (RPE).

Methods: We cultured embryonic stem cell-derived retinal pigment epithelium (hESC-RPE) for 12 weeks. The cells were synchronized by a serum-shock and harvested at 3h intervals for up to 66h. RT-PCR analysis was used to quantify the expression of clock and melanogenesis genes. We performed motif-enrichment search in melanogenesis genes by using online tools (UCSC database, Galaxy and TF Motif View). Pigmentation in hESC-RPE was studied by pharmacology, bright-field microscopy and analyzed by an AI-based trainable WEKA segmentation tool. Bioinformatics was used to analyze published RNA-sequencing data of laser capture microdissected mouse RPE obtained at 4 time-points over 24h.

Results:We found that synchronized hESC-RPE show rhythmic mRNA expression of clock genes BMAL1,CLOCK, CRY2, PER1, PER2, REV-ERBα and melanogenesis-related genes TYR, CDH3, DCT and PMEL. We selected mouse and human conserved promoter regions by using the UCSC database with 6 known time-affected melanogenesis genes (Tyr, Tyrp1, Pmel, Dct, Cdh3 and Rab1a). By TF Motif View, we found over-representation of binding sites for negative regulators of transcription and the ROR binding site, a known regulator of clock genes. Bright-field microscopy revealed decreased pigmentation in hESC-RPE treated with the ROR agonist Nobiletin compared to controls. Conversely, significantly increased pigmentation was observed in hESC-RPE cells supplemented with the indirect ROR antagonist SR9009 compared to controls. Finally, bioinformatics analysis revealed that melanogenesis pathways are enriched in genes up-regulated at night compared to late-afternoon time points in mouse RPE.

Conclusions: Overall, these results suggest that melanogenesis is regulated by the circadian clock. Modulation of the clock could be a potential target for treating blinding pigment deficits such as albinism.

Keywords: retinal pigment epithelium, albinism, circadian clock, melanogenesis, stem cell

Assessing the effect of pan versus isoform specific HDAC inhibition in two models of achromatopsia

Presenter: Annie Miller

Background

Histone deacetylase (HDAC) overactivity has been shown in the photoreceptors of several models of inherited retinal disease (IRD) and has been linked to the death of photoreceptors. Administration of pan-HDAC inhibitors was shown to reduce both rod and cone degeneration in models of retinitis pigmentosa (RP) and in the Pde6ccpfl1 model of achromatopsia, but the role of specific HDAC isoforms is less understood. HDAC6 inhibition has previously shown potential as a neuroprotective target, providing improvements in visual function and cell morphology in a zebrafish model of inherited blindness, and conferring increased cone survival in an ex vivo model of RP. However, the role of HDAC6 inhibition has yet to be investigated in models of cone-only degeneration, such as achromatopsia.

Methods

We tested pan- and isoform-specific HDAC inhibitors in two murine models of achromatopsia carrying mutations in the Gnat2 or Pde6c gene, while also expressing GFP exclusively in cones (referred to as Gnat2.GFP or Pde6c.GFP herein). We intravitreally injected the pan-HDAC inhibitor SAHA or the HDAC6 inhibitor, Tubastatin A, at 12 or 20 days of age (P12 or P20). Eyes were collected four days or twelve days after treatment for histological and molecular analyses.

Results

In Pde6c.GFP mice, SAHA treatment administered at P12 caused an 18% increase in cone numbers compared to controls when eyes were assessed four days after treatment ($n=7$, $P=0.0015$). However, when eyes were collected twelve days after treatment, there were no protective effects noted, suggesting a lack of sustained protection ($n=6$-7, $P>0.05$). Similarly, in Gnat2.GFP mice that were treated with Tubastatin A at P20, retinae that were collected four days after treatment showed an 11% improvement in cone numbers compared to controls ($n=7$, $P<0.0001$), with this effect lost when eyes were collected twelve days post-treatment ($n=5$-6, $P>0.05$).

Conclusion

While we noted significant neuroprotection after administration of SAHA in Pde6c.GFP mice and Tubastatin A in Gnat2.GFP mice, these protective effects were only noted when retinae were assessed four days after treatment but not twelve. As both drugs do not appear to have sustained treatment effect, the next steps in this study involves implementing an ex vivo system to provide sustained delivery of treatments which will allow us to understand whether long term protection of the cone photoreceptors occurs and assess long term toxicity of treatment application.