PRO RETINA FOUNDATION

14th PRO RETINA
RESEARCH-COLLOQUIUM POTSDAM

CONFERENCE REPORT

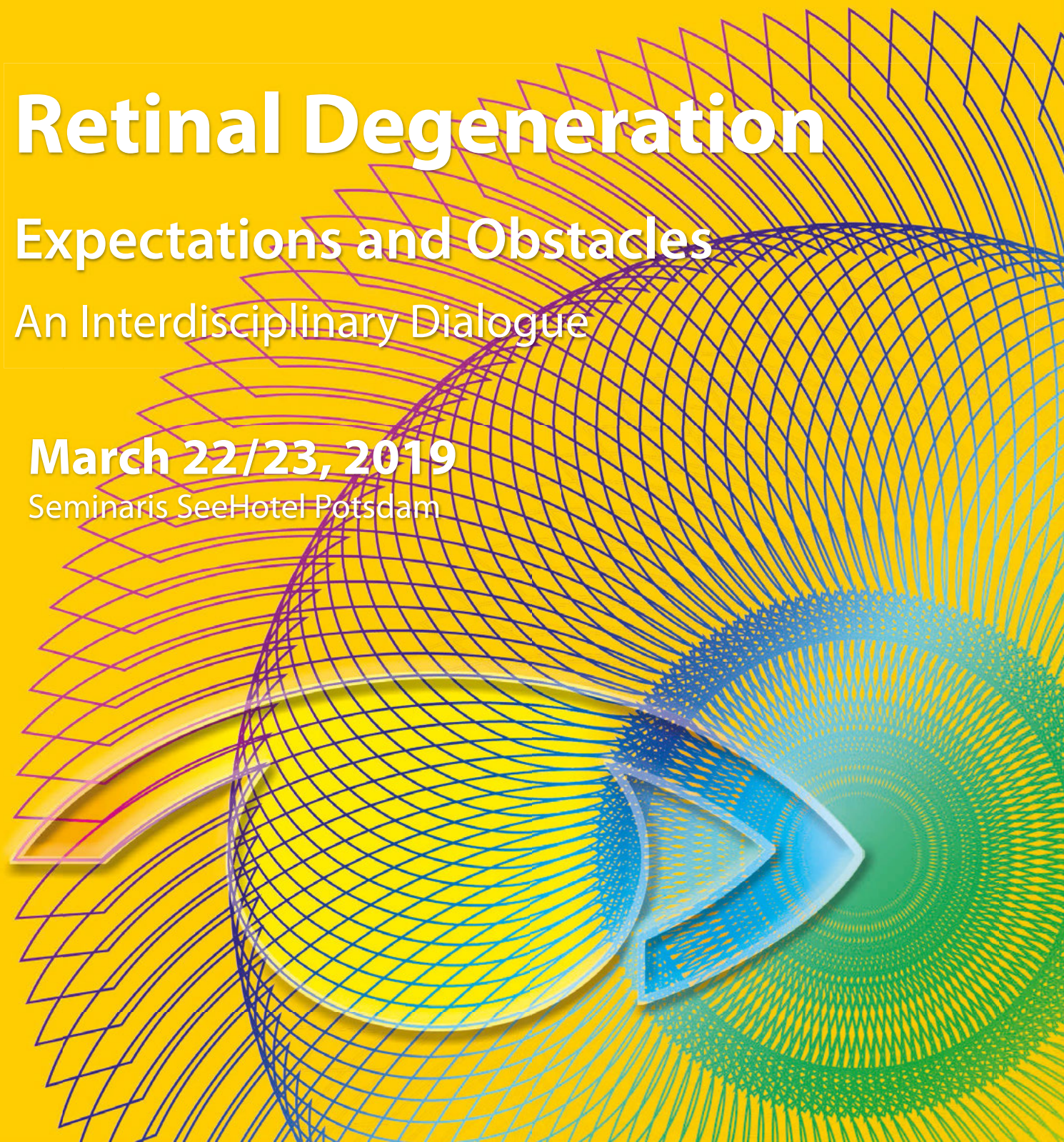
Retinal Degeneration

Expectations and Obstacles

An Interdisciplinary Dialogue

March 22/23, 2019

Seminaris SeeHotel Potsdam



PRO RETINA FOUNDATION

14th PRO RETINA

RESEARCH-COLLOQUIUM POTSDAM

CONFERENCE REPORT

Retinal Degeneration

Expectations and Obstacles

An Interdisciplinary Dialogue

March 22 / 23, 2019

Seminaris SeeHotel Potsdam

PRO RETINA DEUTSCHLAND E. V. & THE PRO RETINA-FOUNDATION FOR PREVENTION OF BLINDNESS

WHO WE ARE

The patient-organisation, "PRO RETINA Deutschland e. V.", was founded in 1977 as „Deutsche Retinitis Pigmentosa-Vereinigung" by patients and their relatives intended to organize help for themselves. The three objectives mentioned in the constitution are to actively support research, to give psychological and social advice for its members and to strengthen public information.
Every member can join one of the 60 regional groups, which are spread throughout Germany. At present (2019), PRO RETINA Deutschland e. V. counts more than 6,000 members. The Board, the counsellors, the leaders of the regional groups and all active members are working on a non-profit basis, but they are supported by a fulltime working staff at our office which is located in Aachen (www.pro-retina.de).

WHAT WE DO IN RESEARCH

The jewel of all this work is the PRO RETINA-Foundation for Prevention of Blindness, which was founded in 1996.
From the early beginning we have created a stable network with researchers and ophthalmologists for joined information and advice. We support research projects with direct financial funding – since the "Foundation for Prevention of Blindness" was established in 1996, more than two million Euro have been donated. We actively initiate research projects and therapy tests and contribute to their implementation.

Every year, we award two research prices and organize and support national and international seminars and conferences on relevant topics. We are financing PhD grants in order to foster research activities and networking between researchers.

We are consulted by a Scientific and Medical Advisory Board ("Wissenschaftlicher und Medizinischer Beirat", WMB) and a Working Group on Clinical Questions ("Arbeitskreis Klinische Fragen", AKF). In this Working Group scientists of different medical and other relevant disciplines are taking part.

The main objective is to secure a long-term support for research activities, e. g. by granting financial means for the development of new research projects or by financing the initial phase of relevant projects.

It is envisaged to increase the capital of the foundation to a minimum of Euro 5,000,000, which are to result in a steady source of funding for the support of research, independent from changing income of donations.
We guarantee that the benefits of the Foundation will only be dedicated to the research of retinal diseases, with the wider objective to develop applicable therapies for the patients.

PROGRAMME

Friday, March 22, 2019

13:00 – 13:05 **Welcome remarks**
Franz Badura (Amberg)

13:05 – 14:30 SESSION 1 Selected poster presentations

Eight abstracts to be selected

14:30 – 15:00 **Keynote lecture**
Rando Allikmets (New York)
Selecting patients for clinical trials based on their specific mutations

15:00 – 15:45 Coffee break

15:45 – 17:25 SESSION 2 Molecular diagnostics and gene therapy in retinal dystrophy

Chair: Bernhard Weber

15:45 – 16:10 Susanne Roosing (Nijmwegen)
Diagnostic and research explorations of WES and WGS for retinal diseases

16:10 – 16:35 Andrea Milenkovic (Regensburg)
Distinct pathomechanisms of the retinal bestrophinopathies – distinct treatment approaches?

16:35 – 17:00 Susanne Kohl (Tübingen)
Achromatopsia CNGA3 clinical trial

17:00 – 17:25 Alex Garanto (Nijmegen)
Critical assessment of gene therapy

17:30 Dinner

19:00 – 20:00 EVENING LECTURE

19:00 – 20:00 Birgit Lorenz (Gießen)
RPE65 deficiency. Challenges in diagnosis and treatment

20:00 – open Swingin' poster session

PROGRAMME

Saturday, March 23, 2019

09:00 – 10:40	**SESSION 3**	**Retina chip und optogenetics**
		Chair: Klaus Rüther
	09:00 – 09:25	Katarina Stingl (Tübingen) *State of the art and expectations of retinal implants*
	09:25 – 09:50	Daniel Rathbun (Tübingen) *Form vision: How good can retina chips actually get*
	09:50 – 10:15	Jens Dübel (Paris) *Optogenetics meets cell replacement – a synergystic approach to repair retinal structure and function*
	10:15 – 10:40	Volker Bußkamp (Dresden) *The potential of optogenetic rhodopsin channels*
	10:40 – 11:15	Coffee break
11:15 – 12:55	**SESSION 4**	**Cell substitution in retinal dystrophy**
		Chair: Olaf Strauß
	11:15 – 11:40	Henry Klassen (Irvine) *Stem cell therapy for RP: Launching a phase IIb clinical trial*
	11:40 – 12:05	Marius Ader (Dresden) *Photoreceptor replacement therapy – pros and cons*
	12:05 – 12:30	Slaven Erceg (Valencia) *iPS cell-derived RPE in disease development and cell therapy. And what about photoreceptors?*
	12:30 – 12:55	Boris Stanzel (Sulzbach) *RPE cell therapeutics: How do you actually get them from the lab into a patient?*
	12:55 – 13:00	**Concluding remarks**
	13:00	Lunch and end of meeting

PRO RETINA
POTSDAM-MEETING

POSTER ABSTRACTS

Evaluation of CNGb1 expression during development and degeneration

Marlly Natalia Achury[1], Maria Pia Polito[2,3], Norman Rieger[2], Soumyaparna Das[1,2], Francois Paquet-Durand[2]

[1] Graduate Training Center of Neurosciences, Eberhard Karls Universität Tübingen
[2] Institute for Ophthalmic Research, Universitäts Klinikum Tübingen
[3] Universidad de Módena y Reggio Emilia, Modena, Italy

Purpose: Retinitis pigmentosa is a hereditary retinal dystrophy characterized by progressive photoreceptors degeneration. Photoreceptors express cyclic nucleotide gated channels (CNGC) in the membranes of outer segment (OS) which allow Na^+ and Ca^{2+} influx during depolarization in the phototransduction cascade. CNGCs over-activation is believed to increase Ca^{2+} influx followed by photoreceptor collapse. Accordingly, CNGCs have been suggested as a promising target to delay photoreceptor degeneration. The goal of this study is to evaluate the expression patterns of the CNGb1 subunit during postnatal development in wild-type (wt) and during degeneration in *rd1* retina.

Methods: In our study we used the *rd1* mouse model, which shows primary loss of rod photoreceptors with a peak of around postnatal day 11 (P11), progressing to almost a complete loss by P21. The results were compared with its corresponding wt. The changes in expression pattern of CNGb1 at the transcriptomic and proteomic level were assessed. We evaluated localization of expressed protein in the retina through immunohistochemical staining on histological preparation, and transcripts (mRNA) by performing RT-PCR from total RNA. For total RNA extraction, retinas from 3 different animals were homogenized together followed by a subsequent cDNA synthesis, which was used as template for the reaction. Both assays were performed on wt and *rd1* mice across different time points namely P9, P11, P13, P15, P18, P22 and P30 (n=2 per time point).

Results: The evaluation and quantification were done considering the spatiotemporal pattern of the retina. Our preliminary results suggest that during development in wt retina, the thickness of the OS, analyzed by CNGb1 immunostaining, increases across time points: about 3.6µm in P11, 14.7µm in P15 and 20.3µm in P30. The outer nuclear layer (ONL) thickness is increased as the photoreceptors mature from 55.9µm in P11 to 69.1µm in P15 and is decreased to 48.1µm in P30. This suggest that during development (P11-P15) the growth of the OS is around three times faster than that of ONL. In contrast, in *rd1* animals there is a decrease in the ONL thickness across time points: 47,2 µm in P11, 21.72µm in P13 and 7.5µm in P30. Additionally, there is also a disruption in the development and a decline in the OS thickness going from 2.7µm in P11, 1.85µm in P13 to 0µm in P30.

Conclusion & Outlook: CNGb1 mRNA and protein products change drastically during *rd1* degeneration. The change in the two expression products is correlated over time. Specifically, the degeneration in the OS and ONL starts around P11 and P13, respectively. These results could be a basis for future studies in retina degeneration. In the context of possible targets for delaying it, the same experiments could be performed in retinal organotypic explant cultures across the different time points to simulate CNGB1 expression *in vitro*. Additionally, the results presented here, could be complemented with protein and mRNA quantification through Western Blot and qPCR, respectively.

ERdj5 overexpression is protective against P23H rhodopsin

Mònica Aguilà, James Bellingham, Dimitra Athanasiou, Dalila Bevilacqua, Yanai Duran, Ryea Maswood, Robin R. Ali, and Michael E. Cheetham

UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK

Mutations in rhodopsin are the most common cause of autosomal dominant retinitis pigmentosa (RP). The majority of mutations, exemplified by P23H, which is the most common change in the USA, lead to protein misfolding, ER retention, degradation and aggregation. ERdj5 (DNAJC10) acts as a co-chaperone for the ER lumenal Hsp70, BiP(HSPA5), which has been shown to protect against P23H, and also catalyses the reduction of disulfide bonds in misfolded proteins. ERdj5 enhances the degradation of P23H rod opsin in transfected cells and following ERdj5 shRNA knockdown, WT and P23H rod opsin were retained in the ER and P23H rod opsin aggregation increased.

In this study, we investigated the role of ERdj5 in photoreceptor homeostasis *in vivo* by using an ERdj5 knock-out mouse crossed with the P23H knock-in (P23H-KI) mouse and by viral-mediated overexpression of ERdj5 in P23H transgenic rats. Electroretinogram (ERG) and optical coherence tomography (OCT) of WT/ERdj5 KO and P23H KI/ERdj5 KO mice showed no effect of ERdj5 loss on retinal function, or photoreceptor survival. Rhodopsin levels and localisation were similar to those of the non-ERdj5 KO animals. AAV8-ERdj5-HA was subretinally injected into the superior hemisphere of P23H-3 rat retinae at post-natal day 15 and their retinal function and survival was assessed at 6 and 12 weeks of age.

Analysis of the full field ERG showed that overexpression of ERdj5 slightly reduced the loss visual function, as both a- and b-wave amplitudes were preserved compared to the control (PBS-injected) eyes at 12 weeks. The preservation in ERG amplitudes correlated with significantly increased photoreceptor survival at 6 weeks. Assessment of the ONL thickness at 12 weeks showed preserved ONL thickness in the superior retina. Overall, these results suggest that manipulation of the ER quality control and ERAD factors could potentially be beneficial for RP caused by misfolding mutations in rhodopsin.

Inhibition of VCP structurally and functionally reconstitutes photoreceptor cells in Rho^{P23H} organotypic retina cultures

Blanca Arango-Gonzalez[1], Merve Sen[1], Tsui-Fen Chou[2], Ray Deshaies[3], Sylvia Bolz[1], Haq Wadood[1] and Marius Ueffing[1]

[1]Centre for Ophthalmology, University Eye Hospital Tübingen, Tübingen, Germany
[2]Department of Pediatrics, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, CA, United States
[3]Discovery Research, Amgen, Inc., CA, United States.

Purpose: Photoreceptor cells are especially vulnerable to defects in protein homeostasis. Protein homeostasis comprises the folding, assembly, and degradation pathways that maintain a balance of functional proteins within cells. Numerous human diseases, including RP, arise from defects in protein homeostasis, marking this process as critical for human health. When exploring the role of ERAD and UPR in retinal degeneration, we built experimental evidence that UPR related stress is controlled by VCP, an ATPase, which functions in conjunction with a large family of adaptors to comprise the 'VCP network' (Griciuc et al., 2011). Being VCP a key player in protein homeostasis, we built experimental evidence for neuroprotective effects of its inhibition in the *Rho^{P23H}* transgenic rats *in vitro*.

Methods: Retinal organ cultures prepared from heterozygous *Rho^{P23H}* transgenic rats as previously described (Arango-Gonzalez et al. 2010) were treated either with Eeyarestatin I (EerI) or with ML240 and then evaluated by photoreceptor cell rows quantification, TUNEL assay, immunohistochemistry and electron microscopy (EM). The impact of the treatment on the retinal visual responses was investigated by light stimulation and simultaneous ganglion cell recordings, utilizing multi-electrode arrays (MEA).

Results: The percentage of TUNEL positive cells in the ONL indicates that VCP inhibition significantly reduced the number of dying cells. More importantly, the ONL of EerI and ML240-treated *Rho^{P23H}* retinas contained more cell rows compared to the controls. The VCP inhibition almost completely restored distribution of rhodopsin immunostaining to that of the normal WT phenotype, which is disrupted in the *Rho^{P23H}* transgenic rat. Ultrastructural analysis by EM confirmed preservation of proper morphology within treated *Rho^{P23H}* rod outer segments and electrophysiological MEA recordings demonstrated improved responses to light stimulation in the treated *Rho^{P23H}* retina.

Conclusions: We found that VCP inhibition stem loss of photoreceptor neurons in *Rho^{P23H}* cultured rat retinas. Additionally, VCP inhibition restored the proper trafficking of rhodopsin into the outer segment of photoreceptors, which is disrupted in this animal model, and improved the outer segments structure in those retinas as well as the electrophysiological response in treated *Rho^{P23H}* retinas.

Biochemical studies of human GCAP2 and a variant (p.Gly157Arg) putatively involved in retinal dystrophies

Anna Avesani [1], Valerio Marino[1], Daniele Dell'Orco[1]

[1]Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Verona, Italy

The phototransduction cascade in rod and cone photoreceptor cells involves a series of biochemical events, in which cGMP plays a fundamental role as a second messenger to keep the cyclic-nucleotide-gated channels open, thereby allowing the influx of ions in the dark state. The capture of photons by rhodopsin triggers the hydrolysis of cGMP, leading to the closure of channels and the hyperpolarization of the photoreceptor membrane. Once photoreceptors have responded to light, they must restore their cGMP to the dark levels to recover their sensitivity to light. This is achieved by retinal guanylate cyclases (GCs), which are regulated by guanylate cyclase activating proteins (GCAPs). At low Ca^{2+} levels, GCAPs activate guanylate GC and stimulates the cGMP synthesis, thereby restoring cGMP levels and leading to re-opening of CNG channels. Consequently, a dysregulation of the Ca^{2+} and cGMP levels is implicated in retinal degeneration.

Retinitis pigmentosa (RP) is a relatively rare inherited neurodegenerative disorder of the retina caused by mutations in genes involved in the phototransduction cascade. Disease progression profile is characterized by rod degeneration followed by cone photoreceptor death. To date, up to 20 point mutations have been found in *GUCA1A*, encoding GCAP1, which have been associated with cone (COD) or cone-rod dystrophy (CORD). Only a single variant in *GUCA1B*, encoding GCAP2, (p. Gly157Arg) has been associated with retinal dystrophy, specifically autosomal dominant RP. The pathogenic role of this individual GCAP2 variant is somehow controversial. On the other hand, recent cell biology experiments with bovine GCAP2 showed that a mutation in the *GUCA1B* (equivalent to human mutation G157R) contributes to pathophysiology by preventing translocation of the protein to the outer segment. To understand the effects of this mutation on the human protein and the putative association with RP, we expressed and purified human recombinant GCAP2 and the G157R mutant and performed a structural and functional characterization *in vitro* of the proteins.

In this work, we report a detailed biochemical and biophysical characterization of the wild-type and G157R variant. This study represents the first biophysical characterization of the human GCAP2 and it might help understanding the molecular basis of disease associated with GCAP2 variants.

Co-inhibition of PGF and VEGF blocks their expression in mononuclear phagocytes and limits neovascularization and leakage in the murine retina

Carsten Balser[1], Anne Wolf[1], Marc Herb[2] and Thomas Langmann[1]

[1]Laboratory for Experimental Immunology of the Eye, Department of Ophthalmology, University of Cologne, Faculty of Medicine and University Hospital Cologne, 50931, Cologne, Germany.
[2]Institute for Medical Microbiology, Immunology and Hygiene, 50931, Cologne, Germany

Background: Age-related macular degeneration (AMD) is a leading cause of visual impairment in the elderly. The neovascular (wet) form of AMD can be treated with intravitreal injections of different anti-vascular endothelial growth factor (VEGF) agents. Placental growth factor (PGF) is another member of the VEGF family of cytokines with pro-angiogenic and pro-inflammatory effects. Here, we aimed to compare single and combined inhibition of VEGF-A and PGF in the laser-induced mouse model of choroidal neovascularization (CNV) with a focus on the effects on retinal mononuclear phagocytes.

Methods: CNV was induced in C57BL/6J mice using a YAG-Laser. Immediately after laser damage antibodies against VEGF-A (aVEGF), anti-PGF (aPGF), aVEGF combined with aPGF, aflibercept, or IgG control were injected intravitreally in both eyes. Three and 7 days after laser damage, the vascular leakage was determined by fluorescence angiography. Lectin staining of retinal and RPE/choroidal flat mounts was used to monitor CNV. In situ mRNA co-expression of Iba1, VEGF and PGF were quantified using in situ hybridization. Retinal and RPE/choroidal protein levels of VEGF and PGF as well as the pro-inflammatory cytokines IL-6, IL1-beta, and TNF were determined by ELISA.

Results: Early (day 3) and intermediate (day 7) vascular leakage and CNV were significantly inhibited by PGF and VEGF-A co-inhibition, most effectively with the trap molecule aflibercept. While VEGF-A blockage alone had no effects, trapping PGF especially with aflibercept prevented the accumulation of reactive microglia and macrophages in laser lesions. The lesion-related mRNA expression and secretion of VEGF-A and PGF by mononuclear phagocytes were potently suppressed by PGF and partially by VEGF-A inhibition. Protein levels of IL-6 and IL1-beta were strongly reduced in all treatment groups.

Conclusions: Retinal inhibition of PGF in combination with VEGF-A prevents vascular leakage and CNV possibly via modulating their own expression in mononuclear phagocytes. PGF-related, optimized strategies to target inflammation-mediated angiogenesis may help to increase efficacy and reduce non-responders in the treatment of wet AMD patients.

Role of Interferon-beta (IFN-ß) and its receptor in retinal degeneration.

Verena Behnke, Thomas Langmann

Department of Ophthalmology, Laboratory of Experimental Immunology of the Eye, University of Cologne, Cologne, Germany

Purpose: Age-related macular degeneration (AMD) is one of the leading causes of blindness among the elderly within developed countries. Overly activation of microglia is associated with retinal degeneration, which makes them a therapeutic target for AMD. Interferon-beta (IFN-β) is a potent immune regulator. Previous work of our group showed a therapeutic effect of IFN-ß administration in a laser model of wet AMD. Here we hypothesized, that modulation of microglia via IFN-β may also dampen mononuclear phagocyte reactivity and thus protect from retinal degeneration in a light-damage mouse model mimicking some features of dry AMD.

Methods: BALB/cJ mice received intraperitoneal injections of 10,000 U IFN-β or vehicle (PBS) every other day, starting at the day of exposure to 15,000 lux white light for 1 h. Spectral domain optical coherence tomography (SD-OCT) was performed to quantify retinal thickness and thereby retinal degeneration. The effect of IFN-β treatment on microglia reactivity was analyzed by immunohistochemically staining of retinal sections or flat mounts and gene expression analysis.

Results: Iba-1 staining of retinal flat mounts and sections of BALB/cJ mice showed infiltration of amoeboid-shaped microglia in in the subretinal space four days post light damage. IFN-ß treated animals displayed a significantly lower number of microglia in this area. However, mRNA expression of complement factors *C3* and *C1qa* as well as pro-inflammatory marker such as *TSPO* were slightly elevated after IFN-ß application compared to vehicle treatment. SD-OCT analysis showed no rescue of retinal degeneration by IFN-ß therapy.

Conclusion: The injection of IFN-β enhanced IFNAR-signalling in the retina and reduced the number of reactivated microglia. However, there was neither rescue of retinal thickness nor decreased expression of complement factors detectable. The underlying mechanisms of these findings are still unclear and need further analysis.

Towards the development of new biomarkers for retinal neurodegeneration

Soumaya Belhadj[1,2], Frank Schwede[3], Norman Rieger[1], François Paquet-Durand[1]

[1]Cell Death Mechanism Laboratory, University of Tübingen, Tübingen, Germany
[2]Graduate Training Center of Neuroscience, University of Tübingen, Germany
[3]Biolog Life Science Institute, Bremen, Germany

Background: Inherited retinal degeneration (RD), such as retinitis pigmentosa or Leber's congenital amaurosis, is a group of diseases characterized by progressive photoreceptor death leading to blindness. These diseases are still untreatable. A key problem for therapy development is the lack of *in vivo* biomarkers which could aid diagnosis, monitor disease progression and detect the patient's response to treatment. The aim of the present study is to promote the development of novel biomarkers that can be used both for an early diagnosis of RD and for the rapid assessment of the *in vivo* efficacy of neuroprotective treatments.

Methods: Novel molecular probes for the detection of cell death related processes were tested initially on unfixed retinal tissue sections (*ex vivo*) and afterwards on live organotypic retinal explant cultures (*in vitro*). The probe development focused on the detection of the activity of poly-ADP-ribose-polymerase (PARP) using a variety of different NAD^+ analogues (6-Biotin-17-NAD^+, 6-Fluo-10-NAD^+, ε-NAD^+). Retinal sections were incubated in a PARP reaction mixture with/wo the NAD^+ analogues and PARP-activity-dependent fluorescence increases were visualized under the deconvolution microscope.

Results: The NAD^+ analogues 6-Biotin-17-NAD^+ and 6-Fluo-10-NAD^+ revealed large numbers of PARP activity positive cells in retinal sections. In sections derived from wild-type animals the number of PARP activity positive cells was relatively low, while in *rd1* sections many positive cells were detected in the photoreceptor layer. This result suggested 6-Fluo-10-NAD^+ as a candidate probe for the assessment of cell death *in vivo*. However, when tested on live organotypic retinal explant cultures no PARP activity could be detected with 6-Fluo-10-NAD^+.

Conclusion and Outlook: The compound 6-Fluo-10-NAD^+ may be used as a new molecular probe to assess PARP activity in *ex vivo* retinal tissue sections. However, this probe does not appear to be suitable for *in vivo* testing since it may not be able to penetrate the cell membrane. Hence, a further development towards an *in vivo* probe may require the use of an appropriate drug delivery system to enable the NAD^+ analogue to reach into photoreceptor cells.

Development of patient-derived retinal organoids as a tool to model Retinitis Pigmentosa

P. Berber[1], A. Milenkovic[1], B. Weber[1]

[1]Institute of Human Genetics, University of Regensburg, Regensburg, Germany

Purpose: Recently, human induced pluripotent stem cells (hiPSC) have been used to generate 3D retinal organoids, which emulate mature retinal tissue in terms of retina-like structure and organ-specific cell type. The ability to grow retinal tissue in vitro offers exciting new pathways of scientific discovery, including the modeling of retinal dystrophies such as Retinitis Pigmentosa (RP, OMIM #268000). Individuals affected by RP experience a worsening of vision in dark environments, which is later accompanied by a constriction of the visual field. The histopathologic correlate to the observed visual deterioration is the progressive loss of light-sensitive rod and cone photoreceptors. Here, our study aims to differentiate retinal organoids from healthy controls and RP patients harboring autosomal dominant mutations in the retinitis pigmentosa 1 (*RP1*) gene, which is required for the correct stacking of photoreceptor outer segment discs. We aim to treat this pathology in vitro by utilizing CRISPR/Cas9 genome editing technology.

Methods: Two previously published methods of retinal organoid development will be tested in this study. Firstly, a protocol developed by Wahlin et al., which utilizes B27 supplement to initiate neural retinal cell fate, and secondly a similar protocol by Zhong et al. which uses N2 supplement. Both protocols generate retinal cups which are then excised for further maturation, and generate mature photoreceptors after a minimum of 150 days.

Results: Adult human dermal fibroblast were obtained from skin biopsies of five RP patients with pathologic *RP1* mutations and reprogrammed into hiPSCs. Sanger sequencing of the genomic locus spanning 6 kb of the *RP1* locus revealed at least one single-nucleotide polymorphism (SNP) in cis with the mutation in each patient. Single guide RNA sequences (sgRNA) targeting these SNPs were selected using the "Benchling CRISPR Design Tool". Editing efficiency and specificity of the sgRNAs are currently being tested in HEK293 cells using an established fluorescence-based assay.

Conclusion: We aim to grow retinal organoids from five RP and WT hiPS cell lines, to establish an in vitro RP phenotype. We will then attempt to ameliorate this pathology by treating the patient hiPSCs utilizing CRISPR/Cas9 genome editing technology to selectively ablate the mutant transcript, while leaving the wildtype transcript intact. Instead of targeting the *RP1* mutations directly, we will attempt a haplotype-specific approach which targets common SNPs in cis with the mutation.

MAP kinase regulators and age-related macular degeneration (AMD) – Investigating the effect of AMD-associated polymorphism rs704 on vitronectin function and disease-related cellular processes

Fabiola Biasella, Claudia Karl, Felix Grassmann, Bernhard H. F. Weber, Ulrike Friedrich

Institute of Human Genetics, University of Regensburg, Germany

Background: Age-related macular degeneration (AMD) is a degenerative disease of the retina influenced by genetic background, age, gender, and other individual/environmental risk factors. The molecular pathology of AMD is not fully understood, although a defective complement regulation appears to play a crucial role. Besides, there is increasing evidence for a contribution of aberrant MAP Kinase signaling in AMD pathogenesis (e.g. Dridi et al., 2011, Tao et al., 2016). The latest genome-wide genetic association study by Fritsche et al. (2016) identified AMD-associated polymorphisms in MAP kinase signaling associated genes, such as the genes encoding for vitronectin (*VTN*), BRCA1-associated protein (*BRAP*), or neurturin (*NRTN*). Here, we report on our work elucidating the influence of rs704 in exon 7 of the *VTN* gene on protein function and retinal homeostasis.

Methods: HEK293 cells were transfected with expression vectors for risk (VTN_rs704:T) and non-risk (VTN_rs704:C) variants of VTN. Differences in expression, secretion, and binding to retinal (ARPE-19, Y79, Weri-RB1, BV2) and non retinal (HUVEC, HEK293) cell lines were assessed in Western Blot analysis. The effect of VTN risk and non-risk on H_2O_2-stimulated autophagy and MAP kinase signaling was investigated *via* Western Blot analyses in ARPE-19 cells following LC3 conversion and ERK1/2 phosphorylation, respectively. An effect on phagocytosis was addressed following the uptake of fluorescently labelled bioparticles by ARPE-19 cells.

Results: Genetic variant rs704 affects the endogenous proteolytic cleavage at amino acid position 398 of the protein. Protein expression of the VTN risk variant is increased about 2-fold compared to the non-risk variant. VTN binds to ARPE-19, Y79, Weri-RB1, and HUVEC, but not to BV2 and HEK293 cells, in line with the expression profile of the VTN receptor $\alpha_v\beta_5$ integrin in these cell lines. Quantification revealed significantly decreased binding of VTN risk compared to VTN non-risk protein isoforms. After H_2O_2 stress, VTN slightly decreased autophagy induction compared to control treatment, while an effect of VTN on ERK1/2 phosphorylation was not observed. Preliminary tests indicate a stimulating effect of VTN on phagocytosis.

Conclusion: The AMD-associated variant rs704 in *VTN* exerts striking effects on protein expression, processing and binding. First functional assays suggest an involvement of VTN in cellular processes like autophagy and phagocytosis. Further studies are under way to assess the consequence of rs704 on protein function and an involvement in AMD pathogenesis.

Proposing the targeting of Müller cells for complement modulating gene addition therapy in a mouse model for Stargardt disease type 1

[1]Josef Biber, [2]Yassin Jabri, [3]Dwight Stambolian, [2]Diana Pauly, [1]Antje Grosche

[1] Department of Physiological Genomics, Biomedical Center, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany
[2] Experimental Ophthalmology, University Hospital Regensburg, Regensburg, Germany
[3] Department of Ophthalmology, Perelman School of Medicine, University of Pennsylvania, Philadelphia

Purpose: A common feature of Stargardt type 1 (STGD1) and other diseases with underlying macular degeneration is neuro-destructive para-inflammation. In STGD1, a mutation in the ATP binding cassette subfamily A member 4 (ABCA4) causes an accumulation of lipofuscin which leads to an exaggerated reaction of the complement system (CS). While an intact blood-retina barrier ensures that no circulating complement components enter retinal tissue, it still needs unequivocal proof that complement proteins are expressed by retinal cells. Our aim is to adjust the derailed closed circuit CS with long-term efficient gene addition therapy. To decide which complement factor holds potential to be targeted at which time point of disease progression, we first needed to in-depth characterize our ABCA4$^{-/-}$ mouse model in terms of changes in retinal complement expression and progression of disease in the course of aging.

Methods: We established the expression patterns of complement factors in wild type and ABCA4$^{-/-}$ mice through RNAseq and qPCR. Currently, we are developing protocols for immunolabeling to localize the secreted complement factors in healthy, ABCA4$^{-/-}$ and postischemic retinae. The latter serves as positive control, as we could demonstrate massive intraretinal upregulation of complement activity. Analysis of RPE autofluorescence, neuronal cell loss and glial reactivity was performed on eye cup as well as retinal slice and flatmount preparations to characterize degenerative processes in the ABCA4$^{-/-}$ eye from animals 8, 16, 24, 32 – 40 weeks of age.

Results: We show that the main inhibitory complement regulator, complement factor H (CFH), was mainly expressed in retinal pigment epithelium, microglia and vascular cells, while the only known positive regulator, properdin (CFP), was primarily detected in Müller cells, microglia and neurons. Morphometric analysis revealed higher RPE autofluorescence levels in ABCA4$^{-/-}$ than in age-matched controls, as well as enhanced microglia activation beginning in mice 24 weeks of age. No major neuronal degeneration was observed in any of the mouse strains at the ages investigated.

Conclusion: In collaboration with the team of Diana Pauly, we are on our way to develop a gene therapeutic approach for STGD1 that modulates the overshooting CS. We established the background knowledge needed to decide on which CS modulatory protein holds potential to dampen the retinal complement response if overexpressed in Müller cells. Ultimately, the advantage of this approach will be its independence of the underlying genotype and that it can be applied to STGD1 or other inherited retinal degeneration with complicated or unknown genetics.

Neuroinflammation is required as a regenerative cue of the adult zebrafish retina

Oliver Bludau, Anke Weber, Veronika Kuscha, Stefan Hans, Viktoria Bosak, Kristin Dietrich & Michael Brand

DFG-Center for Regenerative Therapies Dresden, Cluster of Excellence, Center for Molecular and Cellular Bioengineering (CMCB), Technische Universität Dresden, Dresden, Germany

Purpose: Chronic inflammatory retinal diseases often cause neuronal loss in mammalian vertebrates. These diseases are marked by leakage of blood vessels, leukocyte infiltration and gliosis leading to persistent and so far irreversible loss of retinal neurons and photoreceptors. In contrast to mammals, in zebrafish neuronal inflammation is crucially important and sufficient to stimulate a regenerative response after traumatic brain injury. Here we tested the hypothesis that the immune system positively supports adult retina regeneration in zebrafish.

Methods: Leukocyte and Müller glia reactivity in response to sterile photoreceptor ablation were monitored using immunohistochemistry and transgenic reporter lines. Further, to investigate the role of the immune system during retina regeneration, we performed immunosuppression studies. Immunosuppressed fish were analysed for the numbers of leucocytes, proliferating Müller glia, activation of NFκB pathway in Müller glia, overall number of proliferating cells as well as the amount of regenerated cells after lesion. To elucidate whether stimulation of the immune system in the absence of a lesion is sufficient to induce Müller glia reactivity, we performed intravitreal injections of different immune stimulating factors. These retinae were analysed for proliferation and expression of regeneration markers. Finally, we addressed the regenerative response in *irf8* mutant, a model for genetic reduction of microglia, to retinal lesion.

Results: We observe a strong activation of the immune system after light lesion such as significant accumulation of leukocytes at the lesion site, morphological changes of microglia and the recruitment of neutrophils. Immunosuppression results in an impairment of the immune cells, significant reduction of cycling Müller glia, a strong decrease of overall proliferation as well as a striking lower number of regenerated cells including UV-cones. Conversely, stimulation of the immune system with flagellin, zymosan A or M-CSF in the absence of lesion resulted in a robust proliferation response and the up-regulation of regenerative marker genes. Moreover, *irf8* mutants show failures in the accumulation of leukocytes and highlight an impaired proliferative response.

Conclusion: We conclude that neuronal inflammation occurs in the zebrafish retina following a sterile lesion. Acute inflammation regulates Müller glia reactivity and positively influences the regenerative outcome. Microglia enhance the formation of proliferative clusters highlighting the beneficial effects of the immune system to the regenerative capacity of the adult zebrafish retina.

Dissecting the role of Lebercilin in cilia disassembly

Karsten Boldt, Md Moniruzzaman, Tobias Leonhard, Wadood Haq, Sylvia Bolz, Katrin Junger, Adem Cakar, Tina Beyer, Marius Ueffing

Eberhard-Karls Universität Tübingen

Purpose: Leber Congenital Amaurosis (LCA) is a severe and early onset blinding disease. It affects children in the first year of life and is the most common cause of blindness at this age. We have previously shown that mutations in Lebercilin cause LCA due to an impaired transport along cilia in photoreceptors which leads to rapid degeneration and thereby blindness. Yet, the molecular function of Lebercilin and especially the consequences of impaired IFT are unclear.

Methods: To gain further insights, CRISP/Cas9 was used to generate hTERT-RP1 knock-out cell lines to analyze the cellular phenotype of a Lebercilin knock-out. Further, an endogenous FLAG tag was introduced in HEK293 cells and FLAG-affinity purification as well as proximity labelling in combination with quantitative mass spectrometry were applied to characterize transient interactions of Lebercilin to understand the phenotype.

Results: The knock-out of Lebercilin in hTERT-RP1 did not result in a ciliogenesis defect but in a strong cilia disassembly phenotype. After induction of cilia disassembly, the cilia number remained high and cilia length was not reduced. To further dissect this mechanism, we used protein complex analysis and, in addition to previously described Lebercilin interactors, several central components of the ciliary disassembly machinery were identified. A knock-out of Lebercilin resulted in altered binding patterns of these proteins, suggesting that Lebercilin has a function in regulating these proteins.

Conclusion: We here demonstrate that Lebercilin is involved in cilia disassembly. This is in accordance with previous experiments where we could detect normal formation of cilia in Lebercilin knock-out animals. How this defect can be connected to the isolated retinal phenotype and if photoreceptors are the only cell type affected remains to be elucidated.

Vitreo-retinal interface abnormalities in an older European population: Results from the AugUR study

Caroline Brandl[1,2,3], Felicitas Borgmann[1], Martina E. Zimmermann[1], Horst Helbig[2], Bernhard H. F. Weber[3], Iris M. Heid[1], Klaus J. Stark[1]*

[1]Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany
[2]Department of Ophthalmology, University Hospital Regensburg, Regensburg, Germany
[3]Institute of Human Genetics, University of Regensburg, Regensburg, Germany

Purpose: Spectral domain-optical coherence tomography (SD-OCT) enables unprecedented assessment of the vitreo-retinal interface (VRI), but SD-OCT based data on VRI abnormalities from epidemiological studies is scarce. Here, we determined frequencies of vitreo-macular adhesion/traction (VMA/VMT), partial/full thickness macular holes (PTMH/FTMH), and epiretinal membranes (ERM) via SD-OCT in older individuals from a population-based study and investigated associations with presence of metamorphopsia as an example of visual function impairment in this cross-sectional data.

Methods: Macular cubes with 49 Raster lines, 20 x 20 °, were acquired via the Spectralis SD-OCT for at least one eye in 508 AugUR participants aged 70-94 years. Presence of VRI abnormalities was manually assessed per eye. Metamorphopsia were assessed per eye via standard Amsler grid applying the participant´s own refractive correction if applicable. Association of ERM on metamorphopsia was tested via logistic regression in one random eye per person, adjusting for age, sex, early and late age-related macular degeneration (assessed on color fundus images).

Results: SD-OCT scans were gradable for VRI abnormalities in 500 right and 498 left eyes from 508 participants (mean age 77.7 ± 5.3 years, 51.2 % women). A total of 221 right eyes (44.2 %) and 231 left eyes (46.4 %) revealed no VRI abnormalities.
VMA were detected in 25 right and 32 left eyes (5.0 % and 6.4 %, respectively). VMT or macular holes were only found in 5 right and 6 left eyes (1 % each).
Approx. half of all eyes showed features of ERM (253 right [50.6 %], 229 left eyes [46.0 %]). However, only a relatively small number of eyes demonstrated major changes of foveal structure due to ERM (i.e. loss of foveal depression: 42 right and 43 left eyes [8.5 % each]).
Association in random eye of ERM on metamorphopsia was not statistically significant when foveal depression was preserved (P = 0.46). However, loss of foveal depression was significantly associated with metamorphopsia ($P = 6.00*10^{-3}$; OR = 2.5 [95 %CI = 1.3-4.9]).

Conclusion: We here provide the first epidemiological data on VRI abnormalities in a European population aged 70+ applying SD-OCT. VMT or macular holes were rare, but features of ERM were present in 50 % of eyes. Visual function impairment due to ERM in the form of metamorphopsia was significantly associated with loss of the foveal structure.

Point mutations in Ca^{2+}-coordination sites of GCAP1 associated with retinal dystrophies deeply affect second messenger homeostasis in photoreceptors

Giuditta Dal Cortivo, Valerio Marino, Daniele Dell'Orco

University of Verona, Department of Neuroscience Biomedicine and Movement Sciences

Purpose: Guanylate Cyclase Activating Protein 1 (GCAP1) is a Ca^{2+}-sensor protein involved in the regulation of Guanylate Cyclase (GC) during phototransduction, the signaling cascade which initiates the visual process. To date, 20 point mutations in GUCA1A, the gene encoding for GCAP1, are known to be associated with Cone and Cone/Rod Dystrophies (COD and CORD), autosomic dominant diseases characterized by progressive central and/or peripheral vision loss. Here we present a thorough biochemical and functional characterization of four mutations directly involved in Ca^{2+} coordination, namely p.Asp100Gly, p.GluE111Val and p.Glu155Ala/Gly.

Methods: After the expression and purification of recombinant human wild-type (WT) and mutant GCAP1 in *E. coli* cells, a combination of techniques was used to characterize the structural and functional effects of such mutations. Structural features were investigated by circular dichroism (CD) while the aggregation propensity was analyzed by time-resolved dynamic light scattering (DLS). Ca^{2+} binding properties were evaluated by combining the mobility shift on SDS-PAGE (gel-shift) and the Ca^{2+}-binding competition between GCAP1 and Br_2-BAPTA chromophoric chelator. Finally, enzymatic parameters for GC activity at different Ca^{2+} concentrations were measured by functional assays.

Results: All the mutants analyzed in this study are correctly folded and change conformation upon addition of ions. The variants do not form large aggregates over time, exception made for E111V in the presence of Mg^{2+}. Both gel-shift and Ca^{2+}-binding assays highlight a reduced affinity for Ca^{2+} for all variants, similarly to most of the previously studied COD/CORD mutants. Functional analyses show a non-physiological Ca^{2+}-dependent regulation of the GC activity. Finally, the presence of increasing concentrations of WT GCAP1 partially mitigates the effects of the mutations on the Ca^{2+}-dependent regulation of the target GC.

Conclusions: COD/CORD-associated point mutations in GCAP1 affecting residues directly involved on Ca^{2+}-coordination are less sensitive to physiological Ca^{2+} fluctuations, leading to severe dysregulation of the GC activity. However, preliminary biochemical results suggest that high concentration of WT GCAP1 may mitigate the dominant effect of the COD/CORD mutants, thus opening new scenarios for protein-therapies.

Ca^{2+}-channel blockers: Effect on photoreceptor Ca^{2+}-levels and calpain activity

Soumyaparna Das[1,3], Michael Power[1-3], Luke Rogerson[2,3], Thomas Euler[2], Francois Paquet-Durand[1]

[1]Institute for Ophthalmic Research, University of Tübingen, Germany
[2]Werner Reichardt Centre for Integrative Neuroscience (CIN), University of Tübingen, Germany
[3]Graduate Training Centre of Neuroscience, University of Tübingen, Germany

Retinitis pigmentosa (RP) is a hereditary retinopathy, which leads to primary degeneration of rods and a secondary degeneration of cones. RP mutations often result in accumulation of cGMP in the photoreceptor outer segments and over-activate cyclic nucleotide gated channels (CNGC), leading to rising levels of intracellular Ca^{2+}. High Ca^{2+} concentrations are believed to induce photoreceptor degeneration by activating Ca^{2+}-dependent calpain-type proteases. Voltage-gated-Ca^{2+}-channels (VGCC) are another major source of Ca^{2+} in photoreceptors. Studies reported improved rod viability and cone function after CNGC knockout, whereas VGCC knockout showed no improvement.

Here, we study the effects of specific inhibitors for CNGC and VGCC in photoreceptor to explore their role in degeneration. L-cis-diltiazem and D-cis-diltiazem (100 µM), known to selectively interact with and inhibit CNGC and VGCC, respectively, were used on organotypic retinal explant cultures derived from *rd1* and wild-type (wt) animals. Their effects on cell death was analysed using calpain activity and cell death (TUNEL) assays. Furthermore, the effects of the compounds on Ca^{2+} dynamics in cone photoreceptors were tested using two-photon imaging.

Our initial results suggest around 330 % higher numbers of calpain activity positive cells per 1000 µm^2 in the *rd1* ONL (1.18 ± 0.08, n = 4), as compared to wt (0.36 ± 0.03, n = 4). On treatment with L-cis-diltiazem, calpain activity positive cell count decreased by 30% in *rd1* ONL (0.91 ± 0.07, n = 3) and D-cis-diltiazem treatment decreased calpain activity positive cells in *rd1* by 34% (0.88 ± 0.07, n = 2). Interestingly, D-cis- diltiazem increased the calpain positive cell count in wt ONL by 45 % (0.66 ± 0.06, n = 2). Ca^{2+}-imaging showed a reduction in Ca^{2+} baseline for both D-cis-diltiazem and L-cis-diltiazem. However, D-cis-diltiazem did not affect the light induced Ca^{2+} response in cones, while L-cis-diltiazem suppressed this response.

Our preliminary data suggests that pharmacological block of both VGCC and CNGC can decrease photoreceptor Ca^{2+} levels and the activity of calpains. The differential effect of L-cis- and D-cis diltiazem on light-induced responses indicates that only CNGC inhibition can reduce Ca^{2+}-levels in photoreceptor outer segments.

Developing a scalable drug screening pipeline for Bestrophin-1 (BEST1)

Lisa M. J. Duerr, Andrea Milenkovic, Bernhard H. F. Weber

Institute of Human Genetics, Regensburg, Germany

Purpose: Human BEST1 is a chloride channel controlled by Ca^{2+} and cell volume and is localized to the basolateral membrane of the retinal pigment epithelium (RPE) in the posterior eye. So far, there is no therapy for BEST1-associated disease of which the most common is Best vitelliform macular dystrophy, also known as Best disease (BD). Our current efforts are directed towards developing drug screening pipelines which target both, BEST1 localization correction (correctors) and BEST1 channel function enhancement (potentiators).

Methods: We established a cellular model, stably expressing wildtype BEST1 or BD-associated BEST1 mutations in MDCKII cell lines. To screen for BEST1 correctors, we designed a BEST1 localization assay based on immunocytochemistry and converted it to a 96 well plate format applying robotic process automation. The microscopy readout and statistical analysis were performed automated by a high content imaging system (Operetta®). To identify potentiator molecules, we developed the halide assay by using the BEST1-expressing MDCKII cell model in the presence of yellow fluorescent protein (YFP)-based halide sensor. Cells were stimulated by extracellular addition of iodide known to pass the plasma membrane through anion channels consequently quenching intracellular YFP fluorescence. Variations in YFP fluorescence levels as a marker for BEST1 function were recorded in 96 well plates by a plate reader setup. A small-scale 2,560 compound library, commercially available as Spectrum Collection (MicroSource Discovery Systems, Gaylordsville, USA) was used for initial screening performances.

Results: Consistent with endogenous BEST1 in human RPE, wildtype BEST1 shows authentic localization at the plasma membrane in the MDCKII cell model, while several BEST1 mutant forms are grossly mislocalized to the cytoplasm. Based on a defined algorithm, wildtype-like plasma membrane and mutant-like intracellular BEST1 localization can reliably be distinguished in a cell monolayer by automated microscopy analysis. The halide assay revealed reproducible halide permeability across wells and, as a control, reliably detected MDCKII cells overexpressing wild type BEST1 by a decrease of YFP fluorescence to 70 % following a 60 second stimulation. Under these conditions, cells expressing mutant BEST1 showed 85 % of default YFP fluorescence. Five compounds were detected enhancing halide permeability without a negative effect on cell layer integrity or default YFP fluorescence intensity. Two of these revealed an enhancing effect on BEST1 channel conductance across several BEST1 mutations.

Conclusion: The current study established two assays well suited for high-scale compound screening for BEST1 correctors or potentiators. These pipelines provide the basis for screening large chemical compound libraries in search for future therapies for BEST1-associated retinal diseases.

Multiomics approaches to uncover the effects of changing zinc homeostasis in the eye

Eszter Emri[1], Sascha Dammeier[2], Franziska Klose[2], Lajos Csincsik[1], David Simpson[1], Jose Sousa[3], Marius Ueffing[2], Eye-Risk Consortium and Imre Lengyel[1]

[1]Centre for Experimental Medicine, Queens University, Belfast, Northern Ireland, United Kingdom
[2]Institute for Ophthalmic Research, Universitaets klinikum Tuebingen, Tubingen, Germany
[3]Advanced Informatics, Faculty of Medicine, Health and Life Sciences, Queens University, Belfast, Northern Ireland, United Kingdom

Cultured retinal pigment epithelial (RPE) cells are widely used to model of age-related macular degeneration (AMD). In AMD, cellular zinc levels decline and it had been shown that zinc supplementation can attenuate the progression to late AMD. Our aim was to identify the regulatory networks affected by zinc supplementation in the RPE cells.

We cultured primary human foetal RPE cells from three individuals on transwell inserts and supplemented with medium containing 125 uM added zinc or left untreated for 4 weeks. Thereafter, apical and basal secretomes and the RPE cells were harvested for downstream analysis, including genotyping, transcriptomics, proteomics, lipidomics, immunolabeling and transmission electron-microscopy.

All cultures reflected in vivo phenotype of RPE. They were well differentiated, identified by cobblestone morphology, extensive pigmentation, high transepithelial resistance (TER), tight junctional (ZO-1) staining and accumulation of sub-RPE deposit on electronmicroscope. When cells were treated with zinc apically or basally, a significant increase in TER values were observed in all cases ($p < 0.05$). Using computational approaches to identify regulatory networks, data were analysed. Using machine-learning algorithms, we found that zinc supplementation led to increased entropy values matching the higher phenotypic differentiation of the cells upon zinc treatment. Ingenuity pathway analysis of significant differences (false discovery rate < 0.05) revealed several networks associated, including those regulated by ERK1/2 signalling.

These results indicate that long-term zinc supplementation may serve as an ex vivo model to eventually understand the beneficial effects of zinc supplementation in AMD patients.

This work was supported by COST TD 1304 'The Network for the Biology of Zinc' and EYE-RISK European Union's Horizon 2020 (Grant Agreement No 634479).

The role of Sphingosine-1-phosphate in neovascularization in the mouse model of oxygen-induced retinopathy

Jeanette Eresch[1], Martin Stumpf[1], Alexander Koch[1], Rajkumar Vutukuri[1], Nerea Ferreirós[2], Yannick Schreiber[2,3], Katrin Schröder[4,6], Kavi Devraj[1], Rüdiger Popp[5,6], Andrea Huwiler[7], Lars-Olof Hattenbach[8], Josef Pfeilschifter[1] and Waltraud Pfeilschifter[9]

[1]*Pharmazentrum Frankfurt, Institute of General Pharmacology and Toxicology, Goethe University Hospital, Frankfurt/Main, Germany*
[2]*Pharmazentrum Frankfurt, Institute of Clinical Pharmacology, Goethe University Hospital, Frankfurt/Main, Germany*
[3]*Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Project Group Translational Medicine and Pharmacology (TMP), Frankfurt, Germany.*
[4]*Institute for Cardiovascular Physiology, Goethe University, Frankfurt/Main, Germany*
[5] *Institute for Vascular Signaling, Goethe University, Frankfurt/Main, Germany*
[6]*German Center for Cardiovascular Research (DZHK), Partner site RheinMain, Frankfurt/Main, Germany*
[7]*Institute of Pharmacology, University of Bern, Bern, Switzerland*
[8]*Department of Ophthalmology, Ludwigshafen Hospital, Ludwigshafen, Germany*
[9]*Department of Neurology, Goethe University Hospital, Frankfurt/Main, Germany*

Purpose: Sphingosine 1-phosphate (S1P), a bioactive sphingolipid synthesized by two sphingosine kinases (Sphk1, Sphk2), is an important signalling molecule involved in the regulation of various cellular processes, such as cell survival, proliferation, differentiation, migration and inflammation. S1P emerged as a key regulator of angiogenesis and vessel stabilisation and as a decisive factor in retinal pathologies, contributing to retinal and choroidal neovascularization (NV). We investigated the role of Sphk2, S1P, and S1P receptors (S1PR) during retinal vascular development and NV using the oxygen-induced retinopathy mouse model (OIR).

Methods: Neonatal C57BL/6J (WT), tgSphk2 (humane Sphk2 expressing mice with C57BL/6J background, leading to a general overexpression of Sphk2) and Sphk2$^{-/-}$ were used in the OIR model. Neonates were subjected to 75 % O2 for 5 days (postnatal day (P) 7 to 12) and then returned to room air. Quantitative Real-time PCR, Lipid analysis by LC-MS/MS, immunohistochemistry on wholemount, and paraffin embedded retina slides were performed on P12, P14 and P17 old retinae. Retinal thickness, avascular areas and neovascularisation (NV) were analysed with Adobe Photoshop and ImageJ. One-way ANOVA was used for statistical analysis.

Results: Under normoxia retinal S1P concentrations were increased in tgSphk2 and Sphk2$^{-/-}$ mice compared to WT. Plasma S1P levels in tgSphk2 mice were similar to WT, while Sphk2$^{-/-}$ lead to an increase. Branching point analysis on P7 retinae revealed a prematurely dense vessel network in tgSphk2 compared to WT, whereas in Sphk2$^{-/-}$ mice the density was reduced. The ONL and INL thickness were reduced at P12 in both animal strains. At P17 only the Sphk2$^{-/-}$ INL showed signifi-

cant reduction. In OIR the avascular area at P12 was significantly reduced in tgSphk2, whereas it was similar to WT in Sphk2$^{-/-}$ mice. While tgSphk2 lead to an increase in NV, it was decreased in Sphk2$^{-/-}$mice. In both strains, the visual appearance of the vessel network differed from WT. MRNA expression studies showed lower VEGFα expression levels in tgSphk2 mice throughout the model, while in Sphk2$^{-/-}$ VEGFα levels were drastically reduced. S1PR4 was the most strikingly regulated receptor revealed in S1P receptors expression studies, with a 10fold higher expression in tgSphk2 compared to WT but did not change significantly upon OIR, while it remained very low in Sphk2$^{-/-}$.

Conclusions: We demonstrate that genetic modulation of Sphk2 leads to a profound alteration of retinal angiogenesis and vasculopathy. The strong interplay between Sphk2/S1P/S1PRs suggest that stage specific pharmacological intervention may offer therapeutic potential to treat retinal diseases involving ischemia and neovascularization.

Characterisation of PDE6D & RAB28 protein network and its connection with glucose metabolism

Siebren Faber[1], Sylvia van Beersum[1], Jeroen van Reeuwijk[1], Katrin Junger[2], Marius Ueffing[2], Karsten Boldt[2], Rob Collin[1], Ronald Roepman[1]

[1] Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands
[2] Division of Experimental Ophthalmology and Medical Proteome Center, Center of Ophthalmology, University of Tübingen, Germany

Purpose: The light sensing outer segment of photoreceptors (PRs) requires renewal every ten days due to its high photoactivity, especially of the cones during daytime vision. This demands a high turnover of biosynthetic compounds (membranes, proteins) as well as a lot of energy. Therefore, PRs are highly dependent on converting glucose into metabolites by aerobic glycolysis. Furthermore, a refined proteostasis network (PN) is crucial for PR viability. The PN and glucose metabolism are both regulated by several intimately intertwined pathways, but details of these pathways in PRs remain largely unknown. Recently, it has been shown that the GTPase RAB28 is involved in GLUT4 trafficking in muscle cells and adipose tissue. Interestingly, RAB28 is the first RAB associated with inherited retinal blindness (autosomal recessive cone-rod dystrophy, CORD18). Furthermore, it has been shown that PDE6D, a protein facilitating intracellular trafficking of prenylated proteins in PRs, interacts with RAB28. Here, we investigated the protein interactome of PDE6D and RAB28 to identify their association with glucose metabolism.

Methods: We used tandem affinity purification (TAP) of the proteins of interest, which were transiently expressed in HEK293T cells, to co-purify their associated interactomes. The co-precipitated proteins were subsequently analyzed by mass spectrometry (MS).

Results: Our preliminary results show the identification of all the known interactors of PDE6D, including ARL3, INPP5E, RPGR and RAB28. Interestingly, we also found a group of newly identified interactors of PDE6D that belong to the transducin family, which are essential for phototransduction. Moreover, several interactors of the Rab28 TAP are involved in energy/glucose metabolism. This connection to the energy/glucose metabolism is confirmed by our interaction screen of GLUT4 identifying several cilia-gold-standard proteins and proteins associated to ciliopathies, including RAB28.

Conclusion: Our results show a preliminary association between PR/cilia specific proteins and glucose metabolism. Unraveling PR/cilium specific networks that associate with glucose metabolism might reveal targets for broadly applicable treatments of inherited retinal diseases.

Transplantation of cone photoreceptors purified from retinal organoids generated with a cone-specific human reporter iPSC line

Sylvia J Gasparini, Madalena Carido, Karen Tessmer, Stephanie Wieneke, Anka Kempe, Oliver Borsch, Manuela Völkner, Volker Busskamp, Mike Karl, Marius Ader

Human vision is particularly reliant on cone photoreceptor dependent high acuity and colour vision. A strategy to restore vision in retinal degenerative diseases is the replacement of lost cells with stem cell-derived photoreceptors, however, efficient human cone-enrichment strategies are lacking. Here, we describe the enrichment and transplantation of purified cones through the use of a human iPSC cone reporter line.

Applying piggyBAC technology, hiPSCs were transduced to express GFP under the control of the mouse cone arrestin promoter and subsequently used to generate retinal organoids. Organoids were dissociated at 140 or 200 days of age and FACS sorted for GFP^+ cells. Sorted cells were transplanted into the subretinal space of wild-type mice and three mouse lines exhibiting aberrant retinas: CPFL1 (cone degeneration), CPFL1; $Rho^{-/-}$ (cone and rod degeneration) and the $Nrl^{-/-}$ line which displays a cone-like cell enriched retina. Mice were immune-suppressed with triamcinolone and eyes were analysed 3 or 9 weeks later.

GFP^+ cells comprised 8 % of all organoid cells at day 140 and 27 % by day 200. Enrichment of cones as well as the cone identity of GFP^+ cells were confirmed by immunostaining. This showed 90% of sorted cells co-express GFP, the pan-photoreceptor marker recoverin and the cone marker ARR3 (GFP negative fraction: < 4 % $ARR3^+$ cells). Grafted cells formed clusters in the subretinal space and expressed human cone markers. While GFP^+ cells were almost absent in the photoreceptor layer of wt, CPFL1, and CPFL1; $Rho^{-/-}$ hosts, in $Nrl^{-/-}$ mice some transplanted cones appeared to incorporate into the host retina. The transfer of cytoplasmic material between donor and host photoreceptors, as was recently observed in mouse-to-mouse transplantation, was not detected.

In summary, FACS of dissociated retinal organoids derived from a hiPSC cone-GFP line yielded a greatly enriched cone population suitable for transplantation. Human cones survived in the murine subretinal space but did not appear to integrate into the recipient tissue or engage in material transfer, with the exception of $Nrl^{-/-}$ hosts, where a subset of donor cones incorporated into the retina. The viability of organoid-derived cones and the specificity of the reporter line suggests the outlined strategy to be a promising approach for further translational studies.

Pharmacological manipulation of oscillatory activity in the retina of the retinitis pigmentosa mouse model rd10 improves efficiency of electrical stimulation

Jana Gehlen[1], Stefan Esser[1], Kim Schaffrath[2], Sandra Johnen[2], Peter Walter[2], Frank Müller[1]

[1]Institute of Complex Systems, Cellular Biophysics, ICS-4, Forschungszentrum Jülich, Germany
[2]Department of Ophthalmology, RWTH Aachen University, Germany

Purpose: To eliminate or suppress pathological electrical activity in the retina that occurs upon photoreceptor degeneration and that may compromise the efficiency of retinal ganglion cell (RGC) stimulation by an electrical prosthesis.

Methods: Electrophysiological recordings were obtained *in vitro* from wild type (WT) retinae and from *rd10* retinae using multi electrode arrays (MEA). Retinae were obtained from animals at the age of 3 – 4 months. Local field potentials (LFP) and spike activity of RGCs were recorded and RGCs were stimulated electrically. The effects of different agonists at inhibitory neurotransmitter receptors on oscillatory activity were investigated.

Results: As described earlier, in *rd10* retinae but not in WT retinae, we observed oscillations in the LFP at a frequency of around 3 – 6 Hz. Often oscillations were observed to wax and wane. Glycine and GABA strongly reduced or even abolished retinal oscillations in a reversible and reproducible way. Moreover, diazepam, flunitrazepam and lorazepam, all allosteric modulators of the benzodiazepine family acting at the $GABA_A$ receptors, also eliminated oscillations.
We found that the efficiency of electrical stimulation – measured as ratio of spike rate after and before the stimulation pulse – was lower in *rd10* retina than in WT retina. Most importantly, treatment of the *rd10* retina with GABA or the different types of benzodiazepines increased the efficiency of electrical stimulation to values similar to those observed in WT retina.

Conclusion: In *rd10* retina, pathological oscillatory activity seems to reduce the efficiency of electrical stimulation. Abolishing oscillations improved stimulation efficiency to values similar to WT retina. This study may open the way to a therapy that supports electrical stimulation by retinal prostheses with pharmacological treatment.

Acknowledgement: The study was supported by the DFG grants MU-3036/3-3, WA-1472/6-3, JO-1263/1-3 and Pro-Re/Projekt/Johnen-Diarra.1-2015.

The role of Müller cell glucocorticoid receptors in diabetic retinopathy

Farhad Ghaseminejad[1], Anna Pfaller[1], Stefanie Hauck[2], Antje Grosche[1]

[1]Department of Physiological Genomics, Martinsried-Germany
[2]Helmholtz-Zentrum München, Neuherberg-Germany

Purpose: Diabetic Retinopathy (DR) is one of the major complications associated with diabetes. Approximately a third of people with diabetes are diagnosed with diabetic retinopathy and a tenth develop vision threatening effects, making DR the leading cause of blindness among working-age adults. One of the main cell types within the retina associated with DR are Müller cells. Localized within the Müller glial cells, glucocorticoid receptors (GCR) are activated in the presence of glucocorticoids or homologous agonists, and are found to be involved in anti-inflammatory responses. GCR is encoded by the Nr3c1 gene which is a highly conserved gene amongst warm-blooded vertebrates and its expression within the retina is quite exclusive to the Müller cells.

Methods: Transgenic db/db mice were used in this study as animal models. Extensive proteomics analysis was performed to identify specific target genes in purified Müller glia of diabetic mice. Immunological stainings were used to compare the expression of GCR in retinal Müller cells of wild type, 12-weeks and 24-weeks old db/db mice. Additionally, western blots were used to evaluate and contrast the GCR protein expression and activation via phosphorylation of Ser220 in the presence and absence of cortisol in retinal organotypic cultures.

Results: The Nr3c1 transcript was significantly downregulated in Müller cells from diabetic mice compared to the wild type. The exclusive expression of the GCR within Müller cells was demonstrated using immunological stainings. In western blot evaluations, a trend of GCR downregulation was detected in 24-weeks old db/db mice compared to wild types. Lastly, evaluation of GCR activation after cortisol treatment of retinal explants via western blot indicated a significant increase in expression of phosphorylated-GCR compared to the inactive form. This increase in expression was found to be consistent over 24 and 48 hours in culture, suggesting that the receptor remains stable in long-term exposure to excess cortisol.

Conclusion: The significant downregulation of the GCR-coding Nr3c1 gene expression in db/db mice, the exclusive expression of this gene within the Müller glial cells of the retina and the anti-inflammatory role of glucocorticoids, all imply that GCR may be a promising target in studying DR pathology. Hence, the subsequent step would be to modulate the expression of GCR in diabetic mice and evaluate its potential ability in interfering with DR pathomechanisms.

Adult choroid endothelial cells secrete Indian hedgehog (Ihh) regulating choroidal homeostasis and immune response

Christin Hanke-Gogokhia[1], Guillermo Lehmann[1], Michael Ginsberg[2], Daniel Nolan[2], Yang Hu[3], Rohan Bareja[3], Alexandre Wojcinski[4], Olivier Elemento[3], Alexandra Joyner[4], Shahin Rafii[5], Ignacio Benedicto[1], Enrique Rodriguez-Boulan[1]

[1]Margaret Dyson Vision Research Institute, Weill Cornell Medicine, New York, NY, USA
[2]Angiocrine Bioscience, San Diego, Ca, USA
[3]Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY, USA
[4]Memorial Sloan Kettering Cancer Center, New York, NY, USA
[5]Department of Medicine, Regenerative Medicine, Weill Cornell Medicine, New York, NY, USA

Purpose: The cellular mechanisms of AMD or other choroidal disorders are poorly understood. We investigated the expression of Indian hedgehog (Ihh) in choroid endothelial cells (ECs) of adult mice and its function as an angio- and immunomodulatory signaling molecule likely relevant to AMD.

Methods: Wildtype RPE/choroid cells were single-cell sorted and scRNAseq was performed to characterize sub-cell types. Bulk RNAseq was used to compare the transcriptomes of purified RPE/choroid from wildtype and Ihh-deficient mice after injury. Tamoxifen-induced EC-specific Ihh knockout mice (*Ihh$^{flox/flox}$;Cdh5-Ert2Cre^{+}*) were generated to examine the function of Ihh. Choroidal neovascularization was induced using matrigel to evaluate the role of Ihh during AMD-like conditions. Photoreceptor function was measured by ERG and OKT and vascular damage was determined by angiography and OCT. Morphology of RPE/choroid and photoreceptors was evaluated by immunohistochemistry and histology.

Results: Single cell RNAseq analysis of adult mouse RPE/choroid tissue identified 13 main cell types including 3 subtypes of ECs. Corroborating with the ssRNAseq data, the transcriptomic analysis of tissue-specific ECs from other organs revealed a markedly increased Ihh expression in choroid ECs, particularly in the choriocapillaries located underneath the RPE. EC-specific *Ihh* KO mice displayed loss of mast cells, and an altered inflammatory and vasculatory response after injury. Additionally, knockout animals showed visual impairment and an altered morphology in RPE/choroid, indicating a novel Ihh-dependent signaling pathway important for choroidal homeostasis and retinal function in adult mice.

Conclusion: EC-derived Ihh plays an important role in choroidal neovascularization and immune response and is likely relevant to atrophic AMD and choroidal disorders.

Label-free detection of rod precursor cells using artificial intelligence

Maik Herbig[1], Tiago Santos-Ferreira[2], Karen Tessmer[2], Marius Ader[2], Jochen Guck[1]

[1]Biotechnology Center, Technische Universität Dresden, Dresden, Germany
[2]CRTD/Center for Regenerative Therapies Dresden, Technische Universität Dresden, Fetscherstrasse 105, 01307 Dresden, Germany

Photoreceptor precursor transplantation was shown to be a promising treatment strategy in animal models of retinal degeneration. Prior enrichment of labeled rod precursors improves transplantation success, yet it cannot be used to transplant human cells in a clinical setting. We thus aim at developing a technology for label free detection of rod precursors for subsequent cell sorting.

To achieve that we are utilizing data obtained with a microfluidic device which allows capturing images as well as a fluorescence signal of single cells at high-throughput (>1000 cells/s). Given the recognition of rods via their GFP reporter expression, we trained machine learning models to classify images either as rod precursors or other cell types based on the bright-field image alone. With deep convolutional neural nets (CNNs) we achieve a rod precursor classification accuracy of 82 %, which would allow their enrichment to 92 %. At the current technological state, the computation time of such complex CNNs requires around 3 ms (on an NVIDIA GTX1080), which would only allow slow analysis and cell-sorting (<150 rods/s). Screening for less complex, faster neural nets revealed two potent candidates. These have computation times around 100 µs on a CPU and an accuracy of 78 %, potentially allowing rod precursor enrichment to 85 % with a throughput of >1000 rods/s.

This shows that the information of the bright-field image is sufficient to distinguish rod precursors from other retina cells. Current consumer PC hardware is capable of performing the classification in real-time, which would allow image based cell sorting. Furthermore, the resulting photoreceptor purity upon image based sorting would be sufficient for transplantation.

Potential therapy of neurodegenerative retinopathies via activation of the BDNF-TrkB signaling pathway by a specific aptamer

José Hurst, M. Löscher, E. Lilou, K.-U. Bartz-Schmidt, S. Schnichels

Universitätsaugenklinik Tübingen, Elfriede-Aulhorn-Str.7, 72076 Tübingen

Purpose: Retinal degeneration processes, such as those associated with age-related macular degeneration (AMD) or retinitis pigmentosa (RP), result in the death of photoreceptors and can lead to blindness. Growth factor-based therapy is an experimentally often successfully studied approach to achieve a slowdown or arrest of the photoreceptor degeneration. A promising neurotrophic growth factor is the brain derived neurotrophic factor (BDNF), which exerts its action via the activation of the TrκB signaling cascade. By using an already published aptamer, which binds specifically to the TrκB receptor, we want to mimic the neuroprotective effect of BDNF and develop a new treatment option for retinal diseases.

Method: Safety of the TrκB aptamer was tested using primary retinal cells from dissociated porcine retinae. The expression of the specific retinal cell markers (TUBB3, GFAP, Opsin, Rhodopsin), markers for cellular stress (IL-1beta) as well as markers of the TrκB signaling cascade (HSP70, pERK, bFGF, GNDF etc.) was investigated in a retinal organ model treated with the aptamer and compared to treatment with the actual ligand BDNF. Residence time and binding efficiency were examined in cell culture and on porcine retinal explants by using a fluorescence labeled TrκB-aptamer. To prove the specificity of the aptamer a control aptamer was carried along. The neuroprotective effect of the aptamer was evaluated on a $CoCl_2$ based retinal degeneration model.

Results: No loss of cell viability or amount was observed 24 hours after administering the aptamer on the primary retinal cells. Binding of the aptamer was confirmed by fluorescence microscopy. In Western blot and real-time PCR analyses we were able to detect the activation of the TrkB signaling pathway after 24 h and after 96 h. Furthermore, an mRNA increase in cell-specific markers such as TUBB3 rhodopsin and opsin was observed. In the degeneration model, $CoCl_2$ induced cell loss could be counteracted by treatment with the aptamer.

Conclusion: The biocompatibility of the TrκB aptamer was confirmed. Our data prove the functionality of the aptamer on retinal cells and retinal explants, as the downstream targets were activated. TrκB receptor activation with an aptamer targets specifically the desired cells, is more stable than the actual ligand, and can be used in much lower concentrations. Furthermore, the neuroprotective effect of the aptamer was demonstrated in an ex-vivo damage model.

Age-dependent complement expression in a mouse model for Stargardt macular

Yassin Jabri[1], Josef Biber[2], Antje Grosche[2], Diana Pauly[1]

[1]University Hospital Regensburg, Department of Ophthalmology, Regensburg, Germany
[2]Department of Physiological Genomics, Biomedical Center, Ludwig-Maximilians-University Munich

Background: Mutations in *ABCA4* gene are associated with fluorescent lipofuscin deposition in the retinal pigment epithelium (RPE), progressive photoreceptor degeneration, oxidative stress and complement activation. The exact role of complement activation in retinal degenerative disease remains unclear. In this study, we investigated the transcription level and protein detection of selected complement components in the retina and RPE of *ABCA4-/-* mice, a model for Stargardt macular degeneration.

Methods: RPE autofluorescent, neuronal loss and glial activity in wild type and ABCA4-/- mice were previously reported by Grosche et al. from aging mice. Here we used five different retinal cell populations from albino wild type and *ABCA4-/-* mice model were used for cellular complement expression profile. Mice's were aged between 8 to 24 weeks old. The different retinal cell types were isolated by immunomagnetic cell separation and RPE cells were scratched from eyecups. Quantitative RT-PCR was performed to determine complement component expression levels. Complement protein levels were determined by Western blot in RPE cells as well as in the retinae. Systemic complement activation was analysed by ELISA.

Results: Overall, we found aging-associated changes in complement expression in the different retinal cell types, which were comparable in both wild type controls and *ABCA4-/-* mouse strains. Our data revealed that *ABCA4-/-* mice expressed increased transcript of *c3* in RPE cells and decreased transcripts of *cfi* in microglia cells compared to wild type controls. Besides that, we detected C3 and CFI protein levels in RPE cells and in the murine retinae. C3a and C5a concentrations in the mouse serum were determined.

Conclusion: The results of this study give a better insight of complement component expression in retinal cell populations and suggest a well-organised complement system regulation in which each type cell might have key function.

Does the retinal photoreceptor composition influence Müller cell heterogeneity?

Lew Kaplan[1], Peter Fuchs[2], Ursula Schlötzer-Schrehardt[3], Christian Grimm[4], Magdalena Götz[1], Stefanie Hauck[5], Antje Grosche[1]

[1]Department of Physiological Genomics, Ludwig-Maximilians-Universität München, Germany
[2]Department of Biochemistry and Cell Biology, Max F. Perutz Laboratories, University of Vienna, Austria
[3]Department of Ophthalmology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany
[4]Department of Ophthalmology, University of Zurich, Switzerland
[5]Helmholtz-Zentrum München, Neuherberg, Germany

Purpose: Being key for accurate vision, the human macula is exceptionally prone to neurodegenerative processes. We aim to elucidate whether a functional heterogeneity of Müller cells, the major macroglia of the retina, may explain part of this macular susceptibility. To clarify the genetic basis of this heterogeneity, we generated and analyzed proteomic data from cone- and rod-rich systems from human and mice.

Methods: Müller cells, microglia, vascular cells and retinal neurons from all cone R91W;Nrl$^{-/-}$ and R91W control mice as well as from human macular and peripheral samples were isolated by immunomagnetic separation and searched for differential protein expression by use of tandem mass spectrometry. Müller cell specificity of proteins of interest was additionally tested by evaluating their expression in retinal scRNAseq datasets (1, 2), while one particularly promising candidate was subjected to immunofluorescence staining followed by superresolution microscopy for validation.

Results: We found significant differences in protein expression between predominantlycone- and rod-associated Müller cells in human as well as in the murine model, strengthening our hypothesis of functional Müller cell heterogeneity in the human retina. Indeed some proteins showed a Müller cell specific expression pattern in both the scRNAseq and our own proteomic data making these candidates especially promising for further research.
We identified Epiplakin (EPPK1) as a Müller cell specific and differentially expressed protein and for the first time showed through immunofluorescent imaging how it is localized along the entire length of the stem processes of murine Müller cells. Superresolution STED microscopy revealed, that *Eppk1* seems to dot-like colocalize with *Gfap* in an ischemia induced gliosis model indicating a possible role in this pathology.

Conclusion: Here we could show that the consistently different expression profile of some genes in human and murine Müller glia yields first interesting candidates that may coin functionally distinct Müller cell subpopulations. In future experiments the physiological relevance of these candidates needs to be validated in the cone-only mouse model and human donor tissue. Ultimately, our studies will help to improve the understanding of why the human macula is so sensitive to

disease-associated changes and probably will open avenues for the development of novel strategies to treat macular degeneration.

References: Macosko et al. Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell. 2015, 161:1202-1214.
Peng et al. Molecular Classification and Comparative Taxonomics of Foveal and Peripheral Cells in Primate Retina. Cell. 2019, 176:1222-1237.

Characterization of genetic variant rs2168518:G>A on 15q24.1 highlights its association with neovascular age-related macular degeneration (AMD) and gender-specificity

Christina Kiel[1], Tobias Strunz[1], Felix Grassmann[1,2], Bernhard H. F. Weber[1]

[1]Institute of Human Genetics, University of Regensburg, Germany
[2]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Sweden

Purpose: Investigations into common diseases represent a challenging task due to their high prevalence, as well as their complex nature of disease risk and pathology. As a complex disease, AMD is remarkable, because genetic variability plays a major role in disease risk. To date, a large number of genetic variants have been shown to be associated with AMD, although for the majority the underlying biological mechanisms are unclear. One of these genetic variants is rs2168518:G>A at 15q24.1, which is of special interest due to its prominent location within a genomic miRNA locus. This could suggest a potential influence of the variant on regulating posttranscriptional gene expression. However, biological proof of the functional consequences of rs2168518 on classical miRNA pathways remains elusive. With this study, our aim is to firstly validate the rs2168518 signal in an extended AMD cohort and secondly to further characterize the genetic locus with extended bioinformatical approaches in an effort to clarify whether regulatory effects other than typical miRNA-related pathways could also drive the association with AMD.

Methods: We replicated the association of rs2168518 with late-stage AMD in a dataset of 63,155 individuals, including the data set of the International AMD Genomics Consortium (IAMDGC) and the Genetic Epidemiology Research on Aging (GERA) Cohort. All variants located within 500 kb up- and downstream of rs2168518 were included in the calculations and tested for their association with late-stage AMD. AMD subgroups and gender were analyzed. Functional annotation of genetic variants in linkage disequilibrium (LD) with rs2168518 (defined as R^2>0.7) was performed with the RegulomeDB browser, which identifies DNA features and regulatory elements in non-coding regions of the human genome.

Results: We replicated the findings from previous studies and now report rs2168518 to be associated with late-stage AMD with genome-wide significance. Subgroup analyses of all variants at this genetic locus revealed mainly an association with the choroidal neovascular complication of AMD but not the dry form also known as geographic atrophy. Separation by gender revealed an association of rs2168518 with AMD exclusively in male patients. Functional annotation of variants in LD with rs2168518 revealed that the binding affinity of eight proteins are most likely affected. This includes SOX9, which is important for sex determination, and CTCF, which is known for its role in vascular development and susceptibility to oxidative stress in endothelial cells.

Conclusion: Taken together, our analysis demonstrates that the genetic locus at rs2168518 is strongly associated with AMD at a genome-wide significant level. Interestingly, this association is

exclusively driven by the neovascular form and by gender-specificity. The study also highlights that this locus may act in multiple ways, by influencing specificity of the seed region of miRNA hsa-miR-4513, but also by influencing gene regulation through altering protein-binding affinities.

A mineralomic study of the retinal pigment epithelium-Bruch's membrane complex in human eyes with age-related macular degeneration

Matthew G. Pilgrim[1,2], Anna C. S Tan[3], Sarah Fearn[4], Elena Tsolaki[5], Sergio Bertazzo[5], Christine A. Curcio[6], Imre Lengyel[1,2]

[1]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, 97 Lisburn Road, Belfast, Northern Ireland, BT9 7BL, UK; [2]Institute of Ophthalmology, University College London, 11 – 43 Bath Street, London, EC1V 9EL, UK; [3]Singapore National Eye Centre/ Singapore Eye Research Institute, 11 Third Hospital Avenue, Singapore 168751; [4]Department of Materials, Imperial College London, Royal School of Mines, Exhibition Road, London, SW7 2AZ, UK; [5]Department of Medical Physics and Bioengineering, University College London, Mallet Place, WC1E 6BT, UK; [6]Department of Ophthalmology, University of Alabama at Birmingham, 1670 University Boulevard, Birmingham, AL 35294, USA.

Aim: Previous histological studies have identified 3 distinct calcifications at the retinal pigment epithelium (RPE)-choroid complex in human eyes with age-related macular degeneration (AMD): spherules, Bruch's membrane (BrM) plaques and large calcified nodules. Whilst the elemental composition and mineral constituents of spherules (PMID: 25605911), and to a lesser extent BrM plaques (PMID: 1543459), have been investigated, the elemental composition and mineral content of large "calcified" nodules remains undetermined (PMID: 9620067). This study aimed to identify the mineral components of BrM plaques and the elemental and mineral composition of large "calcified" nodules.

Materials and Methods: Human cadaveric eyes were dissected and embedded in epoxy resin, sectioned at 0.1 to 2.0 µm and mounted on to glass slides. The elemental composition of BrM plaques and large calcified nodules was investigated using scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDX), and secondary ion mass spectroscopy (SIMS). The mineral components were determined using transmission electron microscopy-selected area electron diffraction (TEM-SAED).

Results: Spherules, BrM plaques and large calcified nodules all contained material of high atomic number, later identified as Ca and P by EDX (Results 2) and SIMS (Results 3). TEM-SAED confirmed that spherules were composed of highly crystalline Mg-substituted whitlockite, whilst BrM plaques and large calcified nodules were composed of amorphous or polycrystalline hydroxyapatite, respectively (Results 4).

Conclusion: Calcium and phosphorus were detected in all types of lesions. Interestingly, this is the first time that Ca and P were confirmed within large "calcified" nodules. It is also the first time that Mg-enriched whitlockite has been identified as a mineral constituent of spherules. Our data highlight the need for further research on metal ion homeostasis, specifically, Ca, P and Mg, at the interface between the RPE and the Choroid. Understanding these processes may aid the development of novel treatment strategies for AMD.

Moritz Lindner

Optogenetic strategies to restore vision in patients who are blind from end-stage retinal degenerations aim to render remaining retinal cells light sensitive once photoreceptors are lost. Here, we assessed to what extent neural retinal responses to complex light stimuli can be restored following intravitreal delivery of red-shifted channelrhodopsin (ReaChR) in the rd1 mouse model of retinal degeneration using an adeno-associated viral vector. By ectopic expression of ReaChR we were able to restore a variety of light response patterns closely paralleling those that can be observed in the retina of healthy mice. In treated retinae, neurons were capable to follow flicker light responses with high temporal precision up to a frequency of 25Hz. Furthermore, we could demonstrate that accurate space encoding can be obtained: Recordings from transduced neurons revealed an average receptive field diameter of 120.36±4.67 µm. However, high light intensities and a contrast of at least 30 % was required to elicit reliable responses. We additionally gathered evidence that depolarization block in response to sustained light stimuli occurs in most of the studied neurons. Though this this does not fully interrupt responsiveness to continuous complex light stimuli over more than 8 minutes, responses became significantly less determined over this interval of time. As clinical trials on optopogenetic vision restoration are commencing, the present data provide important quantitative information on the capacity of optopogenetic tools to restore spatiotemporally encoded vision. Importantly, they also identify potential limitations that will be addressed by engineering optimal optogenetic tools and identifying the optimal target cell population.

Towards genome editing in a special mouse model Rpgr^{tm1St} to treat X-linked RP

Müller B[1], Fühler B[1], Janise A[1], Götzmann D[1], Vössing C[2], Neidhardt J[2], Lorenz B[1], Stieger K[1]

[1]Department of Ophthalmology, Justus-Liebig-University Gießen, Gießen, Germany
[2]Faculty of Medicine and Health Science, University of Oldenburg, Oldenburg, Germany

Purpose: Genome editing represents a potentially powerful tool to treat inherited disorders. Using highly specific endonucleases a DNA double strand break near the mutant site can be induced and subsequently be corrected by cellular DNA repair mechanisms that involve the presence of a wild type template DNA. Here we show for the first time efficient targeting using the homing endonuclease I-SceI enzyme at the genomic locus near *RPGR*. We present a proof of concept in photoreceptors following AAV mediated gene transfer *in vivo* and characterize the DNA sequence alterations following a DSB repair on the X chromosome.

Methods: We used two months old mice (B6J.SV129-Rpgr^{tm1stie}) for transfer of AAV vectors into the subretinal space. A total of 5 different AAV-vectors were applied including AAV2/5 and AAV2/8 that contained either tissue specific Rhodopsin kinase (RK) or general CMV promotor, AAV2/5.CMV.I-SceI-T2A-GFP and AAV2/8.RK.I-SceI-T2A-GFP. One AAV2/8.RK vector also contained a template DNA of the target region, in which the I-SceI site was replaced by the Hind III for screening purposes. Eight weeks after subretinal injection eyes were harvested, retinae dissociated, and the GFP-positive cell population enriched by FACS. PCR fragments were subjected to T7 surveyor assay and subsequently Sanger sequenced to detect DNA sequences changes at the target site.

Results: GFP expression was observed in all injected eyes. Specific PCR of the target region and subsequent surveyor assay revealed DNA-repair activity in all injected eyes and none in the control eyes. Following Sanger sequencing of PCR-clones we observed small insertions and deletions as well as single nucleotide substitutions (altogether between 5 and 16 % of all clones). Deletions and substitutions outnumbered insertions. In retinae that were injected with an all-in-one vector containing the template in addition to the nuclease, we were able to detect replacement of the I-SceI site by the HindIII site with low frequency, indicating the presence of HDR at the target site.

Conclusion: We successfully induced in vivo genome editing in photoreceptors of a mouse model of retinal degeneration following AAV-mediated gene transfer. Both, NHEJ and HDR were detectable. These data represent the basis for further studies regarding regarding the occurrence of DNA sequence changes at target sites in retinal neurons in vivo.

Distinct protein degradation pathways underlying autosomal dominant and autosomal recessive bestrophinopathies

Anna-Lena Nachtigal, Andrea Milenkovic, Bernhard H. F. Weber

Institute of Human Genetics, University of Regensburg, Germany

Purpose: Bestrophin 1 (BEST1) encodes an integral membrane protein localized to the basolateral membrane of the retinal pigment epithelium (RPE). Functional BEST1 is a Ca^{2+}-regulated volume-sensitive chloride channel, composed of five homomeric BEST1 subunits. Pathogenic mutations in the BEST1 gene have been associated with distinct retinopathies, including the autosomal dominant Best Disease (BD) and the autosomal recessive Bestrophinopathy (ARB).

Methods: To understand the functional mechanisms underlying the dominant and recessive forms of BEST1-associated pathology, we generated RPE cells differentiated from induced pluripotent stem (iPSC-RPE) cells from BD patients (heterozygous for Q238R or Ile295del), ARB patients (compound heterozygous for N99K/R141H or A195V/L197PX26) and their healthy parents (heterozygous mutation carriers for one mutation, respectively). We analyzed BEST1 protein stability and degradation pathways by treating the cells with cycloheximide, a potent protein synthesis inhibitor, and selective inhibitors of the endo-lysosomal (e.g. chloroquine) or proteasomal (MG132) degradation pathway.

Results: Upon treatment with cycloheximide Western Blot analysis revealed that normal BEST1 protein is stable for up to 12 hours in control iPSC-RPE cells. However, mutant BEST1 is strongly degraded - with iPSC-RPE cells from the ARB patient revealing even less protein expression than the BD cell lines. In BD iPSC-RPE cells, mutant BEST1 is prone to degradation via the endo-lysosomal degradation pathway whereas the recessive BEST1 mutations are degraded by the proteasome.

Conclusion: Autosomal dominant mutations in BEST1 are degraded via the late endo-lysosomal degradation pathway. As a result, increased formation of non-functional BEST1 channels are likely to occur due to a roughly equimolar incorporation of normal and mutant BEST1 subunits into the channel complex. In contrast, recessive ARB mutations trigger a fast protein degradation process in the proteasome, thereby strongly favoring a decreased stoichiometry of mutant versus normal BEST1 subunits in the assembly of the homo-pentameric BEST1 chloride channel. Our results suggest that the site and speed of subcellular protein degradation account for the distinct retinal disease phenotypes in BD and ARB.

miRNA expression profile of ARPE-19 cells and their extracellular vesicles regulated by oxidative stress

Oltra M[1], Vidal-Gil L[1], Sancho-Pelluz FJ[2] and Barcia JM[2]

[1]Escuela de Doctorado Universidad Católica de Valencia "San Vicente Mártir"
[2]Facultad de Medicina y Odontología, Universidad Católica de Valencia "San Vicente Mártir"

Purpose: Previous studies indicate that oxidative stress (OS) affects the retinal pigment epithelium. Among the responses induced by OS, RPE cells release extracellular vesicles (EV) and their cargo include genetic material such as microRNA (miRNA). We hypothesize that ARPE cells and EV present different miRNA cargo compared to control and stressed cells.

Methods: ARPE-19 cells were cultured at $1x10^6$ cells/cm^2. ARPE-19 cells were treated for 24 hours with 600µM H_2O_2 (using medium with 1% FBS exosome depleted). EV were isolated from supernatant using ExoQuick-TC and RNA was isolated from both ARPE-19 cells and EV using the SeraMir Kit. To analyze the miRNA expression profile microarray assay was performed with 384 well SeraMir Profiler. *In silico* analysis were performed using DIANA TOOLS mirPAth v.3 algorithms to identify related pathways. Quantitative real time PCR (qRT-PCR) using TaqMan™ microRNA Assay was used to validate the expression profile of selected miRNAs in 4 independent samples.

Results: ARPE-19 cells were exposed to 600µM of H_2O_2. 24 hours after, 384 miRNAs were analyzed from supernatant and cell. Two different clusters were observed in both ARPE-19 cells and EV. 306 miRNAs out of 384 were expressed in ARPE-19 cells (control and H2O2). Let-7a, miR-518d-3p, miR-521, miR-338-5p, miR-548b-5p, miR-205 and miR-302c were over-expressed under physiological conditions compared to H_2O_2. In contrast, EV released by ARPE-19 over-expressed miR-302a and miR-122 under physiological conditions. miRNAs differentially expressed by ARPE-19 cells are involved in cell cycle, adherents' junctions and p53 signaling pathway which are related to RPE degeneration. The EV over-expressed miRNAs regulate TGF-beta signaling pathways, FoxO signaling pathway and cell cycle among others. In order to validate these findings, qRT-PCR was performed on ARPE-19 miRNAs (miR-205-5p, miR-521 and miR-302c) and EV miRNAs (miR-302a and miR-122), validating the microarray. To select one of the most interesting miRNAs we analyze the putative target of each miRNA using Target Scan Human software. One of the miR-205-5p target is VEGFA, a regulatory protein of vasculogenesis.

Conclusions: ARPE-19 cells and released EV present differential miRNA expression profile depending on the physiological state. The miRNAs expressed in normal ARPE-19 cells, such as miR-205-5p could be protective against pathological processes.

Artificial intelligence for the prediction of rod- and cone-function based on retinal morphology in geographic atrophy secondary to age-related macular degeneration

Maximilian Pfau[1], Leon von der Emde[1], Philipp T. Möller[1], Sarah Thiele[1], Moritz Lindner[2], Matthias Schmid[3], Steffen Schmitz-Valckenberg[1], Frank G. Holz[1], Monika Fleckenstein[1]

[1]Department of Ophthalmology, University of Bonn, Bonn, Germany
[2]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom
[3]Institute of Medical Biometry, Medical Informatics, and Epidemiology, University of Bonn, Bonn, Germany.

Purpose: To investigate the association of retinal morphology with rod- and cone-function in geographic atrophy (GA) secondary to age-related macular degeneration (AMD) using state-of-the-art artificial intelligence (AI) algorithms.

Methods: Mesopic, dark-adapted (DA) cyan and red sensitivity were measured using fundus-controlled perimetry ("microperimetry"). Test-points were placed along "iso-hulls" at pre-defined distances to the GA boundary using patient-tailored perimetry grids. Retinal microstructure was assessed through spectral-domain optical coherence tomography (SD-OCT) imaging. Fundus-controlled perimetry data were registered to SD-OCT data based on vessel bifurcations. Reflectivity and thickness values were extracted for six retinal layers for each test-point. Using random forest regression, we evaluated (i) the cross-validated (CV) mean absolute error (MAE) with and without patient-specific training data and (ii) increase in out-of-bag meansquared error (% IncMSE) as measure of SD-OCT feature importance.

Results: Thirty eyes of 30 patients (76.4 ± 7.1 years; 16 female) with GA from the prospective natural progression study DSGA (Directional Spread in Geographic Atrophy; NCT02051998) and 40 normal eyes form 40 age-similar subjects were included. For patients with GA, sensitivity was predicted with a MAE [95 % CI] of 4.15 dB [3.39; 4.91] for mesopic, 5.46 dB [4.64; 6.27] for DA cyan and 3.98 dB [3.44; 4.53] for DA red testing in absence of patientspecific data. Partial addition of patient-specific sensitivity data to the training sets decreased the MAE to 2.53 dB [2.49; 2.58], 3.21 dB [3.16; 3.26] and 2.55 dB [2.52; 2.58]. For all three types of testing, the outer nuclear layer thickness constituted the most important feature (70.88, 78.66 and 107.81 % IncMSE). AI-based spatial mapping of "inferred sensitivity" across the whole retina imaged by SD-OCT and comparison to normal data revealed that DA cyan sensitivity loss spatially exceeded mesopic sensitivity loss in eyes with GA.

Conclusions: AI-based "inferred sensitivity" mapping may be applicable as quasi-functional clinical outcome to (partially) substitute for time-consuming psychophysical testing. Rod-function appears to be more severely affected in eyes with GA secondary to AMD and may constitute a potential therapeutic target to prevent downstream cone-degeneration.

An iPSC-derived RPE cell repository with high and low genetic AMD risk as model systems to study AMD pathology *in vitro*

Karolina Plößl, Annie Lauer, Felix Grassmann, Caroline Brandl and Bernhard H.F. Weber

Purpose: Age-related macular degeneration (AMD) is the leading cause of vision loss in developed countries. Currently, the molecular processes underlying AMD are not yet understood, although specific environmental and genetic factors have been recognized as key determinants to a person's individual risk to develop the disease. To gain further insight into the molecular pathology of AMD, we generated an iPSC-derived RPE cell repository with cell lines derived from defined genetic low and high risk AMD background, respectively. Since oxidative stress plays an important role in AMD pathogenesis, we sought to initially determine the effect of Paraquat (PQ) treatment on the different cell lines.

Methods: AMD patients and controls were genotyped for 13 AMD-associated genetic variants at 8 different loci known to be highly correlated with AMD risk. Genetic risk scores for AMD were calculated using the model published by Grassmann et al., 2012. Fibroblast or PBMC cultures were established from patients with very low (category 1) and very high (category 5) AMD risk scores, reprogrammed to iPSCs and differentiated into RPE cells. RPE cell properties were assessed by TEER measurement and immunocytochemistry. Oxidative stress was induced by treatment with PQ and cellular responses were measured by MTT assay, qRT-PCR, and ELISA.

Results: Four unrelated RPE cell lines corresponding to AMD risk score categories 1 and 5 have successfully been established and characterized. All cell lines showed high TEER values above 800 $\Omega*cm^2$ indicating a tight monolayer structure. Staining patterns for ZO1 as a tight junction marker and BEST1 as a RPE specific ion channel were distinct in all cell lines. Oxidative stress induced with 1 mM PQ for 24h significantly decreased cell viability of all cell lines with no differences between cell lines from high risk and low risk genetic backgrounds. Quantitative RT PCR analysis revealed PQ dependent changes in *VEGF* and *CD46* expression, but not in *C3* or *CFH* expression. Secreted VEGF protein in basal supernatants of PQ treated cells was also significantly elevated. Taken together, there were no differences between high and low risk cell lines in any of the parameters tested.

Conclusions: Having established RPE lines with known genetic AMD background and a reproducible protocol to induce oxidative stress in these cells, we now aim to gain deeper insight into molecular processes responsible for regulation cellular responses following oxidative stress and to determine the influence of the genetic AMD risk on molecular disease mechanisms.

Retinal microglia signaling affects Müller cell behavior in the zebrafish following laser injury induction

Ana Maria Quintela Pousa [1, 2], Federica Maria Conedera [1, 2, 3], Nadia Mercader [4], Markus Tschopp [1, 5], Volker Enzmann [1, 2]

[1] Department of Ophthalmology, University Hospital of Bern, University of Bern, Bern, Switzerland
[2] Department of BioMedical Research, University of Bern, Bern, Switzerland
[3] Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland
[4] Institute of Anatomy, University of Bern, Bern, Switzerland
[5] Department of Ophthalmology, Cantonal Hospital Aarau, Aarau, Switzerland

Purpose: Microglia are resident tissue macrophages of the retina. Under pathophysiological conditions, microglia can signal to Müller cells, the major glial component of the retina, affecting their morphological, molecular, and functional responses. Microglia-Müller cell interactions appear to be bi-directional shaping the overall injury response in the retina. Hence, microglia and Müller cell responses to disease and injury have been ascribed both positive and negative outcomes. However, Müller cell reactivity and survival in absence of immune cells after injury have not been investigated in detail in adult zebrafish.

Methods: A model of focal retinal injury combined with pharmacological immunodepletion in zebrafish was employed. The retinal injury was induced by a 532 nm diode laser to damage photoreceptors. Two pharmacological treatments were used to deplete either macrophage-microglia (PLX3397) or selectively eliminate peripheral macrophages (clodronate-liposomes). Retinal degeneration and regeneration was examined at different time points post-injury induction by *in vivo* imaging (OCT) and morphological analysis (H&E staining). Furthermore, immunohistochemical analyses (e.g., GS, GFAP, and PCNA) were used to characterize how microglia affect Müller cell response to the laser injury.

Results: PLX3397 treatment induced immune cell depletion hindering retinal regeneration in zebrafish, which is reversed by microglial repopulation. On the other hand, selective macrophage elimination did not affect the kinetics of retinal regeneration. The absence of retinal microglia and macrophages leads to dysregulated Müller cell behavior. Unlike the untreated fish, where Müller cells were activated after injury induction, immunosuppressed animals showed no activation. However, the re-entry in the cell cycle was not affected in the PLX3397 treated fish.

Conclusion: Without microglia signaling Müller cells can still dislocate into the damage area despite not being activated. This dysregulated Müller cell behavior results in impaired regeneration in the retina of immunosuppressed fish. Thus, microglia and Müller cell signaling is pivotal to unlock the regenerative potential of Müller cells in order to repair the damaged retina.

Influence of mutant bestrophin-1 on L-type channel trafficking and activity in the RPE

Nadine Reichhart[1], Magdalena Cordes[1], Piotr Bucichowski[1], Ahmad S. Alfaar[1], Seba Almedawar[2], Olaf Strauss[1]

[1]Department of Ophthalmology, Charité - Universitätsmedizin Berlin, Germany
[2]Center for Regenerative Therapies, Technical University Dresden, Dresden, Germany

Best's disease is a rare hereditary form of macular dystrophy with early onset. It is caused by mutations in the BEST1 gene coding for bestrophin-1, a protein expressed in the basolateral membrane of the retinal pigment epithelium. Besides its role as a Ca^{2+}- dependent or volume regulated anion channel, bestrophin-1 functions as a modulator of L-type Ca^{2+}-channels. A common feature of bestrophin-1 mutations is a trafficking defect of the mutants to the cell membrane. Thus, we aimed to investigate the influence of bestrophin-1 mutations on L-type channel trafficking and channel activity.

Bestrophin-1 mutations from four mutation hotspots (R218C, F80L, T6P, F305S) and wild-type bestrophin-1 were used in the study. Immunoprecipitation (IP) of heterologously expressed $Ca_V1.3$ subunits and bestrophin-1 in CHO-K1 revealed interaction of R218C-, F80L-, and F305S-bestrophin-1 with L-type channels. Compared to wild-type, the mutants showed significantly weaker co-IP-efficacy; with T6P, co-IP could not be detected. The presence of T6P-, F80L- and F305S-bestrophin-1 led to a reduced plasma membrane localization of L-type channels, as confirmed by calculation of the Pearson correlation coefficient (PCC) of a plasma membrane marker with the pore forming $Ca_V1.3$ subunit. Whole cell patch clamp recordings showed reduced L-type channel activity in presence of the bestrophin-1 mutations. In porcine or iPSC-derived RPE cells with endogenous bestrophin-1 expression, the impairment of L-type channel trafficking by transfected mutant bestrophin-1 was confirmed. GFP- or c-myc tagged plasmids enabled the identification of transfected bestrophin-1. Edge detection showed reduced plasma membrane localization of both $Ca_V1.3$ and total bestrophin-1.

In summary, mutant bestrophin-1 reduces the plasma-membrane localization of L-type channels by heteromerization. Mutant bestrophin-1 that interacts with $Ca_V1.3$ causes directly influences L-type channel activity. Thereby mutant bestrophin-1 might impair L-type channel dependent functions of the RPE such as phagocytosis, ultimately leading to retinal degeneration.

The Usher syndrome type 1G protein SANS regulates splicing of genes associated with retinal disorders

Jessica Ries, Adem Yildirim, Ann-Kathrin Wallisch, Ben Fadl and Uwe Wolfrum

Molecular Cell Biology, Institute of Molecular Physiology, Johannes Gutenberg University of Mainz, Germany

Purpose: The human Usher syndrome is the most common form of inherited combined deaf-blindness and a complex ciliopathy. USH can be classified into three clinical subtypes, namely USH 1-3. 10 USH genes have been identified so far. USH proteins form an USH interactome in retinal photoreceptor cells and inner ear hair cells. Previous studies revealed that USH genes are heavily spliced and that USH1G SANS participates in the spliceosome complex. Alternative splicing has suggested being an important process for the diverse functions of USH proteins and changes in splicing may underlay the pathophysiology of USH. Here, we aim to explore role of SANS in splicing of USH genes and other ciliopathy genes.

Methods: SANS was depleted in HEK293T cells by siRNA-mediated knockdown. SANS knockout (KO) murine IMCD3 cells were generated by CRISPR-Cas9. SANS KO mouse retinas have been previously described and were provided by Dr. El-Amraoui, Paris. RNA-seq human and mouse retina libraries were screened for alternatively spliced regions of USH genes. For analysis of selected genes and regions RNAs were isolated and converted to cDNAs from indicated samples. Subsequently, PCR products of local splice site regions were separated by capillary electrophoresis and quantified in TapeStation.

Results: Our studies revealed that SANS depletion or deficiency causes perturbations in the splicing of both human and mouse USH genes, namely *CDH23, PCDH15, WHRN, myo7A, cdh23, pcdh15, whrn, ush2A and vlgr1*. Comparison of mouse retina and IMCD3 cells also demonstrated a cell specific alternative splicing and indicated the expression of retina cell specific splice variants of *vlgr1* (USH2C) and *ush2a*. Furthermore, SANS has an influence on the splicing of other ciliopathy related genes, namely *ttc8/bbs8* and *cep290* in murine cells and retina.

Conclusion: USH1G protein SANS is relevant for the correct splicing of ciliopathy and USH genes and may also specifically regulate the expression of alternatively spliced isoforms specific for primary cilia and/or the retina. Alteration of USH gene splicing may affect the protein structure and function of the USH proteins and thereby, their interactome. Our data suggests that defective splicing of ciliopathy and USH genes underlay the pathophysiology of USH1 caused by mutations of USH1G gene *SANS*.

Supports: FAUN-Stiftung, Foundation Fighting Blindness (FFB)

Recent advances linking molecular functions between Poly ADP ribosylation and extracellular vesicle activity in photoreceptor cell death

Ayse Sahaboglu[1], Lorena Vidal-Gil[2], Jorge M Barcia[2], Eberhart Zrenner[1], Javier Sancho-Pelluz[2]

[1]Institute for Ophthalmic Research, Tuebingen, Germany
[2]School of Medicine, Catholic University, Valencia, Spain

Purpose: Retinitis Pigmentosa (RP) represents a group of inherited neurodegenerative diseases that result in selective cell death of photoreceptors. Excessive PARP activity is seen in photoreceptor cell death in several rat and mouse models for RP, where it increases the level of PARylation in the degenerating photoreceptors. Extracellular vesicles (EVs) have roles in cell-cell communication by carrying nucleic acids, proteins and lipids that can, in turn, regulate behaviour of the target cells. EVs extensively participate in progression of diverse blinding diseases, such as age-related macular (AMD) degeneration. The EVs activity and mechanism of neuroprotection by PARP inhibition in photoreceptor degeneration is under debate. Here, we investigated the effects of inhibition of PARylation on EVs in rod photoreceptor degeneration.

Methods: Retinal explants from *rd10* mouse were treated with 100nm olaparib. Treatment was started at PN11 and terminated at PN18. TUNEL, PAR, CD9, Rhodopsin, RPE65, EVs isolation, electron microscopy and flow cytometry were performed for the assessment of dying cells, PARylation, CD9, rhodopsin, RPE65 and EVs respectively.

Results: TUNEL analysis showed the minimum number of TUNEL positivity and maximum number of photoreceptor rows for 100 nM olaparib treated group (untreated: 4.13 ± 0.4 SEM, n=6; treated: 2.32 ± 0.09 SEM, n = 4; $p < 0.01$: Row numbers; untreated: 4.8 ± 0.15 SEM, n = 4, treated: 7.1 ± 0.38 SEM, n = 4; $p < 0.01$). PAR positive cells in ONL significantly decreased for olaparib treated group (untreated: 1.11 ± 0.05 SEM, n = 4; treated: 0.62 ± 0.09 SEM, n = 4; $p < 0.01$). CD9 expression was observed at choroid, RPE, inner segment, INL, and GCL of *wt* mice eye at P18. In *rd10* mice at P18, CD9 expression was highly increased in choroid, ONL, and INL, when compared to corresponding *wt* mice. Electron microscope studies showed most of the EVs matched morphology and the size of exosomes. The amount of rhodopsin increased in EVs from olaparib treated group.

Conclusions: The level of retinal EVs changes by PDE6 mutation in rod photoreceptor degeneration. Poly ADP ribosylation may interact with EVs in photoreceptor degeneration. PARP inhibition protects photoreceptors by controlling EVs release in photoreceptor degeneration. This interaction between PARP and EVs activity could open new treatment strategies for a variety of hereditary photoreceptor dystrophies.

Oxidative stress and properdin modulate primary human RPE cells

Nicole Schäfer[1], Renate Foeckler[1], Stephanie Lötscher[2], Volker Enzmann[2], Diana Pauly[1]

[1]University Hospital Regensburg, Department of Ophthalmology, Regensburg, Germany
[2]University Hospital of Bern, Department of Ophthalmology, Bern, Switzerland

Background: The retinal pigment epithelium (RPE) forms the outer blood-retina barrier and is important for the ocular immune privilege. More than fifteen years ago, risk factors within genes of the complement system were found to be associated with retinal degeneration. So far, retinal complement activity has been related to systemic complement components and to retinal microglia/macrophages. Here, we aim to shed light on the local, RPE cell-specific complement system and elucidate the immunomodulatory function of stressed primary human RPE cells.

Materials & Methods: The expression of complement components and inflammation-associated signaling factors was analyzed in genotyped primary human RPE cells using quantitative real-time PCR (qPCR), Western Blot and Multiplex–ELISA. RPE cells were apically stressed either with hydrogen peroxide (H_2O_2) alone or with both H_2O_2 and the complement positive regulator properdin (FP).

Results: Primary human RPE cell cultures from sixteen different donors showed individual single nucleotide polymorphisms (SNPs) in genes of the complement system, which were previously associated with a risk for age-related macular degeneration. SNPs were mainly in the *CFH*, *C2/CFB* and *CFI* genes. On mRNA level, these RPE cells expressed complement components, regulators and receptors. Primary RPE cell treatment with H_2O_2 or combined with H_2O_2 and FP addition resulted in genotype-dependent expression changes. Opposed RNA expression changes in oxidatively stressed and FP-treated RPE cells could be detected for *C3*, *C5* and the complement receptors *C5AR1* as well as *CR3* in half of the tested genotypes. Whereas, the inflammasome-associated transcript of *NLRP3* was down-regulated in all stressed RPE cells.
On protein level, stressed and FP-treated cells showed a higher secretion of C3 and C3b (three of five tested genotypes), C4 and CFB (four of five tested genotypes), and a lower secretion of CFH and CFI (three of five tested genotypes). We could show that the CFI secretion is relative to the secretion of the negative regulator CFH. The complement activation markers C3a and C5a were detected in all primary human RPE cell extracts using Western Blots, independent of stress conditions.

Conclusion: In conclusion, our data unveiled a local complement activity in primary human RPE cells. A genotype-associated complement expression, on both mRNA and protein level, could be detected in stressed RPE cells.

Roadmap to wireless organic photovoltaic neurostimulating platforms for vision restoration

Manuela Schiek[1], O. S. Abdullaeva[1], F. Balzer[2], M. Schulz[3], A. Lützen[3], J. Parisi[1], K. Dedek[4]

[1]Institute of Physics, University of Oldenburg / D
[2]University of Southern Denmark, Sonderborg / DK
[3]Kekulé Institute for Organic Chemistry and Biochemistry, University of Bonn / D
[4]Institute for Biology and Environmental Sciences, University of Oldenburg / D

Purpose: Perspectively, we intend to develop a minimal invasive implantable neurostimulating device for restoration of light sensitivity in degenerated retina. The implementation of organic photoactive materials offers a seamless match to biological tissue, and the photovoltaic principle allows an electrically self-powering, i.e., wireless device design. Currently, we are aiming to engineer a device with a non-phototoxic, purely photo-capacitive stimulation mechanism and are using a cellular model system for optimization.

Materials and Methods: We conduct electrophysiological patch-clamp recordings on murine neuroblastoma (N2A) cells grown on small molecular organic semiconductor based photoactive layers with optional dielectric coating under physiological conditions. We investigate the stimulation of voltage-gated ion channels with light pulses of varying illumination intensity.

Results: Upon pulsed illumination, a rapid transient photocurrent is generated and depolarizes the membrane potential, thereby stimulating fast-responding voltage-gated sodium channels. The dielectric coating is decisive to facilitate an active cellular response. Due to the high irradiance level, another slower and unintended signaling pathway, presumably photothermal heating, activates voltage-gated potassium channels.

Conclusion: It is possible to activate neural model cells with photovoltaic organic devices by a safe photo-capacitive stimulation mechanism. We found that the charge injection efficiency is decisive, and that the dielectric coating turns the tip to facilitate photo-capacitive stimulation. In the next step, the device sensitivity needs to be increased to reduce the irradiance threshold and avoid unwanted heating effects.

Retinoschisin and cardiac glycoside crosstalk at the retinal Na/K-ATPase and consequences on retinal integrity

Verena Schmid, Karolina Plössl, Carina Schmid, Sarah Bernklau, Bernhard H. F. Weber, Ulrike Friedrich

Institute of Human Genetics, University of Regensburg, Germany

Purpose: Mutations in the *RS1* gene cause X-linked juvenile retinoschisis (XLRS), a hereditary retinal dystrophy in young males (Sauer et al., 1997). The retinoschisin protein specifically and directly interacts with the retinal Na/K-ATPase consisting of subunits ATP1A3 and ATP1B2. Na/K-ATPases are membrane spanning ion pumps involved in three major tasks including maintaining the electrochemical membrane potential, regulating intracellular signaling cascades and mediating intercellular adhesion. Their activity is mainly modulated by cardiac glycosides like ouabain (Jorgensen et al., 2003) which were reported to play a major role in retinal development and homeostasis (Lopatina et al., 2008). In this context, heart patients showed retinal dysfunction and visual impairment as a consequence of ouabain treatment (Fraunfelder et al., 2014). In this study, we investigated the crosstalk between retinoschisin and cardiac glycosides at the retina specific Na/K-ATPase with the focus on retinal integrity.

Methods: The effect of ouabain on retinoschisin binding to the retinal Na/K-ATPase was assessed by Western Blot and immunocytochemical analyses in binding assays on Hek293 cells heterologously expressing ATP1A3 and ATP1B2. Vice versa, the effect of retinoschisin on ouabain binding was investigated by testing the capacity of ouabain to inhibit Na/K-ATPase catalyzed ATP hydrolysis in retinal membranes from *retinoschisin*-deficient ($Rs1h^{-/Y}$) and wildtype mice. The reciprocal effect of retinoschisin and ouabain on Na/K-ATPase localization and photoreceptor degeneration was addressed after long-term cultivation (4 days) of $Rs1h^{-/Y}$ murine retinal explants applying immunohistochemical analyses.

Results: Ouabain competitively displaced retinoschisin from the retinal Na/K-ATPase and murine retinal membranes. In contrast, retinoschisin had no effect on the ouabain induced inhibition of Na/K-ATPase catalyzed active ion pumping and thus on the ouabain affinity of the retinal Na/K-ATPase. Ouabain and retinoschisin exerted adverse effects on retinal integrity, i.e. in the presence of ouabain, the retinoschisin induced enrichment of the retinal Na/K-ATPase at the inner segments and protection against photoreceptor degeneration was significantly decreased.

Conclusion: Our findings reveal opposing effects of retinoschisin and ouabain on retinal Na/K-ATPase binding and on retinal integrity, suggesting that a fine tuned interplay between both components is required for maintaining retinal homeostasis.

Possible role of epithelial-to-mesenchymal transition underlying retinal pigment epithelium phenotype in cilia mutant mice

Sandra Schneider[1], Sonja Boxhorn[1], Helen Louise May-Simera[1]

[1]Institute of Molecular Physiology, Johannes-Gutenberg University, Mainz, Germany

Purpose: Primary cilia, microtubule-based organelles, are present on almost every eukaryotic cell type and play important roles in physiological and developmental processes via regulation of signalling pathways. Dysfunction of primary cilia leads to retinal degeneration. So far, most research has focused on the highly specialized primary cilium of the photoreceptor outer segments. However, our previous work showed that primary cilia in the retinal pigment epithelium (RPE) are essential for its development and function. A fully functional RPE, which is indispensable for vision, is critically dependent on its maintenance of an epithelial phenotype.

Methods: As *Bardet-Biedl syndrome* (*Bbs*) genes encode for ciliary trafficking proteins, that are important for ciliary maintenance and function, we used *Bbs*-deficient mice to model the effect of cilia function on RPE maturation and maintenance via gene expression analysis and immunohistochemistry.

Results: Gene expression analysis of visual cycle genes, considered as markers for RPE maturity, revealed a significant downregulation in *Bbs8*$^{-/-}$ mice compared to wildtype littermates, suggesting maturation defects of the RPE. This might be due to misregulation of miRNA-204 and miRNA-211, that are known to regulate RPE gene expression. Targets of these miRNAs are among others, genes associated with cellular polarity and epithelial-to-mesenchymal transition (EMT). EMT denotes the trans-differentiation of epithelial cells into mesenchymal cells and is manifested by loss of cell-cell junctions and apical-basal polarity, the cytoskeletal reorganization, as well as changes in signalling and gene expression.
We saw that *Bbs8*$^{-/-}$ mice showed EMT-like characteristics due to abnormal regulation of EMT hallmark genes. Immunohistochemistry of RPE flatmounts of *Bbs8*$^{-/-}$ mice of different ages revealed changes in epithelial patterning, cytoskeletal arrangement and apical-basal polarity, compared to wildtype littermates.
Unexpectedly, ciliation of mature RPE cells was observed in *Bbs8*$^{-/-}$ mice, possibly suggesting a de-differentiation of the RPE cells.

Conclusion: EMT is initiated and controlled by the convergence of many signalling pathways. Since the main function of the primary cilium is to co-ordinate numerous signalling pathways, it is highly likely that dysfunctional ciliary signalling underlies the possible EMT phenotype in ciliary mutants. These results highlight the important role of primary cilia in the RPE, which has long been ignored but needs to be considered when designing treatment strategies for retinal degeneration and differentiation of iPS-derived RPE.

Modulation of the VCP/ERAD/Proteasome axis in Rho^{P23H} organotypic retina cultures

Merve Sen[1,2], Oksana Kutsyr[3], Sylvia Bolz[1], Tsui-Fen Chou[4], Ray Deshaies[5], Blanca Arango-Gonzalez[1], Marius Ueffing[1]

[1]Centre for Ophthalmology, University Eye Hospital Tübingen, Tübingen, Germany
[2]Graduate Training Centre of Neuroscience, Eberhard Karls University of Tübingen, Tübingen, Germany
[3]Department of Physiology, Genetics and Microbiology, University of Alicante, Alicante, Spain
[4]Department of Pediatrics, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, CA, United States
[5]Discovery Research, Amgen, Inc., CA, United States.

Purpose: The most prevalent mutation related to autosomal dominant retinitis pigmentosa (adRP) is the *Rho^{P23H}*, leading to affect proper folding of rhodopsin and its retention in the Endoplasmic Reticulum (ER). Misfolded proteins are selectively identified and cleared during a process called ER-associated degradation (ERAD) that followed by the progressive loss of photoreceptors. The overall objective of this study was to identify targets for therapeutic intervention in pathways regulating protein homeostasis and stress response and thus be able to discover molecules for drug development towards a pharmacological treatment of retinal degeneration.

Methods: To evaluate the efficacy of ERAD inhibitors we used VCP inhibitor NMS-873 and proteasome inhibitor Bortezomib to modulate VCP-ERAD proteasome axis. The mannosidase inhibitor Kifunensine and Grp94 inhibitor Geldanamycin that control protein integrity or proper folding of proteins were utilized to check other pathways in ERAD. We prepared organotypic cultures from *Rho^{P23H}* retinas of transgenic rats at PN9. Retinal explants were treated with different concentrations of inhibitors, and we fixed the retinas and performed TUNEL assay with DAPI counterstaining at DIV6. We evaluated photoreceptor cell survival in those retinas by counting cell rows and measuring the percentage of TUNEL-positive cells in the ONL. Rhodopsin distribution was checked using a specific antibody.

Results: VCP and proteasome inhibitors significantly reduced the number of TUNEL positive cells and increased the average number of cell rows in the outer nuclear layer in *Rho^{P23H}* rats. Moreover, treatment with VCP inhibitor partially restored distribution of the visual pigment rhodopsin in mutant retinas.

Conclusion: The mechanism between protein homeostasis and photoreceptor cell death is a common degenerative process, which could be an ideal therapeutic way for all the RP patients.

Mutation screen in 480 patients with Retinitis Pigmentosa applying a MIP-based next generation sequencing approach

Maria Solaki[1], Sinja Kieninger[1], Nicole Weisschuh[1], Susanne Kohl[1], Bernd Wissinger[1], Frans P.M. Cremers[2], Muhammad Imran Khan[2]

[1]Centre for Ophthalmology, Institute for Ophthalmic Research, Molecular Genetics Laboratory, University of Tuebingen, Germany
[2]Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Purpose: Retinitis pigmentosa (RP) comprises a clinically and genetically heterogeneous group of inherited retinal diseases (IRD) characterized by night blindness, visual field constriction, and eventually central vision loss. RP can be inherited as an autosomal recessive, autosomal dominant or X-linked trait and is currently attributed to mutations in 88 genes. We aimed to obtain a molecular diagnosis for 480 patients that had been initially diagnosed with either sporadic or autosomal recessive RP. Therefore we analyzed a total of 108 IRD-associated genes applying a targeted next generation sequencing (NGS) procedure based on molecular inversion probes (MIPs).

Methods: MIPs were used to selectively enrich coding exons and flanking intronic sequences of 108 IRD-associated genes in 480 samples, including two common deep intronic variants in *USH2A* (c.7595-2144A>G) and *CEP290* (c.2991+1655A>G). Following next generation sequencing and variant filtering the identified putative pathogenic variants were validated by Sanger sequencing. If possible, biallelism in recessive cases was demonstrated using segregation analyses or allelic cloning.

Results: Putative pathogenic variants were identified in 188/480 cases, resulting in a detection rate of 39 %. Solved cases were classified according to the proposed inheritance mode: 138 likely autosomal recessive, 38 likely autosomal dominant and 12 likely X-linked. The mutation spectrum comprises 230 distinct pathogenic variants in 42 IRD-associated genes, most frequently in *USH2A*, *ABCA4* and *RP1*. Of these, 45 % were novel variants, suggesting that the mutational variety in IRD genes is far from being saturated. 16 % of the cases carried either one heterozygous pathogenic variant in genes associated with autosomal recessive inheritance or variants with an uncertain pathogenicity. 45 % of the cases were lacking any putative pathogenic variants, possibly resulting from insufficient coverage of target regions, mutations in genes not represented in this study, mutations outside the coding regions or not detectable by this exon-focused approach (i.e. deep intronic variants, copy number variations).

Conclusion: MIPs offer a cost-effective technology to perform large-scale targeted sequencing by having a 10-20x lower price tag than other NGS-based procedures. Moreover, we could demonstrate that MIPs provide a sensitive tool for analyzing RP.

Gene expression imputation identifies candidate genes and novel susceptibility loci for age-related macular degeneration (AMD)

Tobias Strunz and Bernhard H. F. Weber

Institute of Human Genetics, University of Regensburg, Regensburg, Germany

Purpose: In late stage age-related macular degeneration (AMD), genome-wide association studies (GWAS) have identified 52 independent gene variant signals in 34 genomic loci. While GWAS do not generally provide detailed information about the biological function of a particular genetic variant, Gamazon et al. (2015) proposed a gene-based association method known as PrediXcan to address this issue. The approach estimates the component of gene expression determined by an individual's genetic profile. Subsequently, it correlates 'imputed' gene expression with the phenotype under investigation. This algorithm has the potential to identify susceptibility loci that were missed in traditional GWAS analyses and to suggest candidate causal genes. Here, we used PrediXcan to estimate tissue-specific gene expression of European individuals, which participated in the latest AMD GWAS (Fritsche et al., 2016).

Methods: We applied PrediXcan algorithms on the genotype information available through the IAMDGC data set and imputed gene expression data for 27 different tissues. The models were built based on gene expression measured in healthy tissues of the Genotype-Tissue Expression (GTEx) Project. Input for gene expression imputation included over 12 million genetic variants from 16,473 late-stage AMD cases and 19,033 healthy controls. After gene expression imputation of up to 9,661 genes, we applied a linear regression model to identify AMD correlated genes.

Results: Overall, we identified 119 genes for which imputed gene expression was significantly correlated (FDR < 0.001) with AMD status in at least one tissue. Among these, 15 genes showed a differential expression in AMD cases and controls in more than 10 tissues, highlighting potential systemic effects like regulation of immune responses (*CFH*, *CFHR1*, *PLEKHA1*, *PILRB*, and *PILRA*). Interestingly, 59 genes show significance in only one or two tissues. These latter signals may indicate tissue-specific effects relevant for AMD pathogenesis. For example, *LIPC* exhibited a higher expression in AMD cases (estimate 0.037, SE: 0.005) compared to controls. This effect was found exclusively in liver tissue, further reinforcing the notion that cholesterol metabolism could play a crucial role in AMD aetiology. Overall, 93 of 119 genes are located within already known AMD Loci, whereas 26 genes point to 17 new loci, which so far did not reach genome-wide significance in recent GWAS.

Conclusions: The PrediXcan algorithm identified 119 genetically regulated genes in the currently largest known data set for AMD cases and controls and correlated gene expression with AMD status. Furthermore, this analysis identified 17 novel susceptibility loci, which harbour potential candidate genes potentially relevant for AMD pathology.

Creation of different biosensors with high affinity to VEGF

Stumpf C, Wimmer T, Lorenz B, Stieger K

Department of Ophthalmology, Justus-Liebig-University Giessen

Introduction: In retinal and choroidal diseases like age-related macular degeneration (AMD), which is the major cause of visual impairment in the population over 50 years, hypoxia and inflammatory processes lead to an upregulation of the vascular endothelial growth factor (VEGF) expression and thereby to pathological neovascularisation with incorrectly formed vessels prone to damage, thus increasing the vascular permeability and the risk of bleeding and edema. State of the art treatment of these diseases are repeated intraocular injections of anti-VEGF medications like Avastin®, Lucentis® or Eyelea®. For developing improved individualized treatment approaches, a minimally invasive, repeatable method for in- vivo quantification of VEGF in the eye is necessary. The aim of this project is to generate biosensors capable of quantifying VEGF that can be used in future in vivo applications.

Methods: Several biosensor variants were generated with different VEGF receptor domains as the VEGF Binding motive fused to a *Renilla* luciferase (Rluc8) and a green fluorescent Protein (GFP2). HEK293-T cells were transfected with biosensor plasmids, and cells were lysed after 24h. Luminescence was measured with cell lysate before and after incubation with VEGF, and the BRET2 ratios were measured in dual luminescence mode using blue/green BRET2 filter set with the Tecan Infinite 1000 Pro. Western Blots were performed with rabbit anti-RLuc-IgG and goat anti-rabbit-IgG-HRP. GAPDH served as loading control. A donor splice site in the Rluc8 was deleted by the generation of a silent point mutation via mutagenesis PCR.

Results: In total 10 different VEGF biosensor were generated based on either single domains or full length of VEGF receptor extracellular regions. Expression of alternative splice variants was eliminated with a sequence change in the donor splice site. Efficient expression of the biosensor variants was verified by Western Blot and GFP2 signalling. Luminescence scans show signals for the Luciferase and a BRET2 signal. A change in the BRET2 ratio after incubation with VEGF could be shown.

Conclusion & Discussion: Generation of functional biosensors with an active GFP2 and Rluc8 is possible. The BRET2 ratio after incubation with VEGF suggests that the extracellular domain 3 of both VEGF receptors shows the best performance in binding VEGF, likely due to a conformational change that allows a change in the energy transfer rate.

Outlook: We are currently expressing the most sensitive biosensor variant on the surface of VEGF-A knockout arpe-19 cells, which could be, encapsulated in micro vessels, represent a carrier system for intra-ocular quantification in in vivo applications.

Effects of subretinal injection of human lipofuscin in murine retina

Nan Su[1], Gerburg Nettels-Hackert[1], Tanja Plagemann[1], Jeannette König[2], Annika Höhn[2], Tilman Grune[2], Constantin E. Uhlig[3,4], Nicole Eter[4], Peter Heiduschka[1,4]

[1]Research Laboratory, Department of Ophthalmology, University Medical Center, Münster, Germany
[2]German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany
[3]Cornea Bank Münster, Department of Ophthalmology, University Medical Center, Münster, Germany
[4]Department of Ophthalmology, University Medical Center, Münster, Germany

Purpose: In the aged retina, lipofuscin accumulates in the RPE and can be released to both basal and apical sides. As microglial cells may migrate into the subretinal space with increasing age, reaction of microglial cells towards lipofuscin may have important implications for AMD development and progression. In recent *in vitro* experiments, we found a strong inflammatory reaction of microglial cells towards lipofuscin. As a next step of our studies, we injected lipofuscin subretinally in mice to check reaction of the microglia *in vivo*.

Methods: Human lipofuscin was isolated from the RPE of human donor eyes. Suspension of lipofuscin was injected subretinally in eyes of adult C57BL/6J mice. Eyes were isolated at 0, 1, 3, 5, 7, 14 or 28 days after injection. Lipofuscin was detected in cryo
sections of the retina at different time points after subretinal injection by its autofluorescence at 488 nm.
Localisation and shape of microglial cells was checked by CD11b and Iba1 immunohistochemistry (IHC) in cryo sections. IHC was also performed to check the retina for different cytokines and factors, and qPCR of the retina and the RPE/choroid complex for the same purpose. In additional experimental groups, the microglial inhibitors minocycline or the peptide Thr-Lys-Pro (TKP) were applied topically onto the eyes of the mice.

Results: After subretinal injection, local retinal detachment could be demonstrated by OCT. The injected lipofuscin was visible in fundus autofluorescence images. Lipofuscin particles were found in the subretinal space as well as in the outer retinal layers. Lipofuscin was not visible any more later than one week after injection.
Immunoreactivity for IL-6, IL-1β, CXCL1, CCL2 and FGF-2 was increased clearly 7 to 14 days after injection. Analysis by qPCR showed a higher expression of inflammatory cytokines in the RPE/choroid compared to the retina, whereas FGF-2 and VEGF-A expression were higher in the retina. Treatment with microglial inhibitors resulted in a decreased number of microglial cells, a decelerated removal of lipofuscin particles and clearly decreased levels of IL-6 and TNF-a.

Conclusions: There is a quick reaction of the microglia upon subretinal injection of lipofuscin by migration into subretinal space and removal of lipofuscin. Levels of inflammatory cytokines IL-6, IL-1β, CXCL1, CCL2 and FGF-2 are increased. Inflammatory processes connected with enhanced load of lipofuscin may be the major source of stress. The importance of slower lipofuscin scavenging upon microglial inhibition for retinal pathology has to be clarified.

Galectin-3 (Gal-3) as a target for retinal immunomodulation

Mona Tabel, Thomas Langmann

Department of Ophthalmology, Laboratory of Experimental Immunology of the eye, University of Cologne, Cologne, Germany

Background: Age-related macular degeneration (AMD) represents a leading cause of vision loss in the western world. Chronically activated microglia contribute to retinal degeneration and therefor their therapeutic targeting represent a potential treatment option for AMD. Galectin-3 is a pro-inflammatory microglia regulator that is induced upon neuronal damage. Here, we hypothesized that modulation of Galectin-3 via the small molecule inhibitor TD139 dampens mononuclear phagocyte reactivity and protects from retinal degeneration in the murine model of light-induced retinal degeneration.

Methods: BALB/cJ mice received intraperitoneal injections of 15 mg/kg TD139 or vehicle for five consecutive days, starting 1 day prior to exposure to 15,000 lux white light for 1 h. The effect of TD139 treatment on microglia reactivity was analyzed by immunohistochemical staining and *in situ* hybridization (RNAscope) of retinal sections and flat mounts. Spectral domain optical coherence tomography (SD-OCT) was performed to assess retinal thickness and thereby determine the extent of retinal degeneration.

Results: The Aif-hybridization by RNAscope assays in BALB/cJ mice shows the infiltration of microglia in the outer nuclear layer (ONL) and in the subretinal space 4 days post light damage. TD139-treated animals displayed a lower number of microglia in these areas. Iba-1-immunolabeled cryosections of BALB/cJ mice shows amoeboid microglia located in the ONL whereas TD139-treated mice only have ramified microglia in inner- and outer plexiform layer.
Immunohistological analysis of Iba-1-stained retinal flat mounts showed an increase of reactivated amoeboid microglia in BALB/cJ mice. The majority of microglia shows a ramified phenology in TD139-treadted mice. BALB/cJ mice have a thinner ONL. In contrast the ONL thickness of TD139-treated mice is compared to healthy control mice.

Conclusion: The inhibition of Galectin-3 by TD139 reduced the number of chronically activated microglia and preserves retinal thickness. Therefore, the inhibition of Galectin-3 could be a potential treatment option for dry AMD.

Testing of novel PKG inhibitors in two different models for hereditary retinal degeneration

Arianna Tolone[1,3], Frank Schwede[2], Norman Rieger[1], and François Paquet-Durand[1]

[1]Institute for Ophthalmic Research, University of Tübingen, Germany
[2]BIOLOG Life Science Institute Forschungslabor und Biochemica-Vertrieb GmbH, Bremen
[3]Graduate Training Centre of Neuroscience, University of Tübingen, Germany

Background: Inherited retinal degeneration (RD) is one of the major causes of severe visual loss and blindness. One feature that several different forms of RD have in common is an excessive accumulation of cGMP in the cytoplasm of the photoreceptor cell. Previous studies have shown that high cGMP levels cause abnormal activity of two cellular effectors: cGMP-dependent protein kinase (PKG) and cyclic-nucleotide-gated-channels. Hence, the manipulation of these cGMP targets may prevent or slow down the course of the disease. In this study, we evaluated the effects on photoreceptor cell death of second generation cGMP analogues inhibiting PKG in organotypic retinal explant cultures derived from *rd1* and *rd10* mice.

Methods: Eyes were extracted from P5 *rd1* and P9 *rd10* and the retina dissected aseptically. The retina was placed on a culture membrane and incubated in complete R16 medium at 37 °C / 5 % CO_2. After two days of culture, retinas were treated with cGMP analogues inhibiting PKG (DF238, DF247; all at 10µM) while the remaining retinas were incubated in R16 as a control. The cultures were concluded at P11 for *rd1* and P17 for *rd10* and fixed with 4 % PFA followed by incubation with sucrose, embedding, and cryosectioning. The protective capacities of the cGMP analogues were evaluated using TUNEL staining, an assay for cell death detection, on 12 µm-thick retinal cryosections.

Results: Our preliminary results showed that compared to *rd1* untreated (100%), retinas treated with the compounds DF238 and DF247 showed a 17% and 10% reduction of TUNEL positive dying cells in the photoreceptor layer, respectively. In *rd10* retinas treatment with DF238 and DF247 showed a 19 % and 18 % reduction of TUNEL positive cells, respectively, when compared to untreated.

Conclusion: The PKG inhibitors tested thus far did not show clear protective capacities at a concentration of 10µM. However, we observed a numerical reduction of photoreceptor cell death in both *rd1* and *rd10* retinal explants. Future studies may reveal whether PKG inhibitors can be photoreceptor protective at higher concentrations.

Epigenetic modifications associated with cone photoreceptor survival

Dragana Trifunović[1], Marijana Samardzija[2], Klaudija Masarini[1], Christian Grimm[2], Marius Ueffing[1]

[1]Institute for Ophthalmic Research, Department for Ophthalmology, University of Tübingen, Germany.
[2]Lab for Retinal Cell Biology, Department for Ophthalmology, University of Zurich, Switzerland.

Purpose: Cone photoreceptor cell death in inherited retinal degeneration leads to devastating loss of color perception and visual acuity. We have previously shown that epigenetic modifications play major role in mutation-induced cone photoreceptor degeneration. In particular, an increased activity of histone deacetylases (HDACs) seems to be a crucial driver of primary cone degeneration, as a pharmacological inhibition of HDAC activity fully prevents cone loss in the cone-photoreceptor-function-loss (*cpfl1*) mouse (Trifunovic et al., 2016). We further investigated the role of epigenetic chromatin marks on cone survival by analyzing histone H3 lysine methylations (H3K27me^3 and H3K9me^3) following the cone survival afforded by intravitreal injection (IV) of TSA.

Methods: To assess if the TSA treatment affected methylation we performed immunostaining against H3K27me^3 and H3K9me^3 in *cpfl1* animals IV treated with 10nM TSA and compared them to sham-treated as well as with *cpfl1* and wt animals as controls. Co-labeling with cone arrestin was performed to identify cone-specific H3K27 and H3K9 methylation patterns. The neuroprotective properties of the Jumonji H3K27me^3/me^2 demethylase inhibitor GSK-J4, were tested on *cpfl1* retinal explants followed by immunohistological detection of cones. *Cpfl1* retinas were explanted at the onset of degeneration at postnatal day 14 (P14) and the effects of GSK-J4 were assessed by quantifying the number of cones in GSK-J4-treated *vs.* sham-treated explants at PN24, the peak of degeneration.

Results: In wt retinas H3K27me^3 and H3K9me^3 staining was observed in different cells within the INL and the GCL layer, while in the ONL only cones were prominently stained. No cone-specific H3Kme staining was detectable in the *cpfl1* retinas at P24. Similarly, loss of cone-specific staining was also present in sham-treated retinas. In contrast, a single IV treatment of TSA at P14 resulted in a restoration of cone-specific H3K methylations. To confirm that the increased H3K27 methylation is associated with increased cone survival we treated *cpfl1* retinal explants with 10µM GSK-J4, a selective inhibitor of the H3K27 histone demethylases JMJD3 and UTX, from P14 onward. GSK-J4 treatment resulted in a 30 % increase in cone survival at P24 compared to sham-treated controls.

Conclusions: We show that primary cone photoreceptor degeneration is also associated with the loss of cone-specific H3K27 and H3K9 methylation. Pharmacological inhibition of Jumonji H3K27me^3/me^2 demethylase can significantly attenuate hereditary cone photoreceptor degeneration, opening new venues for targeted cone neuroprotection and highlighting the importance of epigenetic modifications in inherited cone dystrophies.

Release of retinal extracellular vesicles in a model of Retinitis Pigmentosa

Lorena Vidal-Gil, Maria Oltra, Jorge M. Barcia, Eberhart Zrenner, Ayse Sahaboglu, Javier Sancho-Pelluz

Purpose: The role of retinal extracellular vesicles (EVs) in blinding diseases, such as retinitis pigmentosa (RP), is far from being understood. Extracellular vesicles (EVs) are released from many types of cells and their cargo – lipids, genetic material, and proteins – makes them critical in cell communication. Previous studies demonstrated that cells under stress modify the release of EVs, possibly promoting changes in other cells. We compared EVs release from retinas of *rd1* mice – a well-known animal model for RP –, and retinas from healthy *wt* mice.

Methods: Fresh *rd1* and *wt* retinas were collected at different time-points: post-natal day (P) 11, 13, and 15 (N = 5). Expression of distinctive EV-membrane proteins (CD9 and CD81) was analyzed by means of immunofluorescence in the different retinal layers. Microscopy was performed by a Zeiss Apotome Microscope and fluorescence intensity measured by Image J software (NIH). Statistical analysis was performed by GraphPad Prism 4.01 software. Retinal organotypical explants from *wt* were cultured, mediums collected, and EVs isolated using qEVcolumns (Izon Science). EVs identity was confirmed by electron microscopy (EM) and by nanoparticle tracking system (NanoSight NS300; Malvern Instruments).

Results: Immunopositivity for CD9 and CD81 showed different protein expression among the different layers (ONL, INL, GCL), ages (P11-P15), and type of animal (*wt* and *rd1*). Although expression of CD9 was significantly increased in GCL compared to ONL at P11 and P13 in *wt*, CD9 significantly decreased at P15 compare to P13 in GCL and INL rd1. Also, CD81 expression was reduced in INL and ONL at P15 in *rd1* compared to corresponding *wt*. Moreover, CD81 immunopositivity increased in GCL P13 compared to P11 and in ONL P15 compared to P11 and P13 in *wt retinae*. Furthermore, isolated EVs from *wt* organotypical cultures presented a 100 nm average diameter and a canonical morphology.

Conclusions: The level of EVs is changes in different layers of retina and in course of retina degeneration suggest that EVs might have different roles in cellular signaling in healthy and retina under degeneration. Interestingly, EVs level changes by the age suggesting their possible role in development of retina. It is important to understand different EVs and their actual cargo in *wt* and *rd1* retinae in order to understand their exact roles in health and disease to develop therapy for retinal diseases.

Unexpected localization of the DNA repair protein Ku80 within the murine retina

Wagner F, Müller B, Laucke L, Rheinhard W, Lorenz B, Stieger K

Department of Ophthalmology, Justus-Liebig University Giessen, Germany

Introduction: Genome editing represents a therapeutic option for inherited retinal degenerations (IRDs) using the cell's own proteins for non-homologous end joining (NHEJ), micro-homology mediated end joining (MMEJ) or homology directed repair (HDR), which are the major pathways for DNA double strand break (DSB) repair. The Ku80 protein is a key player in the NHEJ pathway. This protein recognizes the DSB, protects it against nucleolytic degradation by binding the ends of broken DNA double strands tightly, has catalytic activity and recruits other core NHEJ factors, like DNA-PKcs. In the present study, we examined the expression and localization of Ku80 in the murine retina, since there is almost nothing known about the activity of the DNA repair machinery in photoreceptor or RPE cells, either murine or human.

Methods: Retinae of wild type C57Bl6, LBR2 mice, and of an XLRP mouse model (B6J.SV129-Rpgr^{tm1stie}) were investigated regarding the presence of Ku80. Neuroretina explants were prepared and evaluated after 1 to 8 days in culture by qPCR or immunohistochemistry. Uncultured retinae were used as controls. Total RNA was isolated from whole explants or individual retinal layers after laser microdissection (LMD) and processed for qPCR. For histological investigations, explants where harvested and prepared for cryo-sectioning. Ku80 antibodies were used in combination with markers for photoreceptor proteins and analyzed by confocal microscopy.

Results: Analysis of relative Ku80 gene expression by qPCR showed a constant upregulation by up to three-fold during retinal explant culture compared to uncultured retinae. In wildtype mice, this upregulation was higher compared to the LBR2 mouse model and 9-month-old RPGR mice. Only retinal explants of 3-month-old RPGR mice showed a downregulation from day 2 until day 6. With LMD, we were able to compare the Ku80 expression in the ONL and the inner retina with whole retina samples and found an increasing expression during retinal explant culture, especially in the ONL. Strong Ku80 immunoreactivity was detected in the outer plexiform layer (OPL) of the retina in all mouse lines investigated. No nuclear staining was observed in the outer nuclear layer (ONL). Weak Ku80 immunoreactivity was found in nuclei of the inner nuclear layer of retinal explants at most culture time points. During retinal explant culture, Ku80-positive droplets were released into the ONL to some extent.

Discussion: Direct Ku80 protein detection and upregulated relative Ku80 expression in whole retina preparations as well as in individual retinal layers (ONL) demonstrate the presence of this important protein in retinal neurons. The delocalization of Ku80 in the photoreceptor synaptic terminals in the OPL was rather unexpected and needs further investigation. The knowledge about the DSB repair mechanisms in all retinal neurons is the prerequisite for future genome editing strategies.

Characterization of a novel porcine *in vitro* photoreceptor cultivation suitable as AMD model

Wagner N[1], Gammel M[1], Greulich A[1], Reinehr S[1], Hurst J[2], Schnichels S[2], Dick HB[1], Joachim SC[1]

[1]Experimental Eye Research Institute, University Eye Hospital, Ruhr-University Bochum, Bochum, Germany
[2]University Eye Hospital Tübingen, Centre for Ophthalmology Tübingen, Tübingen, Germany

Purpose: The age-related macular degeneration (AMD) is a multifactorial disease. Suitable models for AMD are limited. Therefore, an established porcine degeneration model has been modified to enable improved photoreceptor cultivation and to make it applicable for AMD research.

Methods: Two methods, namely "filter" and "tweezers", were tested to gain porcine neuroretina explants, with the ganglion cell layer facing down. Retinas were cultivated for 4 days and compared to explants gained via an established method, photoreceptors facing down. To characterize the explants optical coherence tomography (OCT; n = 6/group), H & E staining, immunohistochemistry, and qRT-PCR (n = 4/group) were performed. More specifically, morphology of cones (opsin), rods (rhodopsin), amacrine (calretinin), bipolar (Pkcα) and retinal ganglion cells (RBPMS, *β-III-Tubulin*) was evaluated.

Results: OCT analyses revealed a decrease of retinal thickness to a lower extent in "tweezers" compared to "filter" ($p \leq 0.001$) and "established" method ($p = 0.04$). Moreover, measurements of retinal thickness via H & E staining showed for both new methods a significantly improved photoreceptor structure compared to the established method (filter: $p = 0.002$; tweezers: $p = 0.003$). Additionally, the rhodopsin$^+$ area was significantly increased in the "filter" ($p = 0.0005$) and "tweezers" group ($p = 0.048$) in contrast to the established one. In contrast, the number of cones was only higher in "tweezers" method compared to the established one ($p = 0.035$). The amount of amacrine, bipolar and retinal ganglion cells was unaltered. On mRNA level, we revealed an upregulation of *Rhodopsin* (filter: $p = 0.048$) and *Opsin* (tweezers: $p = 0.045$) in both new methods compared to the established one.

Conclusion: This project aimed to develop a more suitable *in vitro* photoreceptor degeneration model. The cultivation by the "tweezer" method led to a significantly improved morphology, which made it more comparable to the *in vivo* situation. Subsequently, to establish the AMD model a co-cultivation of neuroretina and RPE-cells will follow. Consequently, this system may serve as a new model for AMD drug screening.

Funding: PRO RETINA-Foundation for Prevention of Blindness

Towards a therapeutic genome editing approach as treatment option for juvenile neuronal ceroid lipofuscinosis (CLN3)

Weller M, Klekamp P, Wimmer T, Lorenz B, Stieger K

Department of Ophthalmology, Justus-Liebig-University Giessen

Purpose: Juvenile neuronal ceroid lipofuscinosis (JNCL) is a neurodegenerative disorder caused by mutations in the CLN3 gene. While patients develop seizures and CNS degeneration as teenagers, the earliest sign is severe vision loss starting at about 4-6 years of age. About 80% of patients harbor at least one allele with a deletion between exons 6 and 9 that spans 1.02kB, causing absence of exons 7 and 8. The consequences of this deletion are currently not completely known in terms of residual protein expression and pathogenicity. Genome editing represents a promising emerging field in the treatment of monogenic disorders. It is based on the cells own capacity to restore integrity of the genome following a double strand break (DSB) by either non-homologous end-joining (NHEJ) or homology directed repair in the presence of a template with long (HDR) or short homologous sequences (MMEJ). The aim of this project is to develop a treatment approach for JNCL based on re-inserting the missing 1.02kB DNA sequence into its original locus within the CLN3 gene.

Methods: Eight guideRNA (gRNA) sequences were identified within the human CLN3 and five gRNA sequences within the murine Cln3 genomic region in intron 6 and intron 8 using ATUM (Newark, CA). Target and gRNA oligonucleotides were hybridized and cloned into px459 (Addgene: #62988) and a bioluminescence biosensor (BRET) reporter vector. A HDR/MMEJ shuttle system with a fluorescent reporter was generated to study the HDR and MMEJ frequency. Additionally, synthetic wt-templates containing fused exons 7 and 8 were generated using the GeneArt gene synthesis kit (Thermo Fisher, Dreieich, Germany) and subcloned.

Results: The hybridized DNA fragments were all cloned successfully into the reporter systems and the gRNA/Cas9 vector. For both species, three highly active gRNA/Cas9 complexes in the CLN3/Cln3 intron 6 and 8 were identified and characterized using the BRET biosensor. The synthetic murine wt-templates as well as a fluorescent HDR/MMEJ shuttle system were also successfully generated and cloned.

Conclusions: In this study we completed the first steps towards therapeutic genome editing for CLN3 *in vitro*. With the identified gRNA sequences and the use of wt-templates and a fluorescent reporter system with homologous sequences, we can perform HDR and MMEJ assays in vitro using a murine Cln3deltaEx7/8 cerebellar precursor cell line as well as with a human CLN3deltaEx7/8 cell line derived from pluripotent stem cells.

A Bioluminescence Resonance Energy Transfer (BRET) based reporter as a versatile tool to study endonuclease specificity in genome editing applications

Wimmer T, Bonthu D, Stumpf C, Klekamp P, Moeschl V, Kapygen R, Lorenz B, Stieger K.

Department of Ophthalmology, Justus-Liebig-University Giessen, Germany

Introduction: Genome editing is a promising new approach for treating inherited retinal diseases. Especially the introduction of the easy-to-program RNA guided endonuclease CRISPR/Cas system accelerated the development of therapeutic approaches. However, before translating approaches into the clinics, in vitro reporter systems are necessary to verify the success of the desired strategy in order to overcome major safety aspects. Most commonly used reporter systems for evaluating the cleavage activity of the guideRNA/Cas9 (gRNA/Cas9) complexes are labor intensive, expensive and do not allow high throughput screening as well as an absolute quantification of the effect. The aim of this study was to generate and validate a Bioluminescence Resonance Energy Transfer (BRET) based, episomal reporter system to validate target specificity of gRNA/Cas9 complexes prior to therapeutic application.

Methods: A shuttle cloning box, containing AvrII and BsiWI restriction sites was introduced between the RLuc8 (renilla luciferase) and GFP2 open reading frame in a splice site optimized BRET plasmid. Endonuclease target sequences were hybridized and cloned into the BRET plasmid. Guide RNAs corresponding to the target sequences were also hybridized and cloned into the Cas9 containing px459 (addgene: #62988) under the control of the U6 promotor. Corresponding target and gRNA plasmids were co-transfected and after incubation and cell lysis, BRET ratios were measured as a quotient of the GFP2 and the RLuc8 signal. A reduction of the BRET ratio corresponds to the activity of the gRNA/Cas9 complex. The BRET sensor was compared with a commonly used FACS reporter and validated in terms of specificity and potential off target activity.

Results: The BRET sensor showed a reduction of the BRET Ratio in response to cleavage by the gRNA/Cas9 complex and NHEJ (non homologous end joining) as DNA repair pathway in different cell lines. Specificity of the system was proven by using unrelated gRNAs. The sensor also allows measurements of NHEJ pathway knockouts and target sequence/gRNA mismatch analysis.

Conclusion: We generated an easy to use, time saving and effective BRET sensor for the validation of the activity of endonucleases with a wide spectrum of possible applications used to improve genome editing strategies.

Localization of glucose transporters in the mouse retina and their initial functional characterization

Ming Yang[1,2], Soumyaparna Das[1], Stavros Vagionitis[1], Kangwei Jiao[2], François Paquet-Durand[1,2], Zhulin Hu[2]

[1] Institute for Ophthalmic Research, Eberhard-Karls-Universität Tübingen, Germany.
[2] Kunming Medical University, Kunming, China.

Purpose: Diabetes is a chronic disease characterized by defective insulin-signaling and exceedingly high levels of glucose in the blood stream. Hence, glucose transporters (GLUTs) may play an important role in the disease pathogenesis. One of the most feared consequences of diabetes is diabetic retinopathy (DR) but little is known about the expression of GLUTs in the retina. The purpose of this study is to investigate the retinal expression of GLUT1-4 and to functionally validate this expression using agents selectively transported by individual GLUTs.

Methods: We used wildtype and *rd1* mice to assess the expression of GLUT1-4 in healthy and in degenerating retina. We performed immunofluorescent staining using antibodies directed against GLUT1-4, calbindin, and rhodopsin to find out where GLUT1-4 is expressed. To functionally confirm the expression of GLUT2 in the retina, we used organotypic retinal explant cultures treated with streptozotocin (STZ), an alkylating antineoplastic agent selectively transported by GLUT2. Wildtype mouse retinal explants prepared at post-natal day (P) 5 were exposed to different concentrations of STZ (1.5 to 15 mM) from P7 to 11, followed by a TUNEL assay to label dying cells, calbindin staining to label horizontal cells, and corresponding quantification.

Results: In the mouse retina, we found GLUT1 to be expressed in RPE cells, while GLUT2 was expressed in photoreceptors and in horizontal cells, and GLUT3 was expressed in photoreceptors. GLUT4 expression was found only in retinal blood vessels. Remarkably, in organotypic retinal explant cultures, we found STZ to have a strong toxic effect on photoreceptors, while producing only minor effects on horizontal cells.

Conclusion: Knowledge on the expression of glucose transporters in mouse retina may be crucial to guide future studies on DR. Importantly, GLUT2 was previously only known to be expressed in insulin-producing beta cells and STZ is widely used to abolish insulin production and generate diabetic animals. The direct adverse effects of STZ on photoreceptors of the retina highlight the need for careful evaluation of research results obtained in STZ treated animals.

THIS MEETING IS ORGANISED BY

Franz Badura
PRO RETINA Foundation,
Research Division
Mühlgasse 1 a
92224 Amberg

and

face to face event

face to face event GmbH
Hohenzollernring 72
50672 Köln
Tel: +49 (0) 17 6 / 32 23 66 82
k.bedenk@facetoface-event.de

THIS MEETING IS FUNDED BY

PRO RETINA
POTSDAM-MEETING

PRO RETINA FOUNDATION FOR PREVENTION OF BLINDNESS

www.potsdam-meeting.de

THIS MEETING IS SUPPORTED BY

NOVARTIS