PRO RETINA FOUNDATION

12th PRO RETINA
RESEARCH-COLLOQUIUM POTSDAM

CONFERENCE REPORT

Retinal Degeneration

A Look at Cutting-Edge Translational Research

An Interdisciplinary Dialogue

April 07 / 08, 2017

Seminaris SeeHotel Potsdam

PRO RETINA FOUNDATION

12th PRO RETINA

RESEARCH-COLLOQUIUM POTSDAM

CONFERENCE REPORT

Retinal Degeneration

A Look at Cutting-Edge Translational Research

An Interdisciplinary Dialogue

April 07 / 08, 2017

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 (2017), PRO RETINA Deutschland e. V. counts nearly 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, April 7, 2017

13:00 – 13:05 **Welcome remarks**
Franz Badura, PRO RETINA FOUNDATION / RESEARCH DIVISION

13:05 – 14:30 Session 1 Selected poster presentations
Eight abstracts to be selected

14:30 – 15:00 **Keynote lecture**
Michael Brand, Dresden
Learning from the zebrafish: Regeneration of the retina is possible – but how does it work?

15:00 – 15:45 Coffee break

15:45 – 17:25 Session 2 Modifying disease processes

15:45 – 16:10 Stephen H. Tsang, New York
Personalized therapeutic strategies for patients with retinitis pigmentosa

16:10 – 16:35 Sandro Banfi, Naples
The role of microRNAs in the human retina

16:35 – 17:00 Bernhard G. Herrmann, Berlin
Long non-coding RNA in development and disease

17:00 – 17:25 Francois Paquet-Durand, Tübingen
Targeting cGMP-signaling in inherited retinal degeneration

17:30 Dinner

19:00 – 20:00 Session 3 Of men and mice in science

19:00 – 19:30 Eberhard Zrenner, Tübingen
Hereditary retinal disorders: A pioneering field in biological research

19:30 – 20:00 Wolfgang Baehr, Salt Lake City
The rd mouse – a long story

20:00 – open Swingin' poster session

PROGRAMME

Saturday, April 8, 2017

09:00 – 10:40	**Session 4**	**An update on disease mechanisms**
	09:00 – 09:25	Peter Zipfel, Jena *12 years after: The search for complement inhibitors in AMD*
	09:25 – 09:50	Florian Sennlaub, Paris *Complement Factor H controls macrophage life-span*
	09:50 – 10:15	Tim Krohne, Bonn *The NLRP3 inflammasome – new therapeutic target in macular degeneration*
	10:15 – 10:40	Najate Ait-Ali, Paris *Mode of action of RdCVF and its origin*
10:40 – 11:15		Coffee break
11:15 – 12:55	**Session 5**	**Innovative approaches to translation**
	11:15 – 11:40	Dr. Sebastian Bultmann, München *Visualization of specific DNA sequences in living mouse embryonic stem cells with a programmable fluorescent CRISPR/Cas system*
	11:40 – 12:05	Elly Tanaka, Wien *Regeneration of complex multi-tissue structures*
	12:05 – 12:30	Kathleen C. Keough, San Francisco *CRISPR/Cas9 genome surgery to eliminate dominant negative disease*
	12:30 – 12:55	Morgan Maeder, Cambridge Massachusetts *Envisioning a gene editing approach to treat inherited blindness*
12:55 – 13:00		**Concluding remarks**
13:00		Lunch and end of meeting

Curriculum Vitae

Prof. Dr. med. Dr. med. h.c. mult. Eberhart Zrenner

A. Personal

Distinguished Professor of Ophthalmology
Centre for Ophthalmology, Institute for Ophthalmic Research
Schleichstr. 12-16 D-72076 Tuebingen
Tel.: +49 7071-29 84786
Fax: +49 7071-29 50 38
e-mail: ezrenner@uni-tuebingen.de
http://www.eye.uni-tuebingen.de/zrenner

Born Oct. 18, 1945
Married, 3 children

B. Qualifications

University of Erlangen and Munich	**Student**	1966 – 72	Medicine (Approbation as Physician 1973) and in Parallel 4 Semesters of Electronic Engineering
Technical University of Munich	**MD**	1973	Medicine (Information Transfer in the Eye) (Summa cum Laude)
Max-Planck-Institute for Physiol. and Clin. Research	**Post Doc**	1973 – 74	Sensory Physiology
National Inst. of Health, USA	**Fogarty fellowship**	1977 – 78	Neurophysiology
University of Giessen	**Habilitation (PD)**	1981	Cell Physiology
University of Munich	**Ophthalmol. board exam**	1985	Ophthalmology

C. Academic Positions

1973	Intership in Surgery and Internal Medicine, Munich
1974 – 1976	Research Fellow at the Max-Planck-Institute for Physiological and Clinical Research, Bad Nauheim, Dept. of Experimental Ophthalmology, and Eye Hospital in Frankfurt and Munich

1977 – 1978	Fogarty International Fellow, NIH, Laboratory of Vision Research
1979 – 1984	Experimental Ophthalmology at the Max-Planck-Institute for Physiological and Clinical Research, Bad Nauheim
1985 – 1989	Associate Professor and Head of a Research Unit of the Max-Planck-Society at the University Eye Hospital, Munich (Prof. Lund) ; Hon. Prof. Univ. of Giessen
1989	Full Professor of Ophthalmology, University of Tuebingen

D. Administrative Positions

1991 – 2007	Executive Director of the University Eye Hospital Tübingen
1992	Offer of Full Prof. at Aston-University, Birmingham, England (declined)
1993	Offer of Head of Dept. Ophthal., McGill Univ. Montreal Canada (declined)
1994 – 1995	Dean of the Medical Faculty of the University of Tuebingen
Since 1995	Coordination of a Consortium "Subretinal Implants"
1998 – 2000	Dean of the Medical Faculty of the University of Tuebingen
Since 2005	Chairman of the European Vision Institute
2007 – 2011	Head of the Strategy Committee of the Medical Faculty, Tuebingen
2007 – 2011	Director of the Centre for Ophthalmology, together with Prof. K.U. Bartz-Schmidt
2007 –	Professor at the Institute for Ophthalmic Research, and Head of Pathophysiology of Vision, University of Tuebingen
2011 –	Chairman of the Center for Neurosensory Systems (ZfN), University of Tuebingen
2013 –	Distinguished Professor at the Center for Integrative Neuroscience (CIN Center of Excellence), University of Tuebingen

E. Honors and Awards

1973	Dissertation Award for an Experimental Study on the Optic Qualities of the Eye
1976	Fogarty Fellowship Award
1979	Franceschetti-Liebrecht-Award of the German Ophthalmological Society
1984	NATO travel award, cooperation with the University of Cambridge, UK
1995	Alcon Research Award (100.000$)
1998	Memorial Medal of Charles University, Prague
1998	Election as Member of the "Heidelberger Akademie der Wissenschaften"
1999	Election as Member of the „German Academy of Sciences, Leopoldina"
1999	MacKenzie Memorial Award
2000	von Graefe-Award of the German Ophthalmological Society
2002	Order of the Federal Republic of Germany, "Bundesverdienstkreuz am Bande"

2006	"Bartimaeus Award" of the Detroit Institute of Ophthalmology awarded at the World Congress of Artificial Vision 2006
2007	Emiko Adachi-Award of the International Society for Clinical Electrophysiology of Vision (ISCEV)
2009	Karl Heinz Beckurts Award
2009	Claire Jung Preis
2010	Pfizer-Award for Visiting Professorship an der State University New York
2010	Retina International: Special Recognition Award
2011	Edridge Green Award
2011	EURETINA Innovation Award
2011	Honorary doctorate, Naresuan University, Thailand
2012	Honorary doctorate Medical University of Lublin, Poland
2013	Hong Leong Visiting Professorship National University of Singapore
2013	Hector Award, Hector Foundation, Weinheim
2014	Reinhard von Koenig Award
2015	Gold Medal of the Saudi Ophthalmological Society
2016	Albrecht von Graefe Medaille

F. Scientific expertise

Physiology and pathophysiology of the visual system; neuro-ophthalmology, functional and neuropharmacological studies in vivo; single cell recording from the mammalian retina for studies on structure and function of the retina, psychophysics, clinical electrophysiology, colour vision, hereditary retinal degeneration, non-invasive techniques of functional diagnostics in ophthalmology; ocular toxicology; subretinal implants.

Curriculum Vitae
Wolfgang Baehr, Ph. D.

A. Personal

Citizenship: USA
Mailing Address: Department of Ophthalmology and Visual Sciences
John A. Moran Eye Center
University of Utah Health Science Center
65 Mario Capecchi Dr.
Salt Lake City, UT 84132-5330
Telephone: 801-585-6643 (office)
Fax: 801-585-1515 (office)
Telephone: 801-585-1482 (laboratory)
Telephone: 801-359-3173 (home)
E-mail: wbaehr@hsc.utah.edu

B. Education

1961 – 1967	Undergraduate education, University of Heidelberg, Heidelberg, Germany Diploma (MS) in Organic Chemistry, Inorganic Chemistry and Physical Chemistry
1967 – 1970	PhD Thesis (Dr. rer. nat.) in Organic Chemistry University of Heidelberg, Germany (summa cum laude). Thesis Advisor: Dr. Klaus Weinges Title of Thesis: Isolation and Structure of Bilobalid A, a Novel Sesquiterpenoid from *Ginkgo biloba L.*
1971 – 1975	Postdoctoral Fellow. Department of Molecular Biology, Max-Planck-Institut for Biophysical Chemistry, Göttingen, Germany (Advisor: Dr. Thomas Jovin)

C. Personal Statement

My education is in organic chemistry, molecular biology, biophysics and enzymology. After my Ph.D. in chemistry, I switched to molecular biology and studied initiation of transcription and enzyme kinetics at the Max-Planck Institute for Biophysical Chemistry in Goettingen, Germany. Since coming to the United States in 1976, I have been involved in retina research. The main topics of research were photoreceptor biochemistry and molecular biology with focus on phototransduction in rods and cones, the retinoid cycle, and more recently, membrane protein transport with molecular motors and photoreceptor ciliogenesis. Since joining the faculty at the Moran Eye Center (1995), my lab has generated numerous transgenic and

knockout models focusing on regulation of phototransduction and the retinoid cycle, cone-rod dystrophies and retintis pigmentosa, and AAV-based gene therapy of dominant and recessive retina dystrophies. I enjoy working with students. I published or co-authored over 200 peer-reviewed articles and reviews, most of which cover topics in retina research.

D. Positions and Honors

Positions and Employment

1975 – 1982	Assistant Research Scientist, Dept. of Biochemistry, Princeton University, Princeton, NJ 08540
1982 – 1986	Associate Research Scientist, Dept. of Biology, Purdue University, W. Lafayette, IN 47907
1986 – 1987	Senior Staff Fellow, National Institutes of Allergy and Infectious Diseases, Laboratory of Microbial Structure and Function, Hamilton, MT 59840
1987 – 1991	Assistant Professor, Dept. of Ophthalmology, Baylor College of Medicine, Houston, TX 77030
1988 – 1995	Jules and Doris Stein Research to Prevent Blindness Professor, Baylor College of Medicine, Houston, TX 77030
1991 – 1994	Associate Professor, Dept. of Ophthalmology, Baylor College of Medicine, Houston, TX 77030
1994 – 1995	Professor, Departments of Ophthalmology, Biochemistry, Molecular Physiology and Biophysics, and Neuroscience, Baylor College of Medicine, Houston, TX 77030
1995 –	Professor, Department of Ophthalmology, University of Utah, Salt Lake City, UT 84132
2001 –	Adjunct Professor, Department of Neurobiology and Anatomy, University of Utah
2001 –	Adjunct Professor, Department of Biology, University of Utah
2015 –	Director of Research, Moran Eye Center

Other Experience and Professional Memberships

1989 – pres.	Association for Research in Vision and Ophthalmology (ARVO)
1989 – pres.	American Association for the Advancement of Science (AAAS)
1990 – pres.	American Society for Biochemistry and Molecular Biology (ASBMB)
2008 – pres.	Society for Neuroscience (SfN)
2003 – 2006	Study section, BDPE (formerly VisC), permanent member
2009	Study section, MIST, 2009, *ad hoc* reviewer
2012	Study section, Biology of the Visual System (BVS) study section, *ad hoc* reviewer, 2012
2013	K99 Study Section
2014	June 5, 2014. K99 Study Section

2015	July 2015 R24 Study Section Bethesda MD
2016	July 2016 R24 Study Section Bethesda MD

Honors

1988 – 1995	Research to Prevent Blindness Jules and Doris Stein Professorship
2000 –	Ralph and Mary Tuck Professor of Ophthalmology, Department of Ophthalmology, University of Utah, Salt Lake City
2002	RPB Senior Investigator Award
2013	ALCON Research Award
2014	ARVO Proctor Medal
2014	RPB Nelson Trust Award

Patents and inventions

1994 Generation of 661W cone-derived cell lines (Dr. Muayyad Al-Ubaidi and Dr. Wolfgang Baehr, Baylor College of Medicine, Houston TX).

PRO RETINA
POTSDAM-MEETING

SPEAKER ABSTRACTS

The role of microRNAs in the retina

Sandro Banfi

Department of Biochemistry, Biophysics and General Pathology, University of Campania "L. Vanvitelli", Naples and Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

MicroRNAs (miRNAs) are a class of small, endogenous RNAs that negatively regulate gene expression post-transcriptionally by binding to target sites in the 3′ untranslated region of messenger RNAs. Although they have been found to regulate developmental and physiological processes in several organs and tissues, their role in retinal function is still largely unknown. To gain more insight into this issue, we first generated a comprehensive expression atlas of miRNAs significantly expressed in the mouse eye by means of RNA *in situ* hybridization and then we determined their expression profiles in the human retina by Next Generation Sequencing procedures. The latter analysis allowed us to a) establish the catalogue of retina-expressed miRNAs and determine their relative abundance. Moreover, we discovered a vast diversity of miRNA variants (isomiRs), encompassing a wide range of sequence variations including seed modifications that are predicted to have an impact on miRNA action. In parallel, we have started a characterization of the functional role of miR-204, which is among the most highly expressed miRNAs in several eye structures including retina and retinal pigment epithelium. We demonstrated, by means of both *in vitro* and *in vivo* studies, that miR-204 regulates multiple aspects of eye development and function and identified some of the most relevant target genes. Finally, we found that a point mutation in miR-204 is responsible for an autosomal dominantly inherited form of retinal dystrophy and bilateral coloboma through a gain-of-function mechanism. These data provide the first example of a microRNA with a pathogenic role in inherited retinal dystrophies.

Targeting cGMP-signalling in inherited retinal degeneration: From basic research to clinical translation

François Paquet-Durand, on behalf of the DRUGSFORD consortium

Cell Death Mechanism Laboratory, University of Tübingen, Tübingen, Germany

Background: Hereditary retinal degeneration (RD) relates to a group of currently untreatable neurodegenerative diseases affecting photoreceptors. Major challenges for RD therapy development include retinal drug delivery and the identification of common targets that can address the large genetic heterogeneity. RD-causing mutations often lead to a dysregulation of cyclic guanosine monophosphate (cGMP), making cGMP-signalling a prime target for the development of new pharmacological approaches. The recently ended EU-funded DRUGSFORD project (www.drugsford.eu) generated a large number of new cGMP analogues to efficiently bind and inhibit cGMP effectors, *i. e.* protein kinase G (PKG) and the cyclic nucleotide gated channel (CNGC). To facilitate transfer of cGMP analogues across the blood-retinal-barrier these were combined with an innovative liposomal drug delivery system.

Results overview: The screening of 250 cGMP analogues in test systems of rising complexity (cell-free assays, cell cultures, retinal tissue cultures, *in vivo*) led to the discovery of a first lead compound called DF003. Using a variety of *in vitro* techniques, DF003 and its liposomal formulation LP-DF003 was shown to efficiently inhibit both PKG and CNGC. LP-DF003 significantly increased photoreceptor viability in the *rd1, rd2, rd10,* and *cpfl1* animal models for RD. In *rd2* and in *rd10* animals increased viability was accompanied by a significant and marked improvement of *in vivo* retinal function. *In vitro* and *in vivo* studies found no signs of toxicity for DF003 or LP-DF003. Likewise, while *in silico* toxicity simulations (DEREK Nexus, Leadscope) revealed an expected, class-typical possibility for clastogenicity, they did not indicate any major organ toxicity. An exploratory *in vivo* safety and tolerability study in non-human primates, using concentrations of up to 100-fold of the estimated human therapeutic dose, indicated no major DF003 or LP-DF003 related adverse effects. Furthermore, a new chemical synthesis process was developed for DF003, allowing efficient production scale-up according to the standards of Good Manufacturing Practice (GMP).

Conclusion: With cGMP analogues, we have introduced a new class of compounds for RD treatment that was inventively combined with an efficient retinal delivery method. With the establishment of good manufacturing practice production and the successful preclinical efficacy as well as safety and tolerability testing, LP-DF003 is now ready to enter clinical trials. The commercialisation of this approach is supported by the filing of three patents for cGMP analogues and their formulation, and an orphan drug designation (ODD) awarded by the European Medicines Agency (EU/3/15/1462). Together, this provides a clear perspective for a clinical translation and could bring the very first pharmacological treatment to patients suffering from RD.

Mode of action of RdCVF and its origin

Najate Ait-Ali

Paris

RdCVF is a translation product made from an unspliced mRNA encoding the exon 1 with an in-frame stop codon of the Nucleoredoxin-like gene *(Nxnl1)* and corresponds to a truncated thioredoxin-like protein with no thiol-oxidoreductase activity. The other product RdCVFL, made by splicing of the *Nxnl1* gene, is an active thioredoxin enzyme that contains the thioredoxin fold as well as the catalytic CXXC domain. RdCVF has expressed by rod and maintained cone photoreceptor alive by stimulating aerobic glycolysis in cones via basigin-1 (BSG1), its cell surface receptor and the glucose transporter GLUT1 (Aït-Ali et al; ***Cell.*** 2015). Downstream of that metabolic signaling, RdCVFL is involved in redox homeostasis. Hence, the two products of the *Nxnl1* gene are involved in the protection of cones through two distinct mechanisms. Cones evolved earlier than rods, the latter appeared during evolution encouraging the reduction in energy consumption. Is this corresponds to the apparition of the inhibition of the Nxnl1 gene's splicing resulting in it's gain of function by production of the trophic factor RdCVF? Or the intron retention of the *Nxnl1* gene precede that event, a case of molecular exaptation? Our goal is to investigate the molecular mechanisms leading to the *NXNL1* gene's alternative splicing, in order to understand if the process occurred in the ancestral gene or appeared during evolution.

Visualization, modulation and modification of (epi-)genetic information by programmable CRISPR/Cas systems

Dr. Sebastian Bultmann

Biozentrum Ludwig Maximilians University Munich, Großhadernerstr. 2, 82152 Planegg-Martinsried

All cell types of multicellular organisms arise from a single, totipotent precursor. While possessing markedly distinct functions and behaviors, each cell type contains the same genetic makeup. The immense diversity of cell types is generated and controlled by a complex interplay between spatial organization of genetic material within the nucleus, epigenetic modulation of transcription and the action of lineage specific factors. To uncover the links between these pathways, we have recently developed CRISPR/Cas-based tools tailored to highlight individual components of this network. Using catalytically inactive Cas9 proteins as a site-specific recruitment platform, we are able to visualize genetic material in living cells as well as modulate their epigenetic information. Furthermore, by combining CRISPR/Cas-assisted gene-editing with site-specific recombination, we can efficiently and systematically study dynamic cellular processes on physiological level. Thus, our approaches further expand the CRISPR/Cas toolbox for the systematic analysis of complex networks with modular and easy-to-use systems.

Nervous system regeneration is possible in a vertebrate

Elly M. Tanaka

Research Institute of Molecular Pathology (IMP), Vienna Biocenter (VBC), Vienna, Austria

We study CNS regeneration in the salamander, which is able to replace all of the neural populations and axonal connections after spinal cord injury. In some salamanders, regeneration of the retina is possible from the RPE. We have been studying the neural stem cells that undergo regeneration and identifying the injury related signals induce them to replace the missing CNS.

CRISPR/Cas9 genome surgery to eliminate dominant negative disease

Keough KC[1,2], Liu AZ[2], Mayerl SJ[2], Moore AT[1], So PL[2], Pollard KS[1,2], Conklin BR[1,2]*

[1]UCSF
[2]Gladstone Institutes

**Corresponding author*

The advent of CRISPR/Cas9 targeted genome editing has renewed hope for curing genetic disease. Double-stranded DNA cuts made by this genome editing system generally lead to DNA repair via one of two pathways: homology-directed repair (HDR) or non-homologous end joining (NHEJ). In general, NHEJ is more active than HDR [1]. This work aims to direct NHEJ towards treatment of dominant negative genetic diseases, such as Best disease, where the disease allele actively interferes with the normally functioning allele.

Best disease is an autosomal dominant progressive macular dystrophy which leads to severe visual impairment [2]. Using CRISPR/Cas9, we aim to perform genetic surgery to inactivate the disease allele of the causative gene, BEST1, to allow unimpeded function of the healthy allele. With >200 different mutations in BEST1 causing Best disease, it is challenging to target Cas9 to each individual mutation; instead we will target the disease allele using common heterozygous DNA polymorphisms (SNPs and indels) identified through bioinformatics analyses of the BEST1 locus in 1000 Genomes data [3].

This talk will present an approach to scoring and experimentally verifying cut sites in the BEST1 locus towards curing Best disease. Findings include ideal predicted cut sites, construction of a BEST1-fluorophore based high-throughput editing assay, and current progress in allele-specific editing of this locus in Best disease cell lines.

1. Miyaoka Y…Conklin BR. (2016) Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing. Sci Rep. Mar 31;6:23549.
2. Yang, T…Tsang, S. H. (2015). BEST1: the Best Target for Gene and Cell Therapies. *Molecular Therapy: The Journal of the American Society of Gene Therapy, 23*(12), 1805 – 9.
3. Auton, A…Schloss, J. A. (2015). A global reference for human genetic variation. *Nature*, 526(7571), 68 – 74.

Envisioning a gene editing approach to treat inherited blindness

Morgan Maeder

Cambridge Massachusetts

Recent successes in clinical trials highlight the potential to treat genetic retinal disorders using viral gene delivery. While subretinal injection of adeno-associated viruses (AAVs) encoding transgenes has been shown to be safe and efficacious, there are many inherited retinal dystrophies that are not amenable to gene augmentation approaches. Gene editing may be capable of addressing inherited forms of blindness resulting from mutations in genes that exceed the packaging size limit of AAV. In addition, mutations in genes that are toxic when overexpressed or must be regulated by their endogenous control elements, as well as autosomal dominant mutations where removal of the dominant allele is required to produce a wild-type phenotype, may also be well-suited to gene editing approaches. The *CEP290* gene, mutations in which account for ~20% of Leber Congenital Amaurosis, far exceeds the packaging limit of AAV and has been suggested to be toxic when overexpressed in retinal cells. The most common mutation in *CEP290* is an intronic point mutation that creates an aberrant splice site, resulting in a premature stop codon. We have developed a gene editing approach, using the CRISPR/Cas9 system, to delete this disease-causing, intronic mutation. Using a dual-guide RNA approach, we demonstrate that targeted deletions result in increased expression of wild-type *CEP290* transcripts and decreased expression of mutant transcripts, as well as increased expression of wild-type CEP290 protein. We have also established improvements and modifications to the CRISPR/Cas9 technology that may allow for alternative targeting strategies which may be used to develop CRISPR-based gene editing approaches for a broad range of currently untreatable, inherited forms of blindness.

PRO RETINA
POTSDAM-MEETING

POSTER ABSTRACTS

The role of the presence of hyperreflective foci in the progression of early to late age-related macular degeneration

Lebriz Altay[1], Vasilena Sitnilska[1], Eveline Kersten[2], Tina Schick[1], Philip Enders[1], Carel Hoyng[2], Anneke den Hollander[2], Thomas Langmann[3], Sascha Fauser[1]

[1]Department of Ophthalmology, University Hospital of Cologne, Cologne, Germany
[2]Department of Ophthalmology, Radboud university medical center, Nijmegen, The Netherlands
[3]Department of Ophthalmology, Experimental Immunology of the Eye, Cologne, Germany

Purpose: To evaluate the role of hyperreflective foci in the conversion of early to late age-related macular degeneration (AMD) including genetic, environmental and phenotypic risk factors (RF).

Methods: This case-control study included 123 subjects out of 337 early/intermediate AMD patients from European genetic database (EUGENDA) with minimum 5 years follow-up. AMD-Staging and phenotypic characteristics were based on color fundus photographs and spectral-domain optical coherence tomography (SD-OCT). Logistic regression analysis for progression to advanced AMD was performed separately for environmental, phenotypic and major genetic RFs (*CFH rs1061170* and *ARMS2 rs10490924*). The conducted parsimonious prediction model was validated using receiving operating characteristics and Hosmer-Lemeshow test.

Results: During a mean follow-up time of 5.59 years 30 patients (24.4 %) developed late AMD. Age (Odds Ratio (OR): 1.10, p = 0.004, 95 % Confidence Interval (95 % CI): 1.03-1.18), *CFH* (OR: 4.20, p = 0.001, 95 % CI: 1.80-9.76), drusenoid pigment epithelial detachment (dPED, OR: 20.25, $p = 1.93 \times 10^{-7}$, 95 % CI: 6.53-62.86), hyperreflective foci (HF) (OR: 89.97, p = 2.20 x 10-6, 95 % CI: 13.97-579.62), central location of HF (OR: 11.81, p = 0.002, 95 % CI: 2.49-56.11), central drusen location (OR: 10.83, $p = 8.70 \times 10^{-5}$, 95 % CI: 3.30-35.60), reticular drusen (OR: 6.25, p = 0.002, 95 % CI: 2.02-19.40) and pigment abnormalities (OR: 9.14, $p = 3.85 \times 10^{-5}$, 95 % CI: 3.19-26.21) were independently associated with AMD-progression. The prediction model including age, *CFH*, dPED and HF had the highest prognostic value for transition to late AMD (area under the curve: 0.935) and showed an adequate calibration (Hosmer-Lemeshow test chi-squared = 2.74, p = 0.950).

Conclusions: Patients with simultaneous occurrence of HF and dPED in SD-OCT have an increased likelihood of progression to late AMD during 5 years. In those cases intensified monitoring seems advisable to detect conversion and treat as early as possible.

Impact of *in vitro* pharmacological inhibition of p97/VCP on the survival of photoreceptors cells in Retinitis Pigmentosa

Blanca Arango-Gonzalez[1], Merve Sen[1], Tsui-Fen Chou[2], Ray Deshaies[3], Marius Ueffing[1]

[1]Institute for Ophthalmic Research, Centre for Ophthalmology, Eberhard Karls Universität Tübingen, Tübingen, Germany
[2]Division of Medical Genetics, Department of Pediatrics, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute , Torrance, California, United States
[3]Howard Hughes Medical Institute, Division of Biology and Biological Engineering, Caltech, California, United States

Numerous human diseases, including inherited retinal degenerations, arise from defects in protein homeostasis. Retinitis Pigmentosa (RP) is one of the degenerative diseases in humans that is primarily characterized by progressive loss of photoreceptor neurons leading to blindness. Recent evidence suggest that inactivation of p97/VCP suppresses retinal degeneration in *Drosophila*. Our approach is to search for effects of manipulation of protein homeostasis by small molecule inhibitors of p97/VCP on the survival of photoreceptor cells in animal models for RP.

We have carried out a protocol in which retinal organ cultures are sustained in serum-free medium. We cultivated retinas from PN9 Rho^{P23H} rats for 6 days until the peak of degeneration at PN15 was achieved as well as WT retinas (Crl:CD(SD) rats, PN9DIV6). We tested the effect of two chemically unrelated, validated p97/VCP inhibitors: Eeyarestatin I and ML240. Cell death was assessed by TUNEL assay and photoreceptor cell survival by counting the number of remaining photoreceptor cell rows. In order to determine effects of p97/VCP inhibition on signal transduction we performed immunostaining using antibodies against rhodopsin, transducin, PDE6β, arrestin and recoverin.

Expression of the Rho^{P23H} in organ cultures results in enhanced photoreceptor cell death and regression of the ONL, resembling the process of retinal degeneration *in vivo*. Both p97/VCP inhibitors significantly reduced the number of TUNEL positive cells and increased the average number of cell rows in the ONL in Rho^{P23H} rats. Moreover, treatment with these inhibitors partially restored distribution of phototransduction proteins in mutant retinas. On the other hand, ML240 significantly reduced the number of dying cells in WT retinas.

p97/VCP inhibitors showed a neuroprotective effect on Rho^{P23H} and WT cultured retinas, and influenced protein homeostasis in visual signal transduction suggesting that the compounds may provide a new treatment option for retinitis pigmentosa. Identification of the impact of *in vitro* pharmacological inhibition of VCP/p97 will provide invaluable insights into underlying mechanism of the retinal degenerations and will potentially open new avenues for treatment.

A new multiplex assay for the analysis of angiogenic regulators in the vitreous of patients with diabetic retinopathy

Sui Ping Ong[1], Kelvin Teo[2]*, Marcus Ang[3]**, Tanja Endermann[4]**, Pearline Teo[1], Verena Arndt[4], Anna Tan[2], Tien Yin Wong[2], Nobuyo Yawata[3,5,6]***& Markus Zumbansen[4]****

[1]AYOXXA Living Health Technologies Pte. Ltd, Singapore
[2]Singapore National Eye Centre, Singapore
[3]Singapore Eye Research Institute, Singapore
[4]AYOXXA Biosystems GmbH, Cologne, Germany
[5]Ophthalmology and Visual Sciences Academic Clinical Program, Duke-NUS Medical School, Singapore
[6]Singapore Institute for Clinical Sciences (SICS), Agency for Science, Technology and Research, A*STAR, Singapore

*,**,*** These authors contributed equally.

Retinal angiogenic diseases (RAD) are characterized by the growth of abnormal new blood vessels in the eye. Vascular endothelial growth factor (VEGF) and more recently also inflammatory cytokines and chemokines have been identified as key players in disease development and progression. As such, these factors may not only represent therapeutic targets but also effective biomarkers for patient stratification or monitoring. The analysis of protein signatures from vitreous humour in the clinical setting is, however, limited by the low volume of samples and the non-negligible risk of retinal detachment. In this study we validated a novel bead-based protein microarray platform for simultaneous analysis of IL-1β, IL-2, IL-6, IL-8, TNFα, VEGF-A from only 5 µl of vitreous humour. The new multiplex immunoassay showed good assay performance with comparative results to a previously used multiplex assay which utilises higher sample volumes. In addition, quantitative measurement of intravitreal cytokines in diabetic retinopathy corresponded well to reported cytokine levels in vitreous humour as well as to respective patient classifications. In summary, the presented multiplex immunoassay could provide a new tool to analyse protein biomarker signatures in ocular fluids.

Neuroinflammation is critically required as a cue for regeneration of the adult zebrafish retina

Oliver Bludau, Veronika Kuscha, Anke Weber, Michael Brand

TU Dresden, CRTD – DFG-Center for Regenerative Therapies Dresden – Cluster of Excellence, and Biotechnology Center, Fetscherstraße 105, 01307 Dresden, Germany

Purpose: Neuroinflammation in mammalian vertebrates is accompanied by gliosis, glial scarring and secondary loss of neurons. In disease, chronic inflammation leads to a progressive neuronal loss causing an aggravation of diagnostic findings. In contrast to mammals, in zebrafish neuroinflammation is crucially important and sufficient to stimulate a regenerative response after traumatic brain injury. Here we investigate the immune system activation during zebrafish retina regeneration to understand its dynamic role and contribution to photoreceptor regeneration.

Methods: To analyse whether leukocytes are activated following sterile photoreceptor ablation, we used transgenic zebrafish reporter lines for microglia, neutrophils and T-cells in combination with immunohistochemical staining. To study the effects of immune suppression, animals were immersed in dexamethasone. Subsequently, we ablated the photoreceptors and investigated possible changes in regeneration. To this end, we quantified the numbers of leucocytes and proliferating Müller Glia cells, as well as the overall number of proliferating cells. In addition, we analysed the expression pattern of matrix metalloproteinase 9 *(mmp9)*, a novel marker that we found to be upregulated in Müller Glia shortly after ablation of photoreceptor.

Results: After injury, leukocytes accumulate at the site of lesion. Specifically, microglia leave unlesioned areas and populate the lesion area. In addition, neutrophils enter the retina and form neutrophil extracellular trap-like structures at the retinal-pigmented epithelium/photoreceptor outer segment layer. Notably, we do not observe T-Cell accumulation at the lesion site. Upon immunosuppression, the number of microglia decreased in the regenerating retina. Furthermore, we found that the number of Müller Glia re-entering cell cycle to initiate regeneration, as well as overall proliferation, is significantly reduced following immunosuppression. In addition, the expression level of *mmp9* was decreased, as shown by in situ hybridisation. After long-term dexamethasone treatment (38 days) we observed almost complete ablation of leukocytes within the retina as a further consequence of immunosuppression.

Conclusions: We conclude that neuroinflammation occurs in the zebrafish retina following photoreceptor ablation, as seen by activation of microglia and attraction of neutrophils. Moreover, we provide the first evidence to demonstrate a beneficial effect of neuroinflammation during the initial steps of the photoreceptor regeneration program, in particular for proliferation of Müller Glia and the generation of neuronal progenitor cells.

Common eye phenotypes in the elderly: Data from the German AugUR study

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

Background: In Germany and central Europe, a lack of epidemiological data on common eye diseases in the elderly and their treatment has been recognized. However, knowledge about the general ophthalmologic health status and information on prevalence, incidence, and risk factors for disease onset and progression could establish important determinants for patient health care, health service research, the development of prevention- and treatment options, as well as science coordination.

Methods/Design: The German AugUR study (Age-related diseases: understanding genetic and non-genetic influences – a study at the University of Regensburg), is a prospective study in the mobile elderly population of Caucasian ethnicity in and around the city of Regensburg, Bavaria. With its main focus on eye phenotypes, the study program includes a standardized interview-based questionnaire regarding subjective vision status, existing self-reported diagnoses of common eye diseases as well as treatment history. Extensive ophthalmological examinations include testing of retinal function (e.g. visual acuity) as well as retinal imaging (e.g. standardized color fundus photography).

Results: During the period between 2013 and 2015, a total of 1,133 individuals were included in the AugUR baseline survey. Of those, 97.3 % (n = 1,102) participants reported the use of eye glasses either for reading, distance or both and 68.8 % (n = 495, 719 with available data) had a good visual acuity of at least 0.10 logMAR (better eye, with own refractive correction if applicable). Importantly, only 1.3 % (n = 15) of all study participants stated to have low vision equipment (e.g. magnifiers, screen-reading devices). On the other hand, 3.6 % (n = 39, 1,098 with available data) claimed to be unable to read the newspaper (despite appropriate refractive correction) and 5.3 % (n = 58, 1,086 with available data) described their difficulties to recognize people when passing them on the streets.
Possible reasons for visual impairment, as assessed via the interview-based questionnaire, were the following: About half of the study sample (49.0 %, n = 555) reported to have cataract, 69.9 % of these had already undergone cataract surgery (n = 391; 34.5 % of overall study participants). A total of 7.3 % (n = 83) stated to suffer from glaucoma, 1.2 % (n = 14) from diabetic retinopathy and 27.9 % (n = 316) from other eye diseases such as dry eye syndrome.
Remarkably, the self-report of age related macular degeneration (AMD) raised concerns as one third of those participants with late AMD diagnosed on color fundus images (n = 63) were

unaware of having AMD when being asked in the standardized interview (24 of 63 individuals). By validating AMD diagnosis with the treating ophthalmologist, 6 participants (10 % of participants with late AMD) were detected with "latent", i.e. undiagnosed, AMD.

Discussion: AugUR is the first study to provide epidemiological information on general ophthalmological conditions in the elderly German population. Of note, our survey is not representative for the total elderly population, but for the mobile elderly, willing and able to visit our study centre and to undergo the study program of approximately 2.5 hours duration. Our data indicate an undersupply with low vision equipment – patient organizations need to be aware that better information on the existing possibilities of visual aids is required. Importantly, the reasons for participants not being aware of their late AMD diagnosis need to be evaluated further.

Chemical compound screening in Best Vitelliforme Macular Dystrophy (BVMD)

Lisa M. J. Braun[1], Andrea Milenkovic[1], Bernhard H. F. Weber[1]

[1]Institute of Human Genetics, Regensburg, Germany

Purpose: Human Bestrophin-1 (BEST1) is a chloride channel controlled by Ca^{2+} and cell volume and is localized at the basolateral membrane of the retinal pigment epithelium (RPE). So far, there is no therapy for the BEST1-associated diseases, of which the most common is Best vitelliforme macular dystrophies (BVMD). In this study, we developed an assay targeting BEST1 localization and function with an application range for high and small-scale compound screening.

Methods: To assess BEST1 channel function we developed the halide assay. Briefly, MDCKII cell lines were established, stably expressing wildtype BEST1 or BVMD-associated BEST1 mutations together with a yellow fluorescent protein (YFP)-based halide sensor. In polarized MDCKII cells, wildtype BEST1 and BEST1-R218C localize regularly at the basolateral plasma membrane (PM) while BEST1-L224M and BEST1-Y227N are significantly reduced in protein quantity and are grossly mislocalized to cytosolic compartments. Cells were stimulated with extracellular addition of iodide known to pass the PM through anion cannels and, as a consequence, intracellularly quench 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 screening. Positively tested compounds were reanalyzed by Immunocytochemistry, Western blot analysis and whole-cell patch clamp recordings.

Results: 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 60 seconds iodide stimulation. Cells expressing mutant BEST1 showed 85% of default YFP fluorescence after the same time interval.

Conclusion: The current study established an assay appropriate for high-scale compound screening to address BEST1 localization and function. This assay is well suited to screen for compounds in mutant cells lines BEST1-L224M and BEST1-Y227N for their ability to improve trafficking to the PM or correcting protein folding to enhance ion permeability.

mES-derived double fluorescent photoreceptors as a tool to study outer segment formation

Madalena Carido, Lukas Donix, Konstantinos Anastassiadis, Thomas Kurth, Marius Ader

Retinal degenerative diseases like age-related macular degeneration or retinitis pigmentosa lead to photoreceptor cell loss and eventually blindness. Such diseases affect millions of people worldwide and have a tremendous impact on quality of life. Up to date there is no treatment available; however, different approaches are currently being investigated at a pre-clinical level, including cell-based therapies. This therapeutic option requires a considerable amount of a donor cells, namely rods and cones. Pioneering studies have shown that it is possible to obtain several retinal populations, including photoreceptors, from pluripotent stem cells (PSC) using a 3D retinal organoid system, that recapitulates retinogenesis *in vitro*. Furthermore, it has been shown that 3D derived photoreceptor cells can be isolated and used as a cell source for transplantation studies. One concern regarding retinal organoids is the degree of maturity achieved *in vitro*, given the inability to maintain them for prolonged culture periods. Outer segment (OS) production is of specific interest, since this structure is essential for light detection and thus proper photoreceptor function. Here, we took advantage of a mouse embryonic stem cell (mES) double reporter line expressing rhodopsin-GFP fusion protein, besides red fluorescence protein (dsRed) in the cell body to access OS formation both *in vitro* and *in vivo*, following transplantation of rod photoreceptors into mouse models of retinal degeneration. Rhodopsin::eGFP/actin-dsRed mES cells were first analyzed for their capacity to form retinal organoids. Generation of different retinal populations including retinal progenitors, retinal ganglion cells, Müller glia and photoreceptors was accessed by immunohistochemistry, showing recapitulation of mouse retinogenesis. Flow cytometry analysis showed that up to 75 % of cells were rod precursors (CD73+) and that within this population, up to 40 % were GFP+. Rod precursors enriched by magnetic activated cell sorting (MACS) were transplanted into mice with advanced stages of retinal degeneration. Four weeks after transplantation, presence of donor cells and their degree of maturation was accessed.

The influence of Juvenile Neuronal Ceroid Lipofuscinosis (jNCL) on microglial reactivity in (non) light damage conditions

Dannhausen, K.[1], Cotman, S.[2], Hansen J. N.[3,4], Halle A.[3,4] and Langmann, T.[1]

[1]Department of Ophthalmology, University of Cologne, Cologne, Germany
[2]Department of Neurology, Massachusetts General Hospital, USA
[3]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany
[4]Max-Planck research group Neuroimmunology, Research center caesar, Bonn, Germany

Background: Juvenile neuronal ceroid lipofuscinosis (jNCL) is an inherited autosomal recessive lysosomal storage disorder. Affected children exhibit seizures and mental retardation preceded by early visual impairment between the ages of 2–10 years ultimately followed by vision loss and death in the third decade of life. The causative defect results from mutations in the CLN3 gene which encodes Battenin, a transmembrane protein of unknown function. Because of this early involvement of the eye we aimed to analyzing the pathogenesis of this disease in the retina with a focus on microglia as part of the local innate immune system.

Methods: Primary microglia (pMG) were isolated and cultured from P0 CLN3$^{\Delta ex7/8}$ mouse brains. Samples were treated with Lipopolysaccharide (LPS) and Ceramide (C6). Quantitative real-time PCR was used to analyze changes in the expression of pro-inflammatory marker genes. Microglial morphology was analyszed using MotiQ. Two month old Cln3$^{\Delta ex7/8}$ mice were exposed to 10,000 or 15,000 lux white light for 30 or 60 min to induce light damage. The retinal damage was analyzed using a Spectralis OCT, immunohistochemistry (IHC) and qRT- PCR at day 7.

Results: pMG from Cln$^{\Delta ex7/8}$ mice showed higher basal expression of the pro-inflammatory markers TNFα, IL1β, AMWAP, IL6 and CASPASE 11. A combined stimulation with LPS and C6 lead to an increase in these inflammatory mediators. Microglial morphology of Battenin-deficient cells was slightly modified regarding ramification index (RI), numbers of branching points and junctions, spanned area and the total tree length. Accumulated mitochondrial subunit C of ATP synthase (SubC) could be shown in battenin-deficient microglia. Retinas of Cln3$^{\Delta ex7/8}$ mice showed a higher susceptibility to light induced damage compared to wild type controls resulting in a stronger thinning of retinal thickness. IHC using retinal sections showed activated microglia and an increase in TSPO and GFAP as well as accumulation of ATP synthase Subunit C in light damaged Cln3$^{\Delta ex7/8}$ mice. SubC staining showed enriched accumulation in phagocyting microglia somata.

Conclusion: Battenin deficiency leads to a higher light susceptibility and earlier microglial activation in light induced retinal damage. This model could be used to test therapeutic effects.

Inhibition of CNG channel activity for the treatment of retinitis pigmentosa

Soumyaparna Das[1, 2], Tobias Bonifert[2, 3], Klaudija Masarini[1], Norman Rieger[1], François Paquet-Durand[1]

[1]Cell Death Mechanism Laboratory, University of Tübingen, Tübingen, Germany
[2]Graduate Training Centre of Neuroscience, University of Tübingen, Germany
[3]Molecular Genetics Laboratory, University of Tübingen, Tübingen, Germany

Purpose: Retinitis pigmentosa (RP) is caused by mutations in rod photoreceptors and currently there is no effective treatment available for the disease. High levels of Ca^{2+} are thought to cause or contribute to the neurodegenerative process and in photoreceptors. Cyclic nucleotide gated channels (CNGCs) are one of the major sources of Ca^{2+}. A knockout study of CNGC showed an improvement of rod viability in the *rd1* mouse model for RP. Here, we target CNGCs using pharmacological blockers and antisense oligonucleotides (AON) mediated knockdown to try and prevent rod photoreceptor degeneration.

Method: We selected C3H *rd1* and C3H wild-type mice as animal models for RP and control, respectively. Organotypic retinal explant cultures of *rd1* were treated with L-cis-diltiazem, a CNGC blocker, from postnatal day (P) 7 to P11. Dose response data was collected using the TUNEL assay for the quantification of cell death. Furthermore, we developed an alternative AON approach, based on an exon skipping mechanism in the *CNGB1* gene, targeting genomic sequences conserved between mice and human. To allow for a rapid analysis of efficacy, human retinoblastoma cell lines WERI-RB1 and Y-79 were tested for the expression of *CNGB1* by PCR of cDNA derived from their total RNA. In addition, the expression of *Cngb1* was checked several different mouse tissues.

Results: L-cis-diltiazem was found to reduce photoreceptor degeneration on retinal explants derived from *rd1* animals as compared to untreated [Average percent TUNEL positive cells – Treated 7.3008; n=10, Untreated 9.0847; n=15, p=0.0233]. The analysis of the mouse and human *Cngb1/CNGB1* gene sequences identified 10 exons and 37 candidate AON sequences with conserved sequences at internal, donor, and acceptor regions. The selected regions are expected to give increased exon skipping efficiency and easy translation from mouse to human. Human retinoblastoma cell lines WERI and Y-79 were found to express *CNGB1* and may thus serve to rapidly evaluate the knock-down efficacy of different AONs.

Conclusion: We found that the known CNGC blocker L-cis-diltiazem can reduce photoreceptor cell death in *rd1* retina *in vitro* confirming the importance of CNGC for photoreceptor degeneration. Further CNGC targeting compounds and AONs will be tried *in vitro,* and the most successful of these will then be tested *in vivo.* Since several CNGC targeting drugs are already in clinical use (e.g. L-cis-diltiazem), the establishment of CNGC as a therapeutic target could facilitate a rapid translation into new treatments for RP patients.

Preservation of cone function in a murine rhodopsin knock-out model of retinitis pigmentosa by rhodopsin gene delivery

Daniyar L. Dauletbekov[1-3], Alun R. Barnard[1], Michelle E. McClements[1], M. Dominik Fischer[1-3], Robert E. MacLaren[1]

[1]Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, United Kingdom,
[2]University Eye Hospital and
[3]Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany

Introduction: Rhodopsin-linked retinitis pigmentosa (RP) is the most common form of autosomal dominant RP, an inherited retinal degeneration, which leads to loss of vision and blindness. Some studies suggested that dominant-negative mutations of rhodopsin can indeed be treated by increasing the ratio of wild-type to mutated rhodopsin. We hypothesized that the supplementation of wild-type rhodopsin via Adeno-Associated Virus (AAV) vector could slow down rod and secondary cone loss in a murine model of RP lacking endogenous rhodopsin – the rhodopsin knockout mice.

Methods: Subretinal injection of self-complementary AAV vector containing the coding sequence of the human rhodopsin gene driven by the human rhodopsin promoter (scAAV2/8.hRHOp.hRHO) at postnatal week three (PW3) into rhodopsin knockout mice with transgenic expression of the green fluorescent protein (GFP) in cones (Opn1.GFP rho-/-). One group received single unilateral injections, another group received bilateral injections (treatment vs. sham). Dark and light adapted electroretinography (ERG), confocal scanning laser ophthalmoscopy (cSLO) was performed at 2, 3, 4 and 5 months postnatally and when eyes were collected for histology analysis.

Results: The ERG results showed the restoration of a- and b-waves in dark-adapted ERG corresponding to the functioning rod photoreceptors, which were maintained up to three months post-injection. The light-adapted ERG demonstrated the preservation of cone-elicited responses up to 3 months post-injection. The cSLO imaging showed preservation of fluorescent cones up to 3 months after injection. The histology analysis confirmed the preservation of the ONL in the treated eyes compared to untreated and sham-injected eyes. The analysis of GFP-cones on the eye sections revealed also the preservation of cone numbers in the treated eyes compared to untreated and sham-injected eyes.

Discussion: The presented data confirmed a functional benefit of delivering of the rhodopsin gene on rod and secondary cone degeneration in a rhodopsin knockout murine model of RP.

GDNF and CNTF potently and synergistically attenuate the loss of axotomized retinal ganglion cells in adult mice

Kai Flachsbarth, Wanda Jankowiak, Katharina Kruszewski, Simon Dulz, Jorna Boswinkel, Mahmoud Bassal, Elke Becker, Sabine Helbing, Stefanie Schlichting, Susanne Bartsch, Udo Bartsch

Department of Ophthalmology, University Medical Center Hamburg-Eppendorf, D-20246 Hamburg

Purpose: Glaucoma is a neurodegenerative disease of the inner retina that is characterized by a progressive apoptotic degeneration of retinal ganglion cells (RGCs). Neuroprotection is among the strategies that are being explored to establish treatments for this condition. Here, we analyzed the impact of a simultaneous cell-based intravitreal administration of glial cell line-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF) on the survival of axotomized RGCs in a mouse optic nerve crush model.

Methods: Polycistronic lentiviral vectors were used to establish a panel of clonally derived neural stem (NS) cell lines expressing either GDNF (GDNF-NS cells) or CNTF (CNTF-NS cells) together with a fluorescent reporter protein. Cells for control experiments (control-NS cells) were modified with a vector encoding a reporter gene only. To evaluate the neuroprotective effects of these cell lines on axotomized RGCs, cells were intravitreally grafted into adult mice one day after an intraorbital optic nerve crush.

Results: Intravitreal transplantations of the different GDNF- or CNTF-NS cell lines resulted in significant attenuation of axotomy-induced RGC loss. From these cell lines, we selected a GDNF-NS and a CNTF-NS clone that displayed a similar efficacy in protecting RGCs from lesion-induced cell death. Transplantations of either cell line resulted in the survival of ~4-fold more RGCs than in control eyes 2 months after the lesion. When these two cell lines were grafted as a 1:1 mixture, the number of surviving RGCs increased markedly and was significantly higher than the calculated value for a presumed additive neuroprotective effect of both factors. Of note, we found 14.3-fold more surviving RGCs in eyes with grafted GDNF- and CNTF-NS cells than in eyes with grafted control-NS cells 2 months after the lesion, corresponding to ~38 % of the normal RGC population.

Conclusion: Combinatorial neuroprotective approaches might represent a strategy to effectively attenuate RGC loss in glaucoma. Experiments have been initiated to evaluate whether the robust synergistic neuroprotective effect of GDNF and CNTF persists long-term, and to study the impact of a simultaneous administration of both factors on RGC survival in mouse models that more closely mimic the pathomechanisms and the slow disease progression of glaucomatous optic neuropathies.

Interaction of recoverin isoforms and G-protein-coupled receptor kinases in the cone-dominant visual system of zebrafish

Dana Elbers, Alexander Scholten, Karl-Wilhelm Koch

Department of Neuroscience, Biochemistry Group, University Oldenburg, 26111 Oldenburg, Germany

Purpose: Phototransduction starts with the photoexcitation of rhodopsin. Consequently, the concentrations of the two second messengers cGMP and Ca^{2+} decrease. The decreasing [Ca^{2+}] is sensed by a Ca^{2+}-sensor protein, namely recoverin. Recoverin is part of the recovery mechanism of phototransduction and interact with the G-protein coupled receptor kinase (GRK) in a Ca^{2+}-dependent manner. The phototransduction in cones is less well understood than in rods. The faster light responses of cones, their lower sensitivity and wider operation range under changing background illumination intensities likely originate from specific features of the visual transduction machinery. A widely used model for cone vision research is the zebrafish *(Danio rerio)*, which has a cone-dominant visual system. In the zebrafish genome many photoreceptor proteins are duplicated. For example, two paralogs of G-protein-coupled receptor kinase 1 (GRK1a & GRK1b) and GRK7 (GRK7a & GRK7b) are expressed in the photoreceptors and four different isoforms of zebrafish recoverin (zRec1a, zRec2a, zRec1b, zRec2b). So far, the operation mode and physiological meaning of the different zebrafish GRKs and recoverins is unclear. Our aim is to investigate protein-protein-interaction of recoverin and GRK isoforms as well as the specific Ca^{2+}-sensing properties of the different recoverin isoforms.

Methods: The interaction of recoverin and GRK isoforms and different Ca^{2+}-sensitivities of recoverin isoforms were investigated with different biochemical methods. Surface Plasmon Resonance Spectroscopy (SPR) enables investigations of protein-protein-interactions in real time by an immobilization of one protein followed by a titration of a second protein. To investigate the different Ca^{2+}-sensitivities, a $^{45}Ca^{2+}$-binding assay is in progress.

Results: The recoverin isoforms were expressed in *E.coli* and purified by a Hydrophobic-Interaction-Chromatography except of zRec1b, which is purified by an Ammonium-Sulfate-Precipitation followed by a Size-Exclusion-Chromatography. The GRK paralogs were also expressed in *E.coli* followed by an Immobilized-Metal-Ion-Chromatography Purification. Previous experiments showed that based on Ca^{2+}-binding all recoverin isoforms are functional after the purification procedure. The SPR interaction studies indicated heterogeneous binding modes of recoverin isoforms and GRK targets.

Conclusion: The Ca^{2+}-sensitivities of recoverin forms indicate a differential mode of target binding and regulation. Ongoing experiments are designed to identify which pair of recoverin and GRK is operating in which photoreceptor cell and whether different Ca^{2+}-binding modes reflect a step-by-step activation/inhibition of the target GRKs.

Leber congenital amaurosis (LCA): Prevalence of mutations in a large German cohort and clinical characterization of the *CEP290* subgroup

Britta Feldhaus[1], Susanne Kohl[2], Fadi Nasser[1], Eberhart Zrenner[1,3], Ditta Zobor[1]

[1]Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany.
[2]Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany
[3]Werner Reichardt Center for Integrative Neuroscience, University of Tübingen, Germany

Background: Leber congenital amaurosis (LCA) is the most severe and earliest form of all inherited retinal diseases (IRDs). Starting at birth or in the first year of life, clinical features include severe visual loss and undetectable ERG. So far, over 20 genes have been associated with LCA, with *CEP290* being frequent in Northern Europe. Promising gene therapy is becoming available, however, genetic testing and clinical characterization of patients remain essential to identify suitable candidates.
In Germany, there are approximately 2000 patients with LCA. Many of these have been seen in the Institute for Ophthalmic Research, University of Tübingen, and valuable data for this rare disease has been accreted.

Purpose: To describe the prevalence of mutations in LCA-associated genes in a large German cohort, and to give detailed clinical information about a frequent subgroup *(CEP290)*.

Methods: Internal databases were queried for 1) patients with a clinical diagnosis of LCA and/or 2) patients with already identified mutations in LCA-associated genes, despite clinical diagnosis. Patients with mere clinical diagnosis were invited for genetic research testing. Patients with mutations in *CEP290* were invited for anew ophthalmological examination, including psychophysical tests (best corrected visual acuity (BCVA), color vision, visual field) and electrophysiology (fullfield and multifocal ERG). Additionally, fundus photography, autofluorescence (FAF) and spectral domain optical coherence tomography (SD-OCT) recordings were performed.

Results:
General LCA group: 109 patients (54 m, 55 f; age: mean: 30.1yrs, spread: 3 – 76yrs) with mutations in 16 LCA-associated genes *(AIPL1, CABP4, CEP290, CRB1, CRX, IFT140, IQCB1, LCA5, LRAT, NMNAT1, RD3, RDH12, RPE65, RPGRIP1, SPATA7, TULP1)* were included. The largest genetic group was for *CEP290* (n = 24), followed by *CRB1* (n = 23) and *RPE65* (n = 16). The most common clinical diagnoses were LCA (n = 55) followed by RP (n = 46), but also other IRDs were seen (CRD (n = 5), CD (n = 1), CSNB (n = 2)). Of all clinical LCA cases, 29 % were caused by mutations in *CEP290* (n = 16), thereof 87.5 % (n = 14) showed the deep intronic mutation c.2991+1655A>G (p.C988*) (37.5 % homozygous (n = 6); 50 % compound heterozygous (n = 8)).

CEP290 subgroup: 68 % had symptoms starting at birth or in the first year of life (n = 15), 14 % as adults (n = 3). Typical symptoms were nystagmus (75 %, n = 18), photophobia (58 %, n = 14), night blindness (58 %, n = 14) and color vision disturbances (58 %, n = 14). BCVA was severely reduced (mean: 0.13 / 0.18 for the right and left eye, respectively) with 33 % having no light perception (NLP), residual light perception (LP) (n = 8) and 25 % only hand motion vision (HM, n = 6). Exceptions were RP cases with a BCVA of 0.8 (n = 3). All patients showed severely reduced or no visual fields. ERG was typically undetectable, only three patients showed preserved ERG responses. Additional findings were cataract (46 %), keratokonus (12 %), strabismus (29 %) and oculo-digital sign (17 %). Fundus examination showed typical signs of IRD (optic disc pallor, attenuated arterioles, pigment epithelium atrophy), but with clinical variability. Interestingly, pigmentation – if present – was subtle and delicate. SD-OCT revealed centrally preserved photoreceptors or a complete photoreceptor loss. Central foveal thickness was reduced (mean: 176 µm). FAF was decreased with a parafoveal ring of mildly increased FAF. Interestingly, in patients with homozygous c.2991+1655A>G mutation FAF was absent, and patients with ?compound? heterozygous c.2991+1655A>G status showed a patchy pattern of reduced FAF in the periphery.

Conclusion: *CEP290* is the most frequently mutated gene in patients with LCA and, of all LCA genes – accounting for various IRDs – it is the most common. The *CEP290* c.2991+1655A>G mutation plays an important role in LCA patients originating from Germany. However, LCA is highly heterogenic and shows clinical variability, so overlaps with other IRDs exist. For future gene therapy, the affected gene might be more important to look for than the clinical diagnosis.

Diverse effects of retinoschisin on retinal homeostasis may have implications for initial steps in the development of X-linked juvenile retinoschisis

Karolina Plößl, Melanie Royer, Bernhard H.F. Weber, and Ulrike Friedrich

Institute of Human Genetics, University of Regensburg, Germany

Background: X-linked juvenile retinoschisis (XLRS) is a hereditary retinal dystrophy affecting males at young ages. It is caused by mutations in the *RS1* gene and results in a functional loss of the encoded RS1 protein, termed retinoschisin (Sauer et al., 1998, Molday et al., 2012). The function of retinoschisin, and the molecular mechanisms underlying XLRS pathogenesis are still unresolved. Studies on retinal development of Rs1h-deficient (*Rs1h*$^{-/Y}$) mice revealed increased MAP kinase signaling and apoptosis (Gehrig et al., 2007) as well as disease-associated mislocalization of the retinal Na/K-ATPase, a direct interaction partner of retinoschisin (Friedrich et al., 2011). Aim of this study was to investigate a role of retinoschisin on retinal homeostasis in the *Rs1h*$^{-/Y}$ retinal explant model system.

Methods: Retinal explants of *Rs1h*$^{-/Y}$ mice were exposed to recombinant retinoschisin and a retinoschisin mutant, RS1-C59S. Activation of MAP Kinase signaling was assessed *via* immunoblotting against activated constituents of the ERK pathway, c-Raf and Erk1/2, as well as *via* quantitative real-time (q) RT-PCR against the MAP kinase target genes *c-Fos* and *Egr-1*. The effect of retinoschisin on apoptosis was investigated by analyzing expression of apoptosis marker *Bax* via qRT-PCR, as well as by following cone and rod degeneration on retinal cryosections. Immunohistochemistry was applied to analyze the effect of retinoschisin on Na/K-ATPase localization in *Rs1h*$^{-/Y}$ retinal explants.

Results: Exposure to retinoschisin, but not to RS1-C59S, significantly reduced activation of the MAP kinase signaling in *Rs1h*$^{-/Y}$ retinal explants. It also protected *Rs1h*$^{-/Y}$ retinae against photoreceptor cell death. Finally, retinoschisin binding to *Rs1h*$^{-/Y}$ retinae led to an enrichment of the retinal Na/K-ATPase at the inner segments.

Conclusion: Our data demonstrate an important role for retinoschisin in the regulation of retinal MAP kinase signaling, apoptosis and Na/K-ATPase localization. We cannot exclude other cellular processes influenced by retinoschisin, but suggest that defective control of the identified processes could contribute to the loss of retinal homeostasis as observed in XLRS pathogenesis.

AAV8 can induce innate and adaptive immune response in the primate eye

*F. F. Reichel[1-2], A. Fritz[1-2], R. Klein[3], T. Peters[4], I. P. Seitz[1-2], B. Wilhelm[4], M. Ueffing[2], M. Biel[5], B. Wissinger[2], S. Michalakis[5], K. U. Bartz-Schmidt[1], M. D. Fischer[1-2,4,6]**

[1]*University Eye Hospital and*
[2]*Institute for Ophthalmic Research, Centre for Ophthalmology, University Hospital Tübingen, Tübingen, Germany*
[3]*Immunopathology Laboratory, Department of Internal Medicine II, University Hospital Tübingen, Tübingen, Germany*
[4]*STZ eyetrial at the Center for Ophthalmology, University of Tübingen, Tübingen, Germany*
[5]*Center for Integrated Protein Science Munich (CIPSM) at the Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany*
[6]*Nuffield Laboratory of Ophthalmology, Nuffield Department of Clinical Neurosciences, University of Oxford, UK.*

Ocular gene therapy has evolved rapidly into the clinical realm due to promising pre-clinical proof of concept studies, recognition of the high unmet medical need of blinding disorders and the excellent safety profile of the most commonly used vector system, the adeno-associated virus (AAV). With the high number of trials exposing subjects to AAV, investigators independently report about cases of clinically evident inflammation in the treated eye in the preclinical and clinical setting after application of AAV2 or AAV8 and despite the concept of ocular immune-privilege. Here, we provide a detailed analysis of innate and adaptive immunity in non-human primates against clinical grade AAV8 and compare this to preliminary clinical data from the first retinal gene therapy trial for CNGA3 based achromatopsia (NCT02610582).

Developing new antisense oligonucleotide-based therapeutic approaches for inherited retinal dystrophies

Alejandro Garanto[1], Silvia Albert[1], Sarah Naessens[2], Lonneke Duijkers[1], Riccardo Sangermano[1], Miriam Bauwens[2], Elfride De Baere[2], Frans P.M. Cremers[1], Rob W. J. Collin[1]

[1]Department of Human Genetics and Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands
[2]Center for Medical Genetics, Ghent University and Ghent University Hospital, Ghent, Belgium

Background: Inherited retinal diseases (IRDs) are a group of genetically heterogeneous disorders characterized by a progressive photoreceptor degeneration. Recent adeno-associated virus (AAV)-based gene augmentation therapy trials were shown to be safe and moderately effective, which has provided hope for many visually impaired individuals worldwide. Yet, gene augmentation still entails several restrictions. An attractive alternative encompasses the use of antisense oligonucleotides (AONs), small RNA molecules that complementary bind to their target pre-mRNA and can modulate splicing without altering normal gene expression levels. We have successfully developed an AON-based therapy for the most recurrent LCA-causing mutation and here aim to expand the use of AON molecules to treat other subtypes of IRD.

Approach: We have selected different intronic mutations in two IRD-associated genes: *ABCA4* and *CHM*. Some of these variants were not previously characterized. To assess the nature of the splice defects, fibroblast, lymphoblast and iPSC-derived photoreceptor cells were employed. Alternatively, minigene constructs containing the region of interest were generated in order to elucidate the effect of the variant at RNA level. Several AONs were designed for each target and their efficacy to redirect splicing was assessed by co-transfection with minigenes in HEK293T cells or by direct delivery to patient-derived cells.

Results: RNA analysis of cells treated with AONs (both patient-derived cells or minigene-transfected HEK293T cells) revealed that the pseudoexons inserted by the different intronic mutations could be skipped by using an AON-based approach. This rescue was observed for the different variants assessed in *ABCA4* and *CHM*. We are currently studying whether the correction at RNA level correlates with an increase at protein level.

Conclusions: Together, we show the efficacy of AONs to restore splicing defects caused by intronic mutations and reveal the promising therapeutic potential that these molecules hold for future treatment of IRDs.

A mega-analysis of expression quantitative trait loci (eQTL) reveals the regulatory architecture of gene expression variation in the liver

Felix Grassmann[1], Tobias Strunz[1,2], Javier Gayan[2], Satu Nahkuri[2], Everson Nogoceke[2], Debora Souza da Costa[2], Cyrille Maugeais[2], Sascha Fauser[2] and Bernhard H. F. Weber[1]

[1]Institute of Human Genetics, University of Regensburg, Regensburg, Germany
[2]Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland

Genome-wide association studies (GWAS) in late stage age-related macular degeneration (AMD) have identified at least 52 independent gene variant signals in 34 loci with genome-wide significance. Although these associated signals explain over half of the genetic variability of AMD, in most loci, the causative genetic variant is unknown. Expression quantitative trait loci (eQTL) in disease relevant tissues provide an excellent approach to shed light on association signals and hitherto on the true underlying disease mechanisms. As a proof-of-principle, we explored the contribution of AMD associated variants to liver gene expression modulation. To this end, we extracted publicly available datasets to compute the largest eQTL analysis for liver tissue to date. Genotypes from all studies underwent standard quality control and were imputed using the 1000 Genomes reference panel. The expression values were normalized across all samples using quantile normalization and ComBat, an empirical batch correction algorithm. MatrixeQTL algorithms helped to assess the influence of genetic markers on local (cis) gene expression. In total, liver tissues from 588 individuals (with unknown AMD status) were analysed and 202,489 significant eQTL variants affecting 1,959 genes (Q-Value < 0.001) were identified. In addition, a further 101 independent eQTL signals were identified in 93 of the 1,959 eQTL genes. Overall, the eQTL variants were highly enriched in introns and other intergenic regions as well as in likely functional RegulomeDB classes compared to non-eQTL variants. Importantly, two independent AMD associated variants are significant eQTL for two genes involved in HDL metabolism (P-Values < 8.48×10^{-05}). Therefore, our results reinforce the notion that high density lipoprotein (HDL) metabolism plays a role in AMD pathogenesis. Taken together, by combining four different studies we propose a first regulatory landscape of gene expression in liver and provide further evidence that lipid metabolism is one of the causative pathways involved in AMD disease.

Genetic pleiotropy between age-related macular degeneration (AMD) and 17 complex diseases and traits

Christina Kiel[1], Felix Grassmann[1], Martina E. Zimmermann[2], Klaus Stark[2], Iris M. Heid[2], International AMD Genomics Consortium, Bernhard H.F. Weber[1]

[1]Institute of Human Genetics and
[2]Institute of Epidemiology, Department of Genetic Epidemiology, University Regensburg, Germany

In recent years, genome-wide association studies (GWAS) have identified thousands of single-nucleotide polymorphisms (SNPs) contributing to a defined risk of developing a complex disease. Our and other studies have indicated that many complex diseases and traits share genetic risks with other diseases. This is in line with an antagonistic pleiotropy theory of aging, which hypothesizes that pleiotropic genetic factors may be beneficial at younger ages while possibly unfavorable later in life well beyond the reproduction age. The identification of pleiotropic SNPs has the potential to highlight overlapping functional disease mechanisms. This, in turn, may help to define new treatment options. Alternatively, it may emphasize contrasting risk directions within shared pathways pointing to the need to be cautious when applying drug medication in multimorbid individuals.

To investigate the association of AMD with different complex diseases and traits we extracted the data of 3,164 SNPs associated with 81 complex diseases and traits at genome-wide significance. We calculated genetic risk scores of the 81 complex diseases and traits in the SNP dataset of 43,566 subjects from the International AMD Genomics Consortium (IAMDGC) and assessed their association with AMD. In addition, we evaluated the association of the 3,164 SNPs with AMD.
We found significant associations between AMD and 17 diseases/traits (Q-Values < 0.01, P-Values from $< 1.0 \times 10^{-16}$ to 1.9×10^{-3}). Notably, an increased risk for AMD was associated with reduced risk for cardiovascular diseases, increased risk for autoimmune diseases, higher HDL and lower LDL levels in serum, lower bone-mineral density as well as an increased risk for skin cancer. Within distinct disease groups, like autoimmune diseases and cardiovascular diseases, multiple scores are associated with AMD. Therefore, we analyzed the association of compound genetic scores computed from all variants associated with 11 defined disease groups with AMD and found a significant association of 4 genetic scores. Our results highlight the association of AMD with autoimmune diseases (OR = 1.004 [1.003-1.006], Q-Value = 3.2×10^{-8}), cardiovascular diseases (OR = 0.993 [0.990-0.996], Q-Value = 1.1×10^{-4}), other eye diseases/traits (OR = 0.989 [0.987-0.992], Q-Value = 3.1×10^{-15}) and diverse skin cancer types (OR = 1.010 [1.005-1.015], Q-Value = 2.3×10^{-4}). Furthermore, we found that 32 out of 3,164 SNPs used to calculate the scores are significantly associated with AMD (Q-Values < 0.01). These SNPs are independent of known AMD loci and located within 25 new, pleiotropic AMD loci, increasing the number of AMD associated loci to 59.

Taken together, our data reveal genetic associations between AMD and a number of diseases/traits suggesting an overlap to some extent in complex disease pathways. In addition, 25 novel pleiotropic AMD loci greatly extend our knowledge of the genetic architecture underlying AMD pathogenesis.

Electrically imaging retinal neurons using high-density micro-electrode arrays

Meng-Jung Lee[1,2], Florian Jetter[1], Guenther Zeck[1]

[1]Neurophysics Research Group, Natural and Medical Sciences Institute at the University Tübingen, Reutlingen, Germany
[2]Multi Channel Systems MCS GmbH, Reutlingen, Germany

Purpose: Microelectrode arrays (MEAs) have been widely used in the study of neuronal physiology. The advantages of high sampling rate, multiple recording sites and transparency make it an ideal tool to study not only single neuronal electrophysiology but also network activity in the retina or in brain slices. Here with a newly developed CMOS-based high-density microelectrode array (CMOS MEA), we were able to increase the spatial resolution to a level that enables us to electrically image retinal neurons, and which will lead to a better understanding of the signal transduction in the retina.

Method: Here, we interfaced isolated healthy C57BL/6 and photoreceptor degenerated rd1 retinae in either flat-mount or as vertical slices onto poly-l-lysine coated planar CMOS MEAs (electrode distance: 16 µm). The healthy retina was stimulated using brief light stimuli (customized LED setup) and the neuronal activity recorded by 4225 electrodes simultaneously. Recordings from healthy and photoreceptor-degenerated retinae were analyzed and visualized using customized python-based software SOMA and MATLAB.

Results: First we recorded from the flat-mount retinae from healthy mice and were able to electrically image the light-induced activity from more than one hundred retinal ganglion cell (RGC) per square millimeter, including the signal propagation along the axons. We then provided short light stimulus to the vertical slices from healthy mice retinae to observe the vertical signal transduction. In this preparation, we electrically imaged the propagation of signals from photoreceptor side to the RGCs side; furthermore, we also imaged the horizontal signal propagation within the vertical slices. Finally, we recorded from photoreceptor degenerated retinae in flat-mount configuration and imaged oscillatory field potentials and rhythmic RGC spiking.

Conclusion: Our results prove that with high-density CMOS MEAs, it is possible to electrically image retinal neurons in healthy retina to reveal the functional circuits and, more importantly, to reveal functional changes in photoreceptor-degenerated retinas. This technology will provide a fast and convenient way for neurological study in the future.

HDAC6 as a novel target for retinal degeneration?

Janina Leyk and Christiane Richter-Landsberg

Department of Neuroscience, Molecular Neurobiology, University of Oldenburg, D-26111 Oldenburg, Germany

Purpose: Retinal degenerative diseases are characterized by the progressive loss of photoreceptor cells leading to visual impairment and blindness. In retinitis pigmentosa (RP) primary rod degenera-tion is followed by secondary cone degeneration. Knowledge about the underlying molecular mechanisms is still scarce, but oxidative stress and defects in protein degradation mechanisms, such as macroautophagy (from here autophagy) and the ubiquitin proteasome system, have been implicated to be involved.
Recent studies suggest a role for histone deacetylases (HDACs) in neuro- and retinal degeneration. In mouse models for RP and cone dystrophies it was shown that the inhibition of HDACs prevented photoreceptor degeneration. HDAC6 is a unique member of the HDAC family since its main substrates are non-histone proteins, such as α-tubulin, cortactin, HSP90 and peroxiredoxin 1. It is involved in cellular stress responses by modulating the heat shock response, protein ag-gregate formation, autophagic degradation and redox reactions. The present study aimed to elucidate the effects of HDAC6 inhibition, using the specific inhibitor tubastatin A (TST), on cone photoreceptor-like 661W cells subjected to oxidative or proteolytic stress. Moreover, we investigated whether the autophagic pathway was affected by HDAC6 inhibition.

Results: Morphological data and cell viability assays demonstrated that HDAC6 inhibition by TST pro-tected 661W cells against oxidative stress. TST further induced beneficial effects, such as induc-ing the heat shock response and elevating the activity of peroxiredoxin 1, a redox regulatory protein mediating protection against oxidative damage by reducing peroxides. Also autophagic activity was enhanced in response to TST treatment. This was shown by an increase of the autophagosome marker LC3-II, a decrease in the level of the autophagic substrate p62 and by treating the cells with TST in combination with the autophagy inducer Rapamycin or with the autophagy inhibitor Bafilomycin A1. In contrast thereto, TST preincubation led to cytotoxic effects and to a reduction in heat shock protein levels, when cells were subjected to proteolytic stress, exerted by proteasomal inhibition.

Conclusion: Hence, HDAC6 inhibition might be of therapeutical value in retinal diseases, which are not char-acterized by a reduction in proteasomal activity.

Low molecular weight polysialic acid (avDP20) inhibits complement activation via interaction with properdin

Yuchen Lin[1], Harald Neumann[2], Peter F. Zipfel[1,3] and Christine Skerka[1]

[1]Department of Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany
[2]Institute of Reconstructive Neurobiology, University Hospital Bonn, University of Bonn, Bonn, Germany
[3]Friedrich Schiller University, Jena, Germany

Background: AMD represents the most common cause of blindness in developed countries and is characterized by the formation of drusen at the macular followed by the degeneration of the retinal pigment epithelium (RPE) with photoreceptors. Genome-wide association studies have clearly shown that genetic variants regulating the complement system are associated with AMD. Sialic acids as the terminal caps of the sugar branches play an essential role in self recognition and were shown to modulate alternative complement processes in a factor H dependent manner. Previously we showed that PolySia with an average degree of polymerization of 20 (avDP20) reduced inflammatory neurotoxicity of phagocytes in mouse and human co-culture systems via the immunoglobulin-like lectin-11 (SIGLEC11) receptor and that avDP20 specificly reduced complement activation via the alternative pathway. However, the mechanism how avDP20 interferes with alternative complement activation was still unclear.

Methods: PolySia with an average degree of polymerization 20 (avDP20) was purified via HPLC system from α2.8 – linked polysialic acid after fragmentation and hydrolysis. Complement activation was followed by C3b deposition on a titer plate. Interaction of avDP20 with purified and serum properdin was analyzed by ELISA and in real time by biolayer interferometry. Convertase competition assays were performed by ELISA.

Results: Here we show that avDP20 interacts with the alternative pathway complement activator properdin. Binding of avDP20 to properdin inhibits properdin in stabilizing the C3 convertase and to amplify complement activation. Inhibition of properdin by avDP20 explains the previously described alternative complement pathway regulation by avDP20 and supports the potential use of avDP20 for the treatment of AMD.

Generation of mESC–derived cone photoreceptor precursors

Sílvia Llonch

CRTD / DFG-Center for Regenerative Therapies Dresden, Cluster of Excellence, Fetscherstraße 105, 01307 Dresden, Germany

Cell-based strategies by means of photoreceptor transplantation have been shown to be a feasible therapeutic approach for retinal degeneration diseases. Indeed, after transplantation of primary rod/cone-photoreceptor precursors, some visual restoration has been observed in different mouse models of retinal degeneration. Nonetheless, in the human system, primary photoreceptor precursors are not the ideal cell source for such treatment approach, since transplantable photoreceptor precursors can only be obtained at embryonic stages of development, which is ethically and legally concerning. Therefore, derivation of photoreceptor precursors from pluripotent stem cells (PSC) is being currently developed as an alternative cell source. Several studies have already demonstrated the ability of mouse/human embryonic/induced pluripotent stem cells (m/h E/iPSC) to differentiate into 3D retinal tissue, so called retina organoids, in a self-organized manner. Rod photoreceptor precursors have been isolated from retinal organoids via fluorescent/magnetic-activated cell sorting (FACS/MACS respectively) and have been shown to survive and mature after transplantation into the sub-retinal space of retinal degeneration mouse models. In contrast to the nocturnal mouse, human vision highly depends on cone photoreceptors that are active in daylight conditions. As rods represent the vast majority of photoreceptors in the mouse retina and PSC-derived retina organoids, factors required for cone photoreceptor cell fate acquisition are not well studied. This lack of knowledge leads to a delay in the development of cell therapies for cone degeneration diseases compared to those for rod degeneration. Therefore, we are currently developing and optimizing protocols and tools that allow us to increase the amount of cone photoreceptor precursors and detect them in retinal organoids for further development of cell therapies tailored for cone degeneration diseases.

Measurement of systemic VEGF-A in neovascular disorders: Important aspects to be considered

Claudia Carolina López Yomayuza, Knut Stieger, Birgit Lorenz

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

The expression of Vascular Endothelial Growth Factor (VEGF) increases during the pathological process of eye diseases characterized by retinal neovascularization as in the case of wet Age related Macular Degeneration (AMD). In these cases the intravitreal injection of an anti-VEGF agent as Bevacizumab, Ranibizumab, Aflibercept or Pegaptanib is the treatment of choice. After treatment, systemic VEGF neutralization occurs as a consequence of systemic exposure to the agent. In order to monitor both circulating VEGF and systemic VEGF neutralization after intravitreal anti-VEGF therapy, the VEGF concentration is measured in either serum or plasma samples. However, VEGF is stored in platelets alpha-granules and is released upon activation. Hence, the measurement of free circulating VEGF should be conducted in plasma samples obtained by using a proper anticoagulant to avoid platelet activation and VEGF discharge. The purpose of this study is to compare the efficacy of the anticoagulants Sodium Citrate and Citrate-Theophylline-Adenine-Dipyridamol (CTAD) in the measurement of free circulating VEGF.

Blood samples from healthy adults (n = 10) were collected. Serum (S), serum from recalcified citrate blood (rS), citrate plasma (CB_P), Citrate-Theophylline-Adenine-Dipyridamol (CTAD) plasma (CTAD_P) and platelet suspensions after either activation or lysis were obtained. Levels of VEGF-A, platelet factor-4 (PF-4), angiopoietin-1 (ANG-1) and -2 (ANG-2) were quantified by ELISA (DuoSet ELISA, Fa. R&D Systems).

No significant differences between the concentrations of VEGF-A in CB_P and CTAD_P were found. Values of VEGF-A, PF-4 and ANG-1 in serum, rS and platelet suspensions were significantly higher compared to plasma. VEGF-A and PF-4, ANG-1 and ANG-2 levels in serum and rS correlated significantly.

Both Sodium Citrate and CTAD anticoagulants are suitable for the measurement of free circulating VEGF-A. Platelet activation can be monitored by PF-4 or ANG-1 measurements and should be included in the assessment in order to avoid potentially misleading data outcome.

Supported by Novartis, Germany

Blockade of adenosine A_{2A} receptor modulates complement cascade and inflammasome pathways in human microglial cells

M. H. Madeira[1,2,3], S. Hermann[3], K. Rashid[3], A. F. Ambrósio[1,2,4], A. R. Santiago[1,2,4], T. Langmann[3]

[1]Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, Portugal
[2]CNC.IBILI Consortium, University of Coimbra, Portugal
[3]Laboratory for Experimental Immunology of the Eye, Department of Ophthalmology, University of Cologne, Germany
[4]Association for Innovation and Biomedical Research on Light and Image (AIBILI), Coimbra, Portugal

Purpose: In age-related macular degeneration (AMD) evidence points to reactive microglia as a source of inflammatory mediators, such as cytokines, accumulation of complement system (CS) components and the activation of inflammasome pathways, which contribute to the pathological process. Ours and others findings show that adenosine A_{2A} receptor ($A_{2A}R$) blockade controls retinal microglia-mediated neuroinflammatory processes, affording protection. However, the contribution of $A_{2A}R$ activity to the microglial CS and inflammasome pathways remains to be elucidated.

Methods: Human immortalized microglial cells were pretreated with 50 nM SCH58261 (selective $A_{2A}R$ antagonist) and challenged with zymosan (50 µg/mL) and phorbol 12-myristate 13-acetate (PMA; 100 nM) for 6h.

Results: Challenge with zymosan and PMA up-regulated the expression of $A_{2A}R$ and activated immune response in microglial cells. $A_{2A}R$ antagonist reduced the increase in the mRNA levels of chemokine (C-C motif) ligand 2 (CCL2) and tumor necrosis factor (TNF), and nitric oxide levels elicited by zymosan and PMA. Further, microglia phagocytic efficiency was reduced by SCH58261. Notably, $A_{2A}R$ blockade prevented the up-regulation the mRNA levels of complement component 3 (C3) and complement factor B (CFB). Moreover, SCH58261 prevented the IL-18 mRNA levels up-regulation in stimulated human microglial cells, suggesting a possible role of $A_{2A}R$ in the modulation of the inflammasome pathway.

Conclusion: $A_{2A}R$ blockade impacts human microglial cell reactivity, preventing activation and expression of inflammatory markers and CS components. Still, more studies are necessary to clarify the role of $A_{2A}R$ in the CS and inflammasome pathways. Yet, these results show for the first time $A_{2A}R$ blockade impacts human microglial CS, suggesting that it might be envisaged as a potential therapeutic strategy for degenerative diseases such as AMD, whose pathogenesis include microglia reactivity and CS alterations.

Support: DFG research unit FOR2240 (Lymph) Angiogenesis and cellular immunity in inflammatory diseases of the eye. Foundation for Science and Technology (PEst UID/NEU/04539/2013), COMPETE-FEDER (POCI-01-0145-FEDER-007440), Centro 2020 Regional Operational Program (CENTRO-01-0145-FEDER-000008: BrainHealth 2020). M.H. Madeira is a short-term fellow from the Federation of European Biochemical Societies.

TSPO agonist XBD173 protects photoreceptors in a murine model of retinal ischemia

Kristin Mages[1], Herbert Jägle[2], Antje Grosche[1]

[1]Institute of Human Genetics, University of Regensburg, Germany
[2]Department of Ophthalmology, University of Regensburg, Germany

Purpose: Protective effects and a modulatory role on microglial activation of XBD173, an agonist of the mitochondrial translocator protein (18 kDa) (TSPO), were described in a light damage model. We asked how XBD173 affects the macro- and microglial response in an experimental model of transient retinal ischemia and how this may influence neurodegeneration.

Methods: Retinal ischemia in mice was induced by raising the intraocular pressure to interrupt blood supply for 60 min. XBD173 (10 mg/kg body weight) or vehicle was i.p. injected twice at the day before and the day of surgery and once each day post-surgery. Cells were quantified in DAPI stained retinal slices. Microglia morphometry was investigated in Iba1-labeled flatmounts. Glial activation markers were analyzed by quantitative PCR on samples from microglia, neurons, endothelial and Müller cell samples enriched by magnetic activated cell sorting and retinal pigment epithelium (RPE)/choroid samples prepared 3, 7 and 14 days (d) after ischemia. Retinal function was assessed by full-field electroretinography (ERG) 14 d post-surgery.

Results: TSPO was expressed in control retinae and up-regulated after ischemia in RPE, Müller, endothelial and microglial cells, but not in neurons. After initial cell loss (7 d post-surgery, 76.3 ± 4.3 % surviving cells compared to control eye; n = 3), photoreceptor degeneration stopped in XBD173-treated mice (75.6 ± 6.3 %; n = 5, 14 d after ischemia), while it proceeded in untreated mice from 70.8 ± 5.9 % (n = 4; 7 d) surviving cells to 53.3 ± 9.0 % (n = 5; 14 d). Less microglia invaded into the outer nuclear layer of XBD173-treated mice. In accordance, microglia from treated mice expressed less F4/80 and CD11b at 7 d and pro-inflammatory TNFα at 14 d post-surgery than untreated controls. Differences of M1/M2 differentiation markers were also observed in RPE/choroid samples. M2 markers associated with wound healing were higher expressed 14 d post-ischemia in RPE/choroid of treated mice. We also observed a modified Müller reaction. Although GFAP was up-regulated to similar levels, a better recovery of glutamine synthetase expression and a strongly enhanced expression of DBI, the endogenous ligand of TSPO, was detected in Müller cells 14 d post-surgery in treated mice. RPE65 expression was more stable in RPE/choroid samples of treated mice. ERG recordings revealed only minor differences between both treatment groups.

Conclusions: We demonstrate that XBD173 impacts on the glial response pattern in multiple ways, but may also influence RPE functions. It needs to be specified which of those changes accounts for the protective effect of XBD173 treatment in the postischemic retina. Of note, XBD173 very specifically improves photoreceptor survival, while inner retinal neurons do not appear to benefit from enhanced TSPO-signaling.

BBS proteins facilitate ciliary disassembly during RPE maturation

Sarita Rani Patnaik[1], Viola Kretschmer[1] and Helen May-Simera[1]

[1]Johannes Gutenberg-Universität Mainz, Germany

Purpose: In the visual system the retinal pigment epithelium (RPE) is a monolayer epithelium that borders the retina, and is vital for visual function. We previously showed that cells in the RPE contain a primary cilium, which is critical for maturation of the tissue. Primary cilia disassembly is crucial for organogenesis and homeostasis in numerous tissues. The process of ciliary disassembly has been characterised in RPE cell models, however cilia disassembly *in vivo* RPE has not been described. We questioned whether cilia disassembly might be important for maturation of the RPE.

Methods: We used various microscopy (immunohistochemistry and transmission electron electron microscopy) and real time expression (qPCR) analyses to investigate ciliary expression in mouse RPE at different stages of development. siRNA was used to knock down cilia genes *BBS8* or *BBS6* from hTERT-RPE1 cells. Western blotting, real-time PCR and flow cytometry analyses were performed to investigate ciliary disassembly signalling pathways.

Results: We see transient expression of the primary cilium in the developing mouse RPE. As the RPE matures, primary cilia retract, which is accompanied by altered expression levels of HEF1, AuroraA and HDAC6, key mediators of cilia disassembly. BBS proteins alter this ciliary disassembly pathway via recruitment of Inversin and results in activation of Wnt signaling.

Conclusions: Disassembly of primary cilia is required for RPE maturation. Disruption may contribute to ciliopathy phenotypes. We are beginning to elucidate how BBS proteins facilitate ciliary retraction via regulation of downstream signalling molecules. Targeting these pathways may help to develop therapeutic approaches.

Optical coherence tomography angiography (OCT-A) in an animal model for laser-induced choroidal neovascularization

Johanna Meyer[1], Petra P. Fang[1], Tim U. Krohne[1], Frank G. Holz[1], Steffen Schmitz-Valckenberg[1]

[1]University of Bonn, Department of Ophthalmology, Germany, Ernst-Abbe-Str. 2, 53127 Bonn

Purpose: Optical coherence tomography angiography (OCT-A) is a new diagnostic imaging modality for noninvasive three-dimensional visualization of the retinal and choroidal vascular structures without fluorescent dye injection. In this study, we compared OCT-A with conventional fluorescein angiography (FA) in an animal model of laser-induced choroidal neovascularization (CNV) in rats.

Methods: Pigmented rats (Dark Agouti) underwent argon laser photocoagulation to induce CNV. In vivo imaging using combined confocal scanner laser ophthalmoscopy (cSLO) and OCT-A imaging (Heidelberg Spectralis, Heidelberg Engineering, Germany) was performed before and directly after as well as at day 2, 7, 14 and 21 following laser treatment. OCT-A en-face images were compared to cSLO images obtained by conventional FA. Analysis included measurements of CNV area, vessel density and pixel intensity within/without the laser lesions. In addition, post-mortem analysis in retinal flatmount preparations was performed by immunohistochemistry of retinal and choroidal blood vessels.

Results: OCT-A allowed for high-resolution imaging of the retinal and choroidal blood vessels. Detailed non-invasive visualization of the superficial, intermediate and deep retinal plexus as well as choroidal blood vessels in rats by OCT-A was possible. Within laser lesions, signs of CNV formation occurred at day 7 with progression in size and number of small vessels until day 21. Development of CNV was associated with localized dye leakage on FA. New blood vessels were visible by OCT-A especially in the deep vascular plexus, in the avascular zone (outer nuclear layer and ellipsoid zone) and the choroid but not by conventional FA. Due to leakage and staining effects, CNV area appeared larger in FA compared to OCT-A images ($p \leq 0.0001$ for all tested layers).

Conclusions: This study demonstrates that in vivo OCT-A imaging can be performed in small animals like rats. Detailed and high-contrast images of the retinal and choroidal vasculature can be accurately visualized without invasive dye injection by OCT-imaging in vivo. Compared with OCT-A, invasive FA imaging did not allow for a detailed visualization of the formation of new small vessels within the laser lesions. OCT-A imaging may allow for a more precise, spatial analysis of new blood vessel formation in CNV animal models as compared with conventional FA.

Allele-specific suppression of dominant-negative Bestrophin 1 mutations

Andrea Milenkovic[1], Matthias Hupfer[1], Bernhard H. F. Weber[1]

[1]Institute of Human Genetics, Regensburg, Germany

Purpose: Retinal pigment epithelium (RPE) differentiated from human induced pluripotent stem cells (hiPSC) demonstrated degradation and mislocalization of mutant bestrophin 1 (BEST1) protein in autosomal dominant Best vitelliforme macular dystrophy (Best disease, BD). Importantly, mutated alleles revealed a dominant-negative effect leading to an impairment of volume-regulated chloride transport, the basic function of the homo-pentameric BEST1 channel. Here, our study aimed for a proof-of-concept to treat BD by selectively eliminating BEST1 mutant transcripts in patient-derived hiPSCs prior to RPE differentiation via the CRISPR/Cas9 genome editing technology.

Methods: Adult human dermal fibroblast were obtained from skin biopsies of BD patients and reprogrammed into hiPSCs. Single guide RNA sequences (sgRNA) targeting 7 BEST1 mutations were selected by the "Optimised CRISPR Design Tool" (Zhang Lab, MIT 2015). Editing efficiency and specificity of designed sgRNAs were tested in HEK293 cells using an established fluorescence-based assay. After transfection of hiPSCs the occurrence of insertion/deletion (indel) formation at on- or off-target sites was determined by Sanger-based sequencing. Cas9-treated stem cell populations were further analyzed for pluripotency and selected for full genome sequencing before differentiation to RPE cells.

Results: Computational design of 7 disease-causing BEST1 variants (N11K, V86M, S108R, Q238R, A243V, I295del and T241N) offered at least one sgRNA with predicted high quality per mutant allele. The guide sequences were cloned into the Cas9-expressing plasmid PX459 and co-transfected with pCAG-EGxxFP plasmids containing genomic fragments of ~500 bp of either the mutant or the wildtype sequence to the corresponding sgRNA. As targeted Cas9 cleavage results in reconstitution of the EGFP expression cassette by homology dependent repair, the efficiency and specificity of Cas9 cleavage was evaluated in a plate reader assay by quantifying EGFP fluorescence after 72 h of transfection. As a result, sgRNAs from N11K and I295del showed high allele-specificity and both sequences were used for targeted genome editing in hiPSCs. Full length sgRNA was generated with the GeneArt™ Precision gRNA Synthesis Kit and complexed with recombinant GeneArt™ Platinum™ Cas9 Nuclease to form a stable ribonucleoprotein (RNP) complex for electroporation with Amaxa Nucleofector™ Technology. Indel formation of on- and off-targets was determined.

Conclusion: To date, there is no treatment for BD although the molecular pathology of BEST1-associated maculopathy is reasonably well understood. Our proof-of-concept study aims to determine whether haploinsufficiency of normal BEST1 protein is sufficient to fully or partly restore cellular function in cells of primary BD pathology, namely the RPE.

Comparison of DNA damage response in immature mouse retina and murine retinal explant cultures

Brigitte Müller[1], Franziska Wagner[1], Francois Paquet-Durand[2], Birgit Lorenz[1], Knut Stieger[1]

[1]Department of Ophthalmology, Justus-Liebig-University Giessen, Friedrichstr. 18, 35392 Giessen, Germany
[2]Institute for Ophthalmic Research, University of Tuebingen, Roentgenweg 11, 72076, Tuebingen, Germany

Purpose: The purpose of this study was to detect DNA-DSB-repair proteins in vivo in mouse retina and organ culture of adult murine neuroretina.

Methods: Eyeballs from immature mice between p07 and p30 were harvested and conserved as whole eyes and prepared for cryo-sectioning. The following mouse strains were investigated: CH3, rd1, C57bl6 and rd10. Neuroretina explants were prepared from wild type C57Bl6 mice and evaluated after 1 to 10 days in culture by immunohistochemistry or qPCR. Fresh retinae were used as controls. Explants were cultured in culture plate inserts containing complete culture medium with the photoreceptor layer facing the supporting PCTE-membrane. Eventually total RNA was isolated from explants and processed for qPCR with mouse primers against individual DNA-damage and DNA-repair proteins. For histological investigation explants where harvested, conserved and prepared for cryo-sectioning. Subsequently, retinal tissues were counterstained by immunohistochemical markers for DNA-damage response and analyzed by confocal microscopy.

Results: We localized individual DNA-damage and DSB-mediator/adapter protein in retinal cells with antisera against γH2AX and 53BP1. In retinal explant culture, immunoreactivity of γH2AX occurred prominently as pan-nuclear staining and increased during the first week. In developing retina γH2AX immunoreactive foci were found in all nuclear layers. γH2AX immunoreactive foci correspond to DNA-DSB. Pan-nuclear staining of γH2AX might be viewed as a first step towards apoptosis. Of all investigated retinal tissues neurons of the INL and GCL showed pan-nuclear staining of γH2AX more prominently. 53BP1 immunoreactivity in developing retina and retinal explant culture is much higher in the inner retina. The gene expression of 53BP1 was up-regulated for two days and then down-regulated by two-fold in retinal explant culture.

Discussion: The expression of DNA damage sensing proteins varies in the retina depending on the state of degeneration and, more importantly, the cell type. This observation highlights the importance to study DNA repair protein distribution in the retina as an important part in the development of therapeutic applications.

Investigation of retinal damage dependent Müller glia proliferative gliosis

Robert Münch[#,1,2], Sheik Pran Babu Sardar Pasha[#,2], Alex Sykes[3], Yiqing Zhu[2], Mike O. Karl[1,2]

[1]TU Dresden, Center for Regenerative Therapies Dresden (CRTD), Fetscherstr. 105, 01307 Dresden, Germany
[2]Deutsches Zentrum für Neurodegenerative Erkrankungen e.V. (DZNE), Arnoldstr. 18, 01307 Dresden, Germany
[3]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany
[#]These authors equally contributed to this work.

Retinal regeneration can be found throughout many lower vertebrates, whereas it is highly limited in mammals. Origin of this regeneration are Müller glia (MG), which have adult stem cell potential: to re-enter the cell cycle upon retinal damage and to give rise to progenitors, which restore neuronal cell loss. Stimulation of epidermal growth factor (EGF) signaling is sufficient to stimulate complete regeneration in zebrafish. In contrast, MG in the mouse retina upon EGF treatment regenerate at best very few neurons and instead produce mostly glial progeny. This process, called proliferative reactive gliosis, provides a very promising model for studying the limitations of retinal regeneration in mammals. However, proliferative reactive gliosis is also known as source for scarring and proliferative disorders. The relationships between neurodegeneration (specifically defined cell death mechanisms), proliferative gliosis, scar formation and regenerative competence are incompletely understood.

In this study we use our previously established mouse retina regeneration ex vivo assay to investigate the association of retinal damage models and cell death mechanisms to MG reactivation and proliferation. We observed that EGF receptor inhibition significantly reduced activation of the extracellular signal-regulated kinase (ERK) and proliferation of MG. We studied these two major hallmarks of proliferative reactive gliosis in correlation to different damage models to gain further insight. We found that hypothermic pretreatment during eye and retinal dissection reduces neuronal damage, which results in lower amounts of MG activation and proliferation. In contrast, we induced retinal damage via a stab wound injury, which significantly increased reactive gliosis. Strikingly, combined (but not single) application of chemical inhibitors for defined apoptotic and non-apoptotic (necrotic) signaling pathways are sufficient to reduce neuronal cell death and associated MG proliferation.

In summary, our data suggest a defined relationship between retinal damage and EGF dependent MG proliferative gliosis. Future studies will be aimed at identifying the neuronal cell death dependent factors that instruct differential non-proliferative versus proliferative MG reactive gliosis. We hope that this work may contribute in the understanding of both, early mechanisms of induction of gliotic behavior of MG in diseases and acute damages, specifically to identify novel therapeutic targets to control proliferative disorders, scarring and regeneration.

Hippo signaling in the regenerating retina of adult zebrafish: A play of Müller Glia proliferation and differentiation

Anja Naacke, Anke Weber, Veronika Kuscha, Stefan Hans, Michael Brand

DFG-Research Center for Regenerative Therapies and Cluster of Excellence, CRTD, and Biotechnology Center, TU Dresden, Germany

Purpose: The adult zebrafish retina can, in contrast to mammalian retina, undergo full regeneration following ablation of photoreceptors and other lesion types. The molecular mechanisms underlying this extraordinary ability are not well understood. Hippo signaling is an evolutionarily conserved growth control pathway that is known to play a fundamental role in organ size homeostasis, stem cell maintenance, progenitor differentiation and tumor suppression in various tissues. Recently, the Hippo pathway has been identified to be crucial in controlling the balance of self-renewal and differentiation of retinal progenitor cells during early zebrafish development. Due to the similar cellular activities during eye development and the neurogenic response of Müller Glia cells (MG) after acute retinal damage, the aim of the project is a thorough analysis of the regulation of the Hippo pathway with a view to MG reactivity in retina regeneration of adult zebrafish.

Methods: Retina-specific temporal and spatial expression of individual Hippo signaling pathway members during adult zebrafish regeneration after light lesion was assessed by Polymerase chain reaction, *in situ* hybridization and immunohistochemistry. A reporter line will be used as a tool to monitor endogenous Hippo signaling activity, revealing cell type specificity and dynamics of the Hippo pathway in terms of retina regeneration. Manipulation of Hippo signaling via small-molecule modulators will be used to analyze its function in tissue repair and regeneration following injury.

Results: Core components of the Hippo pathway are present in the adult zebrafish retina and are up-regulated during regeneration. Interestingly, high expression levels of the Hippo downstream targets Yap1 and Tead1a can be found in the inner nuclear layer at around 2 to 3 days post lesion. At this time, regeneration is initiated by the dedifferentiation and cell cycle re-entry of MG that generate proliferating and migrating neuronal progenitors to restore lost photoreceptor cells.

Conclusion: Our data support that the Hippo pathway is conserved in zebrafish and activated during retina regeneration. Based on the reported role of Hippo signaling in the regeneration of other organs, eg. liver and heart, it is surmised that transient activation of Yap/Tead also promotes tissue repair and regeneration of the adult zebrafish retina following injury. In summary, we aim to understand the role of Hippo signaling in regeneration in order to potentially reveal novel therapeutic strategies for regeneration of the normally non-regenerating mammalian retina.

An *ex vivo* model to study choroidal neovascularization (CNV) in macular degeneration

Anna-Lena Nachtigal, Heidi Stöhr, Felix Grassmann

Institute of Human Genetics, University of Regensburg, Germany

Purpose: Choroidal neovascularization (CNV) describes the growth of abnormal blood vessels from the choroid into the subretinal pigment epithelium (sub-RPE) or subretinal space and is typically accompanied by severe vision loss. CNV is a hallmark of wet age-related macular degeneration (AMD) but also of Sorsby fundus dystrophy (SFD), a hereditary macular degeneration caused by autosomal dominant mutations in the gene encoding the tissue inhibitor of metalloproteinases-3 (TIMP3). This study aimed at the development of an *ex vivo* model to further investigate CNV in SFD.

Methods: An *ex vivo* choroid sprouting assay using the choroid/sclera-tissue of wild-type mice, TIMP3-deficient *(knock-out)* mice and mice carrying the SFD-related TIMP3 mutation Ser156Cys *(knock-in)* (CD1 background) was performed and evaluated by a computerized method. Microvessels were identified by isolectin GS-IP4 staining. Vascular sprouting from the choroidal explants isolated from mice at various ages (4, 6 and 9 weeks) was quantified.

Results: Tissue isolated from peripheral regions consistently showed increased sprouting when compared to explants from the central part. Quantification of the sprouting area of 12 – 14 animals per genotype, however, revealed no significant difference between wild-type mice and TIMP3 *knock-out* or TIMP3 *knock-in* animals. Whereas we did not observe an impact of age or gender, the mean sprouting area of the choroid samples obtained from different litters showed high variability.

Conclusion: We have successfully established an *ex vivo* angiogenesis assay imitating CNV *in vivo*. The method can be used to expand the current study and include for instance, animals of different genetic backgrounds and age groups. Furthermore, it is extremely useful to test the influence of environmental conditions and pharmacologic substances on the formation/prevention of CNV.

Ignore the nonsense: Translational read-though as therapy for non-sense-mutation caused ciliopathies

Ananya Samanta[1], Fabian Möller[1], Lisa Wiechers[1], Inessa Penner[1], Timor Baasov[2], Pieter Gaillard[3] & Kerstin Nagel-Wolfrum[1]

[1]Molecular Cell Biology, Institute of Molecular Physiology, Johannes Gutenberg- University of Mainz, Germany
[2]Technion-Israel Institute of Technology, Haifa, Israel
[3]2-BBB Medicines BV, Leiden, The Netherlands

Purpose: Hereditary retinal dystrophies are a genetically heterogeneous group of rare disorders that causes vision defects, for which currently no effective cure is available. Patient screenings predict that ~12 % of all pathogenic variants identified in patients are nonsense mutations. Therefore, a therapy that targets nonsense mutations has great potential to be beneficial for a substantial cohort making the approach both practical and economical. Nonsense mutations introduce a premature termination codon in the coding sequence of genes and lead to the expression of truncated, non-functional protein. Translational read-through mediates the over-read of nonsense mutations and thereby induces the expression of functional full-length proteins.

Methods: We studied the biocompatibility of different translational read-through inducing drugs (TRIDs) in organotypic murine retina cultures. Read-through efficacies were analyzed on different nonsense mutations causing retinal dystrophies with the spotlight on ciliopathies, such as the Usher syndrome, the Bardet Biedl syndrome and the Senior-Loken syndrome in cell culture and one USH2A-patient derived cell line by Western blot and immunfluorescence analysis. *In vivo* studies were performed in the NPHP4$^{nmf/nmf}$ mouse model.

Results: Our data show that in comparison with classical aminoglycosides (e.g. gentamicin), designer aminoglycosides as well as Ataluren exhibit significant better retinal biocompatibility. Our studies conclusively revealed read-through of different nonsense mutations causing the Usher syndrome (*USH1C:* p.R155X and *USH2A: p.G3142X*)), the Bardet Biedl syndrome (*BBS1:* p.G73X) and the Senior-Loken syndrome (*NPHP4:* p.L104X) in cultured cells. In addition, we validated read-through in an USH2A patient-derived cell line. Furthermore, our preliminary data indicate that topically applied Ataluren slow down the retinal degeneration *in vivo* in mice.

Conclusion: In summary, the excellent biocompatibility combined with the robust read-through efficacies of TRIDs emphasize their potential as a treatment option for retinal disorders caused by in-frame nonsense mutations.

Funding: PRO RETINA Deutschland e. V., FAUN Stiftung, Tistou and Charlotte Kerstan Foundation

Non-inferiority of subretinal versus intravitreal injection regarding photoreceptor layer thickness

Ochakovski, Guy Alex[1]; Peters, Tobias[3]; Michalakis, Stylianos[2]; Wilhelm,Barbara[3]; Bartz-Schmidt, K. Ulrich[1]; Fischer, M Dominik[1, 3]

[1]University Eye Hospital, Centre for Ophthalmology, University Hospital Tuebingen, Tübingen, Germany
[2]Center for Integrated Protein Science Munich CiPSM at the Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany
[3]STZ eyetrial at the Centre for Ophthalmology, University Hospital Tuebingen, Tübingen, Germany

Purpose: Despite ever growing adoption of subretinal (SR) and intravitreal (IVT) injection methods for vector delivery in ocular gene therapy trials, concerns regarding the SR approach in terms of possible deleterious effects on the outer retina are yet to be cleared. We tested the SR injection for quantifiable and qualitative structural changes in the outer retina and compared them for non-inferiority to the IVT approach in non-human primates.

Methods: In a fully GLP compliant toxicology study, 22 cynomolgus monkeys (ages 4 – 5, 11 male) underwent single intraocular injections with rAAV2/8 or vehicle. 18 animals received SR injections with vehicle (n = 6) or vector (n = 12), 4 animals received vector as IVT injection. Optical coherence tomography was used to visualise retinal layers before and at two timepoints after the injection (2 and 13 weeks). Outer nuclear layer (ONL) thickness was used as outcome measure to quantify change from baseline for a non-inferiority analysis. Clinical significance threshold for inferiority (M1 value) was set to an ONL reduction equivalent of three line vision loss based on previous studies (Matsumoto et al., Am J Ophthalmol. 148(1):105, 2009). Preservation of the physiological elongation of photoreceptor outer segments in the fovea (foveal bulge) was used as a secondary, qualitative measure of visual outcome.

Results: In the SR group the average ONL change from baseline after 2 weeks was - 6.54 ± 5.16 µm (mean ± s.d). In the IVT group the average change at 2 weeks was +1.50 ± 4.36µm. At 13 weeks, the SR group maintained a difference of -6.54 ± 9.66 µm from baseline while IVT group gained +1.00 ± 4.24 µm in ONL thickness on average. The foveal bulge was preserved in 9 out of 13 eyes (69 %) in the SR group and in all eyes in the IVT group.

Conclusions: The SR injection has proven not to be inferior in a clinically significant manner to the IVT injection in terms of ONL thickness loss and estimated VA losses. Although SR injection was shown to be followed by some limited degree of ONL thinning, the observed effect including the 95 % confidence intervals were under the predefined clinical significance threshold both after 2 and 13 weeks.

Complement components showed a time-dependent local expression pattern in constant and acute white light induced photoreceptor damage

Diana Pauly[1], Antje Grosche[2], Sabrina Schmitt[3], Barbara M. Braunger[3], Nicole Schäfer[1]*

[1]Department of Ophthalmology, University Hospital Regensburg, Regensburg, Germany
[2]Institute of Human Genetics, University Regensburg, Regensburg, Germany
[3]Institute of Human Anatomy and Embryology, University Regensburg, Regensburg, Germany

Background: Photoreceptor cell death due to extensive light exposure and induced oxidative-stress are associated with retinal degeneration. A correlated dysregulation of the complement system amplifies the damaging effects, but the local and time-dependent progression of this mechanism is not thoroughly understood.

Methods: Light-induced photoreceptor damage (LD) was induced in Balb/c mice with white light illumination either for 24 h with 1000 lux (constant model) or 0.5 h with 5000 lux (acute model). Complement protein and mRNA expression levels were compared at 1 d and 3 d post LD for C1s, complement factor B (CFB), mannose binding lectin A (MBL-A), mannose-binding protein-associated serine protease 1 (MASP-1), C3, C4, C9 and complement factor P (CFP) in retina and RPE/choroid. Histological analyses visualized apoptosis, microglia migration, gliosis and deposition of the complement activation marker C3d. Systemic anaphylatoxin serum concentrations were determined using an ELISA.

Results: Apoptosis, gliosis and microglia migration into the outer nuclear layer showed similar patterns in both models. Local complement factor expression revealed an early upregulation of complement factor mRNA in the acute and constant light regimen at 1 d post treatment for *c1s, cfb, masp-1, c3, c4* and *c9* in the RPE/choroid. However, intraretinal complement mRNA expression for *c1s, cfb, c3* and *c4* was increased at 1 d in the constant and at 3 d in the acute model. A comparable regulation on protein level in the retina following both LD models was observed for C3, which was upregulated at 1 d and correlated with increased C3d staining in the ganglion cell layer and at the RPE. In the RPE/choroid C1s-complex protein detection was increased at 3 d after LD.

Conclusions: LD in mouse eyes is correlated with local complement activity. The time-dependent local progression of complement regulation in two different eye tissues, retina and RPE/choroid, on mRNA and protein levels were comparable in the acute and constant LD model. With the exception for the intraretinal, time-dependent mRNA expression. Knowing the relative time courses of local complement expression and cellular activity can help to elucidate novel therapeutic options in retinal degeneration indicating at which time point of disease complement has to be rebalanced.

Secondary cone degeneration is associated with increased HDAC activity

Eleni Petridou[1], François Paquet-Durand[1], Dragana Trifunović[1]**

[1]University of Tübingen, Germany
** Both authors should be considered as equal corresponding authors.*

Purpose: Mutation-independent secondary cone degeneration is a cause of legal blindness in Retinitis Pigmentosa patients. Numerous mouse models reproduce this human phenotype of profound rod photoreceptor degeneration followed by secondary cone death. The *rd10* mouse is characterized by an early-onset and fast progression of rod photoreceptor cell death. However, the molecular mechanisms of secondary cone degeneration still remain enigmatic. We investigated the progression of cone cell death in *rd10* mice, focusing on the over-activation of histone deacetylases (HDAC), which was previously implicated with rod cell death.

Methods: We analyzed cone photoreceptor density by counting cone-arrestin positive cells at different postnatal (PN) stages ranging from PN14 to PN150. Additionally, we also assessed the progression of HDAC activity with an *in situ* activity assay. To determine if HDAC positive photoreceptors were rods or cones, we performed triple co-labeling of HDAC activity with rod- and cone-specific markers.

Results: In the *rd10* mouse, cone loss started after the extensive decline in photoreceptor rows. The first substantial decrease in cone density occurred at PN33. After PN50 the cone photoreceptor population decreased very slowly, with cones present as long as 13 months postnatal. Increased HDAC activity in *rd10* retinas, showed two peaks, at PN18 and PN59, suggesting HDAC involvement in both primary rod and secondary cone loss. Co-labeling experiments confirmed HDAC activity in rods at early PN stages (*i.e.* PN18-PN40), as well as in cones at late stages.

Conclusions: Our results suggest that the secondary cone degeneration in the *rd10* mouse is a very slow process when compared to the rapid loss of rods (Arango-Gonzalez *et al.*, Plos One, 9:e112142, 2014). Remarkably, *rd10* cones survived primary rod loss for more than a year, suggesting that the window-of-opportunity for the preservation of cones was substantially larger than for rods. Furthermore, increased HDAC activity appeared to be associated with both primary rod and secondary cone degeneration, creating an exciting opportunity for the development of a unique therapeutic approach that could delay the primary rod degeneration and as well as restrain the secondary degeneration of cones.

Expression profile of Müller cells from the diabetic mouse retina

Anna Pfaller[1], Felix Grassmann[1], Thomas Pannicke[2], Stefanie M. Hauck[3], Nora Klöting[4], Bernhard H. F. Weber[1], Antje Grosche[1]

[1]Institute of Human Genetics, Regensburg, Germany; [2]Paul Flechsig Institute, Leipzig, Germany; [3]Helmholtz-Zentrum München, Neuherberg, Germany; [4]IFB Obesity Diseases, Leipzig, Germany

Purpose: Müller cells, the major retinal macroglia, are in contact with virtually every retinal cell type, blood vessels, vitreous and subretinal space. This enables them to perform a wide range of functions crucial to support regular retinal function. To further elucidate the complex mechanisms underlying the diabetic retinopathy (DR), we investigated transcriptome and proteome profiles of Müller cells and other retinal cell types in a murine model of diabetes.

Methods: Microglia, neurons, Müller and vascular cells were enriched by magnetic activated cell sorting from retinae of C57BLKS-Lepr^{db} 24-weeks-old diabetic and wildtype mice. RNA was extracted and sequenced to a depth of 25-45 million reads per sample. In parallel, label-free liquid chromatography mass spectrometry was done for proteome analysis. RNA and protein profiles of Müller cells from wildtype and diabetic mice were compared. Flotillin 2 was chosen as first candidate to be validated via Western blotting and immunofluorescence labeling. The DR phenotype of 24-weeks old diabetic mice was confirmed by morphometric analysis of retinal vasculature in retinal flatmounts and demonstration of Müller cell gliotic activation on basis of patch-clamp recordings and analysis of gliosis markers at protein level.

Results: We phenotyped the diabetic mice and found hallmarks of DR including loss of pericytes and features of Müller cell gliotic activation such as a reduced Kir4.1-mediated potassium conductance. Pathway enrichment analysis on RNA sequencing data focusing on genes differently expressed in Müller cells revealed an up-regulation of genes associated with inflammation, oxidative stress defense and intermediate filaments, while genes involved in blood vessel integrity, energy supply for neurons, homeostatic Müller cell functions and platelet-derived growth factor-mediated signaling were downregulated. Comparison of the Müller cell transcriptome to proteome data was performed and screened for genes with a consistent regulation pattern at transcript and protein level. A first candidate we identified was flotillin 2 which is involved in a plethora of cellular processes such as membrane receptor signaling, phagocytosis and endocytosis. In line with this, we localized flotillin-2 in subcellular compartments of Müller cells resembling vesicular/exosomal-like structures in wild type and diabetic retinae.

Conclusion: Having analyzed a large set of expression data proofed crucial to understand the complex alterations of Müller cell functions in the context of multifactorial diseases such as DR. Validation of flotillin 2 as a first interesting candidate demonstrates the feasibility of our approach to shed light on novel Müller cell-specific genes involved in retinal pathology. Further experiments are needed to elucidate the functional role of flotillin 2 in the disease process.

Retinoschisin is associated with retinal Na/K-ATPase signaling - impact on understanding disease pathology of X-linked juvenile retinoschisis

Karolina Plößl[1], Melanie Royer[1], Neslihan Tavraz[2], Thomas Friedrich[2], Bernhard H. F. Weber[1], and Ulrike Friedrich[1]

[1]Institute of Human Genetics, University of Regensburg, Germany
[2]Institute of Chemistry, Technical University of Berlin, Germany

Background: Genetic alterations in the *RS1* gene cause X-linked juvenile retinoschisis (XLRS), a degenerative disease of the central retina (Sauer et al., 1998). Still, the molecular processes underlying XLRS pathogenesis are not fully understood. Previously, we have shown that retinoschisin, the protein encoded by *RS1*, is a regulator of Erk signaling and apoptosis in retinal cells (Ploessl et al., 2016). Also, we identified the Na/K-ATPase as an essential interaction partner of retinoschisin. In this study, we aimed to investigate a role of retinoschisin on retinal Na/K-ATPase functions, namely active ion transport and signal transmission.

Methods: Active ion transport was analyzed in *Xenopus leavis* oocytes *via* atomic absorption spectroscopy of Rb^+ as a congener of K^+. Furthermore, Na/K-ATPase activity and substrate affinity were assessed following Na/K-ATPase specific (ouabain sensitive) ATP hydrolysis in retinal membranes from Rs1h-deficient ($Rs1h^{-/Y}$) and wildtype mice. The effect of retinoschisin on Na/K-ATPase associated signaling pathways (Erk, Ip3/Akt and Ca2+ signaling) was investigated on $Rs1h^{-/Y}$ murine retinal explants and a human retinoblastoma cell line (Y-79). After treatment with retinoschisin and retinoschisin mutant RS1-C59S, as well as with specific signaling inhibitors, immunoblot analyses for marker proteins in different signaling pathways were performed. Finally, immunohistochemistry was applied to identify colocalization of the retinoschisin-Na/K-ATPase complex with intracellular signal transmission complexes.

Results: Our experiments revealed no influence of retinoschisin on active ion transport of the retinal Na/K-ATPase heterologously expressed in *Xenopus leavis* oocytes. In addition, no effect of retinoschisin deficiency was observed on enzymatic activity and substrate affinity of the Na/K-ATPase in retinal membranes. However, we identified an influence of retinoschisin on Na/K-ATPase regulated signaling cascades: Retinoschisin treatment decreased activation of Src and Erk (markers for Erk signaling) in $Rs1h^{-/Y}$ retinal explants and Y-79 cells. In addition, CamKII activation in $Rs1h^{-/Y}$ retinal explants was reduced. Immunohistochemical staining of retinal cryo-sections revealed a colocalization of retinoschisin, the Na/K-ATPase, and constituents of intracellular signaling complexes, such as caveolin and the IP3 receptor.

Conclusions: Together, our data suggest that retinoschisin is an important regulator of the retinal Na/K-ATPase signalosome complex. Disturbances in this interaction likely represent an initial step in XLRS pathogenesis, possibly pointing to novel target pathways for innovative treatment options.

In vitro studies to investigate the therapeutic approach of replacing the defective human RPGR-ORF15 gene through MMEJ

Surya Prakash Goud Ponnam[1,2], Mert Yanik[1], Isabel Gambert[1], Annabella Janise[1], Birgit Lorenz[1], Knut Stieger[1]

[1]Department of Ophthalmology, Justus-Liebig-Universität, Gießen, Germany
[2]Department of Molecular Biology & Biotechnology, Tezpur University, Napaam, Assam India

Introduction: Mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene have been associated with X-linked retinitis pigmentosa (XLRP). More than 80 % of the mutations have been identified in the terminal exon ORF15 of the RPGR gene. Till date, there are very limited treatment modalities available for patients with XLRP. The common gene editing strategies are homologous recombination (HR) and non-homologous end-joining (NHEJ) in combination with highly specific nucleases in mitotic cells. In the current study, we aim to develop a strategy to test and increase the efficacy of a third genome editing mechanism, microhomology mediated end-joining (MMEJ) post CRISPR/Cas9 mediated cleavage in cell culture, which could serve as the basis for treatment of XLRP patients.

Methods: A fluorescence based reporter system for MMEJ was generated and tested in HEK293 cells containing the murine RPGR-ORF15 sequence under the control of the ubiquitous CMV promoter (HEKmORF15 cells). We transfected the cells with a vector containing the luciferase gene flanked with micro-homologous sequences (mHS) that are complimentary to the mouse RPGR-ORF15 gene. The DNA double strand breaks (DSB) were induced by co-transfection of the CRISPR/Cas9 cleavage system employing 4 guide RNAs. Upon the DSB induction, the ORF15 exon is replaced by the luciferase sequence based on the mHS sequences provided, generating a full luciferase expression cassette. The generated luminescence was measured using a plate reader (Tecan). We tested and compared four different mHS sequence length (10, 15, 20 and 30 bp).

Results: The episomal fluorescence based reporter system revealed strong luciferase signal upon co-transfection with template plasmid and endonuclease system. Highest values were observed when the mHS were 15, 20 or 30 bp long, while shorter mHS sequences revealed 5 times lower luciferase signal.

Conclusion: The fluorescence / luciferase reporter system is a valuable tool to study MMEJ efficacy and is the basis for finding the right length of mHS sequences to be used as a source template in order to effectively replace the mutant ORF15 sequence in human patients. Currently, knock-out/down strategies are tested to modify DNA repair protein expression to further increase MMEJ activity.

Simultaneously imaging of calcium and calcium-dependent enzymatic activity in degenerating cone photoreceptors

Michael J. Power[1,2,3], Manoj Kulkarni[1,2,3], Timm Schubert[1,2], François Paquet-Durand[1], Thomas Euler[1,2]

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

Calcium levels are strictly regulated within neurons and, thus, calcium dysregulation has been proposed to be linked to cell death in the nervous systems. Recent research has highlighted how various mechanisms of calcium dysregulation may be connected to both primary and secondary cone photoreceptor (cone) cell death in the mammalian retina (*e. g.* Arango-Gonzalez *et al.*, PLoS One, 9:e112142, 2014).

Here, we examine the activation patterns of the calcium-dependent cysteine protease calpain in comparison with cell death patterns seen in mouse models of photoreceptor degeneration. We determine the spatial activation of calpain at several time points in the secondary cone degeneration model *rd1* (and later *rd10*), and the primary cone degeneration model *cpfl1*. Our preliminary data suggest that calpain activity is markedly increased in all these disease models (P30: *rd1,* 1.01 ± 0.113 calpain-positive cells in ONL/1000 µm^2, n = 45 observations from 3 mice; *rd10,* 0.869 ± 0.068, n = 45 observations from 3 mice; wild-type, 0.086 ± 0.023 n = 45 observations from 3 mice).

To link calpain activation to calcium dynamics within the cones, we use the transgenic *HR2.1:TN-XL* mouse line that expresses a calcium biosensor in cones to study calcium levels and, where applicable, light-evoked responses in an acute retinal slice preparation (Kulkarni *et al.*, J Vis Exp. 6:e52588, 2015). We use two-photon microscopy to measure calcium dynamics in retinas of *HR2.1:TN-XL* mice as our wild-type control, as well as in the retinas of *rd1* and *cpfl1* which have been stably crossbred with HR2.1:TN-XL mice. By adapting an approach used commonly on thin, unfixed retinal sections, we have developed a protocol for the combination of detecting enzymatic activity of calpain and live-cell calcium imaging.

In using this approach, we are able to simultaneously image two different parameters – here calcium and calpain activity – likely involved in cone cell death, in real-time. Future experimental manipulations (*e. g.* calpain inhibitors, CNG channel blockers) will then allow us to establish if and how these factors are linked to each other and cell death. Data generated in this project may be used to rationally design new therapeutic approaches for the treatment of cone degeneration.

Microglia-specific expression of Translocator Protein (18kDa) (TSPO)

Rashid K. [1], Scholz R. [1], Wolf A.[1], Karlstetter M. [2] & Langmann T.[1]

Department of Ophthalmology, University of Cologne, Germany
Bayer AG, BPH-DD-TRG-CIPL, Wuppertal, Germany

Purpose: Microgliosis is a common hallmark in retinal degeneration. Translocator Protein (18kDa) (TSPO) is strongly expressed in activated microglia and serves as an attractive therapeutic target for alleviation of retinal degenerative diseases. However, TSPO transcriptional regulation in immune cells remains largely unknown. This study sought to carry out detailed characterization of the TSPO promoter and identify elements required for microglia-specific expression.

Methods: A 2.8Kb TSPO promoter sequence was amplified by PCR and cloned into the pGL4.10 luciferase reporter vector. Plasmids containing 5′ unidirectional deletions of the promoter were then generated by PCR. BV2 microglia cells were transfected with the reporter plasmids for 24 hours, and an additional 6 hours in the case of lipopolysaccharide (LPS) stimulation. Sequence analysis was performed using Matinspector software and substitution mutagenesis was used to confirm the functional status of the putative transcription factor binding sites. *In vivo* binding of transcription factors was determined using chromatin immunoprecipitation (ChIP).

Results: Deletion mutagenesis indicated that −845 bases upstream of the transcription start site (TSS) was sufficient to reconstitute near maximal promoter activity in BV2 cells. Deletion of sequences extending -593 to -520, which harbour an AP1, Ets.2 (second Ets site) and Nkx3.1 site led to a dramatic decrease in both basal and LPS induced promoter activity *($p<0.0001$)*. Deletion of sequences extending -168 to -39, which contains four SP1/SP3 sites, led to a notable but non-significant decrease in promoter activity. Point mutations of AP-1, Ets.2, Nkx3.1/ SP1/SP3 site and SP1/SP3 sites led to significant decreases *($p<0.01$)* in promoter activity. Notably, results from ChIP-qPCR experiments revealed that the Ets.2, AP-1, Nkx3.1/SP1/SP3 and SP1/SP3 sites are specifically enriched in PU.1, cJUN, cFOS SP1, SP3 and SP4 precipitated DNA.

Conclusion: Consensus binding sequences for AP-1, Ets.2, and Nkx.3.1/SP1/SP3 have to be intact for maximal basal and LPS induced TSPO promoter activity in BV2 microglial cells. Our findings also reveal that SP1/SP3 binding sequences located 125 bases upstream of the TSS constitute positive regulatory elements which have to be intact for maximal basal promoter activity. Furthermore, consistent with a distinct TSPO regulation pattern in microglia cells in the CNS including the retina, we report that TSPO promoter is bound by PU.1 as well as cJUN, cFOS, SP1, SP3 and SP4.

An iPSC-based cell culture model to investigate the pathomechanism of age-related macular degeneration (AMD)

Rosendahl, S. M. G.[1]; Grassmann, F.[1]; Brandl, C.[1,2]; Weber, B. H. F.[1]

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

Introduction: Age-related macular degeneration (AMD) is the most common cause of vision loss in industrialized countries. AMD is a chronic neurodegenerative disease and affects the central retina comprising the choriocapillaris, the retinal pigment epithelium (RPE) and the overlying photoreceptors. Presently, up to 34 genetic susceptibility loci for AMD have reproducibly been reported (Fritsche et al. 2016) although functional studies have been hampered by the lack of available *in vivo* and *in vitro* model systems. We propose to establish an *in vitro* disease model for the study of AMD pathomechanisms by applying the high-end technology of induced pluripotent stem cells (iPSCs).

Methods: Risk alleles at 13 genetic loci together representing a highly informative genetic risk score (GRS) (Grassmann et al. 2012) were genotyped in over 200 AMD patients and disease-free controls. Probands were recruited at the University Eye Hospital Regensburg. Skin biopsy material from cases and controls was collected for reprogramming into induced pluripotent stem cells (hiPSCs) with subsequent differentiation into cells of the retinal pigment epithelium (hiRPE), the cell that is likely the source of primary disease pathology in AMD. To show functional consequences based on genetic background, the physiologic environment of a RPE cell affected by AMD is mimicked by physical and chemical stimuli like UV light and C2-Ceramides.

Results: From 216 patients screened for their GRS profile, we identified five AMD patients with highly increased GRS intervals (> 3.44) as well as six healthy controls with low GRS (≤ -1.79). From these individuals, five iPSC cell lines (three AMD patients, two controls) were successfully generated, followed by differentiation into hiRPE for three iPSCs lines (two AMD patients, one control). Thorough characterization of hiRPE-AMD and hiRPE-control cell lines demonstrates full functionality and high viability, though transepithelial resistance (TER) measurements reveal a constantly lower level of cell polarity in hiRPE-AMD cell lines.

Conclusion: Here we propose a coherent concept to assess functional consequences of a complex combination of risk alleles in an adequate cellular system. Preliminary experiments show promising prospects and a great potential of our proposed cell-culture model closely reflecting the idea of "a patient in a dish".

References:
Fritsche et al. (2016). Nature Genetics, 48, 134-143.
Grassmann et al. (2012). PLoS One, 7, e37979.

Biomechanical fingerprinting of rod photoreceptor development for label-free sorting

Tiago Santos-Ferreira[1,], Maik Herbig[2,*], Oliver Otto[2,5], Madalena Pereira[1], Mike O Karl[1,3], Stylianos Michalakis[4], Jochen Guck[2], Marius Ader[1]*

[1]CRTD/Center for Regenerative Therapies Dresden, Technische Universität Dresden, Fetscherstrasse 105, 01307 Dresden, Germany
[2]Biotecnology Center, Technische Universität Dresden, Tatzberg 47-49, 01307 Dresden, Germany
[3]German Center for Neurodegenerative Diseases (DZNE), Dresden, Germany
[4]Center for Integrated Protein Science Munich (CiPSM), Department of Pharmacy – Center for DrugResearch, Ludwig-Maximilians-Universität München, Munich, Germany
[5]Centre for Innovation Competence: Humoral Immune Reactions in Cardiovascular Diseases (HIKE), University of Greifswald, Greifswald, Germany

** Tiago Santos-Ferreira and Maik Herbig equally contributed to the work.*

Transplantation of photoreceptors is a promising approach aiming to replace the loss of photoreceptors and showed a certain degree of success in preclinical mouse models. Before transplantation, photoreceptors need to be sorted and purified to avoid engraftment of unwanted cells or teratomas formation. The mechanical properties of the cell have been suggested as label free marker which could be used for cell sorting. These properties can be analyzed in a contactless, high-throughput manner using real-time deformability cytometry (RT-DC). Here we characterized primary rod photoreceptor development and the generation of embryonic stem cell-derived rods at the mechanical level using RT-DC. We showed that, independent of their source, rods become smaller, softer and deform less as development proceeds. Additionally, their mechanical properties are sufficient to separate rod photoreceptor from other retinal cells in an unlabeled heterogeneous population. Hence, this study identified the key rheological parameter for future label-free sorting using RT-DC.

Complement regulator fhr-3 is elevated either locally or systemically in a selection of autoimmune diseases

*Nicole Schäfer[1], Antje Grosche[2], Joerg Reinders[3], Stefanie M. Hauck[4], Richard B. Pouw[5], Taco W. Kuijpers[6,7], Diana Wouters[5], Boris Ehrenstein[8], Volker Enzmann[9], Peter F. Zipfel[10,11], Christine Skerka[10], Diana Pauly[1]**

[1] *Department of Ophthalmology, University Hospital Regensburg, Regensburg, Germany*
[2] *Institute of Human Genetics, University of Regensburg, Regensburg, Germany*
[3] *Institute of Functional Genomics, University of Regensburg, Regensburg, Germany*
[4] *Research Unit Protein Science, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany*
[5] *Department of Immunopathology, Sanquin Research and Landsteiner laboratory of the Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands*
[6] *Department of Pediatric Hematology, Immunology & Infectious Diseases, Emma children's hospital, Academic Medical Center, Amsterdam, the Netherlands*
[7] *Department of Blood Cell Research, Sanquin Research and Landsteiner laboratory of the Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands*
[8] *Klinik und Poliklinik für Rheumatologie und Klinische Immunologie, Asklepios Klinikum Bad Abbach, Bad Abbach, Germany*
[9] *Department of Ophthalmology, University of Bern, Inselspital, Bern, Switzerland*
[10] *Department of Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany*
[11] *Friedrich Schiller University, Jena, Germany*

The human complement factor H-related protein-3 (FHR-3) is a soluble regulator of the complement system. Homozygous *cfhr3/1* deletion is found to be protective in age-related macular degeneration (AMD) and a genetic risk factor for the autoimmune form of hemolytic uremic syndrome (aHUS). The precise function of FHR-3 remains to be fully characterized.
We generated four mouse monoclonal antibodies (mAbs) for FHR-3 (RETC) without cross-reactivity to the complement factor H (FH)-family. These antibodies detected FHR-3 from human serum with a mean concentration of 1 µg/mL. FHR-3 levels in patients were significantly increased in sera from systemic lupus erythematosus, rheumatoid arthritis, and polymyalgia rheumatica but remained almost unchanged in samples from AMD or aHUS patients. Moreover, by immunostaining of an aged human donor retina we discovered a local FHR-3 production by microglia/macrophages. The mAb RETC-2 modulated FHR-3 binding to C3b and oxidative stress epitopes, but not the binding of FHR-3 to heparin. Interestingly, FHR-3 competed with FH for binding and the mAb RETC-2 reduced the interaction of FHR-3 and C3b or oxidative stress epitopes, resulting in increased local FH binding.
Our results unveil a previously unknown systemic involvement of FHR-3 in rheumatoid diseases and a putative local role of FHR-3 mediated by microglia/macrophages in the damaged retina. We conclude that the local FHR-3/FH equilibrium in AMD is a potential therapeutic target, which can be modulated by our specific, chimeric mAb RETC-2.

Artificial organic photoreceptors for photo-electrical stimulation of neuronal cells

*Oliya S. Abdullaeva[1], F. Balzer[2], K. Habashy[1], M. Schulz[3], J. Parisi[1], A. Lützen[3], K. Dedek[1], M. Schiek[1]**

[1]University of Oldenburg
[2]University of Southern Denmark
[3]University of Bonn
**manuela.schiek@uni-oldenburg.de*

In many blindness-causing diseases, photoreceptors degenerate whereas second order and projecting neurons are largely unaffected. Thus, one promising avenue to restore vision in affected patients is to develop artificial photoreceptors for retinal prosthetic devices [1]. Artificial photoreceptors based on organic semiconductors emerged as promising alternative for inorganic materials due to increased biocompatibility and the feasibility of direct optical stimulation [2]. We follow an electrophysiological patch clamp approach to conduct fundamental mechanistic studies on a model system, which consists of murine neuroblastoma (N2A) cells grown on a textured small-molecule organic semiconductor thin film under physiological conditions. We have chosen a custom-made anilino-squaraine dye, shortly named SQIB, blended with a commercial fullerene derivative as active layer of the artificial photoreceptor. Patch clamp recordings showed, that photoexcitation of the system with short light pulses stimulated fast capacitive transmembrane currents in the N2A cells [3]. The electrical coupling between the artificial photoreceptor and the neuronal cells was fast and direct, but still was only of passive nature. To increase the capacitive coupling, we deposit an additional dielectric coating such as silicon dioxide onto the active layer. We conduct a systematic investigation of the impact of the dielectric coating on transient photocurrents within the electrolyte and the consequential transmembrane currents. We anticipate transient photocurrents with increased peak values and extended decay time eventually provoking an active response of the cell. Additionally, we study the stability of the modified artificial photoreceptor in physiological environment under illuminated conditions by atomic force microscopy.

[1] L. Bareket-Keren, Y. Hanein, Int. J. Nanomed. 9 (2014) 65.
[2] D. Ghezzi, M. R. Antognazza, G. Lanzani, F. Benfenati, et al. Nat. Photon. 7 (2013) 400.
[3] O. S. Abdullaeva, M. Schulz, F. Balzer, J. Parisi, A. Lützen, K. Dedek, M. Schiek, Langmuir 32 (2016) 8533.

Characterization of endothelin receptor type b mediated signalling in Müller cells and photoreceptors

Sabrina I. Schmitt[1], Antje Grosche[2], Andreas Neueder[3] and Barbara M. Braunger[1]

[1]Institute of Human Anatomy and Embryology, University Regensburg
[2]Institute of Human Genetics, University Regensburg
[3]Sobell Department, University College London

Study objectives: The endothelin receptor type B (Ednrb) is expressed in Müller cells and neurons and is activated after binding of its ligands, including the neuroprotective molecule Endothelin 2 (Edn2). In this study, we characterized the expression of Ednrb and Edn2 in the mentioned cell types and their potential neuroprotective effects on the retina *in vivo* and *in vitro.*

Methods: We enriched Müller cells and neurons of wildtype mice by magnetic activated cell sorting and characterized mRNA expression levels of *Ednrb* and *Edn2*. To learn about the physiological function of the endothelin signalling pathway, we generated mutant mice with a conditional deletion of Ednrb in neurons and Müller cells *(α-Cre;EDNRB$^{fl/fl}$)*. We analysed the retinal morphology, performed morphometric analyses and investigated the molecular expression levels of *Ednrb, Edn2, fibroblast growth factor 2 (Fgf2)* and *leukaemia inhibitory factor (Lif)*. To examine the function of Ednrb/Edn2 signalling selectively in photoreceptors, we designed three guide RNAs to generate a Crispr/Cas mediated deletion of *Ednrb* in the photoreceptor cell line 661W. The mRNA expression levels of *Ednrb, Edn2* and *Lif* were analyzed.

Results: The mRNA expression level of *Ednrb* was higher in neurons than in Müller cells. The deletion of *Ednrb* in neurons and Müller cells *(α-Cre;EDNRB$^{fl/fl}$)* was confirmed by western blotting and realtime RT-PCR. There were no differences of the outer and inner nuclear layer morphology between *α-Cre;EDNRB$^{fl/fl}$* and control mice. The mRNA expression levels of *Edn2* and *Fgf2* were increased in the *α-Cre;EDNRB$^{fl/fl}$* mice compared to control littermates. The deletion of *Ednrb in vitro* via Crispr/Cas, selectively in photoreceptors, resulted in an up to 87% decreased mRNA level of *Ednrb*. Furthermore, real-time RT PCRs showed a decreased mRNA level of *Edn2* and *Lif*.

Conclusions: The deletion of *Ednrb* in neurons, Müller cells and photoreceptors influences the expression levels of *Edn2, Fgf2 and Lif* in a cell type specific manner. Our findings highlight the importance of Ednrb mediated signaling in the regulation of the expression of neuroprotective factors like Edn2, Fgf2 and Lif and might therefore point towards a new therapeutic avenue to attenuate the loss of neurons in diseases like hereditary retina degenerations or age-related macular degenerations.

L-Type Calcium channels are expressed and regulate VEGF secretion in stem cell-derived retinal pigment epithelium

*Lina Korkka[1], Kati Juuti-Uusitalo[2], Hannele Uusitalo-Järvinen[3], Heli Skottman[2], Jari Hyttinen[1], and Soile Nymark[1]**

[1]Faculty of Biomedical Sciences and Engineering, BioMediTech, Tampere University of Technology, Finland
[2]Faculty of Medicine and Life Sciences, BioMediTech, University of Tampere, Finland
[3]Eye Centre, Tampere University Hospital, Department of Ophthalmology, Faculty of Medicine and Life Sciences, University of Tampere, Finland
**Presenting author*

Purpose: Retinal pigment epithelium (RPE) performs important functions for the maintenance of photoreceptors and vision. Malfunctions within the RPE are implicated in several retinal diseases for which transplantation of stem cell-derived RPE is a promising treatment option. Its success, however, is largely dependent on the functionality of the transplanted cells. This requires correct cellular physiology, which is highly influenced by the various ion channels of the RPE. The purpose of this study was to investigate the L-type voltage-gated Ca^{2+} channels in human embryonic stem cell (hESC)-derived RPE as well as their role in vascular endothelial growth factor (VEGF) secretion.

Methods: The pluripotent embryonic cell lines were derived and maintained using existing protocols in the laboratory. The differentiation of RPE from pluripotent hESCs was performed by spontaneous differentiation method. Characterization of L-type Ca^{2+} channels was performed by whole-cell patch-clamp recordings of Ba^{2+} currents and by immunostainings. The data from hESC-derived RPE was compared to that from mouse tissue. The secretion of VEGF by mature hESC-RPE during 24 h incubation was assessed with enzyme-linked immunosorbent assay (ELISA) with and without the presence of L-type Ca^{2+} channel agonist (-)BayK8644 (10 µM) and antagonist nifedipine (10 µM).

Results: Patch-clamp recordings revealed the presence of slowly inactivating L-type currents in hESC-derived RPE comparable to freshly isolated mouse RPE. The findings were supported by immunostainings from both hESC-derived and mouse RPE: L-type channels $Ca_V1.2$ and $Ca_V1.3$ were detected from both cell types. Interestingly, the localization of the major subtype $Ca_V1.3$ only resembled native RPE at the very late stage of RPE maturation. Pharmacological modulation of the L-type channel activity correlated with the VEGF secretion level in hESC-RPE.

Conclusions: This study demonstrates that functional L-type Ca^{2+} channels are present in fully mature hESC-RPE and that they participate in the regulation of VEGF secretion. Our study increases the understanding of Ca^{2+} channels in stem cell-derived RPE and provides novel information regarding their maturation. The results of the study are promising for the success of stem cell based RPE transplantation therapies.

In search for functional progression markers in early onset retinitis pigmentosa caused by CRB1 mutations

Katarina Stingl[1], Marius Ueffing[1], Laura Kühlewein[1], M. Imran Khan[2], Nicole Weißschuh[1], Eberhart Zrenner[1], Krunoslav T. Stingl[1]

[1]Center for Ophthalmology, University of Tübingen, Tübingen, Germany
[2]Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Background: Mutations in the CRB1 gene are known to cause Leber congenital amaurosis or early onset retinitis pigmentosa. Adults suffering from these diseases are blind or have very low vision and the classical clinical disease progression markers such as visual fields or electrophysiology are not measurable anymore.
Defining progression markers of the natural history in late stages of hereditary retinal diseases represents thus a diagnostical challenge.

Methods: We examined 7 patients with early onset retinitis pigmentosa caused by CRB1 mutations in the Clinic for Hereditary retinal Degeneration (Center for Ophthalmology, University of Tübingen, Germany). The examination included detailed history, visual acuity measurement, perimetry, fullfield and multifocal electrophysiology, fullfield stimulus threshold to blue and red light and pupillography to red and blue light.

Results: The disease duration ranged from 21 to 62 years, calculated from the age of first symptoms based on the history. Visual acuity and visual fields were was measurable in only two patients. Fullfield and multifocal electrophysiology were not measurable in any of the patients. Fullfield stimulus threshold to blue and red light were measurable in five patients; the thresholds for blue light showed a strong correlation with disease duration ($R^2 = 0{,}84$). Pupillary responses were measurable in all patients but one (due to strong nystagmus); the post-illumination pupillary response to blue light showed a strong correlation with disease duration ($R^2 = 0{,}87$).

Conclusion: Fullfield stimulation threshold with blue light and pupillary response to blue light are suitable clinical progression markers for disease progression in late stages of retinitis pigmentosa caused by CRB1 mutations.

The role of ATF6a in achromatopsia: Histological characterization of two *Atf6a*$^{-/-}$ mouse models

Julia Stolz[1], Irene Gonzalez-Menendez[1], Peggy Reuter[1], Susanne Kohl[1], Bernd Wissinger[1]

[1]Molecular Genetics Laboratory, Centre for Ophthalmology, Tuebingen, Germany
Contact: Julia Stolz, Institute for Ophthalmic Research, University Tuebingen
+49 70 71 - 2 98 76 19, julia.stolz@med.uni-tuebingen.de

Purpose: Achromatopsia (ACHM) is a rare autosomal recessive retinal disorder caused by cone photoreceptor function loss. It is characterized by color blindness, nystagmus, photophobia and severely reduced visual acuity. Recently, we identified disease causing mutations in the *ATF6a* gene, a key regulator of the unfolded proteins response (UPR) and endoplasmic reticulum (ER) homeostasis. To clarify the underlying pathomechanism of ATF6a-ACHM, we analyzed the retinas of two different *Atf6*$^{-/-}$ knockout mouse models.

Methods: *Atf6a*$^{tm1.1Rjk}$ knockout mice, caused by deletion of *Atf6* exon 4, and *Atf6a*$^{tm1.Kamo}$ mice, generated by deletion of *Atf6a* exons 8 and 9, as well as control littermates were euthanized at certain ages, and eyes were extracted and embedded for retinal sectioning followed by immuno-(histo)chemical analysis. A pathology score was developed to plot and analyze observed alterations.

Results: *In vivo* examination had indicated a late onset degenerative process with rod and cone defects, and deposition of autofluorescent material in *Atf6a*$^{-/-}$ mice at the age of 18 month. Histological assessment of the retina of this mouse model at the age of 5 – 6 month and 12 – 22 months showed an age- and mutation dependent retinal degenerative phenotype that could be confirmed in a second independent *Atf6a*$^{-/-}$ mouse model. *Atf6a*$^{-/-}$ retinae showed a significant accumulation of intracellular globular autofluorescent epi- and subretinal depositions, as well as RPE swelling and degeneration of cell nuclei. The autofluorescent depositions correlated in their distribution with those observed in the *in vivo* analyses. Cells containing autofluorescent depositions could be identified as microglia (Iba1 positive cells) or activated Müller glia cells (GFAP positive cells). Nuclear staining on retinal sections showed a median reduction of three rows of photoreceptors nuclei in *Atf6a*$^{-/-}$ mice compared to wild-type, and a shortening of (blue/S) cone outer segments by 2 µm.

Conclusion: The characterization of the two independent *Atf6a*$^{-/-}$ mouse models revealed an age- and mutation-dependent degenerative retinal phenotype that differs significantly from that observed in human. While in human loss of ATF6a results in a congenital cone-only function loss, mice present with a very late cone- and rod function loss with significant autofluorescent epi- and subretinal depositions, a finding that was not observed in human ACHM patients. Based on the immunohistochemical stainings we could identify two different cell types, microglia and activated Müller glia cells, that contain those autofluorescent depositions. It is known that microglia can migrate into the subretinal space to phagocitize degenerating photoreceptors to protect the RPE against oxidative stress.

Modelling reactive gliosis in retinal organoids

Manuela Völkner[1] & Mike O. Karl[1,2]

[1]DZNE Dresden, Arnoldstraße 18, 01307 Dresden, Germany
[2]TU Dresden, CRTD, Fetscherstraße 105, 01307 Dresden, Germany
manuela.voelkner@dzne.de

The retina is part of the central nervous system and loss of vision due to retinal degeneration is one of the major causes of disability in industrialized countries. Reactive gliosis is commonly associated with all types of neurodegenerative diseases and may have beneficial and detrimental consequences. Most importantly, the role of glia in scar formation and proliferative disorders of the retina are incompletely understood. Pluripotent stem cell derived retinal organoids offer new possibilities for studies on retinal degeneration and regeneration. We developed an efficient protocol for 3D retina organoid differentiation from mouse embryonic stem cells (mESC) yielding big, stratified retinal tissue reminiscent of postnatal retina in vivo. Strikingly, we found that similar to the healthy retina in vivo the Müller glia (MG) in our retinal organoids do not express glial fibrillary acidic protein (GFAP), a hallmark of reactive gliosis, or other known gliosis associated markers. In contrast, reactive gliosis is well known to be strongly induced upon most retinal diseases and injury and upon animal derived retinal tissue culture. Thus, we hypothesized that the retinal organoid system offers a unique opportunity to study reactive gliosis. We asked if application of neuronal damage-related signaling factors is sufficient to induce reactive gliosis in the organoid system. Combined (but not single) application of defined cytokines significantly induced GFAP expression in MG, increased cell proliferation, as well as a severe loss of retinal stratification. Our results suggest a novel model of reactive gliosis, retinal remodeling and dystrophy. In future work we will compare the reactive gliosis response in the mouse and human retina organoid system – interestingly, proliferative gliosis is frequently observed in human patients but rarely in animal disease models.

Suppression of SNARE-dependent exocytosis in retinal glial cells and its effects on ischemia-induced neurodegeneration

Wagner L[1], Pannicke T[1], Cornelia Volz[2], Johannes Hirrlinger[3,4], Herbert Jägle[2], Philip G. Haydon[5], Frank W. Pfrieger[6], Antje Grosche[7]

[1]Paul Flechsig Institute of Brain Research, University of Leipzig, Liebigstr. 19, 04103 Leipzig, Germany; [2]Department of Ophthalmology, University of Regensburg, Franz-Josef-Strauß-Allee 1, 93953 Regensburg, Germany; [3]Carl Ludwig Institute of Physiology, University of Leipzig, Liebigstr. 27, 04103 Leipzig, Germany; [4]Department of Neurogenetics, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Str. 3, 37075 Göttingen, Germany; [5]Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA; [6]Institute of Cellular and Integrative Neurosciences, CNRS UPR 3212, University of Strasbourg, 5 rue Blaise Pascal, 67084 Strasbourg Cedex, France; [7]Institute of Human Genetics, University of Regensburg, Franz-Josef-Strauß-Allee 1, 93953 Regensburg, Germany

Purpose: Nervous tissue is characterized by a tight structural and functional association between glial cells and neurons. It is well known that glial cells support neuronal functions, but their role under pathologic conditions is less well understood. Here, we ask how exocytotic gliotransmission of the major retinal macroglia, the Müller cells, affects neuronal survival in an in vivo experimental model of retinal ischemia.

Methods: Transmitter release from acutaly isolated Müller cells was measured using fluorimetric enzyme assays based on the Amplex® Red system. These measurements were performed on Müller cells of wildtype and transgenic mice for glia-specific inhibition of SNARE-dependent exocytosis. A model of retinal ischemia/reperfusion was used to investigate the impact of glial dnSNARE expression under pathological stress conditions. Histological and immunohistochemical stainings were performed to demonstrate the ischemia-induced morphometric changes. Functional integrity of the retina was measured via electroretinogram (ERG) recordings.

Results: Transgene expression reduced glutamate release from single Müller cells resulting in an impaired glial volume regulation, while ATP release from Müller cells remained unaltered in transgenic mice suggesting hemichannels rather than exocytosis play a role in ATP release mechanisms. Transgene expression did not affect overall retinal functional integrity in untreated eyes. Interestingly, we found that neuronal dysfunction and death determined by morphometric analysis, TUNEL assays and ERG during the early stages of ischemia was reduced in transgenic mice.

Conclusion: We demonstrate that Müller cells are capable to release glutamate, but not ATP, via exocytosis. Importantly, lack of vesicular glutamate release from Müller cells appears to have positive effects in the ischemic retina. This might be due to a reduced amount of glial glutamate being released and, hence, less detrimental neuronal hyperexcitation associated with ischemia. Our study reveals that SNARE-dependent exocytosis in glial cells contributes to neurotoxicity during ischemia in vivo and suggests glial exocytosis as a target for putative therapeutic approaches.

Photoreceptor Guanylate Cyclase (GC-E) mutations and their functional consequence

Hanna Wimberg[1], Dror Sharon[2], Karl-Wilhelm Koch[1]

[1]Department of Neuroscience, Biochemistry Group, University of Oldenburg, Germany
[2]Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Purpose: Mutations in the *GUCY2D* gene (GC-E) lead to severe retinal diseases in humans like Cone-Rod Dystrophy (CRD) or Leber Congenital Amaurosis (LCA). Both diseases lead to a severe phenotype in affected patients. In CRD, degeneration starts in the cones and leads to loss of the central visual field. When degeneration of rods is followed by those of cones CRD ends in complete blindness. The LCA phenotype appears even more severe, with vision loss emerging very early in life. While more than a hundred mutations in the *GUCY2D* gene were phenotypically described, a link to functional consequences in the enzyme was set just for a small number. In this work, we attempt to biochemically characterize some recently identified mutations and try to relate the phenotype to functional impairments of the enzyme.

Methods: The various GC-E point mutations were cloned into a vector system for transfection and expression in HEK cells. In HPLC-based enzyme assays the potential of GC-E mutants to be activated or inhibited by the according proteins (GCAPs and RD3) was tested. Additionally the ability of GC-E mutants to dimerize shall be determined with crosslinking experiments. Via immunostaining the possibility of GC-E to be transported in a HEK cell system is investigated.

Results: The first results showed different effects on the enzyme activity due to localization in the various regions of the GC-E. The p.A710V and p.P873R mutations interestingly showed no enzyme activity at all. An increase in enzyme activity was found for the p.V902L mutant by affecting the catalytic core of the enzyme. This was rather unexpected, because other described mutations affecting the GC-E catalytic domain are connected to loss of GC-E function causing LCA. Mutations in the dimerization domain are known to cause CRD and often lead to a change in Ca^{2+} sensitive regulation of the protein. For instance, the mutants p.E841K and p.K846N exhibited a need for higher Ca^{2+} concentrations to shut off enzyme activity.

Conclusion: These results provide a route to better understand the negative effects of *GUCY2D* mutations in photoreceptor cell physiology. Differences in biochemical key properties of GC-E mutants might help us to understand why some GC-E mutations lead to a LCA phenotype while others result in CRD.

Targeting the translocator protein (18kDa) (TSPO) prevents microglia/macrophage reactivity in a murine model of AMD-like retinal degeneration

Anne Wolf[1] and Thomas Langmann[1]

[1]Laboratory of Experimental Immunology, Department of Ophthalmology, University of Cologne

Purpose: Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly and associated with chronic reactivity of microglia/macrophages in the retina. Our previous work showed that TSPO is a marker for reactive retinal microglia and that the selective TSPO ligand XBD173 exerts strong anti-inflammatory and neuroprotective effects on microglia in light-induced retinal degeneration. Here, we hypothesized that XBD173 dampens microglia/macrophage reactivity and limits choroidal neovascularization (CNV) in the murine model of AMD-like laser-induced retinal injury.

Methods: The mouse model of laser-induced CNV was used to mimick the exudative form of AMD. Retinal inflammation and CNV were analyzed in DMSO-treated C57BL/6J mice and C57BL/6J mice that received 10 mg/kg XBD173 by intraperitoneal injection using fundus fluorescein angiography (FFA), lectin staining and optical coherence tomography (OCT) 3, 7 and 14 days after laser coagulation. Microglia morphology in laser-induced lesions was analyzed by Iba1-staining of retinal flat mounts. Gene expression of pro-inflammatory and pro-angiogenic markers was determined at different time points after XBD173 treatment using qRT-PCR.

Results: Immunohistological analysis of Iba1-stained retinal flatmounts showed a reduced number of activated microglia/macrophages in the laser lesions in XBD173-treated mice compared to controls. XBD173-treated animals also displayed decreased vascular leakage and CNV compared to controls. Quantification of laser spot size indicates that XBD173-treated mice had a faster wound healing compared to the control group. XBD173 reduced gene transcription of pro-inflammatory and pro-angiogenic markers after laser-coagulation compared to controls.

Conclusion: XBD173 treatment reduced microgliosis and led to decreased vessel leakage and CNV. We conclude that TSPO and its ligands represent promising targets for neuroprotective and anti-inflammatory therapy of wet AMD.

Genome editing as therapeutic approach to treat XLRP

Mert Yanik, Maximilian Lehmann, Lennart Trimborn, Birgit Lorenz, Knut Stieger

Department of Ophthalmology, Justus-Liebig-Universität Gießen, Germany

Background: Mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene cause severe X-linked retinitis pigmentosa (XLRP). More than 80 % of the mutations are located in the terminal exon ORF15 of the *RPGR* gene. The aim of the project is to develop gene therapeutic approaches to treat XLRP through genomic editing. The common strategy for targeted gene editing is based on highly specific nucleases which cleave only once within the complex genome at a chosen position. The DNA gets repaired by two different repair pathways, error prone non-homologous end joining (NHEJ) or homologous recombination (HR) with the help of a donor template. The aim of this study is to develop a gene therapy strategy based on HR for XLRP.

Methods: Eleven sequences within or around the ORF15 gene have been targeted for the induction of double or single strand breaks of the DNA. Ten target sites for CRISPR/Cas9-FokI were chosen, three before, within and four behind the exon, respectively, and one target site for TALE-MutH within the exon. These sequences have been cloned into the traffic light reporter (TLR) gene expression system at the homing endonuclease I-SceI site. The TLR system has been modified to express either GFP in case of successful HR or BFP in case of NHEJ. Efficiency of DNA modification was measured in HEK293 cells by FACS analysis. In addition to the episomal TLR system, the genome of murine C2C12 cells were targeted with TALE-MutH nucleases and analysed by next-generation-sequencing (NGS) for on and off-target activity predicted by bioinformatics. Next to activity, the HR events in combination with TALE-MutH and short Oligos (ssODN) have been analysed in HEK293 cells with stably cloned murine ORF15 sequences with NGS.

Results: Cas9-FokI showed preferences in its activity within the ten target sites with activities well above I-SceI activity, while the one target site of TALE-MutH is as efficient as I SceI. Further analysis of TALE-MutH with NGS revealed a high HR rate with certain ssODN as donor templates and the off-target analysis after NGS has been done with 8 different free available tools with controversial results.

Conclusion: The characterization of the activity and toxicity of the tested endonucleases helped us to identify the most promising tailored nuclease and its target sequence in our gene targeting approach to treat XLRP. Before using mouse retinal explants we are studying the efficacy of our strategy in detail with chosen sites and NGS.

Usher syndrome proteins interact with pre-mRNA splicing factors

Adem Yildirim, Barbara Knapp and Uwe Wolfrum

Molecular Sensory Biology, Institute of Molecular Physiology, Johannes Gutenberg-University of Mainz, Germany

Purpose: Human Usher syndrome (USH) is the most common form of combined deaf-blindness. Here, we aim to enlighten the molecular function of the USH1G protein SANS and the USH2C protein VLGR1. For this, we were searching for potential interaction partners, which may define cellular modules related to USH pathophysiology.

Methods: For identification of putative binding partners, we applied yeast-2-hybrid screens (Y2Hs) of retinal cDNA libraries and tandem affinity purifications (TAPs). We validated the identified interactions by complementary assays such as pull down assays. For subcellular localization of complex partners, we applied immunofluorescence and immunoelectron microscopy. We analysed the role USH proteins in splicing of putative client genes after siRNA-mediated knockdowns. For the measurement of the ratios of each splice variants, we applied our samples to capillary electrophoresis.

Results: We identified pre-mRNA splicing factors (PRPFs) as putative interaction proteins of SANS and VLGR1 by Y2H and TAPs. Pull downs confirmed the interactions of SANS and VLGR1 with PRPF6 and PRPF31, major components of the spliceosome complex, which is processing most of the pre-mRNA splicing in the nucleus. Depletion of both PRPFs and SANS revealed very similar perturbation profiles of splice variants in a set of test genes and the USH1C gene harmonin. Immunohistochemistry revealed co-localization of USH proteins and PRPFs in the nuclear speckles and at the base of primary cilia of cultured cells and retinal photoreceptors.

Conclusions: In human, defects in the PRPFs, PRPF6 and PRPF31 and in USH molecules, SANS and VLGR1 cause a common ophthalmologic phenotype, retinitis pigmentosa (RP). Here we show that USH proteins and PRPFs are part of the same splicing complex in the nucleus and cooperate in the regulation of splicing of pre-mRNAs. We reason that splicing defects may be a common pathophysiologic pathway that underlay the retinal phenotype in subtypes of USH and RP caused by PRPF defects.

Supports:
GeneRED; IPP Programme Mainz; FAUN-Stiftung, Nuremberg; DFG FOR2149 WO548

The clinical phenotype of *CNGA3*-related achromatopsia: Pre-treatment characterisation in preparation of a gene replacement therapy trial

Ditta Zobor[1], Annette Werner[1], Franco Stanzial[2], Francesco Benedicenti[2], Günther Rudolph[3], Ulrich Kellner[4], Christian Hamel[5], Sten Andréasson[6], Gergely Zobor[1], Torsten Strasser[1], Bernd Wissinger[1], Susanne Kohl[1] & Eberhart Zrenner[1,7] for the RD-CURE Consortium

[1]Center for Ophthalmology, University of Tübingen, Germany
[2]Department of Pediatrics, Genetic Counseling Service, Hospital of Bolzano, Italy
[3]Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany
[4]Rare Retinal Disease Center, AugenZentrum Siegburg, MVZ ADTC Siegburg, Germany
[5]Institute of Neurosciences of Montpellier, Hôpital Saint Eloi, Montpellier, France
[6]Department of Ophthalmology, Lund University, Lund, Sweden
[7]Werner Reichardt Center for Integrative Neuroscience, University of Tübingen, Germany

Purpose: To clinically characterize patients with *CNGA3*-linked achromatopsia (CNGA3-ACHM) in preparation of a gene therapy trial.

Methods: 36 patients (age 7 to 56) with complete (cACHM) or incomplete (iACHM) CNGA3-ACHM were examined, including detailed psychophysical tests, extended electrophysiology and assessment of morphology by FAF and SD-OCT.

Results: Mean BCVA was 0.78 ± 0.14 logMAR. Colour vision tests were consistent with a rod-dominated function in every cACHM patient. Microperimetry indicated an overall lowered retinal sensitivity within 20° of visual field. In electroretinography (ERG) photopic responses were non-detectable in cACHM patients, but residual cone responses were observed in the iACHM patients. Scotopic responses were altered referring to anomalies of photoreceptor and postreceptor signaling, while in Voltage vs. Intensity functions, Vmax was significantly below normal values ($p < 0.05$). In contrast, slope (n) and semi-saturation intensity (k) were found to be within normal limits. SD-OCT examination showed no specific changes in 14.7 %, disruption of the ellipsoid zone (EZ) at the fovea in 38.2 %, absent EZ in 17.7 %, a hyporeflective zone in 20.5 % and outer retinal atrophy in 8.9 % of all cases and foveal hypoplasia in 29 patients (85 %). No correlation of retinal morphology with visual function or with specific genotype was found. The severity of morphological and functional changes lacked a robust association with age.

Conclusion: Our extended investigations prove that even among such a genetically homogenous group of patients, no specific correlations regarding function and morphology severity and age can be observed.

THIS MEETING IS ORGANISED BY

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

and

interface

Interface GmbH & Co. KG
Postfach 0455
97301 Kitzingen
Tel: +49 (0) 93 21 / 3 90 73-00
Fax: +49 (0) 93 21 / 3 90 73-99
info@interface-congress.de
www.interface-congress.de

THIS MEETING IS FUNDED BY

PRO RETINA
POTSDAM-MEETING

PRO RETINA-RESEARCH FOUNDATION FOR PREVENTION OF BLINDNESS

www.potsdam-meeting.de

THIS MEETING IS SUPPORTED BY

NOVARTIS