PRO RETINA FOUNDATION

PRO RETINA POTSDAM-MEETING

9th PRO RETINA Research-Colloquium Potsdam

CONFERENCE REPORT

Retinal Degeneration

A Step Back is a Step Forward

An Interdisciplinary Dialogue

April 4th/5th, 2014

Seminaris SeeHotel Potsdam

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HOPE2 HEREDITARY RETINAL DISORDERS From Patients Towards Therapies

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PRO RETINA FOUNDATION FOR PREVENTION OF BLINDNESS

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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 (2014), PRO RETINA Deutschland e. V. counts more than 5,800 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 4th 2014

13:00–13:05 **Welcome remarks**
Franz Badura, PRO RETINA Foundation/Research Division

13:05–13:50 **Keynote-Lecture**
Thomas Rosenberg, Glostrup, Denmark
The "Sleeping Beauty" (Dornröschen) is still dreaming of her prince – Clinical trials in retinal dystrophies

13:50–15:30 **Session 1** **Chairman: Olaf Strauß**

13:50–14:15 Diana Pauly, Regensburg
Antibody therapies in AMD as a treatment option – An overview

14:15–14:40 Glen Jeffery, London
Complement and AMD: Are there lessons from animal models?

14:40–15:05 Steffen Schmitz-Valckenberg, Bonn
Role for the complement system as a therapeutic target in atrophic age-related macular degeneration

15:05–15:30 Bärbel Rohrer, Charleston
Complement-activation and age-related macular degeneration: Generation of novel treatments and diagnostics

15:30–16:30 **Coffee break with scientific chitchat**

16:30–18:10 **Session 2** **Chairman: Bernhard Weber**

16:30–16:55 Muna Naash, Oklahoma City
Nanoparticle-based gene therapy for ocular diseases: An update

16:55–17:20 Daniel Chung, Philadelphia
Update on RPE65 gene therapy clinical trials – Can we halt retinal degeneration?

17:20–17:45 Markus Groppe, Oxford
Gene therapy for choroideremia

17:45–18:10 Armin Göpferich, Regensburg
Targeted nanoparticles

18:10 **Dinner**

19:30–open **Swingin' poster session**

PROGRAMME

Saturday, April 5th 2014

08:45 – 10:25	**Session 3**	**Chairman: Klaus Rüther**
	08:45 – 09:10	Michael Bach, Freiburg *Perception with visual prosthetics*
	09:10 – 09:35	Moritz Helmstaedter, München *Reconstructing the retina: Connectomics of the inner plexiform layer*
	09:35 – 10:00	Jens Duebel, Paris *Restoring vision by using microbial opsins*
	10:00 – 10:25	Mark Greenlee, Regensburg *Vision without a fovea: Results of training eccentric fixation in AMD patients*
10:25 – 11:15	**Coffee break**	
11:15 – 12:55	**Session 4** Eight selected poster presentations, followed by presenting the three 2014 PRO RETINA poster price awards	
12:55 – 13:00	**Concluding remarks**	
13:00	**Lunch and end of meeting**	

SPEAKER ABSTRACTS

The "Sleeping Beauty" (Dornröschen) is still dreaming of her prince – Clinical trials in retinal dystrophies

Thomas Rosenberg

Purpose: To present an overview of all clinical trials to date dealing with retinal dystrophies.

Methods: The presentation with mention the ideas behind clinical trials and the general set-up for a trial. Next the eye as a therapeutic target will be emphasized and different methods for outcome measures reviewed.
A search for "retinitis pigmentosa" OR "retinal dystrophy" in the database www.clinicaltrials.gov was made, resulting in 111 hits. After sorting out 21 irrelevant trials the registered therapeutic modalities are accounted of, including physical treatments, drug treatments, transplantation, stem cell treatments, gene treatments, and prosthetic treatments.

Results: Among 90 trials the 56 included some form of therapeutic intervention. However, it is still not possible to cure any hereditary retinal disorder, and we as well as the patients still have to wait a while before we can go from reasonable hope to reality. The present count is only representing the top of the iceberg as a large amount of new clinical trials will be introduced during the next few years and additional results from the ongoing trials will be published.

Conclusion: The review will conclude the first three decades of clinical trials in nine main points.

Antibody therapies in AMD as a treatment option – An overview

Diana Pauly

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

This year we celebrate the 10th anniversary of the anti-angiogenesis therapy for wet age-related macular degeneration (AMD). In long-term studies, anti-vascular endothelial growth factor (VEGF) therapy yielded a good visual outcome in one third of exudative AMD patients, whereas another third fared poorly. In contrast to the wet form, there is still no specific drug on the market for dry AMD, which accounts for 90% of all AMD cases. The partial success story of anti-VEGF drugs encouraged further development of therapeutic antibodies for the treatment of wet and dry AMD. Over hundred active clinical studies are recruiting for interventional AMD therapies and the ophthalmic market will reach US$20.9bn this year. At least ten of these drugs are antibodies or antibody fragments targeting inflammation, the complement pathway, amyloid beta deposition or cell growth. Recently, the complement cascade, a part of the innate immune system, was highlighted as a key pathway for AMD-treatment. Eculizumab, Lampalizumab and LFG316 are antibodies specific for central proteins of the alternative complement pathway and in clinical phase II studies. Systemic inhibition of complement factor C5 by Eculizumab was well tolerated but did not decrease the growth rate of geographic atrophy (GA). Lampalizumab, an antibody fragment targeting complement factor D showed 20% reduction rate of GA after 18 months intravitreal injections. Potential therapeutic antibodies against regulatory proteins of the complement system are in preclinical development. These drugs offer the opportunity for fine-tuning of the complement system and do not directly interfere with the main cascade. A novel monoclonal antibody against properdin, the only known positive regulator of the alternative complement system, inhibits the complement system *in vitro* at a lower effective concentration compared to anti-C5 antibodies. Other regulatory proteins of the complement system are complement factor H-related proteins 1 and 3 (CFHR1/3) but their function is unknown. Studies revealed a protective effect of CFHR1/3 deficiency for AMD development. CFHR1/3 and properdin represent promising antigens for AMD-antibody therapy in the future.

Complement and AMD: Are there lessons from animal models?

Glen Jeffery

Institute of Ophthalmology, University College London, UK

Purpose: Inflammation is a key feature of ageing and disease, particularly in the retina because of its high metabolic demand. With age and inflammation there is declining mitochondrial function with reduced ATP and increased ROS production. Reducing inflammation is a key target in ocular health to reduce the pace of age related photoreceptor loss and reduce the probability of ageing tipping over into disease.

Methods: We adopt two routes in aged C57 mice and immune compromised mice (CFH-/-). First, systemic administration of vitamin d known to modulate the immune system. Second, shifting mitochondrial function by optically stimulating cytochrome C oxidase (COX) with near infra-red light (NIR) at a low energy of 40 mW/cm^2 which is roughly equivalent to indirect daylight. This is administered for 60 – 90 seconds daily over 5 days or longer.

Results: Vitamin d significantly reduces a range of inflammatory markers in the outer retina along with clearing age related macrophages. This was related to an improved ERG. Brief pulsing with NIR over 5 days resulted in increased mitochondrial fusion and COX up regulation, which was associated with increased mitochondrial membrane potentials and elevated ATP production. This significantly reduced a range of independent inflammatory markers in normal old mice and old CFH-/- animals. The effect can be sustain over 4 months and can be delivered by supplemented environmental lighting. Because the wavelengths are relatively long they can penetrate tissue deeply. Hence, ATP levels were also elevated in the brain.

Conclusion: Maintaining normal levels of vitamin d is important in general health and is likely to be an issue in the retina. Examination of outpatient populations in a London clinic revealed that in around 18 % of cases there was no detectable vitamin d in their serum. NIR light is now widely demonstrated to have an impact on inflammation in diverse tissues form a number of labs. The mechanism is partly revealed and efficacy can now be obtained at 4 mW/cm^2. Device costs are economic and self-administration possible reducing clinical time. One clinical trial is in place for birdshot chorioretinopathy, a mainly inflammatory condition and a large MRC trail has been applied for to treat early AMD.

Role for the complement system as a therapeutic target in atrophic age-related macular degeneration

Steffen Schmitz-Valckenberg

Universitäts-Augenklinik Bonn

Purpose: Atrophic age-related macular degeneration (AMD) is the next challenge following the breakthrough in the treatment of neovascular age-related macular degeneration (AMD). Among various interventional pharmacologic approaches, one promising target is the inhibition of the complement system.

Methods: Atrophic AMD is characterized by the development and continuous enlargement of atrophic patches that are spatially confined to absolute scotomata. The slow progression of the disease and the low sensitivity of central visual acuity measurements compared to progressive visual disabilities require innovative concepts for the assessment of therapeutic efficacy.

Results: Possible clinical endpoints include reduction in drusen burden, slowing the enlargement rate of GA lesion area, and slowing or eliminating the progression of intermediate to advanced AMD. Particularly, retinal imaging technology may serve as a surrogate marker for disease progression and functional loss. Furthermore, high-risk markers for fast progression can be identified. Several lines of evidence suggest that local inflammation and dysregulation of the complement system play a significant role in the progression of AMD. Regulators of the complement systems such as Eculizumab, ARC-1905 or Lampalizumab have been or are being tested in interventional, randomized clinical trials in patients with atrophic AMD.

Conclusions: The complement system is currently one major target for pharmaceutical interventions to slow down disease progression in atrophic age-related macular degeneration. Until efficacy and safety is demonstrated in large-scale clinical trials for any therapeutic strategy, low-vision aids and further rehabilitative measures remain essential for patients with advanced dry AMD.

Complement-activation and age-related macular degeneration: Generation of novel treatments and diagnostics

Bärbel Rohrer[1,2,3], Kannan Kunchithapautham[1], Alex S. Woodell[2], Liudmila Kulik[4], Beth Coughlin[1], Gloriane Schnabolk[3], Joshua M. Thurman[4], V. Michael Holers[4]*

Departments of Ophthalmology[1] and Neuroscience[2], Medical University of South Carolina, Charleston, SC; [3]Research Service, Ralph H Johnson VA Medical Center, Charleston, SC; [4]Department of Medicine, University of Colorado Health Sciences Center, Denver, CO

Purpose: Uncontrolled activation of the alternative complement pathway (AP) is thought to be associated with age-related macular degeneration. During activation of the complement cascade complement C3 protein is hydrolyzed resulting in the generation and fixation of C3 activation fragments on affected tissues. Previously, we have shown that in mouse laser-induced choroidal neovascularization (CNV), C3 fragments are present in the CNV lesions. We have tested whether these fragments can be used as addressable ligands for targeted therapeutics to reduce CNV, and to use novel anti-C3d antibodies to detect complement activation *in vivo* in mouse CNV.

Methods: We have generated an AP inhibitor, which is a fusion protein consisting of a complement receptor 2 fragment linked that recognizes C3 fragments on cell surfaces to a complement inhibitory domain of factor H (CR2-fH). To generate a C3d-specific antibody, C3-deficient mice were immunized with human C3d protein. CNV was induced by argon-laser photocoagulation in C57BL/6J mice. Progression was analyzed using molecular, histological and electrophysiological readouts. *In vivo* C3d imaging was performed using the Micron III imaging system after tailvein injection of FITC-labeled mAb.

Results: (1) The AP inhibitor CR2-fH significantly reduced CNV size, concomitant with a decrease in mRNA and protein for complement factor C3 and VEGF and preserved ERG amplitudes. (2) Bioavailability studies showed targeting of CR2-fH after intravenous administration to the lesion sites. (3) mAbs were identified that preferentially bound to the iC3b, C3dg, and C3d fragments, without binding to or interacting with C3 or C3b. (4) FITC-C3d29, bound to CNV lesions could be imaged *in vivo* 24 hrs after the injection by Micron III retinoscopy, when compared to a nonspecific FITC-labeled mAb.

Conclusions: The data show that the AP pathway plays an important role in CNV development and demonstrate that its specific inhibition may represent a potential treatment for AMD. Importantly, CR2-fH targets to ocular tissues and reduces the development of CNV following intravenous injection in both a preventative and a therapeutic paradigm, a finding that opens a new avenue for the development of treatment strategies for AMD. Antibodies specific to tissue-bound C3 activation fragments can be used to visualize sites of complement

activation. This technique may be employed in the future for diagnostic purposes and to monitor effects of therapeutic agents.

NIH grant EY019320, a Department for Veterans Affairs merit award RX000444 and the Beckman Initiative for Macular Research.

Nanoparticle-based gene therapy for ocular diseases: An update

Muna I. Naash

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Mutations in genes expressed in the photoreceptors and retinal pigment epithelium (RPE) cause a wide range of blinding diseases including Leber's congenital amaurosis (LCA), Stargardt's macular dystrophy, retinitis pigmentosa (RP), and a variety of other forms of macular dystrophy. As many of these diseases result from loss-of-function mutations, we have been exploring non-viral gene replacement therapy with compacted DNA nanoparticles (NPs). These NPs are composed of polyethylene-glycol conjugated polylysine and DNA, and for a typical expression plasmid have a minimum diameter of 9 nm. We have conducted extensive studies demonstrating that they are well-tolerated and safe for use in the eye and can mediate long-term, (often for the life of the animal) phenotypic improvements in mouse models of RP *(rds$^{+/-}$)*, Stargardt's dystrophy *(abca4$^{-/-}$)*, and LCA *(rpe65$^{-/-}$)*. In spite of these positive results, clinical relevance requires further improvements in levels and distribution of expression. In an effort to assess ways to improve distribution, we have expanded testing in large animal models (namely non-human primates) and show that the nanoparticles efficiently express in the retina and RPE in this model. Our testing showed that the DNA in the nanoparticles is subject to extensive epigenetic regulation. Therefore, we are also testing the efficacy of a including a variety of additional DNA elements to take advantage of the nuclear architecture of the cell and prevent silencing and down-regulation of expression. With these advancements, compacted DNA nanoparticles have the potential to become clinically relevant treatments for a variety of inherited ocular diseases.

Update on RPE65 gene therapy clinical trials – Can we halt retinal degeneration?

Daniel Chung, Philadelphia

Introduction/Objectives: One of the most severe forms of inherited blindness in children is a group of diseases known as Leber Congenital Amaurosis (LCA). LCA is a recessively inherited infantile-onset retinal dystrophy that presents at birth with vision loss, nystagmus, and flat electroretinographic responses. Defects in one gene, RPE65, cause type 2 LCA, characterized by moderate vision impairment at infancy with progression to total blindness by early to mid adulthood. The RPE65 gene encodes a retinal isomerase involved in vitamin A metabolism and is responsible for the regeneration of 11-cis retinal after light exposure. Although the absence of this protein causes early profound visual impairment in humans and animal models, the degeneration of the retinal cells themselves is delayed. The history and data of initial clinical trial phases will be reviewed, and an overview of the structure of the ongoing phase III trial will be discussed, as well as the potential for long term functional vision.

Methods: Proof-of-principle for gene therapy for LCA 2 was established in two animal models, the Briard dog, a canine species with a spontaneously occurring RPE65 mutation, and both a spontaneously occurring and a genetically-engineered mouse, the Rpe65-/- knockout. In canine studies of LCA2, an adeno-associated virus (AAV) vector containing RPE65 complementary DNA (transgene) was injected into the subretinal space of one eye. Functional analysis revealed restoration by electroretinography, pupillometry and visual behavior studies. Similar results were obtained in mouse models. Twelve individuals were enrolled in phase I/II clinical trials, and were subretinally injected with an AAV vector carrying the wildtype human RPE65 gene (AAV2-hRPE65v2) in a dose escalation study. Subjects underwent both subjective and objective testing at baseline and in follow-up visits. In the subsequent readministration study, the contralateral eyes were also injected, with similar testing protocols as the initially tested eye.

Results: AAV2-hRPE65v2 was well tolerated and subjects demonstrated sustained improvement in subjective and objective measurements of vision (ie, dark adaptometry, pupillometry, electroretinography, nystagmus, and ambulatory behavior). The greatest degree of improvement was seen in children, all of who gained ambulatory vision. Subjects that received readministration in their contralateral eye, also showed similar improvements on both subjective and objective testing.

Conclusions: The use of AAV-mediated gene therapy for treatment of inherited retinal diseases has been shown to be safe, and efficacious. The stability of the improvement in vision in subjects appears to be sustained within the current study period.

Gene therapy for choroideremia

Markus Groppe[1, 2, 3], Robert E. MacLaren[1, 2, 3]

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Purpose: To report the results of a gene therapy clinical trial using an adeno-associated viral (AAV) vector encoding Rab escort protein (REP)1 in six patients with a diagnosis of choroideremia.

Methods: Patients with genetically confirmed diagnosis of choroideremia underwent pre and postoperative visual function assessment and anatomical changes were assessed with OCT. The gene was delivered by subretinal injection of an AAV viral vector.

Results: The surgical procedure did not cause any adverse events. Six months after surgery, the visual acuity improved by two lines and four lines in the two patients in whom VA was less than 6/12 at baseline. VA returned to within one line of baseline in the other four patients. Despite undergoing foveal detachment, the mean retinal sensitivity of functional retinal areas across the cohort was 7.74 dB before surgery. The sensitivity was unchanged 1 month after surgery (7.54 dB) but improved to 8.85 dB 6 months after surgery. The OCT examination showed complete absorption of the vector one day after surgery and no significant structural changes at 1 m and 6 m.

Conclusions: The recovery of VA and retinal sensitivity after foveal detachment in our study suggest that this method of treatment does not bear any adverse effects. Furthermore in patients with advanced disease an early improvement in function was noted.
The sustained improvements seen show the potential of gene replacement therapy when applied before the onset of foveal thinning and validate the concept of subretinal gene delivery predicted by the preclinical studies over recent years.

Targeted nanoparticles

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Purpose: When drugs fail to reach their target in the organism due to unfavorable physicochemical properties it is an option to load them on nanoparticles to overcome these limitations. By making such drug loaded colloids we trade the drugs' physicochemical properties against that of the colloidal particles. While this strategy alone is sufficient to overcome the problem in some disease areas, nanoparticles may not even recognize their target cell or interact with it in others. This is also true for nanoparticles intended to be used for the therapy of retinal diseases such as wet age-related macular degeneration (wet AMD) and proliferative diabetic retinopathy (PDR). One option to overcome this limitation is to design the nanoparticle corona such that it interacts directly with the target cell. The intention of our work is to explore the feasibility of designing colloidal medicines that allow intravenous administration for the treatment of wet AMD and PDR in the future.

Methods: We designed quantum dot nanoparticles that are able to target the αvß3 integrin receptor or the angiotensin II receptor type 1 (AT1R). To this end we immobilized a cyclic RGD peptide or EXP3174, an AT1R antagonist, on the particle surface. We tested their avidity for the targeted receptors on endothelial cells *in vitro*. Their ability to bind to retinal endothelial cells *in vivo* was investigated by injection of the particles in the tail vein of mice.

Results: In vitro we found that both particle species bind to endothelial cells via a receptor- specific interaction with high avidity. Concomitantly we noticed that the particles bind in a multivalent way to the cells and are hard to displace once they adhere to their target cell. *In vivo* we could demonstrate that the particles bind to endothelial cells of the choroid as well as of intraretinal blood vessels.

Conclusion: Ligand decorated nanoparticles allow for the specific binding of particles to endothelial cells. For the therapy of PDR or wet AMD such particles could either be used for blocking disease relevant receptors or for the delivery of antiangiogenic drugs.

Perception with visual prosthetics

Michael Bach[1] and Robert Wilke[2]

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Visual prosthetics gives us the exciting option to bypass pathologic structures (e.g. photoreceptors) and stimulate the visual pathway electrically. To what degree can this replace physiological processing? We will discuss some thoughts on this, starting from the notion that "the eye is not a digital camera".

The basic principle of electric stimulation is subject to the constraints of the achievable grain in potential field distribution. Calculations on possible local field gradients reveal marked constraints on local resolution as will be demonstrated by simulation results.

The resolution of stimulation is, naturally, limited by electrode count and also by spatio-temporal electric field constraints. Thus spatial aliasing needs to be taken into account: In human eyes, the optical resolution – as constrained by both refractive aberrations, and diffraction due to the finite pupil aperture – matches the receptor mosaic's resolution harmoniously; thus physiological aliasing has only been found in the mid periphery. With prosthetic devices, it will be necessary to match optics to electric resolution, possibly by purposefully degrading the optics.

Furthermore, temporal aliasing is also expected: rods have their CFF at 20 Hz, cones at 50 Hz, while typical chip sampling currently occurs below 10 Hz. This poses obvious limitations on flicker resolution, but also on motion perception, especially with high-frequency targets.

One physiologic concern is the summary stimulation of the on/off channels in the retina. While these two systems are stratified, current methodologies do not stimulate them differentially. Simultaneous stimulation of these two channels, which physiologically are operated in a push-pull mode will markedly alter the ganglion cell output pattern – it will have to be tested experimentally to what degree higher order perceptual mechanisms can deal with this, and to what degree filling in and gestalt perception is possible.

Quantitative assessment of the initially ultra-low vision to be expected with visual prosthetics is available. At the very low end, for instance, the BaLM ("Basic quantitative assessment of visual performance in patients with very low vision") test provides a monotonous measure of function starting at Light Perception. It is based on basic visual dimensions, derived from texture segregation, taking into account that there is more to vision than acuity. In the region of Hand Movement and higher there is an overlap of BaLM and FrACT (Freiburg Acuity and Contrast Test), together providing continuous vision measures up to normal acuity.

Reconstructing the retina: Connectomics of the inner plexiform layer

Moritz Helmstaedter

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The mapping of neuronal connectivity is one of the main challenges in neuroscience. Only with the knowledge of wiring diagrams is it possible to understand the computational capacities of neuronal networks, both in the sensory periphery, and especially in the mammalian cerebral cortex. Our methods for dense circuit mapping are based on 3-dimensional electron microscopy (EM) imaging of tissue, which allows imaging nerve tissue at nanometer-scale resolution across substantial volumes (typically hundreds of micrometers per spatial dimension) using Serial Block-Face Scanning Electron Microscopy (SBEM). The most time-consuming aspect of circuit mapping, however, is image analysis; analysis time far exceeds the time needed to acquire the data. Therefore, we developed methods to make circuit reconstruction feasible by increasing analysis speed and accuracy, using a combination of crowd sourcing and machine learning. We have applied these methods to circuits in the mouse retina, mapping the complete connectivity graph between almost a thousand neurons, and we are currently improving these methods for the application to neuronal circuits in the neocortex using automated image analysis, together with online science games.

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Restoring vision by using microbial opsins

Jens Duebel

Institut de la Vision, Paris

The insertion of light-sensitive microbial opsins into retinal neurons is a promising approach to restore vision in retinal degenerative diseases, such as *Retinitis pigmentosa*. In this disease, rod photoreceptors degenerate early, whereas light-insensitive, morphologically altered cone photoreceptors persist longer. To restore vision in mouse models of *Retinitis pigmentosa,* we genetically targeted a light-activated chloride pump (halorhodopsin) to light-insensitive cone photoreceptors by means of adeno-associated viruses (AAVs). These re-sensitized photoreceptors are able to drive sophisticated retinal image processing, activate neuronal networks in the visual cortex, and mediate visually guided behaviors. In human ex vivo retinas, halorhodopsin can reactivate light-insensitive photoreceptors. Using OCT imaging, we identified blind patients with persisting cones for potential halorhodopsin-based therapy. Currently, we are testing our AAV vectors in the monkey retina. In a first round of experiments, macaque eyes were injected sub-retinally with an AAV vector encoding GFP under control of a cone specific promotor. These retinas displayed strong fluorescence in cone photoreceptors. No off-target expression was observed, indicating high specificity of the vector in the primate retina.

Vision without a fovea: Results of training eccentric fixation in AMD patients

Mark W. Greenlee

University of Regensburg

Hereditary and age-related forms of macular dystrophy (MD) lead to loss of cone function in the fovea, resulting in central scotoma and eccentric fixation at the so-called preferred retinal locus (PRL). We investigated whether perceptual learning enhances visual abilities at the PRL. We also determined the neural correlates (3-Tesla fMRI) of learning success. Twelve MD patients (eight with age-related macular dystrophy, four with hereditary macular dystrophies) were trained on a texture discrimination task (TDT) over six days. Patients underwent three fMRI sessions (before, during and after training) while performing the TDT (target at PRL or opposite PRL) with monocular viewing. Reading speed, visual acuity (Vernier task) and contrast sensitivity were also assessed before and after training. All but one of the patients showed improved performance (i.e. significant change in stimulus onset asynchronies, hit rates and reaction times) on the TDT. Eight patients also showed moderate increases in reading speed, six patients showed improved thresholds in contrast sensitivity and nine patients showed improved thresholds in a Vernier visual acuity task after perceptual learning. We found an increase in BOLD response in the projection zone of the PRL in the primary visual cortex in nine of twelve patients after training. The change in fMRI signal correlated ($r = .8$; $p = .02$) with the patients' performance enhancements when the target was in the PRL. The results suggest that perceptual learning can enhance eccentric vision and cortical processing in MD patients.

POSTER ABSTRACTS

The drusen component Apolipoprotein E interacts with Complement Component C1q

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Purpose: Age-related macular degeneration (AMD) is a frequent, complement associated disease in elderly people. It is characterized by an irreversible loss of vision which is caused by the degeneration of retinal pigment epithelial (RPE) cells. Characteristic for AMD is the appearance of extracellular cell debris, called drusen which induce a state of chronic inflammation. Drusen are composed of many different proteins and lipids, including lipoproteins like apolipoprotein E (ApoE), amyloid-beta peptides (Aβ) and proteins of the complement system. ApoE, a lipid transport protein, is genetically linked to AMD, however the role of ApoE in AMD is still unclear. The human ApoE exists in three isoforms which differ in two amino acids, Arg and Cys, at the positions 112 and 158. A pooled studies analysis found the E4 haplotype to be associated with a decreased risk of late AMD, whereas E2/E2 homozygous individuals carried a significantly increased risk of late AMD. A previous study demonstrated that ApoE deficient mice had a decrease in cell number and cell layer thickness in the retina, indicating degeneration of retinal cell layers. This leads to the assumption that the high occurrence of ApoE in drusen is caused by its function to clear the extracellular debris and to protect the RPE cells from complement mediated damage. To study the role of ApoE isoforms in AMD pathology, binding of ApoE to complement proteins was characterized.

Methods: A complement activation assay based on an enzyme-linked immunosorbent assay (ELISA) was used to investigate Aβ activation of the classical or alternative complement pathway. In addition binding and binding conditions of ApoE isoforms to divers complement proteins were evaluated.

Results: NHS incubated with Aβ leads predominantly to the activation of the classical pathway. ApoE in all three isoforms bind to C1q, the recognition molecule of the classical pathway, in a calcium dependent manner.

Conclusion: The ability of Aβ to activate especially the classical pathway of the complement system suggests that also the classical complement pathway contributes to AMD. This is supported by the identification of ApoE, which is a substantial part of drusen material, as a ligand of C1q, the central component of the classical pathway. The data provide evidence that ApoE and its isoforms are involved in classical pathway activation.

Neuroprotective effect of encapsulated human stem cells in retinal organ cultures of the P23H rhodopsin transgenic rat

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Purpose: The aim of this study was to evaluate whether basic human mesenchymal stem cells (hMSC) or glucagon-like peptide-1 (GLP1) expressing hMSCs, encapsulated in alginate spheres, have a neuroprotective effect on the explanted retina of P23H rhodopsin transgenic rats.

Methods: Retinas from P23H and wild type Sprague Dawley (SD/CD) rats together with the attached pigment epithelium were used to generate explants as previously described (Arango-Gonzalez *et al.* IOVS, 2010). To evaluate the effect of hMSC and GLP-1 encapsulated cells (cell beads), two different paradigms were used: (1) Short-term cultures: Retinas were prepared at postnatal day 9 (PN9) and allowed to develop in organ culture for 6 days (DIV6) and (2) Long-term cultures (PN10 DIV20). Cultures were treated by the presence of 1, 5 or 25 hMSC or GLP-1 cell beads during the whole *in vitro* phase. As control, explants were cultured with empty alginate beads. After fixation, cultured retinas were examined by conventional histological techniques and TUNEL staining.

Results: Both groups, hMSC and GLP-1 treated retinas, showed a significantly decreased number of TUNEL positive cells in the photoreceptor cell layer in the short term explants when compared with control P23H untreated retinas. In harmony with these results, long term P23H cultures treated with hMSC or GLP-1 cell beads, revealed an increased number of photoreceptor cells, as detected by quantification of cell rows in the outer retina. After treatment, P23H retinas presented similar values of TUNEL positive cells and photoreceptor rows as the wild type controls. No significant differences were observed between hMSC and GLP-1 treatments.

Conclusions: Application of alginate-encapsulated hMSC or GLP-1 cells in organ cultures of the P23H retinal degeneration model resulted in a significant increase of photoreceptor cell survival. Since encapsulated hMSC are able to produce physiologic combinations of bioactive compounds, our results confirm their potential for sustained therapeutic use in patients with hereditary retinal degeneration.

In-depth characterisation of retinal pigment epithelium (RPE) cells derived from human induced pluripotent stem cells (iPSC)

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Purpose: To establish and comprehensively characterise retinal pigment epithelium (RPE) cells derived from adult human dermal fibroblasts via induced pluripotent stem cell (iPSC) technology.

Materials and Methods: Adult human dermal fibroblast cultures were established from skin biopsy material and subsequently reprogrammed following polycistronic lentiviral transduction with OCT3/4, Sox2, Klf4 and l-Myc. Chromosomal integrity was assessed by karyotyping. RPE cell differentiation was achieved by induction with RPE medium enriched for nicotinamide and Activin A. After 8 weeks, pigmented clusters of RPE cells were manually excised and subcultured. Human iPSCs were characterised by RT-PCR expression of specific stem cell markers and immunofluorescence. IPSC-derived RPE cells were characterised by RT-PCR expression of mature RPE markers, confocal microscopy, scanning electron microscopy (SEM) and functional analysis, the latter including feeding experiments with porcine photoreceptor outer segments (POS) and measurements of transepithelial resistance (TER).

Results: Fibroblast-derived human iPSCs showed typical morphology and regular karyograms. Furthermore, they revealed distinctive stem cell marker properties based on RNA- and protein-expression profiling. Subsequently, human iPSCs were differentiated into pigmented clusters reminiscent of RPE cells. These cells maintained typical hexagonal RPE-morphology during subcultivation. Starting at passage 6 replicative senescence increased. RNA expression of mature PRE markers RPE65, RLBP and BEST1 were found in comparison to human iPSCs. Confocal microscopy demonstrated localisation of BEST1 at the basolateral plasma membrane while SEM demonstrated typical microvilli at the apical side of RPE cell. With regard to functional aspects, iPSC-derived RPE cells phagocytosed and shredded POS. Finally, TER measurements showed a significant increase and maintained high levels of TER indicating functional formation of tight junctions.

Conclusion: Our data demonstrate the successful reprogramming of human adult skin biopsy-derived fibroblasts to iPSCs and their differentiation to RPE cells structurally and functionally highly reminiscent of true native RPE cells. This will allow a broad application to establish cellular models for RPE-related human diseases.

Complement component C5a primes the NLRP3 inflammasome in RPE cells

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Purpose: Photooxidative damage of the retinal pigment epithelium (RPE) is associated with the pathogenesis of age-related macular degeneration (AMD). In addition, involvement of a chronic immune response in the sub-RPE space including activation of the complement system has been demonstrated. To identify a molecular link between these mechanisms we investigated the capability of activated complement components to prime RPE cells for activation of the NLRP3 inflammasome by lipofuscin phototoxicity.

Methods: Lipofuscinogenesis was induced in primary human RPE cells and ARPE-19 cells by incubation with isolated photoreceptor outer segments following modification with lipid peroxidation products. For inflammasome priming, lipofuscin-loaded cells were incubated in serum-free media or media supplemented with full human serum, C5-deficient serum, or isolated C5a. Specific C5a receptor (CD88) antibodies were used to block C5a binding. Control cells were primed with IL-1α. Following priming, cells were irradiated with blue light for up to 6 hours. NLRP3 inflammasome activation was assessed by measuring IL-1β and IL-18 secretion. Pyroptotic cell death was analyzed using LDH release assay, TUNEL staining, and DNA/histonespecific ELISA.

Results: Priming of RPE cells with full human serum or isolated complement component C5a resulted in a lipofuscin load- and light dose-dependent activation of the NLRP3 inflammasome with secretion of IL-1β and IL-18. Complement heat-inactivation, C5 depletion, or C5a receptor inhibition suppressed the priming effect of human serum. Specific inhibition of caspase-1 or cathepsin B, L, or D likewise prevented NLRP3 activation. Inflammasome activation was followed by RPE cell death by pyroptosis as identified by morphological and molecular characteristics.

Conclusions: Complement component C5a is capable of providing the priming signal for subsequent activation of the NLRP3 inflammasome by phototoxic effects of lipofuscin. This molecular pathway may represent a functional link between hallmark features of AMD such as lipofuscin accumulation, photooxidative damage, chronic immune response, and progressive degeneration of the RPE and may provide a novel target for therapeutic intervention in AMD.

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Nerve growth factor inhibits osmotic swelling of rat Müller glial and bipolar cells by inducing glial cytokine release

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Aim: In addition to vascular hyperpermeability, water accumulation in neurons and glial cells resulting in cellular swelling may contribute to the development of retinal edema and neuronal degeneration in ischemic and inflammatory retinal diseases. Nerve growth factor (NGF) was shown to control the neuronal survival in the retina via activation of high-affinity TrkA receptors, that transmit prosurvival signals, and low-affinity p75 neurotrophin receptors ($p75^{NTR}$) that transmit antisurvival signals. We determined whether NGF inhibits the osmotic swelling of rat retinal Müller glial and bipolar cells.

Methods: Swelling of Müller and bipolar cell somata was induced by superfusion of freshly isolated retinal slices or cells with a hypoosmotic solution (60% osmolarity) containing barium chloride (1 mM) for 4 min. Freshly isolated Müller and bipolar cells were immunostained for TrkA and $p75^{NTR}$. Retinal ischemia was induced in one eye of the animals by increasing the intraocular pressure for 60 min; the animals were killed 3 d later.

Results: NGF inhibited the osmotic swelling of Müller and bipolar cell somata in slices of control and postischemic retinas. On the other hand, NGF prevented the swelling of freshly isolated Müller cells, but not of isolated bipolar cells. This suggests that NGF induces a release of factors from Müller cells that inhibit the swelling of bipolar cells in retinal slices. The inhibitory effect of NGF on Müller cell swelling was mediated by activation of TrkA, but not $p75^{NTR}$, and was prevented by a blocker of fibroblast growth factor (FGF) receptors. bFGF fully prevented the osmotic swelling of freshly isolated Müller cells, but inhibited only in part the swelling of isolated bipolar cells. In addition to bFGF, GDNF and TGF-β1, but not EGF and PDGF, reduced in part the swelling of bipolar cells. Both Müller and bipolar cells displayed TrkA immunoreactivity, while Müller cells were also immunostained for $p75^{NTR}$.

Conclusion: The data may suggest that the neuroprotective effect of NGF in the retina is in part mediated by the prevention of the cytotoxic swelling of glial and bipolar cells. The data also suggest that glial TrkA, but not TrkA expressed by bipolar cells, is coupled to cell volume-regulatory intracellular signaling mechanisms. The inhibitory effect of NGF on the osmotic swelling of bipolar cells in retinal tissues is mediated by inducing a release of cytokines, possibly bFGF, GDNF, and TGF-β, from Müller cells.

Osmotic induction of complement factor C9 in retinal pigment epithelial cells: inhibition by vegetable polyphenols

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Purpose: Polymorphisms of genes of various complement factors affect the risk of age-related macular degeneration. A further risk factor is systemic hypertension. High intake of dietary salt results in elevation of the blood osmolarity and thus in elevated blood pressure. We determined the effect of hyperosmolarity on the expression of complement factors in cultured human retinal pigment epithelial (RPE) cells.

Methods: Hyperosmolarity was induced by addition of 100 mM NaCl or 100 mM sucrose to the culture medium. Hypoosmolarity (60 % osmolarity) was induced by addition of distilled water. Alterations in gene expression were determined by real-time RT-PCR. The intracellular protein level was determined with Western blot analysis.

Results: RPE cells expressed various complement factors including C3, C5, C9, CFH, and CFB. Hyperosmotic media increased highly the gene expression of the complement factor C9 whereas the expression of the other complement factors displayed small or no alterations. Hypoosmolarity induced a small transient increase of C9 mRNA. In addition, $CoCl_2$ (150 µM)-induced hypoxia and H_2O_2 (20 µM)-induced oxidative stress stimulated the gene expression of C9. Various inflammatory and growth factors including VEGF, PDGF, TGF-β1, IL-1β, TNFα, and thrombin induced either no alteration or a decrease in the expression of C9. The hyperosmotic gene expression of C9 was mediated by transcription; the stability of C9 mRNA was not altered by hyperosmolarity. The hyperosmotic expression of C9 was dependent on the activation of p38 MAPK, ERK1/2, JNK, and PI3K signal transduction pathways, as well as of STAT3. Various vegetable polyphenols including apigenin, myricetin, luteolin, and quercetin inhibited the hyperosmotic induction of C9.

Conclusions: The data suggest that the gene expression of C9 in RPE cells is relatively specifically induced by hyperosmolarity, hypoxia, and oxidative stress. It is suggested that the increase in C9 mRNA may play a role in the regulation of the transcriptional and/or translational activities in RPE cells in response to pathological conditions.

In vivo improvement in visually guided behaviour following transplantation of ex-vivo genetically modified photoreceptor precursors

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Purpose: Retinitis pigmentosa (RP) is one of the primary causes of inherited retinal blindness, affecting more than 15 million people worldwide. In this disease an initial loss of rod photoreceptors leads to a cellular cascade that promotes a secondary degeneration of cone photoreceptors. Stem cells and rod photoreceptor precursor cells (PPC) have been widely researched as candidates for cell replacement in RP. Patient-specific induced pluripotent stem cells (iPSc) could provide an autologous expandable source of cells for transplantation. However, the use of patient-derived iPSc would require that the disease-causing mutation be repaired ex vivo before cells are transplanted. Ex-vivo gene therapy of PPC and subretinal transplantation of modified cells are here studied in rhodopsin knockout (Rho-/-) mice.

Methods: Rod PPC were dissociated from $Rho^{-/-Tg\ Nrl.EGFP+}$ mice, where green fluorescent protein (GFP) is expressed specifically in rod photoreceptors. PPC were cultured and transfected in vitro using serotype 2 recombinant adeno-associated virus (rAAV2 Y444F) vector carrying the Rhodopsin gene and a DsRed florescent reporter. Magnetic assisted cell sorting was performed to enrich rod PPC via the cell surface antigen CD73 and repeated washing steps were performed to remove free AAV particles. Cells were subretinally transplanted into the degenerate retinae of adult $Rho^{-/-}$ mice and in vivo assessment was carried out three weeks post transplantation.

Results: A reliable cell culture system was achieved by use of supplemented neuronal growth media and incubation at 34° C. Transfection of PPC was achieved ex-vivo by a florescent reporter rAAV2 and dissociated cell cultures were enriched to over 80 % $Nrl.GFP^+$ rod PPC prior to transplantation. In vivo testing revealed an improvement in behaviour following transplantation of ex-vivo modified cells and survival of co-labelled $Nrl.GFP^+/DsRed^+$ cells in the subretinal space was observed within 21 days.

Conclusions: Prolonged survival of rod PPC in vitro allowed for a sufficient period of time for ex vivo assessment of gene therapy before transplantation. Our results show successful transplantation of genetically modified rod cells in a murine model of retinal disease and afford a foundation for the development of ex vivo gene therapy in human photoreceptor precursors derived from autologous iPSC.

In vivo imaging of microglia in choroidal neovascularizaton

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Purpose: Microglia coordinates pathological events in the retina. In order to assess microglia contribution in choroidal neovascularization (CNV) events, we used *in vivo* imaging to study changes in the microglia population of the retina.

Methods: *Csf1r*-EGFP mice provide a suitable model for studying macrophage-lineage cells due to their easy fluorescent visualization both *in vivo* and *in situ*. CNV was induced by laser lesions in triplicate following a straight line pattern and avoiding main vessels (Argon ion laser: 150 mV, 0.1 sec, 50 µm). The microglia status was monitored at stages: D0, D1, D4, D7 and D14 using a laser scanner ophthalmoscope (LSO) to detect microglia fluorescence. As a standard procedure, at D14 eyes were enucleated and retinas collected for flat-mount preparation. *In situ* analysis of the vessels was assessed by isolectin B4 staining.

Results: *In situ,* we observed a defined population of microglia lying on the main large vessels of the retina. LSO allowed us to distinguish different microglia phenotypes: ramified (surveying), transitional (activated) and ameboid (activated). The follow-up analysis was performed by quantification of activated microglia separate at OPL and IPL, revealing OPL as the layer significantly holding the biggest amount of ameboid-like cells. We didn't detect significant levels of activated cells between 30-60 minutes directly after laser injury. However, the number of activated microglia rose after 24 hours, which level was maintained at least until 4 days after it decreased conserving high numbers by the time until 14 days after.

Conclusions: We concluded that there is a subpopulation of microglia lying on vessels which is probably activated for angiogenesis. After laser injury there was no acute reaction, but it appeared after 24 hours and sustained over 14 days, starting prior to new blood vessel formation (D7). Thus, microglia suggests playing an active role in CNV formation.

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Loss of acid sphingomyelinase activity causes changes in retinal microglial morphology and microglial function in mice

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Background: Niemann Pick disease type A and B are lysosomal storage disorders which are caused by loss of function mutations in the acid sphingomyelinase (aSMase) encoding gene causing symptoms like hepatosplenomegaly and rapid neurodegeneration. Intracellulary loss of aSMase avtivity in aSMase-KO mice leads to an accumulation of sphingolipids in lysosomes, especially in macrophages. Because microglial cells are critically involved in retinal health we aimed to determine the consequence of aSMase deficiency in these immune cells.

Methods: Spectral-domain optical coherence tomography (SD-OCT) and infrared fundoscopy (IR) were used to characterize the integrity of the aSMase-deficient retina. Furthermore retinal whole-mounts and cross-sections of aSMase-KO mice were performed to determine the localization and immune status of retinal microglial cells ionized calcium-binding adapter molecule 1(Iba1) and translocator protein (18kDa) (TSPO) immunostaining. Intracellular accumulation of lipids was determined by Nile red and Filipin III staining.

Results: Although SD-OCT showed no changes in architecture of retinal layers, IR fundoscopy revealed an increased number of hyperreflective spots in the ganglion cell layer of the retina of aSMase deficient mice. Histological examination of Iba1-stained whole-mounts and retinal cross-sections showed equal distribution of microglia throughout the retina. However, we detected an increased microglial cell number with significantly enlarged cell bodies in the aSMase-deficient retina. Specifically, aSMase-decifient microglial cells showed enhanced proliferation and elevated expression of TSPO which points towards increased microglial reactivity. These microglial cells also displayed strong accumulation of lysosomal lipids as detected by Nile red and Filipin III staining.

Conclusion: These results suggest that aSMase-deficiency and hence lipid disturbed metabolism affect the morphology and function of microglia in the retina. Further experiments are necessary to prove that the enhanced lipid accumulation limits microglial function such as the phagocytic clearance of retinal debris.

Unveiling biochemical and physiological consequences of cone dystrophy-related mutations in GCAP1

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Purpose: Cone dystrophies are often associated with altered levels of calcium (Ca^{2+}) and cyclic GMP (cGMP), the second messengers operating in the phototransduction cascade in rod and cone photoreceptors. By using a multiscale approach, we investigated the biochemical and physiological effects of four pathogenic point mutations identified in the guanylate cyclase-activating protein 1 (GCAP1) leading to the amino acid substitutions E89K, D100E, L151F and G159V.

Methods: Structure-function relationships were studied by biophysical methods, including circular dichroism to monitor secondary and tertiary structural changes in GCAP1 variants upon binding of Ca^{2+} and isothermal titration calorimetry to monitor the thermodynamics of Ca^{2+}-binding. Experimental parameters describing the regulation of the target ezyme guanylate cyclase 1 (GC) by each GCAP1 variant were incorporated in into a comprehensive kinetic model of phototransduction, in order to assess the effect of each individual point mutation on the whole cell response.

Results: Wild type and cone dystrophy-related point mutations in GCAP1 showed large differences in Ca^{2+}-binding and GC regulation but, except for E89K, the strucrual effects of all the tested mutations are minor and involve mostly a slight rearrangement of aromatic residues in the Ca^{2+}-bound form. System-level modeling suggests that the main effect of all point mutations on the photoresponse kinetics is a perturbation of the photocurrent shape consisting in increased amplitude and prolonged duration. However, the effect is strongly dependent on the expression levels of pathogenic GCAP1 forms as compared to the wild-type form.

Conclusion: Our data suggest that a multiscale approach combing biochemistry, biophysics and systems biology strategies allows a deep molecular understanding of dysfunctional states in photreceptors in cone-dytrophy conditions. In particular, we conclude that the contribution of GCAP1 to the dynamic synthesis of cGMP in rod cells depends on the expression level of the wild-type form, and in the case of high expression levels of cone-dystrophy GCAP1 mutants it would not contribute at all to shaping the cGMP rate, which becomes dynamically regulated solely by the other present Ca^{2+}-sensor GCAP2.

Reticular pseudodrusen associated with Bruch's membrane pathology in pseudoxanthoma elasticum

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Purpose: To describe the phenotype, prevalence and topographic distribution of reticular pseudodrusen (RPD) in patients with Pseudoxanthoma elasticum (PXE) and their association with Bruch's membrane (BM) pathology.

Methods: Diagnosis of PXE was based on ophthalmologic examination, skin biopsy and/or genetic testing. Presence and phenotypic characteristics of RPD were investigated using a confocal scanning laser ophthalmoscope (Spectralis HRA-OCT, Heidelberg Engineering, Germany). The multimodal imaging approach included near-infrared (NIR) reflectance, fundus autofluorescence (FAF), spectral domain optical coherence tomography (SD-OCT) and late-phase indocyanin green angiography (ICG-A). The distribution of RPD was evaluated based on 9-field NIR reflectance images using a modified Early Treatment Diabetic Retinopathy Study grid.

Results: A total of 54 patients were examined. RPD were detected in 41% of PXE patients. Mean age of patients with RPD was 51 ± 7 years (range: 41–63 years). Prevalence of RPD was highest in the 4th decade with up to 70%. RPD appeared as network of round to oval lesions which were hyporeflective on IR and hypoautofluorescent on FAF images. SD-OCT showed characteristic subretinal deposits anterior to the RPE layer. RPD were most frequently located within the superior quadrant (95.6%) and least frequent within the central macula (4.3%). RPD were always located central to areas with peau d'orange and within an area of hypofluorescence on ICG-A-late phase images.

Conclusions: RPD have a high prevalence in eyes of PXE patients. Although RPD in PXE patients occur at younger age, their distribution and phenotype appear to be similar to RPD associated with age-related macular degeneration (AMD) using imaging modalities relevant for RPD detection. The distribution of RPD followed the peripheral spread of BM pathology in PXE indicating a possible pathogenetic role for the development of RPD.

A circulating microRNA profile is associated with age-related macular degeneration (AMD)

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Purpose: Age-related macular degeneration (AMD) is the leading cause of severe vision impairment among people aged 55 years and older. Although an increasing number of gene variants are continuously identified to be associated with AMD, so far only few studies have actually investigated a functional relationship between a gene variant and disease pathology. In contrast, a biomarker which is dysregulated in disease might point towards processes involved in the underlying pathogenesis and thus could help to find novel therapeutic targets. Recently, circulating microRNAs were found in blood serum/blood plasma as potential novel biomarkers for various diseases.

Methods: We used high throughput RNA sequencing (RNAseq) to elucidate the role of circulating microRNAs in AMD by genome-wide microRNA expression profiling in a discovery study. We validated candidate miRNAs in two replication studies by real-time qPCR and performed sensitivity analyses in order to assess the influence of covariates on the association.

Results: We found three microRNAs to be associated with AMD ($P_{adjusted} < 0.05$). A combined profile of the three microRNAs had an area under the curve (AUC) value of 0.718 and was highly associated with AMD ($P = 2.61*10^{-4}$). By performing pathway enrichment analysis on genes which are predicted to be regulated by these microRNAs, we identified novel pathways involved in AMD pathology. We found the strongest enrichment of genes in the canonical TGFβ, mTOR, VEGFA as well as the canonical neutrophin pathway. By combining the genetic risk score (GRS) and expression data of the strongest associated microRNA, we fitted logistic regression models with a bootstrapped AUC value of 0.887 (95 % CI: 0.882–0.893), which significantly improves upon a classification scheme based on genetic factors alone.

Conclusion: Taken together, our results strongly implicate specific microRNAs as novel biomarkers involved in AMD disease.

Arl3 rod-specific knockout displays RP-like photoreceptor degeneration

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Purpose: Arf-like protein 3 (Arl3) is a small GTPase interacting with lipid-binding proteins in photoreceptors. We generated rod-specific Arl3 knockouts to elucidate the role of Arl3 in transport of membrane-associated proteins.

Methods: Chimeras containing a gene trap in intron 1 of *Arl3* gene were generated at the University of Utah core using a EUCOMM cell line. Breeding with Flp mice followed by mating with iCre75^{+} transgenic mice generated rod-specific knockouts. Photoreceptor function and morphology were analyzed by ERG and immunohistochemistry. An Arl3-specific polyclonal antibody was generated using full-length recombinant Arl3 polypeptide expressed in bacteria.

Results: Immunoblots of WT retina lysates identified 20 kDa retina protein which was reduced significantly in two month-old Arl3$^{flox/flox}$; iCre75^{+} retina. Immunohistochemistry revealed Arl3 localization in WT photoreceptor inner segments. Arl3 immunoreactivity was absent in homozygous rod knockouts, while still present in cones and inner retina. Scotopic and photopic ERGs of PN15 rod knockout and wild-type mice had comparable a- and b-wave amplitudes suggesting normal photoreceptor development. At PN15, knockout mice show slightly shorter ROS compared to wild-type. At PN20, scotopic ERG a-wave amplitudes were reduced (70–80 %) but the photopic ERG was unaffected. One month-old Arl3$^{flox/flox}$; iCre75^{+} mice showed 80–90 % reduction in a-wave amplitude with only 4-5 rows of nuclei surviving in the ONL. In retinas of two month-old knockout mice, scotopic ERGs were extinguished and cone ERGs were highly attenuated. OCT confirmed the rapid loss of photoreceptors in the homozygous rod knockout starting at PN15. Although the Arl3$^{flox/flox}$; iCre75^{+} retina fundi were comparable to wild-type, one month-old mutant retina was on average 100 µm thinner than its wild-type counterpart. Immunohistochemistry performed using retina sections of PN15 and one month-old knockout mice revealed that rhodopsin transport is normal; rhodopsin was undetectable in two month-old conditional knockout mice due to complete photoreceptor degeneration. Rod PDE6 and GRK1 mislocalized suggesting trafficking defects.

Conclusion: Rod-specific knockout of Arl3 revealed a rapidly-progressing photoreceptor degeneration. Rod-specific knockout mice were blind at two months of age. Outer segment development appeared to be unimpaired by Arl3 deletion and rod photoreceptor function was normal at P14.

Early microglial activation and transcriptomic changes in the mouse retina during Experimental Autoimmune Uveoretinitis (EAU)

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Purpose: To analyze the influence of retinal microglia in the development of Th1 / Th17 driven experimental autoimmune uveoretinitis (EAU) in B10.R III mice.

Methods: B10.RIII mice were immunized by injecting human interphotoreceptor retinoid-binding protein peptide 161-180 ($hIRBP_{p161-180}$) in Freund´s complete adjuvant and intraperitoneal pertussis toxin injection. After funduscopic examination mice were sacrificed 7, 14 or 21 days after EAU induction. Untreated mice served as negative controls. Sera, eyes, and spleens were collected. EAU severity was assessed by HE-staining. Presence of $hIRBP_{p161-180}$ specific serum antibodies were measured by ELISA. Furthermore, antigen-specific proliferation (Alexa fluor 647; flow-cytometry) and cytokine secretion (direct ELISA; IL-17, IFNγ, IL-6) of splenocytes were analyzed. Genome-wide transcriptional profiling of EAU retinas at day 7, 14 and 21 after EAU induction was performed using DNA-microarrays and bioinformatic data mining. Quantitative real-time PCR was then performed to validate the expression of microglia-specific genes. To further study the localization and immune status of microglial cells, we performed Iba1 immunohistochemistry in retinal cross-sections and flat-mount preparations of murine retinas and retinal pigment epithelium (RPE)/choroid.

Results: Mice developed a $hIRBP_{p161-180}$ specific Th1 /Th17 cytokine response at day 7 after EAU Induction but were lacking signs of EAU. EAU was fully developed at day 14 and day 21 after induction accompanied by an increase of $hIRBP_{p161-180}$ specific proliferation, serum antibodies, and increased levels of IL-17, IFNγ, IL-6 in spleens. Whereas retinal gene expression was unaltered at day 7, prominent changes in the the retinal transcriptome were identified at day 14 and day 21. Interestingly, numerous microglia-specific gene clusters were differentially expressed. Histological analysis revealed the transmigration microglial cells to the subretinal space at day 7 and the presence of activated microglia in proximity to RPE cells.

Conclusion: This study extends our knowledge about microglial processes during EAU pathology. The presence of activated microglia in the subretinal space before any signs of EAU suggests a role of microglial processes in EAU pathogenesis.

Human opsin-G-protein fusion proteins as potential light sensitizers

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Purpose: Opsins are light-sensitive G-protein coupled receptor proteins, essential for vision, circadian rhythmicity and eye development. Opsins modify cellular function by activating G-protein second messenger systems. Rod and cone opsins activate $G_{\alpha t}$, while melanopsin, the opsin of photosensitive retinal ganglion cells, activates $G_{\alpha q/11}$. If inner retinal neurons, such as bipolar cells, were engineered to become light sensitive, these cells could act as substitute photoreceptors in patients who have lost their photoreceptors.
We have fused human melanopsin to different G_{α}-proteins to test whether such a fusion modifies the coupling of an opsin to a G-protein second messenger system. Such an opsin-G_{α}-protein fusion could provide an optogentic tool for restoring sight in humans.

Methods: Two melanopsin-G_{α}-protein fusion constructs were cloned into pMT4 by removing the stop codon from melanopsin and inserting the in-frame coding sequence of either GNAQ (gene of $G_{\alpha q}$) or GNA11 ($G_{\alpha 11}$). Calcium kinetics were observed using Rhod-2 fluorescent dye. Small interfering RNAs (siRNA) targeting endogenous G_{α}-protein transcripts (including GNAQ and GNA11) were applied to HEK293T cells expressing wild type melanopsin and melanopsin-G_{α}-protein recombinant protein to determine the relative importance of the fused G_{α}-protein to activating the intracellular signalling cascade.

Results: Melanopsin-$G_{\alpha q}$/$G_{\alpha 11}$ fusion proteins exhibited a similar response rate (41 % and 40 %, respectively) and time course of calcium kinetics compared to non-fused melanopsin (48 %; no statistically significant differences between groups on ANOVA with post hoc Tukey HSD). Using siRNA to knock down endogenous levels of G_{α}-proteins in HEK293T cells showed melanopsin-G_{α}-protein fusion transfected cells to have a higher response rate than wild type melanopsin transfected cells (melanopsin-$G_{\alpha 11}$ response rate 36 %, wild type melanopsin, 13 %, $p>0.05$).

Conclusions: Fusing melanopsin to either of its native G_{α} subunits, $G_{\alpha q}$ or $G_{\alpha 11}$, has demonstrated that melanopsin can maintain coupling to the $G_{\alpha q/11}$ second messenger system in the presence of a fused $G_{\alpha q}$ or $G_{\alpha 11}$ subunit. Furthermore, transient expression of melanopsin-G_{α} protein fusions in HEK293T cells with siRNA-induced knock down of endogenous G_{α} subunits suggests that fusing a G_{α} subunit to melanopsin enables greater efficiency of coupling to the second messenger pathway. Melanopsin-G_{α} protein constructs may therefore offer advantages over wild type melanopsin as a potential optogenetic gene therapy for photoreceptor loss.

Polarized cytokine secretion by RPE cells secondary to NLRP3 inflammasome activation

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Purpose: Photooxidative damage to the retinal pigment epithelium (RPE) as well as chronic inflammatory processes in the sub-RPE space are involved in the pathogenesis of age-related macular degeneration (AMD). We have shown that lipofuscin phototoxicity can activate the NLRP3 inflammasome in RPE cells by inducing lysosomal membrane permeabilization (LMP). Here, we investigate the effects of LMP-induced inflammasome activation on the secretion of inflammatory and angiogenic cytokines related to the pathogenesis of AMD.

Methods: LMP was induced in primary human RPE cells and ARPE-19 cells either by Leu-Leu-OMe or by incubation with 4-hydroxynonenal-modified photoreceptor outer segments (HNE-POS) to induce lipofuscinogenesis and subsequent irradiation with blue light (0.8 mW/cm^2) for up to 6 hours. LMP was quantified by flow cytometry by means of acridine orange staining. Cytokine secretion was measured using dot blot antibody arrays (RayBiotech) and specific ELISAs (R&D Systems). Paracrine cytokine effects were investigated by incubating human vascular endothelial cells (HUVEC) with RPE-conditioned media. Polarized cytokine secretion was analyzed in RPE cells cultured on permeable membranes.

Results: Protein secretion levels of 42 inflammation- and angiogenesis-related cytokines were investigated in RPE cells. LMP-induced NLRP3 inflammasome activation resulted in significantly increased secretion of IL-1β, IL-6, IL-18, GM-CSF, and CXCL1/2/3. In contrast, constitutive secretion of VEGF was significantly reduced, and migration and proliferation of vascular endothelial cells incubated with conditioned media of LMP-treated RPE cells was decreased compared to control conditioned media. Specific inhibition of caspase-1 or cathepsin B prevented inflammasome-related cytokine release. In polarized RPE cell monolayers cultured on permeable membranes, IL-1β and IL-18 secretion was predominantly directed to the apical side (70 % and 92 %, respectively).

Conclusions: Activation of the NLRP3 inflammasome by lysosomal membrane permeabilization as induced by lipofuscin phototoxicity in RPE cells results in highly polarized secretion of inflammation-related interleukins to the apical RPE side. Via this mechanism, phototoxic RPE damage may trigger local immune processes such as subretinal macrophages/microglia recruitment and thus contribute to the pathogenesis of AMD.

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Optimal electrical stimuli for activation of Retinal Ganglion Cell populations

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Purpose: Retinal ganglion cells (RGCs) electrical responsiveness is a nonlinear function of both voltage and duration. We looked for the voltage/duration pair that activates the largest possible fraction of RGCs in adult wild-type (wt) and rd10 mice, a delayed onset and slowly progressing degeneration model of Retinitis Pigmentosa (RP).

Methods: RGC spiking responses were recorded *in vitro* from adult (P28-P33) *wt* (C57BL/6) and degenerating (rd10) retinas, using a planar multi-electrode array (60 electrodes, 200 µm pitch, 30 µm Ø, MCS GmbH,). Epiretinal electrical stimuli were delivered via one electrode while the other electrodes served for recording. Stimuli consisted of square-wave, monophasic voltage pulses (cathodic & anodic) in incremental blocks (0.1 V–2.5 V) with randomized pulse durations (.06 ms–5 ms) for each block. From these responses rastergrams, peri-stimulus time histograms, and firing rate response surfaces over the voltage vs. duration stimulus space were generated. We defined 'nearby cells' as cells recorded on the 8 electrodes around the stimulation electrode (stimulation distance ≈100-383 µm). Significance was determined by the Kruskal-Wallis multiple comparisons test ($p<05$).

Results: Threshold voltages did not differ between wt and rd10 at any duration. By sampling a complete voltage/duration panel for each RGC, we were able to determine the fraction of the recorded population that responded to each unique stimulus with a rate both above threshold and below saturation. Pulses of -2.3 V and .84 ms activated the majority (>80 %) of nearby RGCs in both wt and rd10 retinas. At a fixed voltage of -1.4 V (within the water electrolysis window) at least 60 % of RGCs could be activated at 2.4 ms in wt and rd10.We unexpectedly saw a tendency for voltage thresholds to increase with duration from .06 ms up to 1 ms for both mouse strains. While this tendency was not always significant for nearby cells (N≈100), it was significant when cells from additional electrodes (stimulation distance >383 µm) were included (N>1000) reflecting its subtle nature. The majority of cells strongly preferred cathodic pulses in both wt and rd10 mice.

Conclusions: The novelty of this study is, it's the most complete examination to date of electrical response thresholds in rd10 retina. Accordingly, we propose tentative stimulation parameters appropriate for activation of the largest possible fraction of rd10 and wt RGCs in our continued efforts to optimize prosthetic retinal stimulation.

Polysialic acid controls microglial reactivity and reduces vascular leakage after retinal laser-damage

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Background/Aim: The retinal environment in age-related macular degeneration (AMD) contains a multitude of immunogenic stimuli including metabolite deposits, modified proteins and cellular debris. These trigger neurotoxic microglial activation that may promote age-associated degenerative processes. In addition, loss of immune-attenuating neuronal surface ligands provoke similar consequences. As such, polysialic acids constitute the outermost part of the neuronal glycocalyx and serve as ligands for the human-specific inhibitory Siglec-11 receptor which controls microglial activation. A loss of sialic acids may lead to complement factor C1q-mediated microglial activation and superoxide production. Here, we hypothesized that purified polysialic acid (PSA) may reduce microglial reactivity by induction of inhibitory Siglec-11 receptor signaling and thus may attenuate retinal inflammation during AMD pathology.

Methods: We analyzed polysialic acid expression by immunohistochemistry and determined Siglec-11 receptor transcript levels by RT-PCR in human and murine retinas. To study the immune-modulatory effect of PSA on microglial cells, we analyzed phagocytosis of retinal pigment epithelial cell debris and superoxide production of human induced pluripotent stem (iPS)-cell derived microglia after PSA treatment. To assess the immune-regulatory potential of PSA *in vivo,* we intravitreally injected PSA in Siglec-11 transgenic mice after laser-damage and analyzed microglial reactivity and fluorescein leakage after 48 hours.

Results: We found that murine and human retinas contain high amounts of polysialic acid and showed for the first time that the human specific Siglec-11 receptor gene is expressed in the human retina. Application of purified PSA on human iPS-cell derived microglial cells prevented overt production of superoxide and reduced microglial phagocytosis. PSA injected Siglec-11 transgenic mice showed reduced accumulation of microglial cells in the laser spots and a ramified morphology compared to amoeboid cells in vehicle injected controls. Furthermore, intravitreal PSA-injection significantly reduced retinal vessel leakage.

Conclusion: We conclude, that polysialic acid decreases human microglial neurotoxicity *in vitro* and that intravitreal application of soluble PSA decreases pathological features of retinal degeneration. Our findings suggest that PSA may provide a potential novel therapy option for AMD.

Endogenously expressed anoctamin 2 and 4 in RPE and lens cells

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Purpose: Anoctamins are a family of transmembrane proteins with ten members. Their function as ion channel was recently identified. Anoctamin (Ano) 2 functions as a Ca^{2+}-dependent Cl^- channel in photoreceptors.

Methods: We investigated the role of anoctamins in the eye by using whole-cell patch-clamp techniques and immunocytochemistry.

Results: RT-PCR of the fresh tissue showed expression of Ano2 and Ano4 in the retinal pigment epithelium (RPE) and in lens cells. Staining of juvenile human lens cells, cells of the human lens cell line B-3 and human RPE cell line ARPE-19 against Ano4 and Ano2 confirmed the expression of both proteins in the cytoplasmic membrane. Staining of mouse retina against Ano2 and Ano4 indicated localization of these proteins in the basolateral membrane of the RPE. We performed whole-cell patch-clamp experiments with the ARPE-19 cells, B-3 cells and juvenile human lens cells. Under K^+-free conditions with low Cl^- concentration in the intracellular solution, ARPE-19 cells showed an increase in the membrane conductance in response to ionomycin application accompanied with a depolarization of the membrane potential. The current exhibited a reversal potential of +8,97 mV. Both types of human lens cells showed a comparable increase in the membrane conductance in response to ionomycin.

Conclusion: Since cells depolarized under intracellular low Cl^- concentration we can rule out the effect as a Cl^- channel; as a result of the K^+-free conditions maxi-K channels were not involved in this current response. These data and previous publications let us suspect that the current was a Ca^{2+}-dependent cation current.

The major proinflammatory cytokine interleukin-1β and ARMS2 are sorted through the same unconventional secretory pathway

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Purpose: Age-related maculopathy susceptibility 2 (ARMS2) is a small (11 kDa), primate-specific protein implicated in the pathogenesis of age-related macular degeneration (AMD), a leading cause of blindness in elderly. We have previously reported that ARMS2 is actively secreted, although it lacks a typical signal peptide. Here we report that autophagy-related processes are instrumental in conveying ARMS2. Autophagosomes are dynamically formed and serve as a bulk degradation pathway. However, emerging evidence suggests that they are also involved in the biogenesis of transport organelles destined to export a specific group of proteins including ARMS2.

Methods: Several human cell lines were transfected with plasmids coding for ARMS2. Plasmids coding for pro-interleukin (IL)-1β and ARMS2 carrying the desired substitutions were constructed by molecular cloning. The intracellular trafficking of the synthesized protein was monitored by co-staining of well-established markers for different cellular compartments. Digital images of immunostained cells were acquired on a Zeiss Axioscope. The classical secretory pathway was inhibited by using brefeldin A (BFA) in some experiments. ARMS2-positive carriers were isolated for proteomic analysis by native electrophoresis.

Results: Golgi reassembly stacking protein proteins (GRASPs) are the only known markers for unconventional protein secretion. ARMS2-positive vesicle-like structures proved to be positive for this marker. Furthermore, the major proinflammatory cytokine interleukin-1β (IL-1β) is a prototypical example of secretory autophagy. Co-expression of ARMS2 and IL-1β leads to the redistribution of these two proteins into the same vesicle-like structures suggesting a common secretory pathway. On the other hand, co-expression of ARMS2 and HTRA1 (classically secreted protein) or eGFP (non-secreted protein) does not give rise to the colocalization of the two proteins (data not shown), indicating an active cargo selection mechanism. Critical residues within ARMS2 for becoming a client of this transport machinery have also been identified.

Conclusions: Our data suggest that ARMS2 belongs to the group of proteins being secreted by autophagy related mechanisms. Strikingly, all other known proteins hauled by this pathway act pro-inflammatory. Accordingly, ARMS2 might exert its physiological function by regulating immune cells within the eye.

Revising the stem cell potential of Müller glia cells in the zebrafish retina

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Purpose: In contrast to mammals, zebrafish are able to regenerate their retina following injury. The stem cells within the zebrafish retina are the Müller glia cells. Up to date, Müller glia cells are thought to generate exclusively rods in the unlesioned retina based on lineage tracing exploiting the GFP persistence.
However, to unambiguously analyse the progeny of Müller glia beyond GFP persistence, lineage tracing experiments based on the Cre/LoxP system are required. In addition, recent studies have shown that specific ablation of a single retinal cell type biases Müller glia dependent regeneration towards the replacement of the lost cell type. However, so far it is unclear whether the putative underlying selection mechanism encroaches already upon the level of cell-type specific progenitor generation.

Methods: Using conditional tamoxifen inducible Cre^{ERT2}, we can drive Cre recombination in a subset of GFAP+ Müller glia cells and follow the progeny of Müller glia in the adult zebrafish retina.

Results: Due to the recombination procedure, we induce a minor damage to the retina that does not result in any obvious damage of retinal lamination and induces only a very limited amount of cell death. To evaluate the extent of cell death, we have compared the number of TUNEL+ cells in retina sections induced by our method with previous published lesion models, such as light lesions and ouabain injection. Diffuse light lesions induce 4-fold more cell death in the ONL and oubain injection induce 100-fold more cell death in INL compared to our protocol.
Without destroying a major portion of any specific retinal cell type, we are able to coax Müller Glia cells to generate all retinal cell types. Although the generation of rods appears highly favoured, we observe Müller glia derived progeny within all lamina and sub-lamina within one week of chase.

Conclusion: Taken together, this data indicate that Müller glia possess the intrinsic property to generate all retinal progenitors independently of a major cell type loss.
Future studies will reveal whether Müller glia cells are able to generate in the absence of any cell death any retinal cell types other than rods. In addition, we will elucidate whether the loss of a specific retinal cell type correlates with the amount of the respective generated progenitors.

Cellular reactions elicited by the laser-induced choroidal neovascularisation in the mouse

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Purpose: Anti-VEGF treatment became the first-line therapy in several neovascular ocular diseases, such as wet age-related macular degeneration (AMD). However, among the patients, there are about 30–40 % who do not respond well to anti-VEGF therapy (so called "non-responders"). Therefore, new targets and treatment modalities have to be discovered. The aim of our study is to identify new signalling pathways in the retina that lead to neovascularisation and stabilisation of the newly formed blood vessels.

Methods: Eyes of wild-type mice were treated by an argon laser (energy 200 mW, diameter 50 µm) to destroy the retinal pigment epithelium (RPE) and to induce a tissue proliferation similar to the wound healing, leading to the ingrowth of choroidal vessels into the subretinal space. In order to characterise the involved cell populations, we isolated the eyes one, two, three or four weeks after laser treatment and prepared paraffin sections for immunohistochemical staining according to standard protocols.

Results: The most abudant cells in the laser spots were the endothelial cells of the newly formed blood vessels, fibroblasts, retinal pigment epithelium cells, microglial cells, detected by antibodies against CD31, vimentin or S100A4, RPE65 and Iba-1, respectively. In addition to VEGF, these cells expressed further cytokines and growth factors to a variable extent, probably contributing to neovascularisation and tissue proliferation, for instance PDGF-β, FGF-1 and interleukin 8 (IL-8). Regarding FGF-1 as an example, we were able to show that it is barely found in untreated eyes. In contrast, it was mainly localised in the RPE at the edges of the laser spots and, to a lower extent, also in fibroblasts after the laser treatment. Similarly, the immunoreactivity (IR) of the FGF-1 receptor increased after laser treatment. FGF-1 receptor IR was found in Müller glial cells and cells of the outer plexiform layer. The highest level was seen after three and four weeks. Similar to FGF-1, the RPE was also positive for the FGF-1 receptor.

Conclusion: The detailed identification of the growth factors and cytokines as well as their receptors will help to understand further the pathological processes in the animal model of laser-induced choroidal neovascularisation and to develop novel therapeutic approaches for the treatment of neovascular diseases.

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Norrin mediates neuroprotective effects on retinal ganglion cells via the induction of leukemia inhibitory factor

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Purpose: To investigate if and how leukemia inhibitory factor (LIF) is involved in the neuroprotective effects of Norrin on retinal ganglion cell (RGC) survival following excitotoxic damage. Norrin is a secreted protein that activates the classical Wnt/β-catenin pathway via specific binding to Frizzled-4. In albino mice, Norrin protects RGC from excitotoxic damage and increases the retinal expression LIF and endothelin-2 (EDN2), as well as that of neuroprotective factors such as fibroblast growth factor-2 (FGF2) and ciliary neurotrophic factor (CNTF).

Methods: Recombinant human Norrin was isolated and purified from conditioned cell culture medium of HEK 293-EBNA cells. To induce excitotoxic RGC death, 3 µl NMDA [10 mM] were injected into the vitreous body of both hetero- and homozygous LIF-deficient mice in a C57/Black6 genetic background. The fellow eye received 3 µl of combined NMDA [10 mM] and Norrin [5 ng/µl]. To determine the degree of RGC damage, TUNEL labeling was performed on meridional sections 24 h after injection, and the number of labeled nuclei was quantified. The expression of mRNA for *Lif, Edn2,* and *Fgf2* was investigated by quantitative real-time RT-PCR of treated retinae.

Results: After injection of wild-type mice with combined NMDA/Norrin approximately 50 % less TUNEL positive cells were observed in the RGC layer when compared to that of NMDA-treated littermates. The protective effect of Norrin was completely lost when homozygous LIF-deficient mice were treated with combined NMDA/Norrin. In addition, in LIF-deficient mice, NMDA induced a substantial increase in excitotoxic damage as 50 % more apoptotic cells in the RGC layer were observed when compared to NMDA-injected wild-type littermates. By real-time RT-PCR for the expression of *Lif* and *Edn2* mRNA in retinae of NMDA/Norrin-treated eyes, a significant increase was observed in wild-type mice when compared to eyes that received NMDA only. The Norrin-mediated effect was substantially reduced in heterozygous LIF-deficient mice. In contrast, only moderate changes were observed in the expression of *Fgf2*.

Conclusions: Norrin mediates its neuroprotective properties on retinal ganglion cells via an increased expression of *Lif*. In pigmented mice, the induction of LIF might involve an increased expression of *Edn2* while *Fgf2* plays no or only a minor role.

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Evaluation of autoantibodies against aldehyde-derived protein adducts in age-related macular degeneration

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Purpose: Age-related macular degeneration (AMD) is a leading cause of visual impairment in industrialized countries. In the early stages of the disease, there is an accumulation of extracellular deposits, or drusen, under the retinal pigment epithelium. It is thought that oxidative damage plays a key role in drusen formation and therefore in the causality of AMD. Autoantibodies against oxidation-specific epitopes (OSEs) have been shown to be elevated in AMD patients compared to controls. These OSEs are products of lipid oxidation, and the immune response they elicit has been suggested to cause a chronic inflammation in the macula. The aim of this work is to better understand the association among anti-OSE autoantibodies, genetic risk variants and the pathology of the disease.

Methods: Our first objective is to generate a multiplex assay for the detection of OSE-specific autoantibodies associated with AMD in patients' blood/plasma samples. Autoantibodies against aldehyde-derived protein adducts are being evaluated, including ω-(2-carboxyethyl)pyrrole (CEP, derived from 4,7-dioxohept-5-enoic acid (DOHA)), 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (HHE) and malondialdehyde (MDA) adducts. For the assays, we synthesized CEP-BSA and HHE-BSA adducts, and used commercially available MDA-BSA and HNE-BSA. BSA was used as a blank, and the ratio between anti-CEP-BSA and anti-BSA was calculated. We set up a standard indirect ELISA method for blood/plasma samples.

Results: IgM blood levels against CEP-BSA were significantly higher in early and late AMD patients (2.40 ± 0.11; $n = 32$) compared with age-matched controls (1.79 ± 0.16; $n = 24$) ($p = 0.002$). Preliminary results showed that IgM against MDA-BSA are higher in AMD patients (1.54 ± 0.05) compared to controls (1.38 ± 0.07) ($p = 0.047$), although CEP-BSA showed stronger differences. Levels of anti-HNE-BSA antibodies were not different between the patients (1.01 ± 0.04) and controls (0.96 ± 0.02). Evaluation of antibodies against HHE-BSA is in progress.

Conclusion: Our results indicate that the immune response in AMD may differ among the aldehyde-derived protein adducts. The multiplex assay will include those adducts giving better differences between patients and controls.

Choroidal thickness in geographic atrophy associated with age-related macular degeneration

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Purpose: To compare choroidal thickness (CT) in different subtypes of geographic atrophy (GA) secondary to age-related macular degeneration (AMD) with normal controls.

Methods: A total of 42 eyes of 42 patients (mean age 76.2 ± 6.5 y) with GA and 31 eyes of 31 healthy controls (mean age 75.6 ± 6.4 y) were examined by confocal scanning-laser-ophthalmoscopy (cSLO) and EDI (enhanced depth imaging) SD-OCT (Spectralis, Heidelberg Engineering, Germany). CT was measured at 26 defined points in horizontal and vertical EDI-OCT scans for each patient. GA subtypes were classified based on abnormal FAF patterns surrounding the atrophic lesions as previously published (Holz et al.; Am J Ophthalmol 2007). Total area of GA was assessed using the RegionFinder Software (Heidelberg Engineering).

Results: Mean CT was significantly thinner in eyes with GA (168.47 ± 88 µm) as compared to control eyes (215.5 ± 55.6 µm, p = 0.011). In the GA group, patients with the 'diffuse trickling' subtype (n = 7) exhibited a significantly thinner CT (108.7 ± 31.9 µm) as compared to other GA subtypes ('non-diffuse trickling' GA, n = 35, 180.7 ± 91.0 µm, p = 0.047). The total GA area in the 'diffuse trickling' group was significantly higher than in other GA subtypes (16.1 ± 6.8 μm^2 vs 6.1 ± 4.8 μm^2). Difference in CT between the 'non-diffuse trickling' GA eyes and control eyes were substantially less pronounced.

Conclusions: The results indicate that the choroid in eyes with GA is thinner compared to normal eyes of similar age. However, this effect appears to be mainly driven by a specific GA subtype ('diffuse trickling'). As this subtype – in accordance to earlier works (Holz et al.; Am J Ophthalmol 2007) – yields larger GA areas further analysis will be necessary to identify if CT also correlates directly with GA size or uniquely with the GA subtype. However refined phenotypic classification of eyes with advanced dry AMD appears prudent when choroidal thickness is assessed and compared. GA-subtype related differences in choroidal thickness may reflect a heterogenous underlying pathogenesis.

Behaviour of microglial cells in an animal model of laser-induced choroidal neovascularisation

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Purpose: Laser-induced choroidal neovascularisation (CNV) is a commonly used animal model to investigate pathological processes that occur during wet age-related macular degeneration (AMD) and to test potential measures against neovascularisation. Microglial cells are the intrinsic immune cells of the central nervous system and also of the retina. During the last years, involvement of microglial cells in the pathology of AMD became of increasing interest. We therefore checked the behaviour of microglial cells in the laser-induced CNV with respect to the expression of various neurotrophic factors and cytokines.

Methods: Eyes of adult wild-type mice were treated by an argon laser to induce CNV. After one week, eyes were isolated, and cryosections were prepared for immunohistochemistry by standard protocols. Microglial cells were stained using CD11b and F4/80 antibodies. Double staining of microglial cells was performed against VEGF, FGF-1, PDGF-β, TGF-β1, PEDF, TNF-α, the interleukines -1β, -6, -8 and -17, MMP-9 and MKi67.

Results: Numerous microglial cells were detected in the region of the laser spot. A subpopulation of microglial cells showed immunoreactivity (IR) for the growth factors VEGF, PDGF-β and FGF-1 as well as for the typical pro-inflammatory factors TNF-α and IL-6. No IR was observed for PEDF and TGF-β1 as well as for IL-1β, IL-8, IL-17, MMP-9 and MKi67. Notably, there were slight differences in co-localisation behaviour depending on whether microglial cells were labelled by CD11b or F4/80.

Conclusion: Microglial cells are present at a high number at the sites of lesion in the model of laser-induced CNV. One week after injury, they express a limited number of growth factors and cytokines. Lack of MKi67 IR indicates a low level of proliferation, leading to the conclusion that at least most of microglial cells were migrating into the laser spot. Further investigation will deal with possible subpopulations of retinal microglia, and we will check expression pattern of the microglia at other time points after laser injury.

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Anti-inflammatory effect of interferon ß signaling in a murine model of AMD-like retinal degeneration

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Purpose: Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly. Chronic activation of the innate immune system, including microglial cells, is a hallmark of AMD. Here, we studied the role of IFNß signaling and microglial activation in an experimental model of AMD-like retinal degeneration.

Methods: Laser-rupture of Bruch's membrane was used as murine model for AMD. Microglial morphology in laser-induced lesions was analyzed by Iba1-staining of flatmounted retinas. Retinal inflammation and choroidal neovascularization (CNV) was analyzed in IFN-alpha/beta receptor knockout ($IFNAR^{-/-}$) mice and wild type controls using lectin-staining, optical coherence tomography (OCT) and fundus fluorescein angiography (FFA).

Results: OCT-analysis showed no influence of laser-induced lesions on retinal thickness. Immunohistological analysis of flat-mounted laser-damaged retinas displayed both, a higher number and a longer presence of activated microglial cells at the sites of damage in $IFNAR^{-/-}$ mice compared to controls. Laser-induced lesions in $IFNAR^{-/-}$ animals showed increased vessel leakage as well as CNV compared to control animals, indicating that $IFNAR^{-/-}$-deficiency enhanced inflammation.

Conclusion: Knockout of IFNAR lead to enhanced microglial activation and retinal inflammation. Therefore, we conclude that IFNß signaling dampens microglial reactivity and is a protective mechanism in retinal degeneration.

CaF_2 nanoparticles and lipid nanovesicles as novel carriers of calcium sensors for targeted retinal therapy

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Purpose: Calcium (Ca^{2+}) plays a major role in various cellular processes and variations in its concentration are detected by calcium sensor proteins, which change their conformation to regulate molecular targets. Calcium sensors like Guanylate Cyclase Activating Proteins are involved in different retinal diseases like retinopathies and cone dystrophies. In this study CaF_2 nanoparticles and nanovesicles with lipid composition similar to that of membranes of rod outer segments were probed as biocompatible carriers of Ca^{2+}-sensors by assessing the structural and functional effects of the interaction with the nanodevice. In particular, Recoverin (Rec) and Calmodulin (CaM) were incubated with 20–25 nm CaF_2 nanoparticles or 70–80 nm nanovescicles and their structure-function was investigated.

Methods: Circular dichroism spectroscopy was employed to investigate changes in protein secondary and tertiary structure and in thermal stability, both in the presence and in the absence of free Ca^{2+}. Isothermal titration calorimetry was used to estimate the stoichiometry and thermodynamics of binding. Variations in hydrodynamic radius of the nanodevices upon protein binding were monitored by dynamic light scattering. The residual functionality of the Ca^{2+} sensor was investigated using fluorescence spectroscopy, by monitoring the exposure of aromatic residues upon Ca^{2+} or nanoparticle binding.

Results: Binding of Rec on lipid nanovesicles was reversible without significant perturbation of either structure or function. Results for CaF_2 nanoparticles were instead protein-dependent. While Rec did not significantly change its structure in the presence of CaF_2 nanoparticles and the binding was only partially reversible, CaM preserved both its secondary and tertiary structure, and the binding to the nanoparticle was fully reversible depending on the level of free Ca^{2+}.

Conclusion: Lipid nanovesicles are widely used as carriers for encapsulated proteins, but our data show that the high area-to-volume ratio conferred from the nanoscale can be proficiently used to carry high concentrations of Rec on their surface for targeted delivery. CaF_2 nanoparticles on the other hand are promising tools for biomedical purposes, but the ability to carry proteins with preserved function is system-dependent. Our study sets the basis for a potentially successful delivery of recombinant proteins in eye disease and equally in cases where a limited administration frequency and high efficiency are highly recommended.

Identification of mutations in retinal disease genes using a panel-based Next Generation Sequencing approach

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Purpose: Retinal dystrophies (RD) are a group of blinding diseases with marked genetic and clinical heterogeneity. Until recently it had been a difficult task to determine the causative mutations in RD since mutations in more than 180 genes have previously been associated with the different disease entities. Therefore, next generation sequencing (NGS) with its massive parallelization is currently considered the most efficient approach to identify mutations in RD genes.

Methods: We screened 20 patients with different forms of RD (Achromatopsia, Macular dystrophy, Cone or Cone-rod dystrophy) for mutations in 105 RD-associated genes using hybridization-based enrichment and SOLiD-based NGS. Most of the patients had been tested negative upon screening for mutations in frequently affected genes applying Sanger sequencing and/or high-throughput mutation microarrays (Asper chips). All putative disease-causing variants identified by our NGS approach were subjected to validation by Sanger sequencing. Sanger sequencing was further used to analyze whether a variation segregated with the disease phenotype in the corresponding family.

Results: We were able to identify mutations in six different RD genes causing the disease phenotype in seven patients. In the remaining 13 patients (65 %) we did not detect sequence alterations that could explain the disease phenotype. Among the unsolved autosomal recessive cases, we found some patients who carry sequence alterations in one of the frequently affected genes (e.g. *ABCA4*), but lack a second clearly pathogenic variant. The disease phenotype in unsolved cases might be caused by pathogenic deep intronic variants which were not detected due to the targeted enrichment of the exonic regions for our NGS-based genetic testing. Another explanation is of course that these patients harbor mutations in yet unknown disease genes. To elucidate this, unsolved cases are potential candidates for whole exome sequencing.

Conclusion: We performed a NGS-based mutation analysis of the vast majority of all currently known retinal disease genes in a genetically pre-selected cohort of 20 patients with RD and were able to identify causative mutations in 35 % of cases. Unsolved cases are promising candidates for the identification of novel disease genes using whole exome sequencing.

In-vivo imaging of choroidal neovascularization using a fluorescent labeled multivalent polymer targeting L- and P-selectin

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Purpose: Selectins are expressed on the vascular endothelium and leukocytes, mediating the migration of leukocytes during inflammation. The novel multivalent synthetic dendritic polyglycerol sulfate (dPGS) inhibits selectins and suppresses inflammation. Aim of the study was to investigate fluorescently labeled dPGS as imaging probe in an animal model of laser-induced choroidal neovascularization (CNV).

Methods: For in-vivo imaging, dPGS was covalently attached to an indocyanine green type dye (6S-ICG), yielding soluble conjugates. Using confocal scanning laser ophthalmoscopy (cSLO), in-vivo reflectance and fluorescence imaging was performed in Dark Agouti rats that had undergone argon laser photocoagulation to induce CNV. Retinal uptake and fluorescence were recorded following intravenous and intravitreal injection of dPGS-6S-ICG. The distribution and accumulation of dPGS-6S-ICG were measured and frozen sections as well as flatmounts were prepared.

Results: Immediately following intravenous and intravitreal injection a strong fluorescence was visible. Twenty-four hours following injection an accumulation of dPGS-6S-ICG within the laser lesions were observed. Furthermore, multiple fluorescent spots were visible up to 56 days following intravenous injection and for up to 100 days following intravitreal injection of dPGS-6S-ICG. Over time, a continuous decrease of the fluorescence intensity was observed. Post-mortem analysis revealed a distinct accumulation to macrophages and/or microglia cells in flatmounts (after staining with CD68 and Iba1).

Conclusion: Pharmacokinetics of fluorescent dPGS can be investigated in-vivo following intravenous and intravitreal injection. The in-vivo and ex-vivo observations are in accordance with an immune mediated response following laser treatment. Fluorescent dPGS may be a potential biomarker for the in-vivo assessment of inflammation and leukocyte migration in retinal disorders.

ARMS2 is expressed in macrophages and anchors complement on apoptotic cells to enhance opsonization

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Purpose: Age-related macular degeneration (AMD) is the most common cause of blindness in developed countries. The disease is characterized by the degeneration of retinal pigment epithelial cells due to the accumulation of deposits (drusen) at the macula. The *ARMS2* variant at 10q26 (A69S or 201G>T, rs10490924) has been repeatedly significantly associated with AMD. However, reports about cellular expression and localization of the ARMS2 protein are inconsistent and the physiological role of this protein is still unclear. Therefore we aimed to determine the functional activity of ARMS2 and expressed recombinant ARMS2 in *Pichia pastoris*.

Methods: Native *ARMS2* expression was evaluated in phagocytes by RTPCR. *ARMS2* cDNA was cloned into the *Pichia pastoris* expression vector, expressed and purified by his-tag chromatography. Rabbit-antibodies were raised to the recombinant ARMS2 protein to specifically detect the protein. Binding of ARMS2 to diverse cell surfaces, as well as heparin beads was determined by flow cytometry, and interaction of ARMS2 with complement proteins was determined by ELISA.

Results: ARMS2 expression was identified in blood derived macrophages and stem cell induced microglia cells, which are responsible for the clearance of apoptotic cells in the subretinal space. Recombinant ARMS2 did not bind to living T cells or erythrocytes, but ARMS2 specifically binds to modified human surfaces such as apoptotic cells. Cell binding was mediated by heparan sulfate as demonstrated with heparin beads and with cells which are deficient in heparin synthesis. ARMS2 attached to cell surfaces recruited properdin, the only known activator of complement. This interaction resulted in enhanced opsonization of cellular surfaces with the complement activation product C3b, which is known to increase phagocytosis by macrophages.

Conclusion: Here we provide evidence that blood macrophages and microglia cells express ARMS2. We show that ARMS2 binds specifically to apoptotic cells, recruits the complement activator properdin, which subsequently activates complement and phagocytosis. The AMD associated *ARMS2* which leads to reduced presence or functions of ARMS2 likely affect clearance of apoptotic cells and cell debris. The data provide important mechanistic insights into the role of ARMS2 and underline the pivotal role of innate immunity in retinal hemostasis and prevention of inflammation.

Wnt/β-catenin signaling in microvascular endothelial and Müller cells is essential for retinal vascular development and repair

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Purpose: Activation of Wnt/β-catenin signaling by several factors is essential for normal retinal vascular development. Here we wondered which role microvascular endothelial and Müller cell-derived Wnt/β-catenin signaling has on the development of the retinal vasculature and its changes during pathological vascular processes.

Methods: Mice with an inducible conditional β-catenin deficiency in microvascular endothelial ($Cdh5^{CreERT}/Ctnnb^{fl/fl}$) and Müller cells ($Slc1a3^{CreERT}/Ctnnb^{fl/fl}$) were generated. To analyze vascular changes, mice were perfused with FITC-coupled dextran and retinal whole mounts were isolated. Further on, an oxygen-induced retinopathy (OIR), the model of retinopathy of prematurity in mice, was induced. In addition, mRNA expression of IGF-1 and angiopoietin-2 in retinae from $Slc1a3^{CreERT}/Ctnnb^{fl/fl}$ mice was investigated.

Results: During development of$Cdh5^{CreERT}/Ctnnb^{fl/fl}$ mice, a retarded development of the superficial and deep vascular plexus was observed compared to control littermates. In contrast, in $Slc1a3^{CreERT}/Ctnnb^{fl/fl}$ mice, only slight changes in the developing retinal vasculature, but a delayed regression of the hyaloid vasculature were detected. Following an OIR in mice with a β-catenin deficiency in microvascular endothelial or Müller cells, a reduced vessel regrowth into vaso-obliterated areas and a retarded development of intraretinal vessels were detected at post natal day (P) 17. Further on, in $Slc1a3^{CreERT}/Ctnnb^{fl/fl}$ mice the formation of preretinal tufts was increased by 2.6-fold compared to control littermates. In contrast, in $Cdh5^{CreERT}/Ctnnb^{fl/fl}$ mice the number of tufts was reduced by 25 % when compared to control animals. Further on, following an OIR at P15, in retinae from $Slc1a3^{CreERT}/Ctnnb^{fl/fl}$ mice mRNA levels for IGF-1 and angiopoietin-2 were significantly reduced compared to littermate controls.

Conclusions: Wnt/β-catenin signaling in microvascular endothelial and Müller cells is essential for the development of the retinal vasculature and for vascular repair following OIR. In mice with β-catenin deficiency in Müller cells, the effects are most likely mediated via a decreased expression of angiogenic factors such as IGF-1 and angiopoietin-2.

Key words: angiogenesis, Müller cells, retinopathy of prematurity, β-catenin

Time course of morphological changes in murine retinal explant cultures

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The purpose of this study was to characterize the organ culture of murine neuroretina to establish survival and early degeneration patterns of neuronal cells to prove retinal explants to be suitable for therapeutic applications.

Neuroretina explants were prepared from wild type C57Bl6 mice and evaluated after 2 to13 days in culture. Fresh retinae were used as controls. Explants were cultured in Millicell® culture plate inserts with the photoreceptor layer facing the supporting membrane. Culture medium (DMEM) was maintained in contact with the membrane beneath the explant. Cryostat sections were prepared for TUNEL assay and immunohistochemistry. Neuroretinal modifications were evaluated after HE staining, TUNEL assay, and after immunostaining for neurodegeneration and cell cycle markers, and neuronal cell markers.

Histologically, during the first week in culture, only minor morphological changes were detectable in retinal explant culture apart from disruption and truncation of photoreceptor outer and inner segments. TUNEL-staining revealed isolated apoptotic nuclei in the outer and inner nuclear layer (ONL, INL). Immunolabeling with anti-AIF (apoptose inducing factor) showed no dislocation of AIF into the nuclear layers. Rod bipolar cells stained by PKCα appeared densely packed in the INL. Sprouting of individual dendrites into the ONL was visible from day one in both bipolar and horizontal cells. Up to one week in culture, very little reduction of cells in both nuclear layers was visible. However, DNA-damage marker γH2AX showed a substantial number of damaged nuclei in the ONL and INL at the end of week one. Antibodies against cell cycle enzyme Cdk4 revealed numerous positive neurons in the INL and only very few in the ONL between day 4 and 6. After two weeks in culture TUNEL-staining revealed numerous apoptotic nuclei in the ONL and INL, which was in line with translocation of AIF into the nuclear layers.

Our results indicate that retinal explants are in a “close to healthy” condition during the first five days of *in vitro* culture. Degeneration processes at the end of the first week seem to reproduce cellular modifications occurring *in vivo* after retinal damage. Therefore murine retinal explant culture represent a suitable model system for therapeutic applications like retinal gene therapy. In addition, this approach can help reduce the numbers of animals used for gene therapeutic experiments.

Functional relevance of autofluorescence patterns in Stargardt disease

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Purpose: Stargardt disease is characterized by distinct patterns of increased and decreased signals on near-infrared (NIR) and short wavelength (SW; blue) fundus autofluorescence (AF) imaging. The aim of this study was to evaluate the structure-function correlation between patterns on fundus AF images and maps of retinal sensitivity derived from microperimetry testing.

Methods: Fifty eyes of 25 patients with Stargardt disease underwent functional testing using fundus controlled perimetry (MAIA, CenterVue, Italy). A confocal scanning laser ophthalmoscope (HRA2, Heidelberg Engineering, Germany) was used for recording NIR-and SW fundus AF. Disease-related patterns on AF images were categorized as follows: 1) no pattern, 2) granular pattern, 3) bright > dark flecked pattern, 4) dark > bright flecked pattern, 5) dark pattern and 6) atrophic lesions. Retinal sensitivity along a horizontal line of 15° eccentricity through the fovea was compared between these regions.

Results: Pattern-related retinal sensitivity was not different between both eyes of each patient (ANOVA; $p = 0.16$ and $p = 0.54$ for SW- and NIR-fundus AF, respectively). Borders between consecutive patterns on NIR-fundus AF images were more eccentric compared to equivalent borders on SW- fundus AF images. For both SW- and NIR fundus AF retinal sensitivity was different between patterns (ANOVA, $p < 0.0001$; post hoc test, $p < 0.0001$ to $p = 0.047$) except for the comparison between patterns 1 and 2 ($p = 0.06$ and $p = 0.98$ for SW- and NIR fundus AF, respectively), as well as between patterns 2 and 3 ($p = 0.42$ and $p = 0.05$ for SW- and NIR fundus AF, respectively). The largest drop in retinal sensitivity was observed at the border to regions with predominantly dark pattern in both SW- and NIR- fundus AF (mean retinal sensitivity [dB ± SEM], SW fundus AF: $1 = 23.33 \pm 0.81$, $2 = 19.94 \pm 1.45$, $3 = 16.60 \pm 1.53$, $4 = 9.27 \pm 1.43$, $5 = 4.13 \pm 1.18$, $6 = 0.58 \pm 0.35$; NIR fundus AF: $1 = 24.08 \pm 0.58$, $2 = 23.66 \pm 0.67$, $3 = 19.38 \pm 1.33$, $4 = 12.62 \pm 1.48$, $5 = 2.97 \pm 0.67$, $6 = 0.71 \pm 0.41$).

Conclusion: Structure-function correlations reveal consistent functional deficits of fundus AF patterns in Stargardt disease. In both, SW- and NIR fundus AF the transition to a predominantly dark pattern is associated with a marked impairment of retinal sensitivity. Areas of equivalent patterns on NIR fundus AF images exceed those on SW fundus AF, suggesting superiority of NIR- fundus AF imaging to indicate early functional alterations.

A European young investigators network for Usher syndrome

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The Usher syndrome (USH) is the most common form of inherited deaf-blindness affecting the two major senses in human. It is a complex disorder divided into three clinical types, which are genetically heterogeneous, making diagnosis and treatment challenging. So far, ten causative genes and one genetic modifier have been identified. Molecular analyses revealed that all USH1 and 2 proteins are organized in protein networks in the eye and inner ear. Although this has provided insights into the function of USH proteins and explains why defects in proteins of different families result in USH, the exact pathomechanisms in the retina remain unclear. EUR-USH (www.eur-ush.eu) is composed of three overlapping components. In component A we aim to improve diagnosis. So far, we have compiled a multinational clinical protocol for a prospective observational longitudinal Usher cohort study. The data of the clinical examinations and molecular analyses will be uploaded in the EUR-USH database. Combination of data will improve diagnosis and provide more details to genotype/phenotype correlations. In component B we will gain more insight into the molecular pathogenesis. For this we adopted proteomics analyses in transgenic zebrafishes to extend to USH protein networks. Furthermore we analysed USH protein expression and interaction of USH proteins with different imaging methods in human donor retinas. In component C we evaluate gene-based therapy options for the retinal degeneration. For this, we generated minigenes for gene replacement in USH2A. Further we analysed the read-through efficacy of different USH causing nonsense mutations and compared the retinal biocompatibility of different read-through inducing drugs.

Translational read-through of in-frame nonsense mutations for the treatment of hereditary retinal dystrophies

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Purpose: Hereditary retinal ciliopathies are a genetically heterogeneous group of disorders that cause vision defects, for which currently no effective cure is available. Patient screenings predict that ~12 % of all pathogenic variants identified for human hereditary disorders are nonsense mutations. Nonsense mutations lead to premature termination of translation and to truncated, non-functional proteins. Recent studies demonstrate translational read-through (TR) as a therapeutic approach for targeting nonsense mutations. Here we aim to analyse the read-through efficacy of different translational read-through inducing drugs (TRIDs) in a series of nonsense mutations causing syndromic disorders associated with retinitis pigmentosa (RP), namely Usher syndrome (USH), the most common form of combined inherited deaf-blindness; Bardet-Biedl syndrome (BBS), a ciliopathy disorder characterized by e.g. obesity, retinal degeneration, and polydactyly, and Senior-Loken syndrome, a combination of nephronophthisis and RP.

Methods: We compared efficacy of TRIDs, namely PTC124, aminoglycosides and the designer aminoglycosides NB84 and NB124, in transfected HEK293T cells by quantitative immunofluorescence microscopy and Western blot analysis. Retinal toxicity of TRIDs was assessed in mouse retinal explants by TUNEL-assays and immunofluorescence analyses applying molecular markers for retinal integrity.

Results: We generated cDNA constructs coding for the disease-related nonsense mutations in *USH1C* isoform harmonin a1 (p.R155X), *USH3A* (p.Y63X; p.Y176X), *BBS1* (p.G73X; p.Q291X), and *NPHP4* (p.L104X), respectively. We observed various read-through efficacies in HEK293T cells transfected with nonsense mutations in the affected genes after application of PTC124, NB84 or NB124, respectively. TUNEL assay demonstrated comparable biocompatibility of NB84 and NB124 to aminoglycosides but an excellent biocompatibility of PTC124 in retinal cells.

Conclusion: The research showed the therapeutic potential of PTC124, NB84 and NB124 for patients with RP caused by the various nonsense mutations. Restoration of protein might stop or slow down progression of retinal degeneration and greatly improve the life quality of RP patients.

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dTGR: A new rat model for systemic hypertensive retinopathy

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Purpose: Systemic and local RAS play important roles in the development of hypertensive end-organ damage. To understand the pathomechanisms underlying hypertensive retinopathy, we used a double transgenic rat model (dTGR), which expresses both the rat and human RAS. The model leads to severe end-organ damage with animals dying by week 7 due to hypertension and overstimulation of systemic RAS. Purpose of the study is to assess whether systemic hypertension affects retinal structure and function.

Methods: By using flatmount preparations, immunohistochemistry of paraffine sections and PatternERG we analysed the retinal phenotype of dTGR.

Results: Flatmount preparations of the retina revealed focal avascular lesions as well as pathological vessels with irregularities in shape and diameter compared to control animals.
In sagittal sections of the retina we detected a focal loss of retinal ganglion cells. GFAP staining revealed activation of astrocytes in the ganglion cell layer (GCL) and astrocytosis of the optic nerve. VEGF-positive staining could be detected in the ganglion cell layer (GCL) and the inner nuclear layer (INL).
To further substantiate the loss of ganglion cells detected in the paraffine sections, Pattern ERG was carried out. By using different pattern sizes (15° and 5–7°) at 2.4 hz of the appearing black and white checkerboard we showed that the positive component after about 50 ms (P50) of the Pattern ERG was significantly smaller in dTGR compared to control rats at 5–7° of the displayed pattern whereas the P50 at 15° remained unchanged. This indicates a loss of ganglion cells in the retina.

Conclusion: Thus we present a first animal model for hypertensive retinopathy showing functional and structural alterations of the retina. Astrocytosis and VEGF-A production in the inner retina indicate possible pathomechanisms for further investigation.

Novel insights into visual information processing of human retina

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Purpose: Retinal information processing has been studied extensively in animal models. Many specialized circuits have been identified that provide information about specific aspects of the visual scene. It is often thought that such sophisticated image processing might be absent in human retina. However, there is a lack of appropriate measurements: retinal information processing in the human retina has not been studied with *in-vitro* methodology. Further, development of targeted treatment options against visual impairment also requires more detailed knowledge about the human retina. We thus aimed to studying human retina function at the cell and system levels *in-vitro*. Additionally, we evaluated the usability of *post-mortem* human retinal tissue for tests of novel treatments against blindness.

Methods: Human retinas were provided by patients who had to undergo medically indicated enucleation of one eye, and by *post-mortem* cornea donors. Pieces of these retinas were placed ganglion cell side-down on 60-electrode multi-electrode arrays, and we recorded spikes produced by retinal ganglion cells in response to light stimuli.

Results: In 8 out of 15 donated retinas we could measure abundant light responses. The recorded cells showed diverse properties, including ON-, OFF- and ON-OFF-responses, and different response latencies and transiencies. We found that human ganglion cells are tuned to higher speeds and higher temporal frequencies compared to previously recorded mouse ganglion cells. Further, we show that *post-mortem* human retina survives up to 27 hours after death.

Conclusion: We show that it is possible to perform *in-vitro* electrophysiology and to measure diverse light responses with human retina. The observed differences in speed tuning (human vs. mouse ganglion cells) might be explained by the higher angular velocities to which human ganglion cells are exposed in the bigger human eye. This pinpoints at the importance of species-specific light stimulation when tracking down sophisticated retinal processing. We found species-specific properties in one such retinal property, namely differences in temporal frequency tuning between mouse and human ganglion cells. Finally, we show that ganglion cells can remain active in *post-mortem* human retinas for up to 27 hours, which allows for future *in-vitro* treatment tests on such retinas.

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Daylight vision repair by cell transplantation

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Human daylight vision depends on cone photoreceptors and their degeneration results in visual impairment and blindness as observed in several eye diseases including age-related macular degeneration or late stage retinitis pigmentosa, with no available cure. Pre-clinical cell replacement approaches in mouse retina are focused on rod dystrophies, due to the availability of sufficient donor material from the rod-dominated mouse retina, leaving the development of treatment options for cone degenerations not well studied. Thus, an abundant and traceable source for donor cone-like photoreceptors was generated by crossing the neural retina leucine zipper-deficient ($Nrl^{-/-}$) mice with an ubiquitous GFP reporter line. Cone-like photoreceptors were enriched by CD73-based magnetic associated cell sorting and transplanted into the subretinal space of adult wild-type, cone-only $Nrl^{-/-}$ or cone degeneration (Cpfl1) mice. Donor cells correctly integrated into host retinas, acquired mature photoreceptor morphology, expressed cone-specific markers and survived for up to six months, with significantly increased integration rates in the cone-only $Nrl^{-/-}$ retina. Integration rates were not influenced by subretinaly located macrophages or monocytes. Individual retinal ganglion cell recordings demonstrated the restoration of photopic responses in cone degeneration mice following transplantation suggesting, for the first time, the feasibility of daylight vision repair by cell replacement in the adult mammalian retina.

The role of CFHR3 and CFH-autoantibodies in age-related macular degeneration

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Purpose: Age-related macular degeneration (AMD) is the most common cause of blindness in the elderly. Activation of the complement system has been identified as a central mechanism for the pathophysiology of AMD. In contrast, deletions of the complement factor H-related 1 and 3 *(CFHR1/3)* genes have been shown to be associated with a decreased risk of AMD although the functions of the CFHR proteins still remain unclear. Autoantibodies against CFH have been shown to be associated with CFHR1/3 deficiency in other diseases. The aim of our work is to elucidate the effect of CFHR3 deletion in AMD patients. We will generate a monoclonal antibody against human CFHR3 to investigate the function of CFHR3, and we will determine anti-CFH autoantibody titer in AMD.

Methods: For the generation of anti-CFHR3 antibodies different immunization strategies in mice were used. Full-length CFHR3 and the short consensus repeat 5 domain (SCR 5) of CFHR3 were recombinantly expressed. Additionally, specific peptides for CFHR3 were identified *in silico* via AbDesigner. The immunized mice antibody titers were analyzed by direct enzyme-linked immunosorbent assay (ELISA). Blood samples of AMD patients and healthy controls were tested for CFH-autoantibodies in an ELISA.

Results: *In silico* analyses were performed to determine specific epitopes of CFHR3. The most immunogenic peptides for CFHR3 were located in the SCR 5 domain. Two peptides based on the highest IgG-score and uniqueness-optimized ranks were synthesized: peptide 1 *[5'-NKNNIKLKGRSD-3']* and peptide 2 *[5'-RSDRKYYAKTGD-3']*. Additionally, CFHR3 full-length protein with STREP-tag has been expressed in HEK293T cells and detected by Western Blot. For the detection of anti-CFH autoantibodies from blood samples an ELISA was established and optimized. Preliminary results showed that the CFH-autoantibody titer is not increased in blood samples of AMD patients compared with healthy age-matched control samples.

Conclusion: These initial results encourage further investigation into the functional role of CFHR3 in AMD. Further mouse immunizations are necessary to generate anti-CFHR3 antibodies. Our preliminary CFH-autoantibody results are consistent with another study from the literature. Further analyses with a higher number of samples will be performed to compare different CFH-autoantibodies (IgG, IgM).

Stimulation of Müller glia proliferation and progeny generation in the mouse retina

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Purpose: In the human and mouse retina Müller glia (MG) are well known to undergo gliosis in all major types of retinal diseases – which sometimes may even lead to scar formation due to proliferative gliosis. Some studies suggest that in the mouse retina MG derived neuronal regeneration can be stimulated, but only to a very limited extent. Here, we started to find out, if conditional immortalization might stimulate MG derived proliferative gliosis and/or neuronal regeneration.

Results: In the juvenile mouse retina, after retinogenesis is finished, some Müller glia shift from a quiescent differentiated state into a proliferative state upon damage of retinal explants ex vivo. This process is inducible by mitogens. EdU (S-phase marker) experiments revealed a tremendous increase in MG proliferation within the first two days, a peak of EdU positive cells at day 4 (14+-2SEM) and a decrease until day 6 (4+-0.4SEM,) per ROI. These results were consolidated with QPCR. Next, we used transgenic mice with tightly and temporally controlled expression of the proto-oncogene SV40 large T-antigen (cSV40LT). It is well reported that cSV40LT binds several proteins including the tumor suppressor p53 and retinoblastoma and bypasses cell cycle checkpoints. Induction of the cSV40LT for 6 days ex vivo led to an overall increase in proliferation compared to control. The number of active cycling (Ki67+) cells was 6-fold increased (SV40LT:32+-6SEM, CTRL:5+-1SEM) per ROI. It was even possible to keep a cycling population until 10 days cSV40LT.
To allow future manipulation of retinal cells in large scale we tested primary cell culture approaches. MG show same characteristics like in organ culture (damage induction, cell cycle re-entry followed by decline in proliferation). Further we established protocols to FACSort MG with a high purity (>80 %). We tested and confirmed its suitability for downstream applications (MG cell culture, transcriptome analysis, transplantations).

Summary & Conclusion: Our results so far suggest that induction of cSV40LT not only overcomes the proliferative restriction of Müller glia but also maintains its progeny in the cell cycle over extended period of time. Surprisingly, major parts of the generated cell progeny formed gliotic cell clusters, which were all located within the boundaries of the retina. We further developed tools which allow manipulation of retinal cells and especially MG in a large scale.
In our current and future work we study the Müller glia and its derived progeny to find out the underlying mechanisms that enable neuronal regeneration and prevent gliotic scar formation.

Optimization of subretinal electrical stimulation parameters

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Purpose: We sought to estimate the optimal voltage amplitude and duration of a square-wave electrical pulse with which to stimulate the mouse retina by looking for the stimuli that would activate the maximum number of retinal ganglion cells (RGCs).

Methods: Multielectrodearray recordings of extracellular RGC spikes were made from the isolated mouse retina (MultiChannel Systems, Reutlingen, Germany). For stimulation, a square-wave voltage pulse of varying amplitude and duration was presented. Amplitudes ranged from -2500 mV to 2500 mV in 500 mV steps; and 8 durations were presented ranging from 300 to 5000 µsec. Only voltage-duration pairs that fell below the safe charge injection limit were presented.
To smooth the noisy response surfaces of firing rate (in Hz) plotted vs. both voltage (V) and duration (µsec), we processed the surface with a weighted LOESS fit where the weight for each average response was determined by the reliability of the response across 5 repetitions (Victor & Purpura 1997). The chosen weighting metric calculates the mean Euclidean distance between spike train pairs for all pairwise combinations at a given stimulus setting. When the distance is less (more reliable), the weight given to the response is higher.

Results: Fits for individual RGCs were averaged across all cells to obtain a population average with a peak that drivesa maximal population response at approximately -2000 mV and 1500 µsec. However, there is little indication of what fraction of the population is driven by this stimulus. Alternatively, we identified all stimuli that drove a given cell within the 10–90 % range (from spontaneous to maximal firing rate). These stimuli were assigned a value of 1 and all other stimuli a value of 0. By averaging the resultant binary surfaces, we were able to determine the fraction of cells that were driven within the 10–90 % range for each stimulus. Surprisingly, the stimuli activating a maximal fraction of the cell population differed noticeably from the peak response stimuli found above.

Conclusion: Population averages do not always reveal the optimal stimulus for activating a maximal fraction of the RGC population. We will next extend this method to identify iterative stimuli that could collectively activate an even greater fraction of the population.

Victor JD, Purpura KP "Metric space analysis of spike trains theory algorithms and application." Network 1997

Reduced pattern ERG in mouse model for neuronal ceroid lipofuscinosis

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Purpose: The Cln3 murine model for juvenile neuronal ceroid lipofuscinosis (Batten disease) shows a mild retinal degeneration in elder animals (age higher than 12 months) with alterations in the CNS including an Optic nerve degeneration. Purpose of the study is the investigation of the ganglion cell function as mechanism for early onset loss of vision.

Methods: Ganzfeld ERG (photopic according to ISCEV standard) and pattern ERG (pattern size: between15°and 4°; frequency: 0.8 Hz, 2.4 Hz and 6.7 Hz) were recorded. The density of retinal ganglion cells was compared between wild-type (Cln3+/+, n = 5) and mutant (Cln3-/- n = 8) siblings at the age of 9 months by means of immune histochemistry.

Results: Cln3 mice showed at the age between 9 and 12 months normal photopic Ganzfeld ERG b-waves when compared to the wild-type control littermates. The pattern ERG showed at the pattern sizes 15° and 10° no differences of the P1 amplitude whereas at the pattern sizes between 4°–9° degrees the P1 amplitudes were significantly smaller in the Cln3 mice in comparison with their wild-type littermates. In contrast histochemical analysis of sagittal sections of the retina revealed to differences in the ganglion cell density between Cln3 and wild-type mice.

Conclusion: The Ganzfeld ERG confirms previously published data that this model shows retinal degeneration at ages higher than 12 months. The same applies for retinal ganglion cells which showed no abnormalities at the age of 9 months. However, at this age we found strong alteration of the retinal ganglion cells function. Thus changes in visual function in Batten disease already occur by decreased retinal ganglion cell function before onset of ganglion cell loss.

Assessment of foveal sparing of reticular drusen in patients with age-related macular degeneration

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Purpose: To evaluate foveal sparing of reticular drusen (RDR) in patients with early age-related macular degeneration (AMD) using confocal scanning laser ophthalmoscopy (cSLO) and spectral-domain optical coherence tomography (SD-OCT).

Methods: Simultaneous combined near-infrared (NIR) cSLO and SD-OCT imaging (Spectralis, Heidelberg Engineering) was performed in 27 eyes of 21 patients (mean age 81 years, range 52–90) with RDR and early AMD. Early AMD was defined by the appearance of drusen or pigmentary changes but without signs of late AMD. The appearance and the distribution of RDR within the center macula were analyzed.

Results: RDR were detectable in NIR images as small hyporeflective lesions and larger hyporeflective rings with a hyperreflective center. In the corresponding SD-OCT scans, a wave-like pattern in the outer retina was noticed. A sparing of RDR with no visible individual lesions in the central macula was observed in 21 eyes, whereas the spared area circle size varied between 800 µm to 2000 µm. In 6 eyes, a few single RDR lesions were found within the 800 µm to 2000 µm diameter central circle, while RDR density (number of individual RDR per involved retinal area) was much higher towards eccentricity. In 12 eyes, foveal sparing of RDR with a horseshoe or incomplete ring appearance was present. The highest density was typically seen in the superior part, while the density tended to be lowest temporal and inferior to the fovea.

Conclusions: Foveal sparing of RDR is found in patients with nonexudative AMD. The findings are in accordance with a dynamic process of RDR evolution and involvement of the fovea not until later in the disease process. Factors for temporary sparing of the foveal retina are yet unknown, but may relate to different photoreceptor density and predominance of cones. Longitudinal studies with larger populations are warranted to further determine the natural history of variations in RDR distribution over time.

Visual responses in rd retina driven by rod bipolar cell targeted CatCh

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Purpose: Retina degenerative diseases cause blindness and life condition impairment of many people. Suitable therapeutic approaches, like gene therapy, exist only for a few of these diseases. Recently, optogenetics was developed as a new approach which provides new opportunities for treatment of retinal degenerative diseases. In this project, we evaluated the expression of a Channelrhodopsin variant called CatCh, which is 70-times more light sensitive than ChR-2, specifically in rod bipolar cell.

Methods: We prepared a plasmid in which the CatCh gene was put under the control of the rod bipolar cell specific promoter L7. We used a shortened 1kb promoter sequence (sL7), around 1/3 of the promoter size used so far with retina. Retinas of wild-type and retinal degeneration (rd1) mice were transfected with this plasmid at postnatal day 1 (P1) through in-vivo electroporation. Multi-electrode array (MEA) recordings of isolated retina obtained between P21 and P72 were used to evaluate functional expression of sL7-CatCh. We applied blue and orange flashes, combined with application of 20 µm APB (blocking input to ON bipolar cells), to distinguish between CatCh-driven and photoreceptor-driven light responses.

Results: sL7-driven expression levels were generally very low. Co-injected and co-electroporated control plasmids (EF-1alpha or CMV-promoter driven fluorescent proteins) usually showed much stronger expression. Nevertheless, MEA experiments showed CatCh driven responses, i.e. ON responses that were selective to the blue light stimulus (but not orange), and that remained in the presence of APB. We observed such results in wild-type mice as well as in rd1 mice (age of 72 days).

Conclusion: The channelrhodopsin-variant CatCh is very promising for vision restoration. Despite weak expression retinal responses could be recorded. In vivo electroporation as a transfection technique is successful, but too restricted in terms of the transfected retinal area. Thanks to the small promoter size of sL7, the whole DNA sequence encoding promoter and CatCh sequence is below 4kb and can be packaged into an adeno-associated virus. Virus-mediated gene transfer of the L7-based CatCh construct will be tested in the future.

Identification of a novel neurotrophic factor from primary retinal Müller cells using SILAC

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Retinal Muller glial cells (RMG) have a primary role in maintaining the homeostasis of the retina. In pathological situations, RMG execute protective and regenerative effects, but can also contribute to neurodegeneration. Cultured primary RMG have recently been recognized to secrete pro-survival factors for retinal neurons for up to two weeks in culture, but this ability is lost when RMG are cultivated for longer durations. In our study, we investigated RMG supernatants for novel neuroprotective factors using a quantitative proteomic approach. Stable isotope labeling by amino acids in cell culture (SILAC) was used on primary porcine RMG. Supernatants of RMG cultivated for two weeks were compared to supernatants from cells which had already lost their protective capacity. Using this approach, we detected established neurotrophic factors such as transferrin, osteopontin (SPP1), and leukemia inhibitory factor (LIF), and identified C-X-C motif chemokine 10 (CXCL10) as a novel candidate neuroprotective factor. All factors prolonged photoreceptor survival *in vitro*. *Ex-vivo* treatment of retinal explants with LIF or CXCL10 demonstrated a neuroprotective effect on photoreceptors (PR). Western blots on CXCL10 and LIF stimulated explanted retina and PR lysates indicated activation of pro-survival Signal Transducer and Activator of Transcription (STAT) signaling and B-cell lymphoma (BCL) pathways. These findings suggest that CXCL10 contributes to the supportive potential of RMG towards retinal neurons.

Survival of retinal ganglion cells is mediated by PEDF from Müller glial cells

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Purpose: It was our aim to show that pigment-epithelium derived factor (PEDF) of glial origin protects retinal ganglion cells (RGC) from pathologic external influences and which pathways are utilized.

Material & Methods: Homotypic and coCulture experiments using immunoisolated primary RGC and cultured Müller glial cells were conducted for 24 hours under normoxic (95 % air; 5 % CO_2) and hypoxic conditions (0 % O_2; 5 % CO_2; 95 % N_2). PEDF was substituted in different concentrations to homotypic cultures and blocked using the appropriate antibody in coCulture experiments. NFκB activity was detected using immunohistochemical staining methods.

Results: After 24 hours of normoxic incubation RGC survival rate was 54.04 ± 0.03 % in homotypic and 68.52 ± 0.03 % in coCultures. Hypoxic incubation led to a decrease of RGC survival with 32.84 ± 0.02 % in homotypic and 44.83 ± 0.02 % in coCultures. Supplementation of PEDF led to an increase of RGC survival in homotypic cultures whereas addition of neutralizing antibody led to a decrease of RGC survival in coCultures. Immunohistochemical staining of NFκB showed PEDF-dependent activation under normoxic and hypoxic conditions. Inhibition of NFκB decreased RGC survival under normoxic and hypoxic conditions.

Conclusion: Our experiments demonstrate the neuroprotective properties of PEDF of glial origin and its mode of action through the NFκB-pathway.

Efficiency of retinal cell fate induction from mouse embryonic stem cells and hPax6-GFP reporter expression

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The differentiation of pluripotent stem cells into retinal cell types opens new possibilities for studies on retinogenesis, retinal degeneration and regeneration. Recently, several protocols for the differentiation of pluripotent stem cells into retinal cell types have been developed. Here, we modified a recently developed mouse embryonic stem cell (mESC) three-dimensional differentiation approach (Eiraku et al, Nature 2011), to make it independent of a transgenic eyefield reporter, and investigated the efficiency of mESC derived retinogenesis at all steps of the protocol. Further, we characterized the GFP expression pattern of a novel hPax6-GFP transgenic mESC reporter in retinogenesis.
We achieved efficient eyefield induction (82 ± 12 SD % of aggregates, N = 7), as well as patterning into RPE and neural retina domains. However, optic cup-like structures were not formed. Further, upon randomly cutting aggregates into three evenly sized pieces, the majority (86 ± 6 SD % of aggregates, N = 2) generated big, stratified retinal tissue, reminiscent of early postnatal retina in vivo. Each aggregate retina had between 0.41 to 1.9 mm (1.4 ± 0.4 SD) photoreceptor layer circumferential length. This novel approach makes the generation of mESC derived, stratified retina simpler and independent of an eyefield transgenic fluorescent reporter. Using this 3D retinal differentiation approach, we found that the hPax6-GFP reporter construct is expressed in a major amacrine population (Pax6+, Brn3-, HuCD+, AP2a+, bHLHb5+, Ebf3+), as well as a retinal progenitor or precursor subpopulation (Pax6+, PH3+, Rx+, Chx10+, Crx+) of the Pax6 lineage. Interestingly, two different mESC clones carrying this reporter construct showed striking differences in GFP expression, which started later in clone 2 and with less cell types being GFP+ compared to clone 1. Therefore, these novel mESC reporter lines will be a useful tool to study lamination, amacrine lineages and subtypes, wiring and patterning and degeneration of amacrine neurons in mESC derived retina.

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Gliotransmitter release from retinal Müller glial cells and its effects on neuronal survival after transient ischemia

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Purpose: There is an ongoing debate whether glial cells are capable to release neuroactive transmitters via regulated exocytosis and thereby fuel the neuron-glia crosstalk. A prerequisite for such kind of glia-neuron interaction is a tightly regulated fast transmitter release from both neurons and glia cells. Glutamate, adenosine-5'-triphosphate (ATP) and adenosine are set free in the context of the volume regulatory signalling cascade of retinal Müller glial cells. Although the action of these gliotransmitters on Müller cells is well described, little is known about release mechanisms. We aimed at characterizing the latter in more detail and to investigate the impact of a disrupted exocytotic gliotransmitter release during retinal pathology.

Methods: We modified a fluorometric enzyme assay based on the Amplex® Red system to visualize glutamate, ATP and adenosine release from acutely isolated murine Müller cells. These measurements were performed on Müller cells of wildtype and mice that express specifically in glial cells a dominant-negative SNARE (dnSNARE) protein that blocks the vesicular release. A model of ischemia/reperfusion was applied to investigate the impact of glial dnSNARE expression under pathological stress conditions.

Results: We demonstrate that Müller cells are capable to release glutamate via regulated exocytosis as significantly less glutamate was liberated from Müller cells of dnSNARE mice. Additionally, at least one alternative calcium-dependent pathway is involved in glutamate release from Müller cells. Putative candidates are hemichannels and NPPB-sensitive big anion channels. Next, we modified the assay enabling the detection of ATP and this provided first direct evidence for their release by Müller cells. Pharmacological data suggest that hemichannels rather than exocytosis play a role in ATP release. Finally, we compared neuronal survival after transient ischemia in wildtype and dnSNARE mice. Interestingly, less neurodegeneration was found in the latter.

Conclusion: We demonstrate that Müller cells are capable to release glutamate via exocytosis, while ATP is liberated via hemichannels. Importantly, lack of vesicular 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.

In vivo imaging of the adult zebrafish retina by Optical Coherence Tomography (OCT)

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Purpose: The zebrafish retina is a prominent model to study both degeneration in disease models and regeneration after injury. *In vivo* imaging by means of optical coherence tomography (OCT) allows to study morphological changes within the same retina over weeks and is therefore a powerful tool to mechanistically dissect retinal degeneration and regeneration.

Methods: To improve OCT imaging in zebrafish, we developed a zebrafish eye model (Gaertner et al., 2014) using geometrical data obtained by *in vivo* dispersion encoded full range OCT as well as a dispersion comprising gradient index (GRIN) lens model based on refractive index data found in the literature. With the aid of the zebrafish eye model, the optics of the OCT scanner head in our customized OCT setup were adjusted to provide high-resolution retinal images with a field of view of 30° x 30°.

Results: We have developed an optimized OCT setup for high quality OCT imaging of retinal degeneration and regeneration in living adult zebrafish, employing two different light lesion paradigms and comparison to histology data (Weber et al., 2013). Moreover the UV cone photoreceptor mosaic, as well as light adaptation of the retinal pigment epithelium can be visualized by OCT. In the future, a hybrid system that allows simultaneous high-resolution spectral domain optical coherence tomography (OCT) in the near-infrared and single-photon excited fluorescence (SPEF) detection in the green and red visible wavelength range (Cimalla et al., 2011) will be further optimized for *in vivo* detection of fluorescently labelled retinal cells.

Conclusion: Using a customized OCT setup, we demonstrate the application of OCT in zebrafish, allowing us improved retinal *in vivo* imaging of the degenerating and regenerating adult zebrafish retina.

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