376 Ocular gene expression, proteomics and lipidomics
Tuesday, May 09, 2017 3:45 PM–5:30 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 3559–3574/B0118–B0133
Organizing Section: Biochemistry/Molecular Biology

Program Number: 3559 **Poster Board Number:** B0118
Presentation Time: 3:45 PM–5:30 PM
Samd7, a photoreceptor-specific component of the polycomb repressive complex, plays an essential role in repressing non-rod genes expression through H3K27me3 regulation in rod photoreceptors
Takahisa Furukawa[1], Shun Kubo[1], Mayu Furuhashi[1], Akiko Ueno[1], Tetsuo Kon[1], Taro Chaya[1], Shinji Ueno[2], Yoshihiro Omori[1].
[1]Molecular and Developmental Biology, Osaka Univ. Inst for Protein Rsrch, & JST, CREST, Suita, Japan; [2]Nagoya Univ School of Med, Nagoya, Japan.
Purpose: Functions and molecular mechanisms of multiple transcription factors in rod and cone photoreceptor-specific gene regulation have been well studied. On the other hand, the functional mechanisms underlying the epigenetic regulation in photoreceptor cell type specification has been poorly understood.
Methods: In the current study, we analyzed biochemical and biological functions of Samd7, a rod photoreceptor-specific SAM domain protein.
Results: We observed that various non-rod genes including S-opsin are ectopically expressed in rod photoreceptor cells and multiple rod genes were significantly down-regulated in the Samd7-null retina. We found that Samd7 physically interacts with components of polycomb repressive complex 1 (PRC1), Polyhomeotic homologs 1-3 (Phc1-3). Chromatin immunoprecipitation assay revealed that multiple genes up-regulated in the Samd7-deficient retina exhibited a significant decrease of the H3K27me3 marks.
Conclusions: Our results suggest that Samd7 plays an essential role in repressing non-rod genes expression through H3K27me3 regulation in rod photoreceptors.
Commercial Relationships: Takahisa Furukawa, None; **Shun Kubo**, None; **Mayu Furuhashi**, None; **Akiko Ueno**, None; **Tetsuo Kon**, None; **Taro Chaya**, None; **Shinji Ueno**, None; **Yoshihiro Omori**, None
Support: Japan Science and Technology Agency, Grant-in-Aid for Scientific Research

Program Number: 3560 **Poster Board Number:** B0119
Presentation Time: 3:45 PM–5:30 PM
Age-related transcription changes in photoreceptor neurons are light-dependent
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Purpose: Epigenetic mechanisms have been proposed to play key roles in the pathogenesis of ocular diseases associated with aging, such as diabetic retinopathy and age-related macular degeneration. An unanswered question is whether age-related changes in chromatin and gene expression drive the transition from aging to early disease state in ocular diseases of aging. Long-lived photoreceptor neurons might be uniquely vulnerable to the effects of aging because they must maintain the expression of genes important for the survival as well as cellular function throughout the adult lifespan. To characterize the mechanisms involved in age-dependent changes in gene expression, we sought to first characterize the transcriptome of aging photoreceptor neurons in *Drosophila*.
Methods: To identify genes that change expression profiles upon aging, we labeled and isolated nuclei from adult photoreceptor neurons and examined the transcriptome using RNA-seq at five time-points between 10 and 40 days post-eclosion.
Results: Using this approach, we identified 1200 genes with age-dependent changes in expression profiles. Genes that are upregulated with age are functionally-enriched for GO terms involved in stress-response and protein synthesis, whereas genes that are downregulated with age are enriched for GO terms such as ion transport, cell adhesion and neuronal function. Strikingly, the downregulated genes show maximal expression changes at the earliest time points examined, while the upregulated genes change later in life.
Conclusions: This suggests that defects in transcription activation precede and may contribute to the increased stress response and cellular dysfunction in aging photoreceptor neurons. Since light is known to lead to increased oxidative stress in the eye, we tested the contribution of light to the age-dependent changes in gene expression. Notably, exposure of young flies to blue light induces similar changes in gene expression in photoreceptors to those that occur during aging, consistent with the hypothesis that the age-related changes in photoreceptor gene expression are dependent on light. Thus, blue light provides us with an experimental system to identify the epigenetic mechanisms involved in the transcriptional decline observed during photoreceptor aging.
Commercial Relationships: Vikki Weake
Support: NIH Grant 1R01EY024905

Program Number: 3561 **Poster Board Number:** B0120
Presentation Time: 3:45 PM–5:30 PM
Heterochromatin Protects Retinal Pigment Epithelial Cells from Oxidative Stress
Lili Gong[1], Ruili Qi[1, 2], Qian Sun[1], Fang-yuan liu[1], Zhong-wen Luo[1], QIAN Nie[1, 2], Xiao-Dong Gong[1], Yun-Fei Liu[1], Lan Zhang[1], Xiangcheng Tang[1], Yizhi Liu[1], David W. Li[1, 2]. [1]Zhongshan Ophthalmic Center, Sun Yat-sen University, GUANGZHOU, China; [2]College of Life Sciences, Hunan Normal University, Changsha, China.
Purpose: Oxidative stress (OS)-induced retinal pigment epithelium (RPE) cell apoptosis is critically implicated in the pathogenesis of age-related macular degeneration (AMD), one of the leading causes for blindness in the elderly. The highly condensed, repressive heterochromatin is recently found to paly critical roles in mediating diverse stress response. How RPE heterochromatin is regulated upon OS exposure remains elusive. This study is aimed to investigate heterochromatin structures and functions upon OS exposure.
Methods: We used a mouse model and cultured human RPE cells to investigate heterochromatin upon OS exposure. Oxidative stress was generated by retro-obital injection of sodium iodate (70 mg/kg) in mice or exposure of glucose oxidase (10 U/ml) in RPE cells. The heterochromatin status was assessed by micrococcal nuclease digestion, immunofluorescence microscopy, western blot, quantitative RT-PCR and chromatin immunoprecipitation analysis. To determine the biological functions of heterochromatin in OS, both pharmaceutical and genetic approaches were applied. MTT assay, flow cytometry and permeability measurement were employed to determine the RPE cell viability, apoptosis and barrier functions, respectively.
Results: OS led to increase heterochromatin formation, as indicated by upregulation of trimethylation at histone3 lyscine9 (H3K9me3) and suppression of heterochromatin satellite genes. Disruption of heterochromatin by chaetocin, a selective inhibitor of H3K9 methyltransferases SUV39H1, led to increased cell apoptosis and degeneration of RPE upon OS exposure. Enhance heterochromatin formation by resveratrol treatment or overexpression of SUV39H1 increased cell viability in oxidative injury.

Conclusions: Heterochromatin protects RPE cells from OS-induced cell damage. Targeting heterochromatin may provide a potential strategy for AMD treatment.
Commercial Relationships: Lili Gong, None; **Ruili Qi**, None; **Qian Sun**, None; **Fang-yuan liu**, None; **Zhong-wen Luo**, None; **QIAN Nie**, None; **Xiao-Dong Gong**, None; **Yun-Fei Liu**, None; **Lan Zhang**, None; **Xiangcheng Tang**, None; **Yizhi Liu**, None; **David W. Li**, None
Support: National Natural Science foundation of China 81500738

Program Number: 3562 **Poster Board Number:** B0121
Presentation Time: 3:45 PM–5:30 PM
Exploring the protein-protein interactions of the p53 apoptosis effector protein PERP
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Purpose: PERP protein is a specific p53-regulated plasma membrane apoptosis effector, which is downregulated in aggressive tumours, including monosomy 3 uveal melanoma. This study aims to define the protein-protein interactions of PERP, to give insight into the signalling pathways involved in its trafficking and apoptosis role. The knowledge gained may help identify novel molecular targets for the treatment of aggressive tumours.
Methods: PERP cDNA was cloned into the N-terminal mammalian Halotag vector (Promega) between EcoRI and NotI sites, and the correct, in-frame fusion was confirmed by sequencing. For construct functional validation, Mel202 cells were transfected with Halotag or Halotag-PERP using Turbofect and protein localisation was assessed by live cell imaging using the TMRDirect Ligand. Protein lysates were also collected for Western blot analysis to determine whether previously characterised signalling pathways were induced by Halotag-PERP. Protein pull-downs were performed from lysates of transfected Mel202 cells; protein interacting partners removed from the resin using ProTEV Plus (Promega) were digested within an SDS-PAGE gel using Trypsin Gold, identified by LC-MS/MS, and analysed using Maxquant proteomics software. Mass spectrometry results were validated by Western blotting.
Results: Halotag-PERP localised to the endoplasmic reticulum (ER) and plasma membrane of Mel202 cells, correlating with an increase in protein levels of endogenous PERP and specific phosphorylation of p53, as previously characterised. A total of 21 proteins were identified as having a fold change of 1.5 or higher in at least 2 independent Halotag-PERP pull-down experiments relative to the Halotag only control, and 6 proteins were identified exclusively in Halotag-PERP pull downs in all experiments. The interaction of PERP with 4 proteins was confirmed by Western blot. Interestingly, two proteins with a fundamental role in maintaining endoplasmic reticulum homeostasis, and one protein involved in plasma membrane protein trafficking were identified as potential interacting partners.
Conclusions: The Halotag protein pull-down system was validated as an appropriate system to study the interactions of PERP, with no effect on protein function and localisation by the Halotag. Three novel protein-protein interactions have been identified, indicating a possible role for PERP in ER stress induced apoptosis.
Commercial Relationships: Samantha J. McDonnell; **Ian Prior**, None; **Luminita I. Paraoan**, None
Support: Humane Research Trust, UK

Program Number: 3563 **Poster Board Number:** B0122
Presentation Time: 3:45 PM–5:30 PM
Mining for novel genes related to uveitis in a large network with a shortest path algorithm
Jian Zhang. Shanghai First People's Hospital, Shanghai, China.
Purpose: Uveitis is an intraocular inflammation disease, which can cause blindness for both young and middle-aged individuals. In west, 10-15% of the blindness are caused by uveitis. Up to now, its pathological processes are still uncovered, inducing difficulties for designing effective treatments. Identification of genes related to uveitis as complete as possible is an important way to understand its pathological processes.
Methods: In this study, some possible novel uveitis related genes were discovered using a computational method. To execute the method, we first retrieved known uveitis related genes from the UniprotKB database and references in PubMed, and constructed a large network using protein-protein interaction information reported in STRING. Then, the shortest path algorithm was applied to search possible novel genes based on known genes related to uveitis. To make the obtained genes more reliable, a permutation test was adopted to exclude false positives and the linkages between possible genes and known ones were used to select essential possible genes.
Results: Twenty-one possible genes were identified by the computational method. They are deemed to be related to uveitis.
Conclusions: According to the analyses of obtained genes, eleven genes have evidences to be related to uveitis. The rest obtained genes required further validation.
Commercial Relationships: Jian Zhang, None

Program Number: 3564 **Poster Board Number:** B0123
Presentation Time: 3:45 PM–5:30 PM
Peripherin-2 and Rom-1 Have Opposing Effects on Rod Outer Segment Targeting of Two Misfolded Peripherin-2 Mutants in Rods
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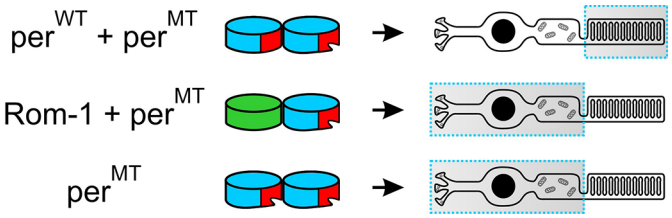
Purpose: The tetraspanins peripherin-2 and Rom-1 can form homo- and heteromeric complexes in photoreceptor outer segments (OS). Mutations in peripherin-2, but not in Rom-1, lead to autosomal dominant retinitis pigmentosa (adRP) and the vast majority of these mutations is located in the D2 loop domain. How different complexes assembled by various combinations of wild type (per^{WT}), mutant peripherin-2 (per^{MT}) or Rom-1, which can be formed in heterozygous patients, contribute to the pathophysiology of adRP remained elusive so far. Here, focusing on the two adRP-linked peripherin-2 D2 loop mutants, per^{P210L} and per^{C214S}, we analyzed the binding characteristics, subunit assembly, subcellular localization, and rod OS targeting of per^{WT}-per^{MT}, and Rom-1-per^{MT} complexes in HEK293 cells and in murine rod photoreceptors.
Methods: Binding characteristics were analyzed by co-immunoprecipitation, protease accessibility assay, peptide competition assay, and quantitative FRET measurements. Subunit assembly of the single complexes was addressed by sucrose density gradient centrifugation. Rod OS targeting and subcellular localization of the single complexes was examined on retinal slices from wild type mice injected with AAV-vectors expressing different combinations of per^{WT}, per^{MT}, and Rom-1.

Results: per^{P210L} and per^{C214S} are misfolded and lead to decreased binding to per^{WT} and Rom-1 due to impaired binding kinetics. The per^{WT}-per^{MT} binding was stronger affected when compared to the Rom-1-per^{MT} combination. In contrast to per^{P210L}, per^{C214S} was mislocalized throughout the rod OS in presence of transgenic per^{WT}. Both mutants preferentially formed non-covalent dimers with per^{WT} and Rom-1. However, only per^{WT}-per^{MT} complexes could be targeted to rod OS.
Conclusions: We show that per^{WT}-per^{MT} dimers can be targeted to rod OS. Moreover, we unravel unexpected opposing roles of per^{WT} and Rom-1 in the pathophysiology of peripherin-2 mutants. We postulate a novel function of Rom-1 in rods with high relevance for the design of future gene-based treatments of peripherin-2-linked adRP.



Rod OS targeting of per^{MT} dimeric complexes. The rod photoreceptor localization of different per^{MT}-containing dimers (per^{WT}-per^{MT}, Rom-1-per^{MT} and per^{MT} only) is symbolized by a dashed rectangle. The position of the D2 loop is highlighted in red and the D2 loop misfolding caused by per^{MT} is symbolized by an incision.
Commercial Relationships: Elvir Becirovic, None; **Sybille Böhm**, None; **Lisa Maria Riedmayr**, None; **Andreas Gießl**, None; **Christian Schön**, None; **Stylianos Michalakis**, None; **Martin Biel**, None
Support: Deutsche Forschungsgemeinschaft, grant number BE 4830/1-1

Program Number: 3565 **Poster Board Number:** B0124
Presentation Time: 3:45 PM–5:30 PM
The β2-subunit of the Na,K-ATPase is lipid modified by palmitoylation in retinal neurons
Emily Sechrest[2], Joseph Murphy[1], David Sokolov[3], Saravanan Kolandaivelu[1]. [1]Department of Ophthalmology, West Virginia University Eye Institute, Morgantown, WV; [2]Department of Pharmaceutical Sciences, West Virginia University, Morgantown, WV; [3]West Virginia University, Morgantown, WV.
Purpose: Maintenance of Na^+ and K^+ gradients by the Na,K-ATPase is crucial for proper cellular function and survival. Na,K-ATPase is a heterodimer, made up of a catalytic a and b subunit, integrated into the plasma membrane. In photoreceptors, the Na,K-ATPase maintains the photocurrent required for transduction of visual signals to downstream neurons. Localized to the inner segment, photoreceptor Na,K-ATPase is comprised predominantly of ATP1β2 and ATP1α3. Genetic ablation of *Atp1b2* results in rapid degeneration of photoreceptors, and loss of retinal ATP1α3 expression. This phenotype is not rescued by substitution of ATP1β2 with ATP1β1, highlighting a unique requirement for ATP1β2 in photoreceptor function and survival. The specific role of ATP1β2 in photoreceptor neurons remains unclear and is the focus of this study. Our lab has identified that ATP1β2 undergoes palmitoylation, a reversible, post-translational lipid modification.
Methods: Palmitoylation of ATP1β2 was assayed by acyl resin-assisted capture (acyl-RAC) in wild-type murine retina. Palmitoylation was further confirmed in mammalian cell culture by metabolic labeling with 17-ODYA, a palmitoyl chemical analog. Palmitoylation prediction software CSS-Palm was used to design the mutant ATP1β2-C10S constructs. HEK293 cells were transiently transfected with wild-type or mutant mouse ATP1β2 constructs. Expression and stability of ATP1β2 and ATP1α3 was monitored using immunoblot. Association between wild-type or mutant ATP1β2 and ATP1α3 was analyzed using co-immunoprecipitation, while trafficking and membrane association was assessed using membrane fractionation and immunocytochemistry.
Results: Using acyl-RAC, we have determined that retinal ATP1β2 is palmitoylated. CSS-Palm predicts the 10th amino acid (Cys-10) in ATP1β2 to be palmitoylated, which is unique to the β2-subunit of the Na,K-ATPase. 17-ODYA labeling in HEK293 cells further confirms this cysteine residue to be palmitoylated, as we observe that a cysteine to serine (C10S) mutation results in loss of palmitoylation of ATP1β2. Additionally, we see that wild-type ATP1β2 and ATP1α3 are enriched at the plasma membrane, while mutant ATP1β2 and ATP1α3 mislocalize to the cytosol in cell culture.
Conclusions: Our findings suggest that palmitoylation of ATP1β2 plays a major role in its association and ATP1α3, as well as proper trafficking of this heterodimeric enzyme to the plasma membrane.
Commercial Relationships: Emily Sechrest; **Joseph Murphy**, None; **David Sokolov**, None; **Saravanan Kolandaivelu**, None

Program Number: 3566 **Poster Board Number:** B0125
Presentation Time: 3:45 PM–5:30 PM
Epigenetic modifications associated with Hyperhomocysteinemia; potential role in Blood Retinal Barrier dysfunction
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Purpose: To study Homocysteine (Hcy) induced epigenetic modifications as potential mechanisms of blood retinal barrier (BRB) dysfunction.
Methods: Retinal tissues isolated from 3 weeks old mice with elevated levels of Hcy due to lack of the enzyme cystathionine-β-synthase (*$cbs^{+/-}$ and $cbs^{-/-}$*) and wild type (*$cbs^{+/+}$*) mice as well as Human Retinal Endothelial cells (HRECS) and Human retinal pigmented epithelial cell line (ARPE-19) treated with or without Hcy (20,50 and 100mM) for 24 hours were subjected to evaluation of 1)- Histone deacetylases (HDAC) using HDAC Activity Colorimetric Assay Kit 2)- DNA methylation using EpiQuik DNA Methyltransferase (DNMT) Activity/Inhibition Assay Kit. 3) - miRNA analysis from retinas of mice with elevated Hcy level (cbs mice) was done, briefly, 12 mouse retinal RNA samples (4 *$cbs^{+/+}$*, 4 *$cbs^{+/-}$* and 4 *$cbs^{-/-}$*) were assayed using Affymetrix GeneChip miRNA 3.0 array. Expression data was analyzed using Partek Genomics Suite. List of differentially expressed miRNAs were generated and imported into Ingenuity Pathway Analysis (Qiagen) for detection of predicted target genes and associated pathways.
Results: Hcy induced significant increase in HDAC and DNMT activity in HRECs, ARPE-19, *$cbs^{+/-}$ and $cbs^{-/-}$* mice retina. In addition miRNA profiling of *$cbs^{+/-}$ and $cbs^{-/-}$* detected 127 miRNAs in *$cbs^{+/-}$* (49 downregulated and 78 upregulated) and 39 miRNAs in *$cbs^{-/-}$* (20 miRNAs downregulated and 19 upregulated) were statistically significant and differentially expressed in comparison to *$cbs^{+/+}$*. miRNAs pathway analysis showed their involvement in ER stress, oxidative stress, inflammation, Hypoxia and angiogenesis pathways.
Conclusions: Hcy induced Epigenetic modifications could represent novel biomarkers or therapeutic targets for retinal diseases associated with elevated Hcy such as age related macular degeneration and diabetic retinopathy.
Commercial Relationships: Amany M. Tawfik, None; **Khaled Elmasry**, None; **Yutao Liu**, None; **Mohamed A. Al-Shabrawey**, None

Support: American Hear SDG grant AHASE00116CS

Program Number: 3567 **Poster Board Number:** B0126
Presentation Time: 3:45 PM–5:30 PM
miRNA profile of human retinal endothelial cells in starvation or angiogenic stimulation with and without VEGF inhibitors
Johanna Madeleine Walz[1, 2], Thomas Wecker[1], Pei pei Zhang[1], Bertan Cakir[1], Björn Grüning[4, 5], Hansjuergen Agostini[1], Lothar Faerber[2, 3], Clemens Lange[1], Gunther R. Schlunck[1], Andreas Stahl[1]. [1]Eye Center, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [2]Department of Pharmacology and Toxicology, University of Regensburg, Regensburg, Germany; [3]Novartis Pharma GmbH, Nuremberg, Germany; [4]Department of Computer Science, University of Freiburg, Freiburg, Germany; [5]Center for Biological Systems Analysis (ZBSA), University of Freiburg, Freiburg, Germany.
Purpose: MicroRNAs (miRNAs) are short, single-stranded ribonucleic acids (RNA). Mature miRNAs are about 20-22 nucleotides long, form a subset of non-coding RNAs and attenuate mRNA translation. They are involved in fine-tuning of diverse biological processes including angiogenesis. Retinal microvascular endothelial cells (RMVECs) are instrumental in retinal angiogenesis. The aim of this study was to decipher the miRNA profile of RMVECs with and without angiogenic stimulation and to determine changes upon VEGF inhibition.
Methods: We analyzed miRNA expression profiles of human RMVECs under 5 different conditions: (i) 24h of starvation, (ii) 24h of angiogenic stimulation (AS), (iii - v) AS for 12h followed by 12h of incubation with one of the VEGF inhibitors aflibercept, bevacizumab or ranibizumab. Cells were harvested and miRNA expression was determined using next-generation sequencing (Illumina). Following data analysis, the significantly ($p \leq 0.05$) and relevantly (>2fold) changed miRNAs were validated by qPCR. The confirmed miRNA modulations, as well as unregulated miRNAs that were very highly expressed were further studied by gene ontology-based enrichment analysis (GO) to determine their potential effect on intracellular signaling pathways.
Results: In all groups, miR-21-5p made up 40% of the RMVEC miRNome. Other abundantly expressed miRNAs were miR-29a-3p, miR-100-5p and miR-126-5p/3p. These were equally expressed (<2fold change) in all groups and might represent a RMVEC specific miRNA signature. Ten miRNAs with lower expression rates were significantly different in starvation and AS. Four of them (miR-335-5p/3p and miR-139-5p/3p) were validated by qPCR. GO analysis linked these miRNAs to regulation of angiogenesis, cell migration, apoptotic signaling and hypoxia response. Interestingly, supplementation of VEGF inhibitors did not alter the previously induced angiogenic miRNA expression profile within the short time frame of our experiments.
Conclusions: While the majority of miRNAs remained stable, we identified a regulated subset of miRNAs in RMVECs exposed to angiogenic stimulation. Addition of VEGF inhibitors to these angiogenically activated RMVECs for 12h did not alter their miRNA expression profile. These findings suggest that VEGF inhibition alone does not have acute effects on miRNA expression profiles of activated RMVECs.
Commercial Relationships: Johanna Madeleine Walz, Novartis Pharma Germany (E), Novartis Pharma Germany (R); **Thomas Wecker**, None; **Pei pei Zhang**, None; **Bertan Cakir**, None; **Björn Grüning**, None; **Hansjuergen Agostini**, None; **Lothar Faerber**, Novartis Pharma Germany (E); **Clemens Lange**, None; **Gunther R. Schlunck**, None; **Andreas Stahl**, Boehringer Ingelheim (C), Novartis Pharma Germany (C), Novartis Pharma Germany (F), Bausch & Lomb (C), Zeiss (C), Novartis Pharma Germany (R)
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Program Number: 3568 **Poster Board Number:** B0127
Presentation Time: 3:45 PM–5:30 PM
RPE miR-204 or -211 KNOCK OUT ALTERS CELL MORPHOLOGY AND FUNCTION
Congxiao Zhang[1], Helen Zhao[1], Nathan Hotaling[2], Kiyoharu Miyagishima[1], Raymond Zhou[1], Lijing Dong[3], Arvydas Maminishkis[1], Kapil Bharti[2], Sheldon S. Miller[1]. [1]SERPD/OGVFB, NEI/NIH, Bethesda, MD; [2]OSCTRU/OGVFB/NEI, Bethesda, MD; [3]Genetic Engineering Facility/NEI, Bethesda, MD.
Purpose: miRNA-204 and -211 are highly enriched in retinal pigment epithelium (RPE) relative to the adjacent retina and choroid. These two miRNAs play a fundamentally important role in maintaining epithelial phenotype and RPE physiology (Wang, F., Zhang, C., et al., 2010). In order to better understand the role of these miRNAs, we specifically deleted miR-204 or 211 in postnatal mouse RPE. In these conditional KO models, we are analyzing the underlying cell signaling networks that mediate changes in RPE structure and function.
Methods: Floxed miR-204 (& 211) KO mice were generated by homologous recombination in germline mice and verified by PCR and Southern blotting. Knockout of target microRNAs in RPE was achieved by crossing the floxed alleles into the VMD2-CRE line. Loss of miR-204 or -211 expression in RPE was verified by TaqMan® microRNA assay. Ocular structure was evaluated by fundus examination using OCT (Heidelberg Spectralis HRA+OCT) and histology using both light and electron microscopy. RPE morphology was also studied by employing in - house developed software (REShAPE) for quantitative morphological analysis of ZO-1 distribution in RPE flat mounts. Retinal or RPE physiological changes were assessed by retinal regular ERG or direct coupled (DC)-ERG recordings, respectively.
Results: In the eye of RPE specific miR-204 or -211 KO mice, gross anatomical structures appeared normal (6 weeks to 1 year). However, RPE apical processes were significantly altered. During the first 12 months, fundus autofluorescence images showed increasing hyper-auto fluorescent spots near the RPE/choroid. These structural changes were associated with an increased variation in RPE cell size and morphology. The retinal ERG responses (a- and b- waves) were unchanged, but the c - wave and Fast Oscillation (FO) responses of the DC - ERG were significantly reduced.
Conclusions: The present experiments indicate that miR-204 and -211 in RPE are crucial for maintaining the integrity of apical membrane structure and RPE cell morphology and physiology. These KO mice thus provide a model for the analysis of barrier function and the miRNA - dependent cell signaling pathways that mediate RPE function.
Commercial Relationships: Congxiao Zhang, None; **Helen Zhao**, None; **Nathan Hotaling**, None; **Kiyoharu Miyagishima**, None; **Raymond Zhou**, None; **Lijing Dong**, None; **Arvydas Maminishkis**; **Kapil Bharti**, None; **Sheldon S. Miller**, None
Support: NEI intramural research fund

Program Number: 3569 **Poster Board Number:** B0128
Presentation Time: 3:45 PM–5:30 PM
Optimization of mouse photoreceptor isolation and micro-fluidic single cell capture for downstream molecular analysis
Jonathan Fuerst, Marie-Audrey I. Kautzmann, William C. Gordon, Nicolas G. Bazan. Neuroscience Center of Excellence, Louisiana State University Health New Orleans, New Orleans, LA.
Purpose: Progress in genomics allows us to investigate molecular activity at the single cell level. Development of mouse models for retinal degeneration offers a valuable tool to investigate molecular mechanisms underlying specific gene disruptions causing photoreceptor death. As a whole transcriptome or candidate gene approach, automation of cell sorting for downstream application is key in the fast and systematic processing of individual cells. The purpose of this study is to optimize methods for micro-fluidic single photoreceptor capture for gene analysis.
Methods: Photoreceptor cells were dissociated following previous protocols with minor changes (Wahlin et al, Mol Vis, 2004). 5 U/ml papain containing L-cysteine (2.7 mM) and EDTA (2 mM) was pre-activated for 5 min at 37°C. Retinas from wild-type mouse were collected, cut into 8-10 pieces and transferred in pre-activated papain for 5 min at room temperature. Retina pieces were rinsed without disturbing the pellet then resuspended in 700 µL DMEM containing 10% fetal bovine serum. Tissue was dissociated by trituration and cell suspension allowed to settle for 5 min. Presence of photoreceptor cells from an aliquot of the supernatant was evaluated with bright field microscopy. Once PRC was established, supernatant was transferred to a fresh tube and allowed to settle for 5 min. The cell suspension was loaded into a micro-fluidic chamber and Hoechst, Calcein and Ethidium Homodimer were used to evaluate live/dead status of captured material.
Results: Cell load on the micro-fluidic chamber showed capture of biological material ranging from single to multiple (corresponding to whole cell) segment composed of 1) outer and inner segment, 2) outer segment only, or 3) cell body with inner segment without outer segment. In some cases, multiple cells corresponding to cell aggregates were also captured in individual capture sites. Chemistry for transcript analysis was run and cDNA concentration for each capture site evaluated. We determined that cDNA collected from each individual capture site is in low abundance due to material size, thus requiring low dilution when harvesting cDNA.
Conclusions: This study establishes a method to isolate and capture individual photoreceptor cells in an automated manner that will allow investigation and analysis of gene expression with downstream applications such as RNA sequencing or targeted gene expression.
Commercial Relationships: Jonathan Fuerst, None; **Marie-Audrey I. Kautzmann**, None; **William C. Gordon**, None; **Nicolas G. Bazan**, None
Support: Supported by NEI grant EY005121, NIGMS grant GM103340 the Eye, Ear, Nose and Throat Foundation (NGB) and Research to Prevent Blindness.

Program Number: 3570 **Poster Board Number:** B0129
Presentation Time: 3:45 PM–5:30 PM
Differential effects of mutations in the *miR183/96/182* cluster on zebrafish sensory tissues
Joseph Fogerty[1], Kyle M. Patterson[1, 2], Brian D. Perkins[1]. [1]Ophthalmic Research, Cleveland Clinic, Cleveland, OH; [2]John Carroll University, Cleveland, OH.
Purpose: The *miR183/96/182* cluster consists of three paralogous miRNAs located in a short intergenic region that is highly conserved across metazoans. They account for nearly 70 percent of all expressed miRNAs in the retina, and are also expressed in inner ear hair cells. Several animal models of retinitis pigmentosa show reduced expression of this cluster, suggesting that its dysregulation contributes to retinal disease. The seed sequences of all three miRNAs are highly conserved, with those of *miR96* and *miR182* being identical, suggesting functional redundancy. The purpose of this study is to determine the scope of functional redundancy among these miRNAs within the zebrafish retina.
Methods: The CRISPR/Cas9 system was used to generate mutations in *miR183*, *miR96*, and *miR182* in zebrafish. In vitro luciferase assays were used to test function and target specificity of wild-type and mutant miRNA alleles. Mutants were evaluated by immunostaining for photoreceptor-specific markers and by plastic histology at 5 dpf and 4 months.
Results: We generated mutations at each miRNA locus individually and confirmed that these mutations inhibited miRNA targeting. Wild-type miRNAs targeted only their cognate sequences, indicating a capacity for functional autonomy. Homozygous mutants at any single miRNA locus were viable, fertile, and lacked retinal phenotypes. A *miR183*$^{-/-}$; *miR96*$^{-/-}$ double mutant was similarly unaffected. High-dosage CRISPRs targeting *miR182* in the *miR183*$^{-/-}$; *miR96*$^{-/-}$ double mutant yielded a cohort of fish that developed abnormal swimming patterns, indicating a malfunctioning vestibular system, but the retinas of these animals remained normal at 4 months.
Conclusions: Prior studies in a mouse *miR96* mutant suggest that the abnormal swimming patterns are likely a result of hair cell degeneration. While miRNA target specificity was very high when tested in vitro, the observation that only mutations at multiple loci were sufficient to generate an abnormal phenotype suggests that they may function cooperatively in vivo. The lack of a retinal phenotype despite the clear vestibular defect indicates that the targets of these miRNAs are likely highly tissue specific. Further development and analysis of compound mutations within this cluster as well as identification of target mRNAs will help us to understand its neuroprotective properties.
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Program Number: 3571 **Poster Board Number:** B0130
Presentation Time: 3:45 PM–5:30 PM
Ex vivo expansion and characterization of rat Meibomian gland progenitor cells
chengyou zuo[1, 2], Juan Li[1, 2], Yangluowa Qu[1, 2], Changkai Jia[1, 2], Shangkun Ou[1, 2], Xin He[1, 2], JingHua Bu[1, 2], ZUGUO LIU[1, 2], Wei Li[1, 2]. [1]Eye Institute of Xiamen University, Xiamen, China; [2]Fujian Provincial Key Laboratory of Ophthalmology and Visual Science, Xiamen, China.
Purpose: To investigate the molecular signatures of duct and acinus of Meibomian gland (MG) in vivo and Meibomian gland cells (MGCs) in vitro clonal culture.
Methods: Fresh MG tissues were isolated from eyelids of 8 weeks old SD rats and enzymatically digested into single cells, then clonal cultured with supplemental hormonal epithelial medium (SHEM) with mitomycin C-treated 3T3 cells as feeder layers. MG tissues and MGCs were harvested to examine the specific markers by quantitative real-time PCR (qRT-PCR) and immunofluorescence (IF) staining. Oil Red O staining was performed to detect the lipid deposition of MGCs.
Results: MGCs clones emerged after 3 to 4 days culture and reached confluent around day 7. qRT-PCR results showed that K5, K14 and p63, hair follicle stem cell markers k15, Integrin alpha-6 (Itga6), Lrig1, sox9 and follistatin (FST) were detected in MG tissues and

MGCs after 7 days culture. The lipid metabolism related gene expression of Lipid1, SCD1 and PPAR-γ decreased evidently during ex vivo culture. The IF result shows that K14 was expressed in MG tissue and all MGCs after cultivation. p63 positive cells were located in the basal of acinus and duct in vivo while were all positive after in vitro culture, Oil Red O staining shows there is nearly no lipid droplets can be observed in cultured cells.
Conclusions: MGCs have some characteristic of hair follicle stem cells. The Meibomian gland acini cells cultured in SHEM can maintain the phenotype as the basal cell of acinus. With this culture method we can expand MG progenitor cells which be used to investigate the biology of Meibomian gland.
Commercial Relationships: chengyou zuo; **Juan Li**, None; **Yangluowa Qu**, None; **Changkai Jia**, None; **Shangkun Ou**, None; **Xin He**, None; **JingHua Bu**, None; **ZUGUO LIU**, None; **Wei Li**, None

Program Number: 3572 **Poster Board Number:** B0131
Presentation Time: 3:45 PM–5:30 PM
Tear Cytokine Profile in a Scandinavian Cohort of Congenital Aniridia
Erlend C. Landsend[4], Hilde Rogeberg Pedersen[3], Ø Utheim[4], Hans Christian Aass[1], Behzod Tashbayev[2], Neil S. Lagali[5], Darlene A. Dartt[6], Rigmor C. Baraas[3], Tor Utheim[3, 1]. [1]Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway; [2]The Norwegian Dry Eye Clinic, Oslo, Norway; [3]Faculty of Health Sciences, National Centre for Optics, Vision and Eye Care, University College of Southeast Norway, Kongsberg, Norway; [4]Department of Ophthalmology, Oslo University Hospital, Oslo, Norway; [5]Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden; [6]Department of Ophthalmology, Harvard Medical School, Boston, MA.
Purpose: To investigate the tear cytokine profile in a Scandinavian cohort of congenital aniridia in a case-control study, and to examine the correlation between cytokine levels and ophthalmological findings.
Methods: Thirty-five patients with congenital aniridia and 21 healthy controls were examined. Tear fluid was collected using Schirmer I test and capillary tubes, and the concentration of 27 human cytokines was determined in the fluid from both eyes separately employing a multiplex bead assay. All participants also underwent an extensive ophthalmological examination including systematic slit-lamp biomicroscopy, measurement of tear production and ocular surface staining, and evaluation of meibomian glands (meibography). Differences in cytokine levels between the two groups were analysed, and correlations between levels in the aniridia group and ophthalmological findings were calculated.
Results: Significantly elevated concentrations were shown for 16 cytokines in tear fluid from both right and left eyes of aniridia patients compared to the controls. These 16 were: interleukin 1ß (IL-1ß), IL-4, IL-6, IL-9, IL-12p70, IL-13, IL-15, IL-17A, eotaxin, basic fibroblast growth factor (basic FGF), granulocyte colony-stimulating factor (G-CSF), interferon gamma (IFN-γ), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), and MIP-1ß. Interestingly, IL-1ß and IL-6, which are suggested to play a role in dry eye disease (DED), demonstrated a substantial increase in concentration in the anridia group compared to the control group (IL-1ß: 20.3 pg/ml vs 0.7, $p<0.001$; IL-6: 6.5 pg/ml vs 1.7, $p=0.001$). The level of interferon gamma-induced protein 10 (IP-10) and regulated-on-activation, normal T cell expressed and secreted (RANTES) correlated significantly with stage of aniridic keratopathy ($R=-0.451$, $p=0.018$ and $R=0.435$, $p=0.023$, respectively).
Conclusions: Tear fluid of aniridia patients demonstrated significantly elevated levels of several cytokines related to inflammation compared to control individuals, and a moderate correlation between aniridic keratopathy and concentrations of IP-10 and RANTES was found. Increased inflammation of the ocular surface may be a contributory factor explaining previous reports of high prevalence of dry eye disease in aniridia patients.
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Program Number: 3573 **Poster Board Number:** B0132
Presentation Time: 3:45 PM–5:30 PM
Quantitative proteome study of *Chlamydia trachomatis* ocular serovar B proteins associated with trachomatous trichiasis
Elisabeth Stein[1], Jelena Mihailovic[2], Aleksandra Inic-Kanada[1], Katarina Smiljanic[2], Marija Perusko[3], Sara Trifunovic[3], Nadine Schuerer[1], Dragana Stanic-Vucinic[2], Ehsan Ghasemian[1], Talin Barisani-Asenbauer[1], Tanja Cirkovic -Velickovic[2]. [1]Laura Bassi Centre of Expertise, Medical University of Vienna, Vienna, Austria; [2]Center of Excellence for Molecular Food Sciences, University of Belgrade - Faculty of Chemistry, Belgrade, Serbia; [3]Faculty of Chemistry, Innovation Center, Belgrade, Serbia.
Purpose: The intracellular bacterium *Chlamydia trachomatis* (Ct) is the worldwide leading cause of preventable infectious blindness. The aim of the present study was to provide a proteome analysis of Ct ocular serovar B (CtB) elementary bodies (EB) by shotgun proteomics.
Methods: Relative quantification of trachoma-associated antigens was achieved by label-free-quantification. Four biological replicates were used for the qualitative and quantitative study of the CtB strain HAR-36 proteome. All were resolved by SDS PAGE and subjected to in-gel digestion by trypsin. For quantification purposes two biological replicates (out of the four used for shotgun experiments) were used. Both preparations (lyophilized and in solution) were electrophoretically resolved, gels were cut into 13 pieces each and in-gel trypsin digested. Both sets of peptides were analyzed by nano-LC-MS/MS (143 runs in total) in duplicates.
Results: Extensive profiling of CtB EBs was achieved, resulting in coverage of approximately 60% of the Ct genome. The most abundant proteins found include major outer membrane protein (MOMP), Elongation factor (EF) Tu, 60 kDa chaperonin Hp60 (GroEL_1), a hypothetical protein CT875 and ATP synthase subunit beta. Our findings also indicate that eight Pmps abundantly expressed in CtB EBs undergo extensive proteolytic processing, consistent with their function as autotransporters.
Conclusions: The biggest differences in Pmps relative content between ocular and genital serovars were found in PmpD and PmpI. The antigens most relevant for trachomatous trichiasis discovered by immunoproteomics are also the most abundant proteins of CtB (GroEL_1, MOMP, EF-Tu, Type III secretion system ATPase). Our data add to the understanding of structure and function of proteins of the infectious form of Ct ocular serovar B thereby extending the current knowledge on immunogenic antigens for vaccine development.
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Program Number: 3574 **Poster Board Number:** B0133
Presentation Time: 3:45 PM–5:30 PM
Histochemical and immunohistochemical characterization of lipid synthesis in meibomian glands
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Purpose: Meibum is composed of a complex mixture of lipid species with expression analysis suggesting in situ synthesis in meibomian glands (MG). This study seeks to identify the sites of lipid accumulation and cellular and subcellular location of key lipid synthesis enzymes identified by expression analysis.
Methods: Eyelid tissue, freshly harvested from mice, or human autopsy, were fixed in 4% paraformaldehyde and embedded in OCT or in 10% buffered formalin and embedded in paraffin blocks. Lipids in OCT embedded tissues were stained with either Nile red or HCS LipidTOX stains. Primary antibodies specific to mouse and/or human proteins, including markers for subcellular compartments, were incubated with tissue sections and binding detected using Alexa Fluor® conjugated secondary antibodies. Imaging used both a Zeiss fluorescent and a Leica SP2 confocal microscope.
Results: Lipid staining revealed initial accumulation within meibocytes in ascini in MG with subsequent release into the ascinar ducts upon holocrine rupture. Limited numbers of lipid drops (LD) were seen in basal meibocytes with their numbers increasing in density and size as differentiation progressed. Both PLIN2 and 5 were observed in association with LD. Defining characteristics of meibum lipids are: 1) high abundance of fatty acyl components ≥ C18 requiring endoplasmic reticulum (ER) fatty acid elongation; Both ELOVL3 and ELOVL4 were highly expressed in meibocytes; ELOVL3 (substrates C16-C22) staining was strong in both basal and differentiating meibocytes. ELOVL4 (substrates ≥ C22) in contrast was most strongly expressed in differentiating meibocytes: 2) lipids containing cholesterol; expression of both SQLE and FDFT1 was observed in meibocytes suggesting endogenous synthesis: 3) lipids containing odd-numbered fatty acyl chain residues. Punctate staining throughout ascinar meibocytes for both BCKDHA and DBT, mitochondrial components of the BCKDH complex suggests a role for branched chain amino acids in their synthesis.
Conclusions: The observed cellular and subcellular expression patterns of proteins, whoses mRNAs are highly expressed in the tarsus, and whose functions/enzymatic activities strongly suggest a role in lipid synthesis, start to define the cellular means by which the MG elaborates the meibum lipidome. This provides a base on which to understand the qualitative/quantitative changes in meibum lipids with MG pathologies.
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