378 Cellular mechanisms of retinal diseases and ocular therapeutics
Tuesday, May 09, 2017 3:45 PM–5:30 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 3587–3601/B0146–B0160
Organizing Section: Biochemistry/Molecular Biology

Program Number: 3587 **Poster Board Number:** B0146
Presentation Time: 3:45 PM–5:30 PM
Upregulation of pro-inflammatory genes accompanies photoreceptors demise in canine models of retinal degeneration
Tatyana Appelbaum, Evelyn Santana, Gustavo D. Aguirre. Clinical Studies, U of Penn School Vet Med, Philadelphia, PA.
Purpose: To examine regulation of the immune response in three early-onset canine models of human retinitis pigmentosa (RP): rod cone dysplasia 1 (rcd1), X-linked progressive retinal atrophy 2 (xlpra2), and early retinal degeneration (erd) caused, respectively, by mutations in *PDE6B*, *RPGR-ORF15* (del1084–1085), and *STK38L*, and the late-onset model, xlpra1, caused by a 5-bp microdeletion (del1028-1032) in *RPGR-ORF15*.
Methods: Retinal samples from rcd1, xlpra2, erd and xlpra1 affected and control dogs were used for immunohistochemistry (IHC), western blot and gene expression (GE) analysis. qRT-PCR was used to examine the expression of 39 genes. Expression profiles were analyzed at different time points relevant to the onset and progression of the disease.
Results: Analysis of GE data sets identified early activation of immune response genes, including genes encoding constituents of NLRP3 inflammasome, its substrates (pro-IL1B, pro-IL18), and common components of IL1B, IL18 and TLR4 pathways in rcd1, xlpra2 and erd. In early-onset models, gradual increase in the number of upregulated pro-inflammatory genes and extent of GE upregulation, was most prominent in rcd1 and consistent with disease progression and severity. In late-onset xlpra1 retinas, a subset of the pro-inflammatory genes was highly upregulated long before any disease-related morphological changes. Notably, strong upregulation of the immune response genes was not always followed by overexpression of their proteins. Double labeling with PYCARD and CD18 identified microglia and infiltrating macrophages as a source of the inflammasome in the mutant retinas. Of the two activated caspase 1 cleavage products, IL1B and IL18, only IL1B was detected in rcd1 and xlpra2 thus highlighting the role of this pathway in the disease progression, while precursor IL18 remained unprocessed in the same protein extract. Of *IL4*, *MANF*, *CX3CL1* and *TGFB,* known for moderating immune response, *IL4* was highly upregulated in late-onset xlpra1; *IL4* and *CX3CL1* were slightly upregulated in rcd1, and none were elevated in xlpra1 and erd models.
Conclusions: Expression data indicate an upregulated immune response accompanying the progression of retinal degeneration in animal models of human RP thus pointing out to potential benefits of anti-inflammatory therapy.
Commercial Relationships: Tatyana Appelbaum, None; **Evelyn Santana**, None; **Gustavo D. Aguirre**, None
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Program Number: 3588 **Poster Board Number:** B0147
Presentation Time: 3:45 PM–5:30 PM
***Crx-L253X* mutation produces dominant photoreceptor defects in *TVRM65* mice**
Courtney D. Linne, Philip A. Ruzycki, Anne Hennig, Shiming Chen. Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO.
Purpose: The Cone-Rod Homeobox (CRX) transcription factor is essential for photoreceptor gene expression, differentiation and survival. Human *CRX* mutations cause dominant retinopathies of varying onset and phenotype severity. Frameshift *CRX* mutations that introduce a premature termination codon (PTC) result in inactive truncated proteins that interfere with normal CRX function. Two animal models of early PTC showed mutant mRNA/protein overproduction in heterozygotes, resulting in severe photoreceptor defects. We hypothesize that the pathogenicity of frameshift mutations depends on the PTC position and the overabundance of mutant mRNA/protein. To test this hypothesis, we characterized the *TVRM65* mutant mouse, from The Jackson Laboratory, which carries a late PTC mutation (*Crx-L253X*), initially reported to model recessive blindness. We predict that, like other *CRX* PTC mutants, *L253X* has a dominant phenotype.
Methods: Retinas of homozygous (*L253X/X*), heterozygous (*L253X/+*), and wild-type control *C57BL/6J* (*WT*) mice collected at various ages were assessed for: 1) retinal cell morphology (histology of H&E sections), 2) the relative abundance of mutant CRX protein/mRNA to *WT* (quantitative Western Blot and digital droplet PCR), 3) gene expression (q-RTPCR and IHC) and 4) retinal function differences (ERG).
Results: *L253X/X* showed a lack of photoreceptor function at 1 month and greater reductions in retinal thickness, but similar changes in gene expression to *Crx* null (on the same *C57BL/6J* background), suggesting that the *L253X/X* phenotype is at least as severe as that of null animals. *L253X/+* mutants showed normal retinal morphology at all ages tested. However, both rod and cone retinal responses to visual stimuli were reduced, beginning at 1 month. q-RTPCR analysis described a complex phenotype of developmental delay and an inability to maintain proper gene expression in mature photoreceptors. *L253X* mRNA/protein were overexpressed relative to normal *Crx*, suggesting a pathogenic mechanism similar to other PTC mutants. However, the overexpression is less pronounced, correlating with a relatively mild dominant phenotype.
Conclusions: *Crx L253X* causes a dominant phenotype of altered gene expression and photoreceptor malfunction. The pathogenicity of *CRX* frameshift mutations depends on the positon of PTC (early is more toxic than late), which determines the degree of mutant mRNA/protein overproduction and phenotype severity.
Commercial Relationships: Courtney D. Linne, None; **Philip A. Ruzycki**, None; **Anne Hennig**, None; **Shiming Chen**, None
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Program Number: 3589 **Poster Board Number:** B0148
Presentation Time: 3:45 PM–5:30 PM
TFIIH-ERCC2 Is Essential For Photoreceptor Gene Expression and Development
Xiaodong Zhang, Philip A. Ruzycki, Anne Hennig, Mingyan Yang, Shiming Chen. Ophthalmology and Visual Sciences, Washington University School of Medicine, Saint Louis, MO.
Purpose: ERCC2, an essential component of the TFIIH general transcription factor complex, was identified as a co-factor of photoreceptor transcription factors (PhTFs) including CRX and NR2E3. TFIIH is known to play a role in DNA repair and transcription. Mutations in TFIIH-ERCC2 cause syndromic diseases with ocular defects and photosensitivity. The goal of this research was to use mouse models to determine the role of TFIIH-ERCC2 in photoreceptor gene transcription, development and disease.

Methods: ERCC2 interactions with PhTFs were investigated by co-immunoprecipitation and chromatin immunoprecipitation (ChIP). ERCC2's function in the retina was determined using Cre/LoxP-mediated conditional knockout and phenotype assessment for changes in morphology (histology and immunohistochemistry), function (ERG) and gene expression (qRT-PCR).
Results: ERCC2 co-localizes with PhTFs in photoreceptor nuclei, and physically interacts with them. ERCC2 co-immunoprecipitated with CRX and/or NR2E3 in retinal extracts of wild-type (WT) mice, but not control extracts from mice lacking CRX or NR2E3. ChIP assays on P14 WT mouse retinas showed that ERCC2 binds to the promoter and coding regions of each *opsin* gene along with CRX/NR2E3. ERCC2 target binding was reduced in mutant retinas lacking either CRX or NR2E3, suggesting that ERCC2 is a co-regulator of PhTFs. Conditional deletion of *TFIIH-Ercc2* in retinal progenitor cells using *Chx10-Cre* resulted in irregular morphology of the outer nuclear layer with whorls and rosettes at P14 and 4 weeks of age, while the inner retinal layers appeared less affected. The *Ercc2* null retinas also underwent progressive photoreceptor degeneration. ERG "a" and "b" wave amplitudes were drastically reduced in dark and light adapted 1 month old *Ercc2*-knockout mice, suggesting these mice are blind. qRT-PCR detected altered expression of many photoreceptor genes in mutant retinas.
Conclusions: The TFIIH-ERCC2 co-activator complex is essential for photoreceptor gene expression, structural integrity and function. ERCC2 adopts this cell type specific function by interacting with key PhTFs on target genes.
Commercial Relationships: Xiaodong Zhang, None; **Philip A. Ruzycki**, None; **Anne Hennig**, None; **Mingyan Yang**, None; **Shiming Chen**, None
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Program Number: 3590 **Poster Board Number:** B0149
Presentation Time: 3:45 PM–5:30 PM
Hyper-stability of mutant mRNA causes pathogenic overexpression of mutant CRX in autosomal dominant retinopathies
Inez Oh[1], Xiaodong Zhang[1], Nicholas Tran[2], Shiming Chen[1]. [1]Ophthalmology and Visual Sciences, Washington University School of Medicine, Saint Louis, MO; [2]Harvard University, Cambridge, MA.
Purpose: Mutations in the cone-rod homeobox gene *CRX* have been associated with autosomal dominant (ad) retinopathies, but the underlying disease mechanisms are poorly understood. Class III frameshift human *CRX* mutations create premature termination codons (PTCs), producing truncated proteins that interfere with wild-type (WT) CRX function in heterozygotes. Animal models of Class III *CRX* mutations (*E168d2*, *E168d2neo* and *Tvrm65* mice, and *Rdy* cats) develop retinopathies, and heterozygotes display elevated ratios of mutant to *WT* mRNA and protein, correlating with phenotype severity. We hypothesize that PTCs expose a stability code within the *Crx* mRNA 3' untranslated region (*3'UTR*), producing hyper-stable mutant RNA, which amplifies mutant protein toxicity, leading to global mis-regulation of gene expression and photoreceptor degeneration. To test this hypothesis, the half-life of WT and mutant *Crx* mRNA was measured and *3'UTR* regions that regulate *Crx* mRNA stability identified.
Methods: In retinal explants from P14 *WT* (*C57BL/6J*) or homozygous *Crx-E168d2* mice, the half-life of *Crx* mRNA was assessed by measuring *Crx* mRNA levels (qRT-PCR) at various times after 1) inhibiting RNA synthesis with Actinomycin D, or 2) metabolic pulse-chase [5'-bromouridine-immunoprecipitation-chase (BRIC)]. Critical RNA regions that confer hyper-stability were identified by *in vitro* reporter gene assays in HEK293 cells transfected with serial *Crx-3'UTR* deletions.
Results: Both RNA decay assays revealed at least 2-fold increases in the half-life of mutant mRNA relative to WT, confirming the hyper-stability of mutant mRNA. This was attributed to the coding region-derived *UTR* sequence (*cUTR*) resulting from the PTC. Increased length of the *cUTR* (due to early PTC) correlates with enhanced mRNA stability, recapitulating *in vivo* observations from the *E168d2* (early PTC) and *Tvrm65* (late PTC) mouse models.
Conclusions: The pathogenic overexpression of mutant CRX stems from a novel mRNA stabilization mechanism intrinsic to specific RNA regulatory sequences. Our findings establish a new paradigm for investigating mis-regulation of gene expression in neurological diseases.
Commercial Relationships: Inez Oh, None; **Xiaodong Zhang**, None; **Nicholas Tran**, None; **Shiming Chen**, None
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Presentation Time: 3:45 PM–5:30 PM
Disease-causing mutations of CLDN19 alter gene expression in retinal pigment epithelia (RPE) and diminish visual function
Tao Xu[1, 2], Shao-Bin Wang[1], Ron A. Adelman[3], Shaomin Peng[2], Lawrence J. Rizzolo[1]. [1]Surgery/Ophthalmology, Yale university, New Haven, CT; [2]Aier School of Ophthalmology, Central South University, Chang Sha, China; [3]Ophthalmology, Yale University, New Haven, CT.
Purpose: Mutations of CLDN16 or CLDN19 cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), but only CLDN19 mutations cause visual impairment. To explore mechanisms whereby claudin-19 mutations might affect on ocular function, naturally occurring mutations were expressed in mice and in human RPE cultures.
Methods: Claudin-19 mutations, G20D and R81W, were constructed by site directed mutagenesis. Wild type and mutant CLDN19 plasmids were transiently expressed human fetal RPE (hfRPE) and ARPE-19. AAV viral vectors encoding wide type and mutant CLDN19 were injected in to the subretinal space of 1-month old C57/BL6 mice. The localization of claudins was visualized with immunofluorescent staining. Protein degradation pathways were inhibited and the effects on steady-state levels of claudin-19 were measured with immunoblots. The expression of RPE signature genes was evaluated with Quantitative-PCR. For functional studies, multifocal ERG (mfERG) recordings were obtained, 2-weeks post-transfection.
Results: Two-weeks after transfection, immunostaining of mouse RPE cells revealed wild-type Claudin-19 mainly localized to the cell membrane, but G20D and R81W mutations were distributed in distinct cytoplasmic locations. The mislocalized protein aggregates were ubiquitin positive and their degradation was retarded by inhibitors of proteasomes. Mice injected with wide type CLDN19 show normal mfERG recordings (P1 wave amplitude = 1.1±0.3 μV). In contrast, mice transduced with mutant CLDN19 showed impaired light response (P1 wave amplitude, G20D = 0.48±0.25 μV, and R81W = 0.61±0.26 μV). In primary cultures of human fetal RPE, overexpression of wide type CLDN19 upregulated melansome-associated genes MITF, PMEL and TYR, as well as a tight junction related gene, MMP2. Expression of the G20D and R81W mutations dramatically downregulated MITF, PMEL, TYR, MMP2 and PEDF.

Conclusions: FHHNC mutations of claudin-19 disrupt the trafficking of claudin-19 and adversely affect the expression of genes for melanogenesis. Claudin-19 mutants impaired the light response measured by mfERG.
Commercial Relationships: Tao Xu, None; **Shao-Bin Wang**; **Ron A. Adelman**, None; **Shaomin Peng**, None; **Lawrence J. Rizzolo**, None
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Cold Shock Proteins Expression in the Retina Following Exposure to Hypothermia
Ignacio M. Larrayoz[1], Manuel Rey-Funes[2], Daniela S. Contartese[2], Federico Rolón[2], Aníbal Sarotto[2], Verónica B. Dorfman[3], César F. Loidl[2], Alfredo Martinez[4]. [1]Biomarkers and Molecular Signaling, Center for Biomedical Research of La Rioja (CIBIR), Fundación Rioja Salud, Logroño, Spain; [2]Neuropatología Experimental, Instituto de Biología Celular y Neurociencia "Prof. E. De Robertis" (IBCN), Facultad de Medicina, UBA, Buenos Aires, Argentina; [3]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina; [4]Angiogenesis, CIBIR, Fundación Rioja Salud, Logroño, Spain.
Purpose: Hypothermia has been proposed as a therapeutic intervention for some retinal conditions, including ischemic insults. Cold exposure elevates expression of cold-shock proteins (CSP), including RNA-binding motif protein 3 (RBM3) and cold inducible RNA-binding protein (CIRP), but their presence in mammalian retina is so far unknown
Methods: Two cell lines of retinal origin, R28 and mRPE, were exposed to 32°C for different time periods and CSP expression was measured by qRT-PCR and Western blotting. Neonatal and adult Sprague-Dawley rats were exposed to a cold environment (8°C) and expression of CSPs in their retinas was studied by Western blotting, multiple inmunofluorescence, and confocal microscopy
Results: RBM3 expression was upregulated by cold in both R28 and mRPE cells in a time-dependent fashion. On the other hand, CIRP was upregulated in R28 cells but not in mRPE. In vivo, expression of CSPs was negligible in the retina of newborn and adult rats kept at room temperature (24°C). Exposure to a cold environment elicited a strong expression of both proteins, especially in retinal pigment epithelium cells, photoreceptors, bipolar, amacrine and horizontal cells, Müller cells, and ganglion cells
Conclusions: In conclusion, CSP expression rapidly rises in the mammalian retina following exposure to hypothermia in a cell type-specific pattern. This observation may be at the basis of the molecular mechanism by which hypothermia exerts its therapeutic effects in the retina
Commercial Relationships: Ignacio M. Larrayoz, None; **Manuel Rey-Funes**, None; **Daniela S. Contartese**, None; **Federico Rolón**, None; **Aníbal Sarotto**, None; **Verónica B. Dorfman**, None; **César F. Loidl**, None; **Alfredo Martinez**, None
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Presentation Time: 3:45 PM–5:30 PM
Impaired Reductive Carboxylation of the Retinal Pigment Epithelium in Sorsby Fundus Dystrophy
Abbi Engel[1], Kaitlen Knight[1], Megan Manson[1], Bela Anand-Apte[2], James Hurley[1], Jianhai Du[3, 1], Jennifer R. Chao[1]. [1]Ophthalmology, University of Washington, Seattle, WA; [2]Ophthalmic Research, Cleveland Clinic, Cleveland, OH; [3]Ophthalmology, Western Virginia University, Morgantown, WV.
Purpose: Previous studies have demonstrated the importance of retinal pigment epithelium (RPE) metabolism in countering oxidative stress. Sorsby Fundus Dystrophy (SFD) is a rare disease that affects the RPE and Bruch's membrane, resulting in central vision loss. Our aim is to determine whether SFD RPE have altered metabolism compared to normal controls.
Methods: RPE cell lines were differentiated from three distinct induced pluripotent stem cell (iPSC) clones from each of three normal control individuals and three iPSC clones from an individual with SFD (S181C TIMP3 mutation). After the RPE cells were cultured to maturity, they were incubated with ^{13}C tracers and metabolites were prepared for analysis and quantified by GC-MS and LC-MS. Transmission electron microscopy (TEM) was performed on RPE cells cultured on transwell filter membranes.
Results: Unlike control RPE cells, SFD RPE cultured on transwell filter membranes did not elaborate an ECM, as detected by transmission electron microscopy. Metabolite analysis revealed a 30-fold increase in 4-hydroxyproline, a metabolite produced by collagen degradation, suggesting that metalloproteinases may be more active in the SFD RPE. Further analysis demonstrated that there was a 25% decrease in reductive flux of alpha-ketoglutarate to citrate and an overall decrease in ^{13}C glutamine-derived M3 metabolites such as malate, fumarate, and aspartate compared to controls, indicating impaired reductive carboxylation in SFD RPE. In addition, SFD RPE appeared to be more energy depleted as noted by a decrease in the NAD^+/NADH ratio and an increase in the AMP/ATP ratio. Finally, we determined that SFD RPE have increased susceptibility to oxidative stress that can be rescued by supplementation with nicotinamide mononucleotide.
Conclusions: Collagen degradation, reductive carboxylation, and mitochondrial bioenergetics are altered in SFD RPE. These changes may contribute to their susceptibility to oxidative stress.
Commercial Relationships: Abbi Engel, None; **Kaitlen Knight**, None; **Megan Manson**, None; **Bela Anand-Apte**, None; **James Hurley**, None; **Jianhai Du**, None; **Jennifer R. Chao**, None
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Program Number: 3594 **Poster Board Number:** B0153
Presentation Time: 3:45 PM–5:30 PM
Comparison of complement pathway activation and synapse loss in young and aged mice undergoing light induced retinal degeneration
Sarah W. Gooding[1], Micah A. Chrenek[1], Jana T. Sellers[1], Jeffrey H. Boatright[1, 2]. [1]Ophthalmology, Emory University, Atlanta, GA; [2]Atlanta VAMC, Atlanta, GA.
Purpose: To determine whether aged versus young mice have increased synapse loss and inflammatory response to light-induced retinal degeneration (LIRD).
Methods: Young (61-62 days old) and aged (446-523 days old) Balb/c mice were exposed to four hours of 50 lux (dim), 2500 lux, 5000 lux, or 10,000 lux light and sacrificed two days later or seven days later following scotopic ERG. One eye was embedded in

paraffin for immunohistochemistry. RNA was extracted from the retina of the other eye for use in digital droplet PCR to quantify gene expression. Whole eyes were sectioned and stained with antibodies against several components of complement pathways and against PSD95, a synapse marker. Images of C1q immunostained retina were threshholded and deposition scored to quantify C1q staining. Statistical testing was via two-way ANOVA with Tukey's post-hoc analysis.
Results: Retina function as measured by a- and b-wave amplitudes declined maximally in aged mice with 2500 lux light exposure whereas as 5000 lux or more was required to obtain maximal loss in young mice. C1qa gene expression was 50% greater in naïve aged versus young mice. CFH immunostaining and C1qa, C1qb, Cfh, Cfb, and C3 gene expression increased in both young and aged mice with LIRD. In contrast, C1Q immunostaining significantly increased only in the aged group two days following 10,000 lux light exposure ($p \leq 0.0002$). Aged mice exhibited less PSD95 immunostaining in the outer plexiform layer (OPL) seven days following 2500 or 5000 lux light exposure compared to young mice, even though young mice apparently lost more photoreceptor cell nuclei. PSD95 staining was nearly absent two days following 10,000 lux exposure in both age groups.
Conclusions: Aged mice lost retina function and OPL synapses at lower levels of light exposure, and had greater C1Q deposition in the ONL compared to young mice. It may be that, as in other CNS structures, aged mice have a hyper-inflammatory response to stressors that results in loss of synapses and associated function. These findings may have implications for retinal degenerative diseases whose etiologies include aging and inflammation.
Commercial Relationships: Sarah W. Gooding; **Micah A. Chrenek**, None; **Jana T. Sellers**, None; **Jeffrey H. Boatright**, None
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Program Number: 3595 **Poster Board Number:** B0154
Presentation Time: 3:45 PM–5:30 PM
Modeling Pharmacological Treatment of Retinitis Pigmentosa with Heterozygous Tvrm4 Rhodopsin Mutant Mice
Robin H. Schmidt[1], Preston E. Girardot[1], Jana T. Sellers[1], Micah A. Chrenek[1], Jeffrey H. Boatright[1, 2]. [1]Ophthalmology, Emory University, Atlanta, GA; [2]Atlanta VA Center of Excellence in Vision and Neurocognitive Rehabilitation, Atlanta, GA.
Purpose: Pharmacological treatments for retinitis pigmentosa (RP), a disease characterized by progressive loss of vision, can be challenging to develop and test. The mostly widely-used mouse models of RP are transgenic strains. The Rho(Tvrm4/+) mouse, a model of Class B phenotype RP, carries a single point mutation in the gene that encodes rhodopsin, causing retinal degeneration after brief exposure to bright light. In contrast to transgenic models, the degeneration process that occurs in the Rho(Tvrm4/+) mouse model is inducible, allowing for more precise coordination of drug administration with particular stages in disease progression. We tested the ability of the neuroprotectant tauroursodeoxycholic acid (TUDCA) to prevent retinal degeneration in the Rho(Tvrm4/+) mouse.
Methods: TUDCA (500 mg/kg) was injected i.p. in Rho(Tvrm4/+) mice (N=5 for each group) prior to light exposure. Pupils were dilated by applying a 0.2% atropine solution. After 30 minutes, mice were exposed to 12,000 lux LED white light for 5 minutes. Mice were then returned to maintenance housing and TUDCA administered every 3 days thereafter. Retinal function was assessed by electroretinography (ERG) and visual function assessed by optokinetic tracking (OKT). Eyes were removed at sacrifice for histology and gene transcription analysis by ddPCR. Photoreceptor nuclei were counted in histological sections stained with toluidine blue.
Results: ERG analysis showed that TUDCA treatment completely prevented changes in a- and b-wave amplitude ($p<0.0001$). TUDCA preserved the number of photoreceptors in light-exposed mice ($p=0.0002$). Protection against degeneration coincided with decreased mRNA transcripts of genes regulating the complement system. TUDCA treatment had no effect on retinal structure or visual function in mice that were not exposed to bright light.
Conclusions: TUDCA-treated Rho(Tvrm4/+) mice were protected against loss of visual function caused by brief bright light exposure. TUDCA treatment also showed near-complete preservation of retinal morphology and photoreceptor cell numbers. Our data support the use of TUDCA in RP, and potentially in other retinal degenerative diseases. Additionally, the predictable triggering of retinal degeneration in the Rho(Tvrm4/+) mouse means that this genetic model may be useful for testing other candidate drugs.
Commercial Relationships: Robin H. Schmidt; **Preston E. Girardot**, None; **Jana T. Sellers**, None; **Micah A. Chrenek**, None; **Jeffrey H. Boatright**, None
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Presentation Time: 3:45 PM–5:30 PM
Galectin-1 involvement in neovascular retinopathies
Magali Evelin E. Ridano[1], Paula V. Subirada Caldarone[1], Maria C. Paz[1], Valeria E. Lorenc[1, 2], Juan C. Stupirski[4], Laura Gramajo[3], Jose D. Luna Pinto[3], Diego O. Croci[4], Gabriel A. Rabinovich[4], Maria C. Sanchez[1]. [1]Clinical Biochemistry, CIBICI CONICET, Cordoba, Argentina; [2]Department of Ophthalmology, The Johns Hopkins School of Medicine, Baltimore, MD; [3]VER Foundation, Private Eye Center Romagosa, Cordoba, Argentina; [4]Immunopathology Laboratory, IBYME CONICET, Buenos Aires, Argentina.
Purpose: Vascular Endothelial Growth Factor (VEGF) inhibitors have been established as the mainstay of current treatment of neovascular retinopathies (NVRx), however, it remains an unmet medical need. Galectin-1 (Gal1) has been implicated in pathologies associated with neovascularization (NV) through VEGF independent pathways and by its interaction with N-acetyllactosamine (NAcLc) structures in N- and O- glycans on glycosylated receptors. Here, we hypothesize that Gal1 is participating in experimental and clinical NVRx and independently of anti-VEGF therapy.
Methods: Oxygen-Induced Retinopathy (OIR) mouse model was carried out. Briefly, C57BL/6 mice (OIR, N=33) were exposed to 75% O2 from P7 to P12, after which they were brought to room air for additional five (P17) or nine days (P26). Age-match mice maintained in room air (RA, N=15) were used as controls. Some OIR mice were intraocular injected at P12 with 1,25 µg of anti-VEGF (N=9) or vehicle (N=9). At P12, P17 and P26 mice were sacrificed. Some eyes were fixed to obtain cryosections for immunofluorescences and retinas were isolated to analyze Gal1 expression by Western blots and qRT-PCR. Moreover, aqueous humor of patients with NVRx (N=16) or from control patients (N=6) was used to measure Gal1 levels by ELISA assays. GraphPad Prism program was employed for statistical analysis.
Results: Gal1 protein and mRNA levels were increased in P17 and P26 OIR retinas respect to RA controls ($p<0,05$).

Immunofluorescence assays showed that it was located in the layers closest to the vitreous humor and its expression was most prominent in areas with NV. Moreover, Gal1 colocalization with Glial Fibrillary Acidic Protein (GFAP) and Glutamine Synthase (GS) suggested that it was present in astrocytes and activated Müller cells. In addition, it was within neovessels (OIR) but not in normal retinal vessels (RA) at P17. Byotinilated lectin staining demonstrated that OIR conditions modify the ''glycosylation signature'' of the normal retina showing a glycosylation pattern permissive to Gal1 binding at P17 OIR. Furthermore, anti-VEGF therapy was not able to reduce the OIR Gal1 induction ($p<0,05$). Finally, Gal1 levels were also elevated in aqueous humor of patients with NVRx ($p<0,05$).
Conclusions: We conclude that Gal1 is participating in the pathogenesis of OIR and it is independently of the anti-VEGF therapy. In light of our results, we also suggest that it is involved in the clinical NVRx.
Commercial Relationships: Magali Evelin E. Ridano; **Paula V. Subirada Caldarone**, None; **Maria C. Paz**, None; **Valeria E. Lorenc**, None; **Juan C. Stupirski**, None; **Laura Gramajo**, None; **Jose D. Luna Pinto**, None; **Diego O. Croci**, None; **Gabriel A. Rabinovich**, None; **Maria C. Sanchez**, None
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Presentation Time: 3:45 PM–5:30 PM
Functional assessment of AIPL1 variations identified in Leber Congenital Amaurosis patients
Almudena Sacristan Reviriego[1], James Bellingham[1], Chrisostomos Prodromou[2], Jacqueline van der Spuy[1]. [1]UCL Institute of Ophthalmology, London, United Kingdom; [2]University of Sussex, Brighton, United Kingdom.
Purpose: Mutations in the photoreceptor-expressed gene *AIPL1* cause autosomal recessive Leber congenital amaurosis (LCA). AIPL1 facilitates the correct assembly of retinal cGMP phosphodiesterase (PDE6) acting as a co-chaperone for HSP90. While over 400 variations have been identified throughout *AIPL1*, only a handful have been experimentally validated and the disease-causing status is often based on *in silico* predictions of pathogenic probability. Therefore, the functional assessment and confirmation of likely pathogenic AIPL1 variants in this study is an important step towards an accurate and early diagnosis and treatment of LCA patients.
Methods: Expression and subcellular localisation of AIPL1 variants was examined by western blotting and immunofluorescent confocal microscopy. To test their ability to interact with HSP90, directed yeast two hybrid (Y2H) and quantitative enzyme-linked immunosorbent (ELISA) assays were performed.
Results: The C-terminal HSP90 pentapeptide MEEVD is critical for mediating the interaction with the tetratricopeptide (TPR) domain of AIPL1. The AIPL1 variants p.L17P, p.C89R, p.Q163X and p.E282_A283dup were unable to interact with HSP90 efficiently, whereas p.G64R, p.V71F, p.K214N and p.G262S retained the ability to bind HSP90 in a TPR-dependent manner. *AIPL1* variations located in the coding region, including c.642G>C(p.K214N) and c.784G>A(p.G262S), or in the non-coding regions of *AIPL1* (c.97_104dup, c.98_99insTGATCTTG, c.276+1G>A, c.276+2T>C, c.277-2A>G, c.785-10_786del12) cause aberrant pre-mRNA splicing leading to alternative transcripts that could encode functionally deficient protein isoforms.. Alternative protein isoforms, which included in-frame domain deletions, frameshift stop mutations, and small insertions and deletions, showed a significant decrease or loss of HSP90 binding affinity, with the exception of one isoform with a small insertion in the TPR domain that retained a TPR-dependent HSP90 interaction.
Conclusions: The present study has validated the disease-association and experimentally confirmed the biochemical defects underlying uncharacterised AIPL1 nonsense, missense and intronic variations.
Commercial Relationships: Almudena Sacristan Reviriego, None; **James Bellingham**, None; **Chrisostomos Prodromou**, None; **Jacqueline van der Spuy**, None

Program Number: 3598 **Poster Board Number:** B0157
Presentation Time: 3:45 PM–5:30 PM
Phenotypic Identification of Vitamin D Receptor Agonists as Regulators of Developmental Angiogenesis and miR21 in the Zebrafish Eye
Breandan N. Kennedy[1], Stephanie Merrigan[1], Eugene Dillon[1], Gerard Cagney[1], Sally Hampton[2]. [1]Sch of Biomolecular and Biomedical Sci, University College Dublin, Dublin, Ireland; [2]Tetricus Science Park, KalVista Pharmaceuticals Ltd, Wilts, United Kingdom.
Purpose: Angiogenesis is fundamental for development and problematic in disease. Ocular disorders including neovascular age related macular degeneration and proliferative diabetic retinopathy have an aberrant angiogenesis component. Our in vivo unbiased screen of 465 ICCB library chemicals identified inhibitors of developmental angiogenesis in the zebrafish eye. Such small molecule inhibitors give insight into regulators of angiogenesis and represent potential anti-angiogenic treatments. Here, we followed up on the safety and efficacy of lead hit calcitriol.
Methods: Calcitriol and vitamin D receptor agonists (VDRA) were tested for inhibition of ocular hyaloid vessel (HV) and non-ocular inter-segmental vessel (ISV) developmental angiogenesis in zebrafish larvae. Safety studies in larvae used light microscopy to evaluate retinal morphology and the optokinetic response (OKR) assay to assess visual function in response to calcitriol treatment. Mechanistic understanding was gained through proteomics, ELISA and QRT-PCR studies in treated zebrafish larval eyes and human retinal pigment epithelial cells (ARPE-19). The anti-angiogenic activity of calcitriol was further evaluated in an ex vivo C57BL/6J mouse model of choroidal sprouting angiogenesis.
Results: Calcitriol and 7 VDRA significantly inhibited HV developmental angiogenesis by up to 50 percent ($P\square 0.001$, $N\geq 20$). Absence of phenotypically observable change in pre-established and developing ISV indicated ocular selective anti-angiogenic activity ($N\geq 15$). Calcitriol treated larvae presented with diminished OKR despite appearance of normal retinal lamination/morphology in ocular cross sections ($N\geq 27$, $P\square 0.001$)($N\geq 3$). Treatment with 0.1-10 μM calcitriol induced miR21 expression and treatment with ≥10 μM calcitriol induced vegfaa expression in larval eyes (n=3). Proteomics of calcitriol treated larval eyes revealed 32 differentially regulated proteins ($P\square 0.05$). Preliminary data suggests calcitriol to attenuate TNFα induced IL-8 expression in ARPE-19 cells (n=2). Anti-angiogenic efficacy was validated in the ex vivo mouse choroidal sprouting angiogenesis model (n=3).
Conclusions: VDRA regulate ocular developmental angiogenesis and miR21 expression in the zebrafish eye. VDRA and/or its downstream mediators such as miR21 signify potential targets for the treatment of neovascular diseases.
Commercial Relationships: Breandan N. Kennedy, None; **Stephanie Merrigan**, None; **Eugene Dillon**, None; **Gerard Cagney**, None; **Sally Hampton**, None
Support: Irish Research Council & Health Research Board

Program Number: 3599 **Poster Board Number:** B0158
Presentation Time: 3:45 PM–5:30 PM
Antiviral activity of a 45 nucleotide DNA aptamer during HSV-1 infection of the cornea
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Purpose: Herpes simplex virus type 1 (HSV-1) is the leading cause of infectious blindness in the United States. In this study, we use corneal model of HSV infection to report the high antiviral activity of a 45 nucleotide DNA aptamer directed against HSV-1 gD protein.
Methods: SYBR Green based ELISA was conducted to evaluate the specific binding affinity of the DNA aptamer by incubating 50 µL of multiple concentrations of soluble HSV-1 gD, gB proteins. MTT based cytotoxicity assay for 2 different aptamer concentrations were conducted along with a random DNA sequence and mock PBS. Viral entry and infectious spread were evaluated using β-galactosidase assay, immunoblotting and immunofluorescence assays. Freshly cut pig corneas were infected with HSV-1 GFP virus and its spread was monitored over a period of 10 days in the presence and absence of the aptamer. BALB/C mice (male and female, 3 weeks old) were anesthetized using ketamine (100 mg/kg) and xylazine (5 mg/kg) prior to scarring the right eye with a 30-G sterile needle in a 3 × 3 grid pattern. The scarred eyes were infected with GFP virus and the progress was monitored for a period of 10 days before sacrificing the mice and harvesting the tissue for analyzing the extent of virus infection and the role of cytokines through RT-PCR assay.
Results: Our DNA aptamer showed specific binding affinity towards HSV-1 gD (Kd = 50 nM) and was able to restrict viral entry by 70% at a 2 µM concentration. Immunofluorescence and immunoblotting studies showed 40-50% inhibition of viral entry and viral replication *in-vitro* respectively. *Ex-vivo* studies on pig corneal models showed therapeutic efficacy of the aptamer in reducing productive infection over a period of 168 hours. *In-vivo* experiments on mice models exhibited significant decrease in ocular infection through prophylactic, therapeutic and neutralization treatment of corneal tissue with the DNA aptamer.
Conclusions:
This is the first study to show a comprehensive decrease in HSV-1 infection using a DNA aptamer. Their role in inhibiting cell to cell fusion and consecutively restrict viral spread was also evaluated in this study. Future studies will shed more light on the clinical implications of this aptamer.
Commercial Relationships: Tejabhiram Yadavalli, None; **Neel Thakkar**, None; **Dinesh Jaishankar**, None; **Alex Agelidis**, None; **Deepak Shukla**, None
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Program Number: 3600 **Poster Board Number:** B0159
Presentation Time: 3:45 PM–5:30 PM
Influence of charge, hydrophobicity, and hydrodynamic radius on vitreous pharmacokinetics of large molecules
Susan Crowell[1], Kathryn Wang[2], Leslie Dickmann[3], C. A. Boswell[1], Daniela B. Yadav[1], Mauricio Maia[4], Whitney Shatz[5], Amin Famili[2], Karthik Rajagopal[2], Devin Tesar[2], Robert F. Kelley[2]. [1]Preclinical and Translational Pharmacokinetics and Pharmacodynamics, Genentech, South San Francisco, CA; [2]Drug Delivery, Genentech, South San Francisco, CA; [3]Clinical Pharmacology, Genentech, South San Francisco, CA; [4]Bioanalytical Assay Services, Genentech, South San Francisco, CA; [5]Protein Chemistry, Genentech, South San Francisco, CA.
Purpose: Development of long acting large molecule therapeutics for human posterior segment ocular disease is hampered by an inadequate understanding of the pharmacokinetic drivers following intravitreal injection. Previous work has shown that charge and hydrophobicity can influence systemic clearance rates, and that hydrodynamic radius is linearly correlated with vitreal half-life over the range of 3 – 7 nm. A better understanding of the molecular attributes contributing to vitreal elimination half-life could enable the design of improved therapeutics.
Methods: The contribution of electrostatic charge, hydrophobicity, and hydrodynamic radius to vitreal clearance was assessed for soluble large molecules including antibodies and antibody fragments (Fabs) following intravitreal injection in rabbits and nonhuman primates. A systematic study of molecule charge was accomplished by determining vitreal half-life in rabbit of variants of ranibizumab whereby charge was altered through introduction of amino acid changes in two of the hypervariable regions of the light chain. Charge was assessed by measuring isoelectric point (pI). Hydrophobicity was determined experimentally via analytical hydrophobic interaction chromatography, and hydrophobicity of the variable domain unit (Fv) was also calculated from the amino acid sequence. Hydrodynamic radius was determined using light scattering.
Results: No significant correlation was observed between vitreal half-life and pI or hydrophobicity. Equivalent rabbit vitreal pharmacokinetics were observed for ranibizumab and its charge variants with pIs in the range of 6.8-10.2. A strong correlation was observed between rabbit vitreal half-life and hydrodynamic radius between 2 – 45 nm ($p<0.0001$).
Conclusions: These results indicate that diffusive properties of soluble large molecules, as quantified by the hydrodynamic radius, make a key contribution to vitreal elimination whereas minor or negligible contributions arise from differences in charge or hydrophobicity.
Commercial Relationships: Susan Crowell, Genentech (I), Genentech (E); **Kathryn Wang**, Genentech (I), Genentech (E); **Leslie Dickmann**, Genentech (I), Genentech (E); **C. A. Boswell**, Genentech (I), Genentech (E); **Daniela B. Yadav**, Genentech (I), Genentech (E); **Mauricio Maia**, Genentech (I), Genentech (E); **Whitney Shatz**, Genentech (I), Genentech (E), Genentech (P); **Amin Famili**, Genentech (I), Genentech (E), Genentech (P); **Karthik Rajagopal**, Genentech (I), Genentech (E), Genentech (P); **Devin Tesar**, Genentech (I), Genentech (E), Genentech (P); **Robert F. Kelley**, Genentech (I), Genentech (E), Genentech (P)

Program Number: 3601 **Poster Board Number:** B0160
Presentation Time: 3:45 PM–5:30 PM

Narrow spectrum kinase inhibitors (NSKIs) are superior to corticosteroid in both *in vitro* and *in vivo* inflammatory eye models

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Purpose: Inflammatory eye diseases, such as uveitis and dry eye syndrome, can be debilitating and in severe cases lead to visual impairment. Despite recent therapeutic advances, corticosteroids (CS) followed by steroid-sparing immunosuppressants remain the mainstay treatment options. However, long term use of CS is complicated by the development of cataract and glaucoma. NSKIs are a novel class of small molecule which selectively target key kinases (p38-alpha, Src family kinases and Syk) involved in both innate and adaptive inflammatory signalling cascades. Here, we compare an NSKI to CS in *in vitro* and *in vivo* inflammatory eye models.

Methods: Efficacy and potency of the NSKI TOP1106 was compared to the CS, fluticasone propionate, using *in vitro* cell assays representative of innate immunity (lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMCs) and primary human macrophages), adaptive immunity (anti-CD3/anti-CD28 stimulated PBMCs) and TNFα/IL-1β stimulated primary human retinal pigment epithelial cells (RPE). *In vivo*, TOP1106 and dexamethasone were tested in a rat LPS-induced uveitis (EIU) model using cellular infiltrate into the aqueous humor and inflammatory cytokine levels in eye tissue as endpoints to assess efficacy.

Results: TOP1106 exhibited a broader and more potent *in vitro* anti-inflammatory profile compared to CS. In both innate and adaptive immune responses, TOP1106 is a potent inhibitor (IC_{50}s < 20 nM) of inflammatory cytokine release and was superior to CS both in terms of potency and efficacy. Similarly, in stimulated RPE cells TOP1106 was markedly more potent than CS in inhibiting IL-6, IL-8 and CCL-5 release. In the EIU model, topical administration of TOP1106 (1 mg/ml) was more efficacious than CS (1 mg/ml) in reducing inflammatory cytokines MCP-1, IL-6 and IL-1β in both anterior and posterior tissues of the eye, as well as inflammatory cell infiltrate into the aqueous humor.

Conclusions: This data demonstrates the potential utility of NSKIs in the treatment of ocular inflammation. With the ability to target both innate and adaptive immunity pathways, NSKIs achieve comparable, if not superior, efficacy to CS thereby offering a potential alternative to avoid the complications of long term CS use.

Commercial Relationships: Claire Walshe, Topivert Pharma (E); **Martyn R. Foster**, Topivert Pharma (E); **Yemisi Solanke**, Topivert Pharma (E); **Sameer Sirohi**, Topivert Pharma (E); **Gayathri Devarajan**, Topivert Pharma (F); **John V. Forrester**, Topivert Pharma (F); **Isabel Crane**, Topivert Pharma (F); **Steve Webber**, Topivert Pharma (E)