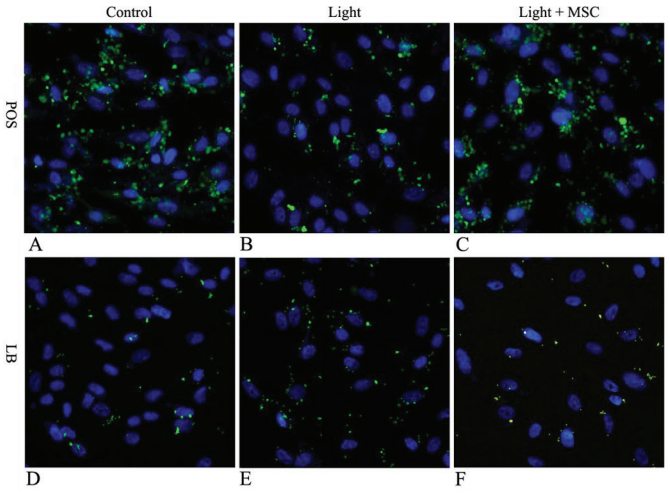
542 Cell Biology of Atrophic AMD
Thursday, May 05, 2016 11:00 AM–12:45 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 6526–6561/D0275–D0310
Organizing Section: Retinal Cell Biology
Contributing Section(s): Biochemistry/Molecular Biology

Program Number: 6526 **Poster Board Number:** D0275
Presentation Time: 11:00 AM–12:45 PM
Effect of Complement Factor H Variants in Regulating AMD-like Pathologies *In Vivo*
Michael Landowski[1], Jindong Ding[1], Mikael Klingeborn[1], Una Kelly[1], Marybeth Groelle[1], Catherine Bowes Rickman[1, 2]. [1]Ophthalmology, Duke University, Durham, NC; [2]Cell Biology, Duke University, Durham, NC.
Purpose: The Y402H polymorphism in the complement factor H (*CFH* gene, FH protein) is associated with increased risk for age-related macular degeneration (AMD), but the role of the risk-associated H402 variant in AMD remains unclear. We generated transgenic mouse lines expressing the same concentration of full-length human normal Y402 or risk-associated H402 variants of the FH protein on a mouse *Cfh* knockout background (*CFH-Y:Cfh-/-* and *CFH-HH:Cfh-/-*, respectively) to mechanistically study the function of FH variants in AMD pathogenesis *in vivo*. We tested the hypotheses (1) that an AMD-like phenotype will develop in old *CFH:Cfh-/-* mice following 8 weeks on a high fat, cholesterol-enriched (HFC) diet and (2) that there is a variant-associated difference in the amount of sub-RPE deposit, visual loss, and RPE damage between these lines.
Methods: 90 week-old male *C57BL/6J*, *CFH-Y:Cfh-/-, CFH-HH:Cfh-/-* and *Cfh-/-* mice were housed conventionally, under ambient conditions and maintained on water *ad libitum* and fed either a normal mouse chow diet (ND) or switched to a HFC diet for 8 wks. ERGs and plasma samples were obtained prior to termination. Eyes were collected and processed for electron microscopy for quantification of sub-RPE deposit load, immunofluorescence to assess RPE damage and routine histology.
Results: Old mice expressing only human FH that were fed a HFC diet developed vision loss, larger sub-RPE deposits, and more multinucleated RPE cells compared to their age-matched, normal diet controls. As we have previously shown, aged *Cfh-/-* mice did not develop an AMD-like phenotype following HFC diet. The phenotype in *CFH-Y:Cfh-/-* mice fed a HFC diet appears to be less severe than in the *CFH-HH:Cfh-/-* mice fed a HFC diet.
Conclusions: *CFH-Y:Cfh-/-* and *CFH-HH:Cfh-/-* mice on HFC diet develop an AMD-like phenotype. The less severe AMD-like phenotype observed in aged *CFH-Y:Cfh-/-* mice on HFC diet compared to aged *CFH-HH:Cfh-/-* mice on HFC diet suggests that the Y402 variant is protective against AMD-like pathology. These models will be useful in elucidating the molecular mechanisms underlying the association of the H402 variant with AMD risk.
Commercial Relationships: Michael Landowski; **Jindong Ding**, None; **Mikael Klingeborn**, None; **Una Kelly**, None; **Marybeth Groelle**, None; **Catherine Bowes Rickman**, None
Support: NEI P30 EY005722; NEI R01 EY019038 and Research to Prevent Blindness

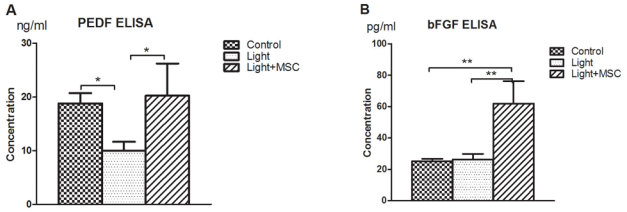
Program Number: 6527 **Poster Board Number:** D0276
Presentation Time: 11:00 AM–12:45 PM
Changes in Genome Expression and Mitochondrial Fission/Fusion Dynamics in RPE J Haplogroup May be Linked to AMD Susceptibility
Ana Rubin[1], Anzor Gvritishvili[1], Anna Salzberg[2], Yuka Imamura[2], Hannah Galvan[1], Lynn Mullen[3], Shari R. Atilano[4], Cristina M. Kenney[4], Joyce Tombran-Tink[1]. [1]Department of Neural and Behavioral Sciences and Department of Ophthalmology, Pennsylvania State University College of Medicine, Hershey, PA; [2]Penn State Hershey Genome Sciences and Institute for Personalized Medicine, Pennsylvania State University College of Medicine, Hershey, PA; [3]Qiagen Bioinformatics, Silicon Valley, CA; [4]Gavin Herbert Eye Institute, Irvine, CA.
Purpose: Mitochondrial DNA (mtDNA) have traceable sets of SNPs that define mtDNA haplogroups. European J haplogroup is linked to increased susceptibility and H to protection against AMD. In this study, we used RPE cytoplasmic hybrids (cybrids) containing J or H haplogroups to determine their effects on genome expression and resilence to oxidative stress.
Methods: RPE cybrids containing the J or H mt haplogroups were seeded out at $1x10^5$ cells/ml in DMEM and cells harvested at day 4. RNA was extracted, cDNA libraries constructed, and samples subjected to RNAseq using Illumina HiSeq 2500. De-multiplexed sequencing was extracted using Illumina Casava pipeline v1.8 and FPKM values calculated using Cufflinks v 2.0.2. Transcriptomes were compared and only significant reliably expressed genes with $p<0.05$, FC>1.5 were included in the analyses and validated by qPCR. RPE cybrids were further exposed to oxidative stress (tbH2O2; 100 mM) for 30min and mt dynamics studied. Tom20 staining and mitotracker uptake were used to visualize the organelles response to oxidative stress and mt function determined by fluctuations in the membrane potential ($\Delta\Psi m$) and calcium uptake ($[Ca^{2+}]m$) using flow cytometry. Stress-induced changes in levels of inflammatory and angiogenic markers were also measured using the bioplex xMAP technology.
Results: Fourteen genes were downregulated with FCs = -1.5-5.16 and forty-four upregulated with FCs = +1.5-3.1 in J compared to H cybrids. Genes involved in mt complex I and V function, mt fusion/fission events, cellular energy homeostasis, anti-oxidant defenses, and inflammatory responses were dysregulated in J cybrids. When exposed to oxidative stress, J cybrids mt showed pathology in fission and fusion events, increased $[Ca^{2+}]m$ uptake ($p<0.05$), and higher levels of IFN-γ, TNF-α and VEGF ($p<0.01$) when compared to H cybrids.
Conclusions: Our results suggest that RPE cells containing the mtDNA J haplogroup are associated with gene expression changes that alter mitochondrial function, resistance to oxidative stress, inflammatory environment and angiogenesis proclivity, which could all influence development of AMD-like pathologies in the retina.
Commercial Relationships: Ana Rubin; **Anzor Gvritishvili**, None; **Anna Salzberg**, None; **Yuka Imamura**, None; **Hannah Galvan**, None; **Lynn Mullen**, None; **Shari R. Atilano**, None; **Cristina M. Kenney**, None; **Joyce Tombran-Tink**, None
Support: Frontiers in Vision and Eye Research

Program Number: 6528 **Poster Board Number:** D0277
Presentation Time: 11:00 AM–12:45 PM
Differential regulation of mitochondrial ferritin in retinal pigment epithelium may differently correlate to the progression of age-related macular degeneration
Xiying Wang[1], Hongkuan Yang[2], Hongyan Ge[1], Ping Liu[1], Ikuo Tooyama[3]. [1]Ophthalmology Department, Eye hospital of Harbin Medical University, Harbin, China; [2]University of Illinois at Chicago, Chicago, IL; [3]Molecular Neuroscience Research Center, Shiga University of Medical Science, Seta, Japan.
Purpose: Mitochondrial ferritin (FtMt) mutation has been reported in cohort of patients with age-related macular degeneration (AMD), which leads to dysfunction of FtMt and is associated with age-related macular degeneration (AMD). Although FtMt is suggested to be implicated in neurodegenerative diseases, the role of FtMt in AMD remains unclear. This study aims to elucidate functions of FtMt in the pathogenesis of AMD.
Methods: Retinal pigment epithelium (RPE)/choroid from young and old mice were dissected and perfused for immunofluorescence, or lysed for western blotting. Human retinal pigment epithelium cell line (ARPE-19) was cultured either under normoxia or hypoxia. FtMt overexpression was undertaken by transient or stable transfection of FtMt-expressing plasmid, and hypoxia-inducible factor-1alpha (HIF-1alpha) knockdown by transfection of exogenous siRNA. Protein levels of FtMt and HIF-1alpha were quantitatively measured with Western blotting, vascular endothelial growth factor (VEGF) in conditioned medium with ELISA, and cell viability with MTT assay.
Results: FtMt expression was significantly increased in aged mice RPE/choroid. Overexpression of FtMt up-regulated the expression of HIF-1alpha and the secretion of VEGF in ARPE-19 cell line, whereas VEGF secretion remained unchanged after HIF-1alpha inhibition by siRNA. Exposure of ARPE-19 cells to hypoxia significantly decreased FtMt expression, while FtMt was less decreased in HIF-1alpha siRNA-transfected cells compared to control cells. For cells cultured under hypoxic condition and treated with H_2O_2, cell viability was dramatically reduced in normal ARPE-19, but not in FtMt stably-overexpressing ARPE-19 cells.
Conclusions: Increased FtMt with age enhances VEGF secretion via stabilization of HIF-1alpha by preventing proteolysis in RPE cells, which may progress AMD to wet form. On the other hand, down-regulation of FtMt under hypoxia is in a HIF-1alpha-dependant manner and increases the susceptibility of RPE cells to oxidative insult, which might be associated with geographic atrophy due to RPE degeneration.
Commercial Relationships: Xiying Wang, None; **Hongkuan Yang**, None; **Hongyan Ge**, None; **Ping Liu**, None; **Ikuo Tooyama**, None

Program Number: 6529 **Poster Board Number:** D0278
Presentation Time: 11:00 AM–12:45 PM
In vitro protective effect of Wharton's Jelly mesenchymal stem cells on light-damaged retinal pigment epithelial cells
Hong Zhuang, Gezhi Xu. Department of Ophthalmology, Eye and ENT Hospital of Fudan University, Shanghai, China.
Purpose: In this study, we investigated whether Wharton's Jelly mesenchymal stem cells (WJ-MSCs) could protect the light-damaged retinal pigment epithelial (RPE) cells in vitro.
Methods: The co-culture system of light-damaged RPE cells and WJ-MSCs was established by Transwell chamber. The RPE cells were divided into three groups: Group A (normal controls), Group B (light-damaged RPE cells), and Group C (co-culture system). The proliferative ability of RPE cells was measured by MTT assay at 24h and 48h after co-culture. The phagocytosis ability of RPE cells was measured by both Photoreceptor outer segments (POS) and Latex beads (LB) phagocytosis assays at 48h after co-culture. ELISA kits were used to measure the levels of PEDF, bFGF in the culture supernatant.
Results: The proliferative ability of light-damaged RPE cells was declined, compared with the normal controls. And their proliferative ability was improved in the co-culture system. The POS phagocytosis ability of light-damaged RPE cells was deceased, compared with the normal controls. And their POS phagocytosis ability was increased in the co-culture system. The LB phagocytosis ability of light-damaged RPE cells was abnormally increased, compared with the normal controls. And their LB phagocytosis ability decreased to normal in the co-culture system. The protein expression of HSP27 was increased in light-damaged RPE cells, compared with the normal controls. Their protein expression of HSP27 decreased to normal in the co-culture system. The secretion of PEDF was decreased in light-damaged RPE cells, compared with the normal controls. And their secretion of PEDF was increased in the co-culture system. The baseline secretion of bFGF in the normal RPE cells was very low, and there was no significant change of bFGF secretion in light-damaged RPE cells. But the level of bFGF in the co-culture supernatant was significantly increased.
Conclusions: Our study demonstrated that WJ-MSCs have in vitro protective effect on light-damaged RPE cells. The possible mechanism is that WJ-MSCs could enhance the level of bFGF in the co-culture supernatant, promote the PEDF secretion by RPE cells, and eventually reduce the HSP27 expression in RPE cells.



The phagocytosis ability of RPE cells was measured by both POS and LB phagocytosis assays.



The levels of PEDF and bFGF in the culture supernatant.
Commercial Relationships: Hong Zhuang; **Gezhi Xu**, None

Program Number: 6530 **Poster Board Number:** D0279
Presentation Time: 11:00 AM–12:45 PM
Effect of epigenetic regulation on fibrotic change in retinal pigment epithelium cells
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Purpose: Proliferative eye diseases such as proliferative vitreoretinopathy and proliferative diabetic retinopathy are a major cause of blindness. Age-related macular degeneration (AMD) is recently on the increase, and it causes central scotoma and severe reduced visual acuity through the progression of choroidal neovascularization and the formation of fibrin at the macula area. Fibroblastic change of retinal pigment epithelium (RPE) cells or the fibroblastic membrane is one of the major causes of proliferative eye diseases. The purpose of this present study was to examine the effect of histone deacetylase (HDAC) and histone acetyl transferase (HAT) on fibrotic change in RPE cells.
Methods: Following the method described in previous reports, ARPE-19 cells (5×10^5 cells/mm^2) were cultured in10% fetal bovine serum (FBS) and then starved in serum-free medium for 24 hours. The culture medium was then replaced with fresh transforming growth factor beta 2 (TGF-β2, 30ng/ml) or/and tumor necrosis factor alpha (TNF-α, 10ng/ml) medium, and fibrotic change of the ARPE-19 cells was induced. Culture medium without TGF-β2 and TNF-α was used as a control. Cell morphology was examined by phase contrast microscopy after 48 hours of incubation. Simultaneously, the expression of α-smooth muscle actin (α-SMA), matrix metalloproteinase-9 (MMP-9), and CD44 were examined by immunostaining, western blotting, and polymerase chain reaction (PCR). The activity of HDAC and HAT were then observed between the following four groups: 1) control, 2) TGF-β2, 3) TNF-α, and 4) TGF-β2+TNF-α.
Results: Fibroblastic change of ARPE-19 cells was observed in all groups except for the control, and the potentiating effect of the change was noted in the medium with TGF-β2+TNF-α. The expression of α-SMA mRNA was enhanced by TGF-β2 ($p<0.05$), yet reduced by TNF-α or TGF-β2+TNF-α ($p<0.01$). The MMP-9 and CD44 mRNA expression was increased by TNF-α or TGF-β2+TNF-α ($p<0.01$). Similar trends were observed in immunostaining and western blotting. The activity of HAT was reduced by TNF-α or TGF-β2+TNF-α ($p<0.05$), and that of HDAC1 was significantly increased by TGF-β2+TNF-α ($p<0.01$).
Conclusions: Our findings indicate that alterations in the activity of HDAC and HAT are likely related to the fibroblastic change of RPE cells. We speculate that HDAC and HAT will become therapeutic targets for proliferative eye diseases and AMD.
Commercial Relationships: Hiroki Hatanaka, None; **Atsushi Mukai**, None; **Kazuhito Yoneda**, None; **Jun Yamada**, None; **Chie Sotozono**, None; **Shigeru Kinoshita**, None; **Junji Hamuro**, None

Program Number: 6531 **Poster Board Number:** D0280
Presentation Time: 11:00 AM–12:45 PM
The Association of Apolipoprotein E and Membrane Attack Complex in Human RPE Cell
Ping Yang[1], Victoria Treboschi[1], Peter Baciu[2], Glenn J. Jaffe[1]. [1]Ophthalmology, Duke University, Durham, NC; [2]Biology, Allergan, Inc, Irvine, CA.
Purpose: Complement activation has been increasingly implicated in the pathogenesis of AMD. Apolipoprotein E (ApoE) and complement activation products such as membrane attack complex (MAC) have been detected in Bruch's membrane (BrM) and drusen from eyes with AMD. We previously reported complement activation induced time-dependent ApoE accumulation in human RPE cells. Herein, we test the hypothesis that the interaction between ApoE and MAC contribute to increased cell-associated ApoE. We also evaluated the relationship between complement activation and ApoE expression in human eyes and mouse models.
Methods: Cultured human RPE cells were primed with a complement-fixing antibody and then treated with either 6% C1q-depleted human serum (C1q-Dep) to elicit cell surface MAC formation or heat inactivated C1q-Dep to block complement activation. Repetitive complement attack was induced every other day for 1 week (three attacks). RPE cells were incubated for 15 minutes with 0.02N ammonium hydroxide 48 hrs after the third complement attack. Western blot was performed on RPE cell-conditioned media, total cellular lysate proteins and extracellular matrix (ECM). ApoE and MAC co-localization were assessed on cultured human RPE cells and human eyes. C3d and ApoE were examined by immunofluorescent microscopy in ApoB 100 transgenic mice and CD55/CD59 double deficient (KO) mice.
Results: After a single complement attack, when compared to controls, decreased cell-associated ApoE was accompanied by increased release of ApoE into conditioned media at days 1, 2 and 3 post complement challenge. Cell-associated S58 priming antibody was decreased on RPE cells in a pattern similar to that observed with ApoE protein. ApoE and MAC were frequently co-localized in complement-treated cells, but not in control cells. ApoE was co-distributed with MAC in sub-RPE deposits and drusen from human eyes. Repeated complement attack dramatically enhanced RPE ApoE accumulation and caused ApoE ECM deposition. C3d and ApoE were detected in BrM of ApoB 100 transgenic mice as well as CD55/CD59 KO mice.
Conclusions: Complement-mediated RPE cell-associated ApoE accumulation and subsequent release may be one of mechanisms that accounts for ApoE in drusen. This information will enhance our understanding of the role that complement activation plays to mediate drusen formation and composition, and may elucidate potential therapeutic targets.
Commercial Relationships: Ping Yang, None; **Victoria Treboschi**, None; **Peter Baciu**, Allergan, Inc; **Glenn J. Jaffe**, None
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Program Number: 6532 **Poster Board Number:** D0281
Presentation Time: 11:00 AM–12:45 PM
Absence of collagen XVIII in mice causes age-related insuffiency in retinal pigment epithelium proteostasis
Niko Kivinen[1], Szabolcs Felszeghy[4], Mari Aikio[2, 3], Kati Kinnunen[1], Niko Setälä[5], Taina Pihlajaniemi[2, 3], Anu Kauppinen[6], Kai Kaarniranta[1, 7]. [1]Department of Ophthalmology, Kuopio University Hospital, Kuopio, Finland; [2]Oulu Center for Cell-Matrix Research, Biocenter Oulu, Oulu, Finland; [3]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [4]Department of Anatomy, Histology and Embryology, University of Debrecen, Debrecen, Hungary; [5]Department of Ophthalmology, Central Finland Central Hospital, Jyväskylä, Finland; [6]School of Pharmacy, University of Eastern Finland, Kuopio, Finland; [7]Department of Ophthalmology, University of Eastern Finland, Kuopio, Finland.

Purpose: Collagen XVIII, which has the structural properties of both collagen and proteoglycan, is a part of basement membranes (BMs). It has been found at the basement membrane/stromal interface and is thought to mediate their attachment. Endostatin, a fragment from Collagen XVIII C-terminal end which is released via proteolytic cleavage, has been reported to have anti-angiogenic effects. Age-related loss of vision in the collagen XVIII mutant mice is accompanied with pathological accumulation of deposits under the retinal pigment epithelium. We recently showed that impaired proteasomal and autophagy clearance associate with the pathogenesis of age-related macular degeneration. In this study we examined quantitatively the staining levels of both proteasomal and autophagy markers in the retinal pigment epithelium (RPE) of different ages of the *Col18a1*$^{-/-}$ mice.
Methods: Eyes from 3, 12 and 18 months old mice were enucleated and embedded in paraffin according to a routine protocol. Sequental 5 µm-thick parasagittal samples were immunostained for autophagy markers SQSTM1/p62 and Beclin proteasomal ubiquitin (Ub). The extent of immunopositivity in the retinal pigment epithelium (RPE) cells was evaluated during confocal microscopic analysis.
Results: Collagen XVIII knock-outs tissue of interest showed age-related RPE degeneration and accumulation of drusen-like deposits. Ub protein conjugate staining was prominent in both RPE cytoplasm and extracellular space whereas SQSTM1/p62 and beclin stainings were prominent in the basal part of RPE cell cytoplasm in the *Col18a1*$^{-/-}$ mice.
Conclusions: Distressed proteostasis regulated by collagen XVIII might be responsible for RPE degeneration, increased protein aggregation and finally lead to choroidal neovascularization.
Commercial Relationships: Niko Kivinen, None; **Szabolcs Felszeghy**, None; **Mari Aikio**, None; **kati kinnunen**, None; **Niko Setälä**, None; **Taina Pihlajaniemi**, None; **Anu Kauppinen**, None; **Kai Kaarniranta**, None
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Presentation Time: 11:00 AM–12:45 PM
Constitutive Activation of Mechanistic Target of Rapamycin Complex 1 (mTORC1) Caused Degeneration of the Retinal Pigment Epithelium (RPE)
Pei Xu[1], Bo Yu[1], Bo Long[1], Zhen-Yang Zhao[1], Alfred S. Lewin[2], Ralph Nichols[3], Theodore G. Wensel[4], Yan Chen[1]. [1]Department of Ophthalmology and Visual Science, University of Texas Medical Branch, Galveston, TX; [2]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; [3]Departments of Ophthalmology, Baylor College of Medicine, Houston, TX; [4]Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX.
Purpose: Signaling pathways mediated by mTORC1 play key roles in regulating cell metabolism, growth, autophagy, and immune responses. Our previous work demonstrated that RPE aging is associated with elevated mTORC1 activity. The causative link between aberrant mTORC1 activation and RPE degeneration has not been established. The goals of this study are to characterize the retinal phenotype of a conditional knockout mice with RPE-specific mTORC1 hyperactivation and to explore the underlying mechanisms.
Methods: Mice with constitutive mTORC1 activation in the RPE were generated by depleting its upstream suppressor protein tuberous sclerosis complex 1 (TSC1), through crossing *TSC1*$^{flox/flox}$ mice with BEST1-Cre transgenic C57BL/6J mice. The RPE-specific knockout of TSC1 and the resulting mTORC1 hyperactivation were confirmed by western blot or immunostaining of RPE flat mounts. The ocular phenotype was examined by fundus imaging, optical coherence tomography (OCT) and scanning laser ophthalmoscopy (SLO) in live animals, and was further validated by histopathology and transmission electron microscopy (TEM). Visual acuity was assessed by measuring the optokinetic responses. Selected downstream targets of mTORC1 were further analyzed by quantitative RT-PCR and western blot analyses.
Results: *TSC1*Δ^{RPE} mice displayed mTORC1 hyperactivation specifically in the RPE, without affecting the mTORC2 pathway. RPE pathology was evident in 5-week-old homozygous knockouts and progressed with age. Between 3 to 6 months, *TSC1*Δ^{RPE} mice showed extensive RPE hypo/hyperpigmentation, lipid droplet accumulation and subretinal cell infiltration. TEM detected membrane whorls between photoreceptor outer segments and apical surface of the RPE, fragmented mitochondria and other signs of defective vesicular trafficking in the RPE. The mRNA level of TFEB and VPS11 was reduced in *TSC1*Δ^{RPE} mice compared to *TSC1*$^{flox/flox}$, BEST1-Cre or wild type mice.
Conclusions: Hyperactivated mTORC1 can lead to RPE and photoreceptor degeneration, by mechanisms that involve disrupted membrane trafficking.
Commercial Relationships: Pei Xu, None; **Bo Yu**; **Bo Long**, None; **Zhen-Yang Zhao**, None; **Alfred S. Lewin**, None; **Ralph Nichols**, None; **Theodore G. Wensel**, None; **Yan Chen**, None
Support: NIH grants EY 019706,the BCM Vision Research Core EY002520,the International Retinal Research Foundation

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Presentation Time: 11:00 AM–12:45 PM
A mouse model to study Aβ-driven pathology in the ageing retina
Savannah A. Lynn, Srini Goverdhan, Rosie Munday, Jennifer Scott, Thomas Freeman, David Johnston, Andrew J. Lotery, J Arjuna Ratnayaka. Clinical and Experimental Sciences, The University of Southampton, Southampton, United Kingdom.
Purpose: The Alzheimer's-linked Amyloid beta (Aβ) peptide is reported to be deposited in aged retinas. Aβ has been implicated in key stages of Age-related Macular Degeneration (AMD), yet its role remains poorly understood. Here we employ a mouse model to study the in-vivo effects of Aβ to delineate its mechanisms of action and to understand how Aβ triggers/drives retinal pathology with age.
Methods: Aβ was characterised by negative stain TEM and immunogold labelling. C57BL/6 mice were subretinally injected with 3µL of oligomeric Aβ1-42 (625nM, n=5) or vehicle control (n=3). At 8 days post-injection eyes were enucleated, OCT-embedded and cryosectioned at 16µM intervals for histological analysis. H&E staining and confocal immunofluorescence analysed retinal morphology in response to Aβ exposure and reported Aβ localisation. Exclusion criteria included a 200µM radius from the injection site to omit areas of mechanical trauma. Morphometric analysis was performed blind using OlyVIA and ImageJ. Data is expressed as means ± SEM with a statistical significance of $*P \leq 0.05$.
Results: TEM and Dot Blot assay enabled us to identify a window in which Aβ is reported to be most toxic. Fundus images showed large areas of pathology in Aβ exposed mice (9582 ± 4831) compared to controls (48.33 ± 8.97) which were indistinguishable from non-injected littermates, p=0.12. Serial line scans of Aβ injected mice revealed a 2-fold increase in RPE hypopigmentation associated with photoreceptor outer segment (POS) loss, RPE disorganisation and RPE hypertrophy compared to controls. Similarly, confocal data showed POS and inner segment disorganisation compared to well-preserved retinal architecture in control retinas. Aβ was detected in multiple retinal locations including POS, the RPE/choroid interface and the outer and inner plexiform layers.

Conclusions: Our findings demonstrate that subretinal Aβ injections faithfully recapitulate key features of early AMD including dysfunctional RPE and damaged photoreceptors. Critically, we found no obvious indication of apoptosis or disruption of the blood-retinal barrier indicating gradual cellular impairment over time. Previously published literature showing static images of Abeta in human post-mortem eyes tantalisingly correlates the presence of Aβ with high drusen-loads and AMD. Our model therefore represents a powerful tool to investigate the dynamic nature of Aβ-mediated pathology in living retinas.
Commercial Relationships: Savannah A. Lynn, None; **Srini Goverdhan**; **Rosie Munday**, None; **Jennifer Scott**, None; **Thomas Freeman**, None; **David Johnston**, None; **Andrew J. Lotery**, None; **J Arjuna Ratnayaka**, None

Program Number: 6535 **Poster Board Number:** D0284
Presentation Time: 11:00 AM–12:45 PM
A primary retinal pigment epithelial (RPE) cell culture model produces lipid- and hydroxyapatite-rich extracellular deposits characteristic of early stage age-related macular degeneration
Matthew G. Pilgrim[1, 3], Antonio Lanzirotti[2], Matt Newville[2], Sarah Fearn[4], Jonathan Knowles[3], Jeffrey D. Messinger[5], Russell W. Read[5], Clyde Guidry[5], Christine A. Curcio[5], Imre Lengyel[1].
[1]Ocular Biology and Therapeutics, University College London Institute of Ophthalmology, London, United Kingdom; [2]Argonne National Laboratory, Chicago, IL; [3]UCL Eastman Dental Institute, London, United Kingdom; [4]Department of Materials, Imperial College London, London, United Kingdom; [5]Ophthalmology, University of Alabama at Birmingham, Birmingham, AL.
Purpose: Accumulation of lipid- and protein-rich drusen and basal linear deposits between the inner collagenous layer of the Bruch's membrane and the basal lamina of the RPE is a hallmark of early age-related macular degeneration (AMD). Hollow spherules composed of lipids and inorganic hydroxyapatite (HAP) have been described within these deposits (PMID 25605911). We report a primary RPE cell culture model capable of producing sub-RPE deposits with these major molecular components.
Methods: RPE cells were isolated from freshly enucleated porcine eyes and cultured on laminin-coated porous Transwell membranes (Millipore). Cells were cultured in Miller medium (PMID 16877436) without addition of photoreceptor outer segments for up to 6 months. Histochemical studies were performed and cultures were examined by light and fluorescence microscopy as well as by scanning (SEM) and transmission (TEM) electron microscopy. Mineral deposition was detected by micro-CT and synchrotron x-ray diffraction (μXRD), trace metal composition was determined using synchrotron x-ray fluorescence (μXRF), and protein and lipid components were identified using secondary ion mass spectrometry (SIMS).
Results: TEM analysis of differentiated and polarised RPE cell monolayers revealed an age-dependent accumulation of diffuse and focal deposits between the RPE cell monolayer and the underlying Transwell membrane. Deposits were highly mineralised and produced diffraction patterns characteristic of the inorganic calcium phosphate hydroxyapatite. Zinc and iron co-localized with hydroxyapatite, but copper signal could not be verified. SIMS analysis confirmed the presence of proteins and lipids in focal deposits.
Conclusions: Healthy primary RPE cell cultures produced focal and diffuse deposits containing lipids, proteins, trace elements and hydroxyapatite, all components of human drusen and basal linear deposits. These deposits formed without the addition of photoreceptor outer segments suggesting that RPE cells are capable of generating nucleation sites for sub-RPE deposit formation, this is consistent with previous in vitro (PMID 21969589) and pathology (PMID 21890786) studies. This cell culture model of early stage AMD provides a novel system for which new therapeutic interventions against early stages of AMD could be trialed.
Commercial Relationships: Matthew G. Pilgrim; **Antonio Lanzirotti**, None; **Matt Newville**, None; **Sarah Fearn**, None; **Jonathan Knowles**, None; **Jeffrey D. Messinger**, None; **Russell W. Read**, None; **Clyde Guidry**, None; **Christine A. Curcio**, Merck (C); **Imre Lengyel**, Unrestricted funding - Optos (F)

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Presentation Time: 11:00 AM–12:45 PM
Anti-proliferative properties of aflibercept on retinal cells in vitro
Volker Enzmann[1, 2], Lorenzo Parisi[1], Sebastian Wolf[1].
[1]Ophthalmology, University of Bern, Bern, Switzerland; [2]Clinical Research, University of Bern, Bern, Switzerland.
Purpose: Secretion of vascular endothelial growth factor (VEGF) is the cause of choroidal neovascularization (CNV), the hallmark of wet age-related macular degeneration (AMD). Intravitreal application of aflibercept suppresses the unwanted vascularization and improves visual function. Herein, we aim to investigate a direct effect of the anti-VEGF substance on different retinal cell types in vitro.
Methods: Concentration-dependent cellular effects of aflibercept (125, 250 μg/ml) were examined using the human Müller cell line MIO-M1, the murine photoreceptor-derived cell line 661W, cultured primary human retinal pigment epithelial cells (hRPE) and murine embryonic fibroblasts (MEF) serving as controls. Cell viability was quantified using the trypan blue exclusion test (400'000 cells/well) after 24 h and 48 h incubation. Cellular growth was detected serial dilution from 5000 to 300 cells/well by XTT-based colorimetric measurement four days after treatment. The anti-VEGF properties of the used substances were assessed by mouse and human-specific ELISAs.
Results: Aflibercept did not decrease cell survival of the tested cell types at the investigated concentrations and time points. However, the treatment decreased cell proliferation of retinal cells but not in the fibroblast controls in a dose-dependent manner. Thereby, the proliferation of MIO-M1 cells was not inhibited by aflibercept 125 μg/ml, whereas 250 μg/ml of the substance reduced the cell growth by 34% (p%0.01). A similar effect was seen in 661W cultures where the proliferation was significantly reduced by 29% (250 μg/ml, p%0.01). The proliferation of hRPE was diminished with both aflibercept concentrations by 12% (125 μg/ml, p≤0.0001) or 30% (250 μg/ml, p≤0.0001). VEGF secretion was significantly inhibited in all trials by ≥ 97% compared to the untreated controls.
Conclusions: The anti-VEGF active substance aflibercept is not toxic for retinal glia, photoreceptors and RPE cells in clinically relevant concentrations. However, it shows a retina-specific anti-proliferative effect.
Commercial Relationships: Volker Enzmann, None; **Lorenzo Parisi**, None; **Sebastian Wolf**

Program Number: 6537 **Poster Board Number:** D0286
Presentation Time: 11:00 AM–12:45 PM
Increased expression of osteopontin in retinal degeneration induced by blue-light-emitting diode exposure in mice
SuJin Park[1], Seung Wook Chang[1], Gyu Hyun Kim[1], Sun-Sook Paik[1], Hyung Il Kim[2], In-Beom Kim[1, 3]. [1]Dept. of Anatomy, The Catholic University of Korea, Seoul, Korea (the Republic of); [2]Gyeongju St. Mary's Eye Clinic, Gyeongju-si, Korea (the Republic of); [3]Catholic Neuroscience Institute, Seoul, Korea (the Republic of).
Purpose: Osteopontin (OPN) is a multifunctional adhesive glycoprotein implicated in a variety of proinflammatory as well as neuroprotective and repair-promoting effects in the brain

pathophysiology. In this study, as a first step to understand the role of OPN in retinal degeneration (RD), we examined changes in OPN expression in a mouse RD model induced by a blue light-emitting diode (LED) exposure.
Methods: RD was induced in BALB/c mice by exposure to a blue LED (460 nm) for 2 hours. Apoptotic cell death was evaluated by terminal deoxynucleotidyl transferase dUTP nick end labeling assay. Immunohiscothcemistry with anti-OPN was performed and compared to that with anti-glial fibrillary acidic protein (GFAP), a commonly used marker for retinal stress condition including inflammation.
Results: OPN expression in RD retinas was markedly increased at 24 h after blue LED, sustained by 72 h, and subsided at 120 h. Increased expression of OPN was found in microglial cells located in the outer nuclear layer (ONL), the outer plxiform layer (OPL), and the subretinal space where inner and outer segments of photoreceptors are located. This expression was restricted to central retina in which photoreceptor cell death occurred. Interestingly, all OPN in the ONL and the OPL, and some OPN plaques in subretinal space were closely associated with microglia, while most of OPN plaques in subretinal space were not. In addition, OPN was more acutely and legion-specifically expressed than GFAP.
Conclusions: These results indicate that OPN could be a useful marker for retinal stress or injury and might acts as a proinflammatory factor produced by microglia and a phagocytosis-inducing opsonin by binding mitochondiria or cell membrane in debris of photoreceptor outer and inner segments in RD retinas, suggesting that OPN plays an important role in RD pathogenesis.
Commercial Relationships: SuJin Park, None; **Seung Wook Chang**, None; **Gyu Hyun Kim**, None; **Sun-Sook Paik**, None; **Hyung Il Kim**, None; **In-Beom Kim**, None
Support: Basic Science Research Program through the NRF of Korea #2013R1A2A2A01014070

Program Number: 6538 **Poster Board Number:** D0287
Presentation Time: 11:00 AM–12:45 PM
Looking into the Crystallin Ball: αB-crystallin is cleaved coincident with inflammasome activation in retinal epithelial cells
Merideth K. Krevosky, Jeffery Bowen. Biological Sciences, Bridgewater State University, Bridgewater, MA.
Purpose: Inhibitors of apoptosis are upregulated in cancer, conferring cellular survival, while downregulated in degenerative diseases. αB-crystallin cleavage and inactivation is coincident with destructive endophthalmitis and loss of retinal function, supporting αB-crystallin's protective role in the retina. αB-crystallin is expressed in retinal pigmented epithelial (RPE) cells and in drusen of patients with age-related macular-degeneration (AMD). While this protein has been suggested as a potential biomarker for AMD, the functionality of this protective protein has not been characterized. Research implicates inflammation in retinal destruction during AMD which involves the inflammasome, a complex which promotes cell death via pyroptosis. Studies herein address the hypothesis that αB-crystallin is cleaved during inflammasome activation in RPE cells.
Methods: RPE cells were activated with IL-1a (10ng/ml) for 18h, treated for 2h with the lysosomal disrupting agent, L-Leucyl-L-leucine methyl ester (LeuLeuOMe, 1mM) and collected for lysosomal, immunocytochemical or Western Blot analysis.
Results: LeuLeuOMe treatment culminates in cell death and activation of the inflammasome, as evidenced by Caspase-1 activation. The number and size of lysosomes increase in response to LeuLeuOMe and αB-crystallin co-localizes in lysosomes, suggesting that this protein is trafficked to and destroyed in lysosomes, potentially preventing its protective role. Western blot analysis supports that αB-crystallin is cleaved in LeuLeuOMe-treated RPE cells suggesting that αB-crystallin is inactivated during inflammasome activation. Taken together, these results support that loss of αB-crystallin correlates with retinal cell destruction.
Conclusions: These studies support that cleavage of αB-crystallin may render it nonfunctional due to localization within cellular lysosomes. As such, cleavage of αB-crystallin likely abrogates its protective effects, which may underlie retinal cell destruction during inflammasome activation. Therefore, ongoing studies will determine whether the protein is exported from the cell, where it may accumulate in retinal spaces resulting in the hallmarks of AMD. Since few therapeutic interventions exist for AMD, modulation of αB-crystallin expression may promote retinal cell viability and prevent vision loss.
Commercial Relationships: Merideth K. Krevosky, None; **Jeffery Bowen**, None
Support: Bridgewater State University, Faculty Librarian Research Grant through the Center for Advancement of Research and Scholarship

Program Number: 6539 **Poster Board Number:** D0288
Presentation Time: 11:00 AM–12:45 PM
Complement is regulated in the RPE by both systemically derived and locally produced CFH
Marisol Cano[1], Lei Wang[1], Sayantan Datta[1], Jasmine Malhi[2], Tongyun Liu[1], Sonny Dike[1], James T. Handa[1]. [1]Ophthalmology, Johns Hopkins University, Baltimore, MD; [2]Johns Hopkins University, Baltimor, MD.
Purpose: AMD is a multifactorial disease with a contribution from genetic abnormalities in Complement factor H (CFH). CFH regulates C3, and is highly abundant in plasma, but can also be produced in the eye. Its origin, whether from the plasma or the RPE, under stress is unknown. This information is essential for designing effective therapy targeting complement regulation in the RPE. The purpose of this study was to determine the major source of CFH in the RPE, and how this source regulates complement C3.
Methods: MX1-cre-CFH$^{fl/fl}$ mice were injected intra-hepatically (IH), intravitreally (Ivt), or both IH and Ivt with ribonucleic acid (plpC) to reduce CFH in the liver, the eye, or both, respectively. Mice were then Ivt injected with 4HNE to activate complement. Total RNA and protein were extracted for RT-qPCR and Western analysis, respectively.
Results: Mice with Ivt injected PlpC had a 93% reduction in mRNA, but only a 33% reduction in CFH protein in the RPE compared to BSS injected animals ($p \leq 0.05$ and $p \leq 0.03$) while CFH mRNA and protein from the liver was unchanged. Mice with IH injected plpC had no change in CFH mRNA or protein in the RPE despite an 81% and 66% reduction in CFH mRNA and protein in the liver compared to BSS injected mice ($p \leq 0.02$ and $p \leq 0.03$ respectively). Mice that received Ivt and IH plpC injections had >90% reduction in CFH mRNA and protein in the RPE and liver (all $p \leq 0.04$), compared to BSS injected mice, while CFH was not reduced in BSS-injected mice. When stimulated with 4HNE, non-knockdown mice had increased CFH and C3d protein in the RPE compared to PBS injected mice ($p \leq 0.04$). In contrast, mice stimulated with 4HNE after Ivt OR IH plpC injection had no change in CFH or C3d protein in the RPE relative to PBS injected CFH knockdown controls. Mice stimulated with 4HNE after Ivt and IH plpC had unchanged C3d in the RPE compared to BSS injected double knockdown controls; however these mice were also depleted of C3 and CFB in the RPE, suggestive of hypocomplementemia.
Conclusions: Complement C3 regulation in the RPE relies on CFH derived from both systemic and ocular sources. Neither source alone

can sufficiently regulate C3. Further studies are required to address how this may affect the overall complement activation.
Commercial Relationships: Marisol Cano; **Lei Wang**, None; **Sayantan Datta**, None; **Jasmine Malhi**, None; **Tongyun Liu**, None; **Sonny Dike**, None; **James T. Handa**, None
Support: EY14005, EY019904, RPB Senior Scientist Award, and and Unrestricted grant from RPB. Dr. Handa is the Robert Bond Welch Professor.

Program Number: 6540 **Poster Board Number:** D0289
Presentation Time: 11:00 AM–12:45 PM
Expression patterns of the short fibulins in the rat retina
Cassandra L. Ondeck, Justin Etzel, Yuanjun Zhao, Alistair J. Barber, Jeffrey Sundstrom. Ophthalmology, Penn State Hershey College of Medicine, Hershey, PA.
Purpose: The fibulin family is comprised of seven glycoproteins (designated FBLN 1-7), thought to be secreted into the extracellular matrix and basement membranes, and binding with fibronectin, laminin and proteoglycans. The fibulins share several common features including repeated epidermal growth factor-like domains and a unique C-terminus domain. A point mutation in the EFEMP1 gene (encoding FBLN-3) is known to be associated with Doyne honeycomb retinal dystrophy. Several mutations in FBLN-5 are also associated with the development of age related macular degeneration. Despite the potential importance of the fibulins in retinal disease there has been little characterization of their expression patterns in the eye. Therefore the purpose of this study was to determine the expression patterns of the short fibulin proteins in the normal rat eye.
Methods: Eyes were enucleated from healthy male Long-Evans rats and preserved by flash-freezing. All procedures were in accordance with the Penn State Hershey College of Medicine IACUC and the ARVO guidelines for use of animals in ophthalmology research. Frozen sections were fixed and probed with primary antibodies to FBLN-3, -4, -5 and -7, and labeled for confocal microscopy imaging (Leica SP8).
Results: Confocal microscopy revealed positive immunoreactivity for FBLN-3 concentrated in the retinal pigment epithelia (RPE) with a punctate distribution, and in the extracellular space between RPE and photoreceptor outer segments (OS). Immunoreactivity for FBLN-4 and -5 appeared similar to each other, with diffuse distribution throughout the retina, concentrated in the plexiform layers and the OS. FBLN-7 immunoreactivity was located exclusively on the surface of the inner retina but did not colocalize with glial fibrillary acidic protein and was especially abundant at the optic nerve head.
Conclusions: Despite their relatively similar molecular structures the fibulins have a diverse distribution in the rat retina. FBLN-3 is exclusively expressed by the RPE and also appears to be part of a matrix in the space between the RPE and OS. FBLN-4 and -5 appear to be abundant throughout the cytoplasm of most retinal neurons and possibly Muller cells. FBLN-7 appears to be exclusively expressed in the axoplasm of retinal ganglion cells and is heavily concentrated at the head of the optic nerve. Given the diverse distribution of fibulins it is likely that these proteins have different functions in the retina.
Commercial Relationships: Cassandra L. Ondeck, None; **Justin Etzel**, None; **Yuanjun Zhao**; **Alistair J. Barber**, None; **Jeffrey Sundstrom**, None
Support: Penn State Hershey Physician Scientist Startup (JMS)

Program Number: 6541 **Poster Board Number:** D0290
Presentation Time: 11:00 AM–12:45 PM
Fibulin-3 expression in healthy and diseased human retina
Justin Etzel, Cassandra L. Ondeck, Yuanjun Zhao, Alistair J. Barber, Jeffrey Sundstrom. Ophthalmology, Penn State Hershey College of Medicine, Hershey, PA.
Purpose: Little is known about Fibulin-3 (FBLN-3) expression in the human retina. A point mutation in the EFEMP1 gene (encoding FBLN-3) is known to be associated with Doyne honeycomb retinal dystrophy, an inherited macular degeneration associated with drusen formation at a young age. However, the role of FBLN-3 in drusen formation and disease pathogenesis remains unclear. The purpose of this study was to determine the expression pattern of FBLN-3 in sections of healthy and diseased human retina.
Methods: Fresh frozen and paraffin-embedded sections of human eyes were procured from NDRI. Donors had either no history of eye disease or were known to have age-related macular dystrophy. Sections were probed with primary antibody to FBLN-3, and labelled for confocal microscopy imaging (Leica SP8). Sections were also labelled for the drusen marker, ApoE.
Results: Confocal microscopy revealed dense FBLN-3 immunoreactivity in the retinal pigment epithelial cells (RPE) in both healthy and diseased retinas. Immunoreactivity for ApoE identified numerous drusen beneath the RPE cells. Punctate FBLN-3 immunoreactivity was located within drusen in some places but generally did not colocalize with ApoE.
Conclusions: FBLN-3 is expressed abundantly within the human RPE and does not appear to be expressed in other parts of the eye. ApoE was a good marker for drusen in this study and FBLN-3 appeared within small vesicle-like structures within the drusen. The abundance of FBLN-3 within RPE suggests that it has an important function there and may accumulate to some degree in drusen.
Commercial Relationships: Justin Etzel, None; **Cassandra L. Ondeck**, None; **Yuanjun Zhao**; **Alistair J. Barber**, None; **Jeffrey Sundstrom**, None
Support: Penn State Hershey Physician Scientist Startup (JMS)

Program Number: 6542 **Poster Board Number:** D0291
Presentation Time: 11:00 AM–12:45 PM
XIAP Involvement in RPE Inflammasome and Cell Death Regulation
Jiangyuan Gao[1], Sijia Cao[1], Aikun Wang[1], Jing Z. Cui[1], Patrick L. McGeer[2], Joanne A. Matsubara[1]. [1]Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada; [2]Kinsmen Lab of Neurological Research, University of British Columbia, Vancouver, BC, Canada.
Purpose: Age-related macular degeneration (AMD) is a neurodegenerative disease that features cell death in retinal pigment epithelium (RPE) and photoreceptor. X-linked inhibitor of apoptosis (XIAP), an anti-apoptotic protein, used in models of retinal degeneration, has recently been implicated in regulating inflammasome activity in non-ocular, immune cells. The purpose of this study is to test the merit of XIAP being a dual regulator of inflammasome activation and cell death.
Methods: Adult Long-Evans rats were divided into three treatment groups. The first group used a complement cascade inhibitor, aurin tricarboxylic acid complex (ATAC). Following our published protocols, naive rats received ATAC in drinking water for 40 days, or drinking water alone, and then sacrificed. The second group used an NF-κB inhibitor, vinpocetine. All rats first received an intraocular injection of Aβ, a component of drusen, followed by either a daily I.P. injection of vinpocetine for 3 consecutive days, or an I.P. injection of

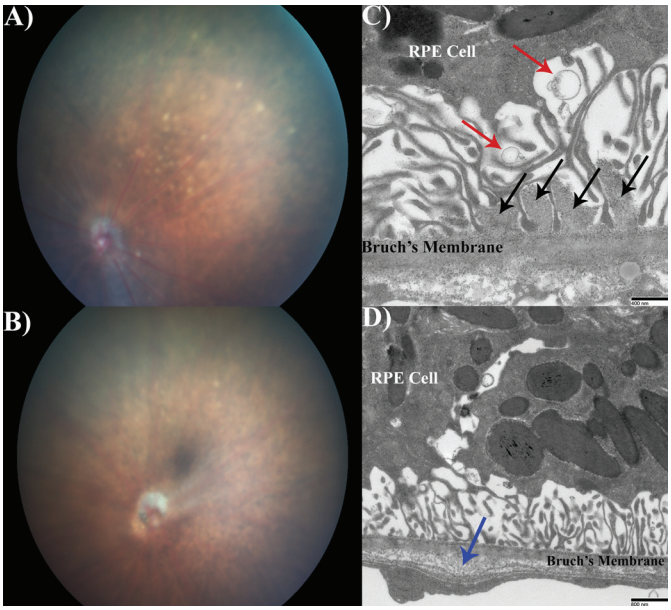
vehicle (DMSO). In the third group, each rat received one intraocular injection of either Aβ or reverse Aβ every 4 days, for 3 times.
Results: Our earlier work demonstrated that inhibition of the NF-κB pathway by vinpocetine reduced Aβ induced caspase-1 cleavage in the eye. We also reported that by suppressing the end product of complement activation, membrane attack complex with ATAC, caspase-1 cleavage is diminished in the eye as well. Here, we showed in the same experimental scenarios, XIAP protein level is lowered by ATAC, but not by vinpocetine, suggesting XIAP might associate with the assembly of NLRP3 inflammasome. Moreover, compared to the control animals, the multiple Aβ treatment group showed a significant decrease in both mRNA and protein levels of XIAP, with a concomitant increase in RIP1, ZO-1, and RPE65 mRNAs, indicating a decline in RPE homeostasis.
Conclusions: Despite the fact that XIAP is a classic anti-apoptotic factor, little is known about its role in regulating inflammatory responses. This study provides proof-of-evidence that XIAP is a potential novel candidate for modulating (suppressing) caspase-1 dependent inflammasome activation. Given that chronic inflammation and RPE apoptosis are features in the pathogenesis of AMD, further investigation to fully characterize XIAP's dual role as an inhibitor of caspase-3 dependent apoptosis and caspase-1 dependent inflammasome activation seems warranted.
Commercial Relationships: Jiangyuan Gao, None; **Sijia Cao**, None; **Aikun Wang**, None; **Jing Z. Cui**, None; **Patrick L. McGeer**, Aurin Biotech Inc (P); **Joanne A. Matsubara**, None
Support: CIHR MOP126195

Program Number: 6543 **Poster Board Number:** D0292
Presentation Time: 11:00 AM–12:45 PM
The combined effect of drusen component amyloid beta and oxidative stress on polarized retinal pigment epithelium
Sijia Cao, Jiangyuan Gao, Jing Z. Cui, Joanne A. Matsubara. Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada.
Purpose: Multiple risk factors contribute to the development of age-related macular degeneration (AMD), but less is known of the combined effect of these risk factors on the retinal pigment epithelium (RPE), the cell type first injured in the disease process. Here we investigated the combined effect of two risk factors – a drusen component (amyloid beta, Aβ) and oxidative stress on RPE in terms of expression of inflammatory mediators and complement genes using a polarized cell model of ARPE19.
Methods: To measure NF-κB activation, ARPE19/NF-κB-luciferase reporter cells were established and cultured on Transwell inserts to form a polarized monolayer. The cells were exposed on the apical surface to oxidative stressor H_2O_2 and/or basolaterally to oligomeric $Aβ_{1-40}$. Gene expression of inflammatory mediators (caspase-1, IL-18, IL-6, IL-8, MCP-1, TNF-α) and complement components (CD46, CD55, CD59, factor B, factor D, factor H, factor I, C3 and C5) was measured by quantitative polymerase chain reaction (PCR). Results were analyzed with Student's t test and significance was set at $p < 0.05$.
Results: The combined stimulation with H_2O_2 apically and Aβ basolaterally promoted NF-κB activation, upregulated IL-18 and MCP-1 (mean fold change normalized to control ± standard error of mean: 2.7±0.1, 3.8±0.3, and 1.5±0.0, respectively, N=3). Stimulation with Aβ or H_2O_2 alone induced an upregulation of IL-18 (2.4±0.5, 4.3±0.5, N=3) and exposure to H_2O_2 alone also caused NF-κB activation (2.6±0.3, N=3). Single stimulation had no effect on MCP-1 expression. Regarding the complement genes, the combined stimulation upregulated CD46, CD55, and C3 (1.5±0.1, 1.5±0.1, and 1.6±0.4, respectively), while stimulation with Aβ or H_2O_2 alone had no effect on any of complement components studied.
Conclusions: The combined effect of drusen component Aβ and oxidative stressor H_2O_2 achieved a greater effect on RPE compared to either stimulus alone. Our results suggest there may be a differential response to stimuli on the apical and basal surfaces and multiple risk factors may contribute to AMD disease processes through regulation of inflammatory and complement genes. Future studies will allow further insight into the interactions between risk factors to better understand AMD pathogenesis.
Commercial Relationships: Sijia Cao, None; **Jiangyuan Gao**, None; **Jing Z. Cui**, None; **Joanne A. Matsubara**, None
Support: CIHR Grant MOP-126195

Program Number: 6544 **Poster Board Number:** D0293
Presentation Time: 11:00 AM–12:45 PM
NAMPT inhibitors are Selectively Toxic to Human Pluripotent Stem Cells (hPSC) and can Minimize Tumorigenic Potential of hPSC-derived RPE cells
Qinghui Hu[1, 3], Amy C. Lo[2, 3], David Wong[3], Kenneth R. Boheler[1, 3]. [1]School of Biomedical Sciences, The University of Hong Kong, Hong Kong, Hong Kong; [2]Department of Ophthalmology, The University of Hong Kong, Hong Kong, Hong Kong; [3]LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, Hong Kong.
Purpose: This study aims to determine the ability of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors to selectively eliminate undifferentiated and tumorigenic cells from hPSC cultures differentiating to retinal pigmented epithelial (RPE) cells.
Methods: We cultured and differentiated hPSCs to neural progenitors and RPE cells, and studied ARPE19 cells. STF31 and FK866, inhibitors of the NAD salvage pathway protein NAMPT, were added in a dose- and time-dependent manner and cell viability assays (Neutral Red and XTT) performed. Teratoma assays are performed in NOD-SCID mice.
Results: Human PSCs differentiated in vitro to RPE cells exhibit epithelial cell morphology, visible pigment from week 4 of differentiation, and contained RNA (BEST1, MITF) markers typical of RPE cells. The putative hPSC-derived RPE cell populations are ~70% pure, and work is on-going to improve RPE purity for functional assessments and for viability testing. To evaluate selective toxicity of NAMPT inhibitors on cells of the neuroectoderm lineage, human ES cells (H7), iPS cells (KB3), PAX 6-positive neural progenitors and ARPE 19 cells were treated. NAMPT inhibitors were highly toxic to hPSCs (0-5% viable cells) within 48-72 hours. PAX6 positive cells showed some decrease in viability (50-60% viable cells) at 72 hours. ARPE19 cell toxicity was minimal. Teratoma assays following 24-hour treatment of hPSCs did not completely prevent hPSC tumour formation, but longer treatment times appear effective.
Conclusions: Elimination of tumorigenicity is a prerequisite for clinical intervention. NAMPT shows selective cell toxicity: hPSCs>>neural progenitors> ARPE19 cells. Purified hPSC-derived RPEs are likely to show limited toxicity to NAMPT, suggesting that treatment of these cultures with NAMPT inhibitors prior to transplantation will effectively eliminate all tumorigenic potential from these cultures – thus advancing efforts to treat Aging-related Macular Degeneration in humans.
Commercial Relationships: Qinghui Hu, None; **Amy C. Lo**; **David Wong**, None; **Kenneth R. Boheler**, None
Support: Seed Funding Programme for Basic Research at HKU and Health and Medical Research Fund, Hong Kong

Program Number: 6545 **Poster Board Number:** D0294
Presentation Time: 11:00 AM–12:45 PM
Identification of low-density lipofuscin in human retinal pigment epithelial (RPE) cells
Rong Wen[1], Ziqiang Guan[2], Shuliang Jiao[3], Byron L. Lam[1], Yiwen Li[1].
[1]Bascom Palmer Eye Institute, University of Miami, Miami, FL; [2]Dept of Biochemistry, Duke University Medical Center, Durham, NC; [3]Dept of Biomedical Engineering, Florida International University, Miami, FL.
Purpose: Lipofuscin and A2E accumulate in human RPE with age and this accumulation is implicated in the pathogenesis of age-related macular degeneration. The present work examines the biochemical and fluorescence properties of low-density lipofuscin-like granules isolated from human RPE.
Methods: Human eyes (donor age 70-90 years) were obtained from Florida Lions Eye Bank. The anterior section and the retina of an eye were carefully removed. The RPE cells were collected in PBS (phosphate buffered saline). The cell suspension was centrifuged at 8,000 g for 10 min to remove the supernatant and the cells were stored at -80°C. To isolate the lipofuscin granules, the cells were homogenized, resuspended in 0.3 M sucrose, and layered onto a discontinuous sucrose gradient of 3 different concentrations: 2, 1.4, and 1.2 M. Four distinguished bands were obtained after centrifugation at 103,000 g for 1 hr, Layer 0 (L0) at the interface of 0.3 and 1.2 M; L1 between 1.2 and 1.4 M, L2 between 1.4 and 2.0M, and M at the bottom. Each band was washed with H_2O, centrifugation at 16,800 g for 10 min, and the supernatant was removed. Sample was air dried until the reflection of the sample surface disappeared. Each sample was then weighed to obtain its wet weight and resuspended in H_2O at 50 µg/µl. The fluorescence spectra in L0 and L1 were examined by a commercial spectrometer together with custom-made excitation delivering and fluorescence detecting optics. The concentrations of A2E of L0 and L1 were measured by normal phase liquid chromatography/mass spectrometry (LC/MS) using electrospray ionization (ESI) in the positive ion mode.
Results: Both L0 and L1 contain high amount of A2E and *iso*-A2E (both detected by MS at *m/z* 592.4), with the content in L0 being more than 5 times of that in L1. The fluorescence spectra of L0 and L1 with 488 nm excitation are identical, but the intensity of fluorescence emission in L0 is 10 times of that in L1.
Conclusions: L1 has been conventionally regarded as to contain most of the lipofuscin granules in the RPE. Our results, however, showed that low-density L0 granules have identical fluorescence properties as L1 and with much higher intensity. In addition, the A2E content in L0 granules is much higher than that in L1 granules. Thus, the previously overlooked L0 granules should be classified as "low-density lipofuscin", and included in the study of the composition and toxicity of lipofuscin.
Commercial Relationships: Rong Wen, None; **Ziqiang Guan**, None; **Shuliang Jiao**, None; **Byron L. Lam**, None; **Yiwen Li**, None
Support: Supported by NIH grants R01EY023666, P30-EY014801, Adrienne Arsht Hope for Vision fund, and an unrestricted grant from Research to Prevent Blindness, Inc.

Program Number: 6546 **Poster Board Number:** D0295
Presentation Time: 11:00 AM–12:45 PM
Generation and Phenotyping of *P2x7r* and *Sod1* Double-Knockout Mice
Kyle Carver, Naheed W. Khan, Cheng-mao Lin, Dongli Yang.
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI.
Purpose: Chronic oxidative stress (OS) contributes to AMD. In mice, chronic OS induced by Cu, Zn-superoxide dismutase (*Sod1)* knockout (KO) leads to features typical of age-related macular degeneration (AMD) in humans. However, the underlying mechanisms of AMD-like pathology in *Sod1* KO mice are not fully understood. The P2X7 receptor (P2X7R) is an ATP-gated ion channel expressed in the retinal pigment epithelium (RPE) and neural retina. It mediates multiple critical signaling pathways in the RPE and AMD. We hypothesized that P2X7R deficiency has a protective role in *Sod1* KO mice. The purpose of this study was to generate and phenotype *P2x7r* and *Sod1* double-knockout (DKO) mice with particular emphasis on microparticle (MP) release, drusen-like deposit formation, and retinal function.
Methods: *P2x7r* KO and *Sod1* KO mice were obtained from Jackson Laboratories, and cross-bred to generate all colonies: wild type (WT), *P2x7r* KO, *Sod1* KO, and *P2x7r*/*Sod1* DKO. MPs were isolated from RPE/choroid complex and quantified by flow cytometry (FC). Drusen-like deposits were examined by fundus imaging and transmission electron microscopy (TEM). Retinal function was examined using the electroretinogram (ERG).
Results: We generated *P2x7r*/*Sod1* DKO and detected MPs isolated from RPE/choroid complex by FC. MP counts were increased in *Sod1* KO mice which were attenuated in *P2x7r*/*Sod1* DKO mice. WT, *P2x7r* KO, and *P2x7r*/*Sod1* DKO littermates had limited evidence of drusen-like deposits in fundus images while drusen-like deposits were present in *Sod1* KO mice. The number of drusen per eye was increased in *Sod1* KO mice compared to all other groups. TEM showed that *Sod1* KO mice developed basal laminar and linear deposits with MP size vesicles present near the deposits. Dark-adapted ERG a- and b-waves were reduced in *Sod1* KO mice but were within normal range in the *P2x7r*/*Sod1* DKO mice.
Conclusions: The results indicate that P2X7R deficiency protects against the release of MPs, formation of drusen, and loss of retinal function induced by OS in *Sod1* KO mice, suggesting that P2X7R could be critical to MP release and drusen formation under chronic OS.



Drusen-like deposits observed in *Sod1* knockout (KO) (A) are attenuated in *P2x7r*/*Sod1* double KO (B) mice. Transmission electron microscopy of basal laminar deposits (C, black arrows) and basal linear deposits (D, blue arrow) in *Sod1* KO mice with microparticle sized vesicles present (C, red arrows).

Commercial Relationships: Kyle Carver, None; **Naheed W. Khan**, None; **Cheng-mao Lin**, None; **Dongli Yang**, None
Support: NIH Grant P30EY007003 (core) and University of Michigan Start-Up Funds (DY)

Program Number: 6547 **Poster Board Number:** D0296
Presentation Time: 11:00 AM–12:45 PM
Inhibition of phagocytic activity of ARPE-19 cells induced by lipofuscin mediated photic stress - effects of donor's age and antioxidants
Magdalena M. Olchawa, Justyna Furso, Grzegorz Szewczyk, Anna K. Pilat, Tadeusz J. Sarna. Biophysics, Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland.
Purpose: It is believed that accumulation of lipofuscin granules (LFG) in the aging retinal pigment epithelium (RPE) may increase the susceptibility of the cells to photic stress and could play a role in pathogenesis of age related macular degeneration (ARMD). In this study we tested if sub-lethal oxidative stress induced by irradiation of ARPE-19 cells containing phagocytized LFG isolated from donors of different age affected the cell phagocytic activity and whether combination of selected antioxidants could modify the observed outcomes by changing phototoxic potential of lipofuscin.
Methods: ARPE-19 cells pre-loaded with LFG, isolated from human RPEs from donors of different age or containing LFG enriched with a combination of zeaxanthin and α-tocopherol (LFG-A) were irradiated with blue light for selected time intervals to produce sub-lethal damage in cells. The specific phagocytosis of FITC-labeled POS isolated from bovine retinas was analyzed by flow cytometry 5.5 h after or 24 h after light irradiation. Photoreactivity of LFG and LFG-A was analyzed by measuring oxygen photo-consumption and by photo-generation of superoxide anion employing electron paramagnetic resonance (EPR) oximetry and spin trapping.
Results: Sub-lethal irradiation or ARPE-19 cells containing phagocytized LFG with blue light induced marked inhibition of the specific phagocytosis of POS with the effect being substantially stronger for LFG obtained from older donors (50-59 y/o) compared to younger donors (18-29 y/o). The inhibitory effect of LFG was significantly reduced by their enrichment with combination of antioxidants. The observed rates of photo-induced oxygen uptake and accumulation of superoxide spin adduct indicated that aerobic photoreactivity of lipofuscin increased with donor's age and decreased after enrichment with antioxidants.
Conclusions: Although phototoxic potential of LFG increases with aging and could contribute to impairment of phagocytic activity of the aged RPE, natural antioxidants such as zeaxanthin and α-tocopherol might help to retain this important RPE function by reducing lipofuscin photoreactivity.
Commercial Relationships: Magdalena M. Olchawa, None; **Justyna Furso**, None; **Grzegorz Szewczyk**, None; **Anna K. Pilat**, None; **Tadeusz J. Sarna**, None
Support: Grant MAESTRO4 2013/08/A/NZ1/00194 from the NCN (TS). Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education.

Program Number: 6548 **Poster Board Number:** D0297
Presentation Time: 11:00 AM–12:45 PM
Early Sub-retinal Delivery of AAV1-*Sod2* Protects the Retina in a Mouse Model of Progressive Retinal Degeneration
Manas Ranjan Biswal, Cristhian J. Ildefonso, Bradley D. Justis, Hong Li, Pingyang Han, Ping Zhu, Lindsey E. Buz, Chulbul M. Ahmed, Alfred S. Lewin. University of Florida, Gainesville, FL.
Purpose: The purpose of our study is to test the sub-retinal delivery of a *Sod2* viral vector in a mouse model of geographic atrophy and to determine how long in the disease course this vector will protect the RPE layer from oxidative damage, induced by the genetic deletion of *Sod2*, once the retinal degeneration is underway.
Methods: We developed a mouse model of retinal oxidative stress (*Sod2*$^{flox/flox}$*/VMD2-cre* mice) caused by RPE-specific deletion of *Sod2,* the mitochondrial isoform of MnSOD. These mice show age-dependent retinal degeneration that recapitulates some of the features of dry AMD. We designed *Sod2 gene therapy vector* by assembling the mouse *Sod2* gene with a Myc epitope under the control of a small chicken beta- actin promoter (smCBA) and this vector was packaged into self-complementary AAV serotype 1 vector (AAV1) for RPE specific transduction. We injected subretinally with AAV1-*Sod2* or AAV1-GFP vector into the opposite eyes of *Sod2*$^{flox/flox}$*/VMD2-cre* mice at 6weeks (10 mice), 3 months (9 mice) and 6 months (6 mice) of age. Over a period of 9 months, we monitored retinal degeneration at different time points by electroretinography (ERG) and spectral domain optical coherence tomography (SD-OCT). We used western blotting to determine exogenous myc tagged *Sod2* expression by an anti-myc antibody.
Results: One month after sub retinal delivery, AAV1-*Sod2* vector resulted in production of myc-tagged MnSOD in the RPE and negligible expression in the neural retina. Fundus imaging showed the widespread expression of GFP by control vector validating the sub-retinal delivery of our vectors. We did not find any adverse functional and structural integrity due to increased expression of *Sod2*. ERG response and thinning of retinal thickness (SD-OCT) was significantly delayed in *Sod2* vector injected eyes to control eye injected with GFP in the eyes of mice treated at 6 weeks compared with those treated at 3 and 6 months of age.
Conclusions: Early delivery of AAV1-*Sod2* vector can be used as a tool to reverse oxidative stress in this mouse model of dry AMD. At later times, damage from oxidative stress may not be reversible.
Commercial Relationships: Manas Ranjan Biswal, None; **Cristhian J. Ildefonso**, None; **Bradley D. Justis**; **Hong Li**, None; **Pingyang Han**, None; **Ping Zhu**, None; **Lindsey E. Buz**, None; **Chulbul M. Ahmed**, None; **Alfred S. Lewin**, None
Support: Florida Biomedical Research program grant e10KG-08; NEI grant R01 EY02025; Vision Core grant EY02172.

Program Number: 6549 **Poster Board Number:** D0298
Presentation Time: 11:00 AM–12:45 PM
The Protective Effect of Bax Inhibitor 1 (BI-1) on Tunicamycin-induced Human RPE Cell Death
Victoria Treboschi, Zhe Ma, Grace Tewkesbury, Ping Yang, Glenn J. Jaffe. Ophthalmology, Duke University, Durham, NC.
Purpose: In eyes with geographic atrophy (GA) associated with age-related macular degeneration (AMD), there is apoptotic and non-apoptotic RPE cell death. Bax Inhibitor 1 (BI-1) is an ER-associated protein that protects against BAX-mediated apoptosis. In the current study, we determined whether BI-1 is expressed in human retinal pigment epithelial (hRPE) cells and if it can protect against tunicamycin-induced cell death.

Methods: Endogenous BI-1 mRNA expression was evaluated by qPCR and protein expression by immunofluorescent staining of cultured confluent hRPE cells. Cell death in hRPE by tunicamycin, a known apoptosis inducer, was evaluated at different concentrations by Western Blot for caspase 3 cleavage. To achieve optimal BI-1 overexpression, hRPE cells were infected with LacZ or BI-1 adenovirus for 24 hours, and BI-1 protein levels were assessed by Western Blot at various times after infection. To test the effect of BI-1 transfection on RPE cell death, LacZ or BI-1 infected cells were treated at 5 days post-infection with 20-30 ug/mL tunicamycin for 0-24 hours in 1% FBS MEM. Cell morphology was assessed by light microscopy. Cell death was determined by DNA fragmentation and cell permeability ELISA assay.
Results: Human RPE cells expressed endogenous BI-1 mRNA and RPE cell BI-1 protein was detected by immunofluorescence. Tunicamycin treatment induced RPE cell death in a dose-dependent manner. After cells were transfected with the BI-1 gene, BI-1 protein was detected by 3 days post-transfection and continued to increase 10-12 days post-transfection. BI-1-transfected RPE cells were resistant to the death inducing effect of tunicamycin; morphologically this BI-1 protective effect was evident after 4 hours of 30 ug/mL tunicamycin treatment and persisted 24 hours later. On DNA permeability ELISA assay, cell permeability was significantly lower in BI-1 than LacZ transfected cells after 24 hours of treatment with 30 ug/mL tunicamycin ($p = .005$), but DNA fragmentation was not significantly different after this treatment.
Conclusions: BI-1 is endogenously expressed in hRPE cells, and, when over-expressed, protects against tunicamycin-induced RPE cell death. BI-1 may serve as a native RPE survival protein that protects cells from endogenous and exogenous death-inducing stimuli. Strategies to up-regulate this molecule may be a novel approach to prevent RPE cell death in eyes with AMD.
Commercial Relationships: Victoria Treboschi; **Zhe Ma**, None; **Grace Tewkesbury**, None; **Ping Yang**, None; **Glenn J. Jaffe**, None
Support: NIH 5P30EY005722 (Core grant), Research to Prevent Blindness, Inc. (RPB)

Program Number: 6550 **Poster Board Number:** D0299
Presentation Time: 11:00 AM–12:45 PM
Stereoselective transcriptional modulation of inflammasome in hRPE cells by the docosanoid Neuroprotectin D1 (NPD1)
Aram Asatryan[2, 1], Nicolas G. Bazan[2, 1]. [1]Ophthalmology, LSUHSC, River Ridge, LA; [2]Neuroscience, LSUHSC, New Orleans, LA.
Purpose: Age-related macular degeneration (AMD) shares pathological features, including uncompensated oxidative stress stimuli and inflammation with other neurodegenerative diseases such as Alzheimer's disease. It has been shown that drusen isolated from human eyes with AMD activates the NLRP3 inflammasome. We found that the lipid mediator NPD1, produced in response to oxidative stress, down-regulates inflammasome-related genes, including AIM2, NLRC4, NOD-2, and Il-1β. NOD-2 is of particular interest since mutations of it are associated with Blau syndrome, which results in uveitis and retinal damage in childhood. We hypothesized that NPD1 regulates the expression of inflammasome components at a transcriptional level and that this modulatory bioactivity is stereospecific.
Methods: To define the effect of NPD1 on inflammasomes, we used human retinal pigment epithelial (hRPE) cells as an *in vitro* model. We have shown previously that oxidative stress triggers activation of certain inflammasome-related genes, including NOD-2. To assess whether this effect is stereoselective, we induced oxidative stress in hRPE cells with 600μM of H_2O_2/TNFα (10ng/ml) and co-treated cells with NPD1, its stereoisomers, resolvin D1, resolvin E1 and lipoxin A4 at 200nM concentration each. Extracted RNA was analyzed using RT-PCR. To understand the scope of NPD1 action, NOD-2 promoter was subcloned into PGL4 luciferase vector and co-transfected into hRPE cells with GFP vector to analyze NOD-2 promoter activity in response to oxidative stress.
Results: As observed previously, oxidative stress triggered the expression of NOD-2 gene. NPD1 had the greatest effect and down-regulated NOD-2 expression more than 2.5 fold. The results of the luciferase assay demonstrate that NPD1 suppressed the transcriptional activation of NOD-2 gene two fold 2h after introduction of oxidative stress.
Conclusions: Our results demonstrate that NPD1 suppresses expression of the pro-inflammatory gene NOD-2 at the transcriptional level and that this effect is stereoselective. NOD-2 is an intracellular pattern-recognition receptor and has a synergistic effect on NLRP3 inflammasome activation. It forms so called "nodosome" that results in the activation of pro-inflammatory NFkB. NOD-2 is also involved in activation of caspase 1 and Il-1β, however this requires the presence of NLRP3.
Commercial Relationships: Aram Asatryan, None; **Nicolas G. Bazan**, None
Support: NEI grant 005121 (NGB), Research to prevent Blindness

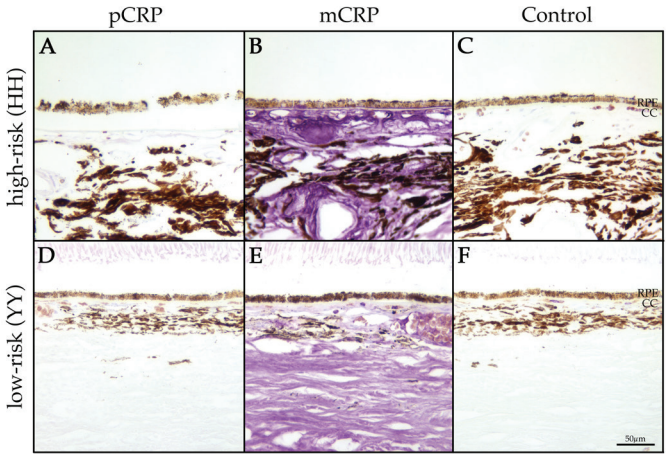
Program Number: 6551 **Poster Board Number:** D0300
Presentation Time: 11:00 AM–12:45 PM
Nucleoside reverse transcriptase inhibitors are anti-inflammatory and inhibit poly I:C-induced retinal pigment epithelium degeneration
Shinichi Fukuda, Junichi Fukuhara, Younghee Kim, Benjamin Fowler, Tetsuhiro Yasuma, Reo Yasuma, Takako Fukuhara, Nagaraj Kerur, Bradley D. Gelfand, Jayakrishna Ambati. Ophthalmology & Visual Science, University of Kentuchy, Lexington, KY.
Purpose: Age-related macular degeneration (AMD) is the leading cause of legal blindness among people aged over 60 years especially in developed countries. Double stranded RNAs including *Alu* RNA and Poly I:C are toxic to RPE cells. We recently demonstrated that *Alu* RNA-induced RPE toxicity, which is mediated by the NLRP3 inflammasome, is prevented by nucleoside reverse transcriptase inhibitors (NRTI), a widely used class of drugs to treat HIV. We and others have previously reported that poly I:C (pIC), a synthetic high-molecular weight dsRNA induces retinal degeneration with large areas of RPE cell loss and apoptosis of adjacent photoreceptors. Since pIC is also known to activate NLRP3 inflammasome, we hypothesized that NRTIs could block pIC-driven inflammasome activation and RPE cytotoxicity in cell culture and a mouse model of RPE degeneration.
Methods: For the mouse model of dry AMD, RPE degeneration was induced by subretinal injection of pIC. Twice daily intraperitoneal administration of NRTI lamivudine (3TC) (75 mg/kg/day) in wild-type male C57BL6/J mice was performed for one week after pIC administration. RPE degeneration was assessed by fundus photography and ZO-1 staining of RPE flat mounts. For cell culture, primary human RPE cells were maintained in DMEM supplemented with 10% FBS and antibiotics. Transfection of pIC was achieved by supplementing the culture medium for 24 hr. Cells were treated with NRTI lamivudine (3TC). NLRP3 inflammasome activation was monitored by western blot for Caspase-1.
Results: NRTI lamivudine (3TC) blocked RPE cell death and inflammasome activation induced by pIC. Intraperitoneal administration of the NRTI lamivudine, at a dose equivalent to what is administered in humans, prevented RPE degeneration in the pIC-induced mouse model of dry AMD.

Conclusions: We have identified that, the novel anti-inflammatory activity of NRTIs can be exploited to prevent the RPE toxicity in a mouse model of dry AMD. Therefore the FDA approved NRTI compounds can be ideally repurposed for therapeutic use in dry AMD.
Commercial Relationships: Shinichi Fukuda, None; **Junichi Fukuhara**, None; **Younghee Kim**, None; **Benjamin Fowler**, None; **Tetsuhiro Yasuma**, None; **Reo Yasuma**, None; **Takako Fukuhara**, None; **Nagaraj Kerur**, None; **Bradley D. Gelfand**, None; **Jayakrishna Ambati**, Inflammasome Therapeutics (I), iVeena Pharmaceuticals (I), iVeena Holdings (I), iVeena Pharmaceuticals (S), University of Kentuchy (P), Inflammasome Therapeutics (S), iVeena Holdings (P), Allergan (R), iVeena Holdings (S), iVeena Pharmaceuticals (P), Olix Pharmaceuticals (F), iVeena Delivery Systems (I), iVeena Delivery Systems (S), iVeena Delivery Systems (P)

Program Number: 6552 **Poster Board Number:** D0301
Presentation Time: 11:00 AM–12:45 PM
Nucleoside reverse transcriptase inhibitors attenuate amyloid-beta-induced retinal pigment epithelium degeneration
Takako Fukuhara, Junichi Fukuhara, Younghee Kim, Tetsuhiro Yasuma, Reo Yasuma, Benjamin Fowler, Shinichi Fukuda, Nagaraj Kerur, Bradley D. Gelfand, Jayakrishna Ambati. University of Kentucky, Lexington, KY.
Purpose: Age-related macular degeneration (AMD) is a leading cause of blindness in the elderly and is projected to afflict up to 200 million people worldwide by the year 2020; it is untreatable in the majority of cases. Recently studies from multiple groups have implicated NLRP3 inflammasome activation in AMD pathogenesis. Nucleoside reverse transcriptase inhibitors (NRTIs) are mainstay therapeutics for HIV that block retrovirus replication. Recently, we made a surprising discovery that NRTIs as a class inhibit NLRP3 inflammasome activation. Amyloid-beta (Aβ) is a drusen component found in AMD eyes and it is reported to induce upregulation of NLRP3, IL-1β, and IL-18 in mouse models. Therefore, we hypothesized that NRTIs block inflammasome activation and RPE cell death in cell culture and in Aβ-induced animal model of dry AMD.
Methods: For the mouse model of dry AMD, RPE degeneration was induced by subretinal injection of fibrillar $A\beta_{1\text{-}40}$. Twice daily intraperitoneal administration of NRTI lamivudine (3TC) (75 mg/kg/day) in wild-type C57BL6/J mice was performed for one week after fibrillar $A\beta_{1\text{-}40}$ administration. RPE degeneration was assessed by fundus photography and ZO-1 staining of RPE flat mounts. For cell culture, primary human RPE cells were maintained in DMEM supplemented with 10% FBS and antibiotics. Stimulation with fibrillar $A\beta_{1\text{-}40}$ was achieved by supplementing the culture medium for 24 hr. Cells were treated with NRTI lamivudine (3TC). NLRP3 inflammasome activation was monitored by western blot for Caspase-1.
Results: NRTI lamivudine (3TC) blocked RPE cell death and inflammasome activation induced by fibrillar $A\beta_{1\text{-}40}$. Intraperitoneal administration of the NRTI lamivudine, at a similar equivalent dose typically administered in humans, prevented RPE degeneration in the $A\beta_{1\text{-}40}$-induced mouse model of dry AMD.
Conclusions: We have identified that the novel anti-inflammatory action of NRTIs blocks Aβ-induced NLRP3 inflammasome activation, and demonstrated their in vivo ability to prevent RPE cell death in a mouse model of dry AMD.
Commercial Relationships: Takako Fukuhara; **Junichi Fukuhara**, None; **Younghee Kim**, None; **Tetsuhiro Yasuma**, None; **Reo Yasuma**, None; **Benjamin Fowler**, None; **Shinichi Fukuda**, None; **Nagaraj Kerur**, None; **Bradley D. Gelfand**, None; **Jayakrishna Ambati**, Inflammasome Therapeutics (I), iVeena Pharmaceuticals (I), iVeena Holdings (I), iVeena Pharmaceuticals (S), Inflammasome Therapeutics (S), University of Kentucky (P), iVeena Holdings (P), Allergan (R), iVeena Holdings (S), iVeena Pharmaceuticals (P), Olix Pharmaceuticals (F), iVeena Delivery Systems (I), iVeena Delivery Systems (S), iVeena Delivery Systems (P)

Program Number: 6553 **Poster Board Number:** D0302
Presentation Time: 11:00 AM–12:45 PM
Measuring mitochondrial flux in RPE under conditions of oxidative stress
Emily Brown, Casey Keuthan, John Ash. Ophthalmology, University of Florida, Gainesville, FL.
Purpose: The goal of this study is to examine mitochondrial dynamics during conditions of oxidative stress using mitochondrial reporter tools. It has been demonstrated that there are changes in mitochondrial morphology, as well as an increase in oxidative stress in patients with age-related macular degeneration (AMD). Mitochondrial reporter tools allow us to examine mitochondrial flux in cell culture and in animal models under various conditions. This provides a mechanism to potentially identify whether an increase in mitochondrial flux provides protection against oxidative stress, and subsequently test therapeutic targets that are possibly capable of increasing mitochondrial flux.
Methods: ARPE-19 cells were transiently transfected with plasmids containing either YFP-tagged mitofusin 2 (Mfn2), mCherry-tagged Dynamin-related protein (Drp1), or mitochondrial-targeted pMito-Timer, with a dsRed mutant fluorophore that switches from green to red once oxidized. Confocal microscopy was used to image cells over time. Cells were treated with various concentrations of paraquat to induce oxidative stress.
Results: Mitochondrial reporter tools demonstrate that there is a shift in mitochondrial flux during oxidative stress in ARPE-19 cells. Our data using pMito-Timer show that oxidative stress increased both oxidation in mitochondria, as well as synthesis of new mitochondrial proteins in these cells. We show that under oxidative stress Mfn-2 and Drp-1 levels are altered as well.
Conclusions: Mitochondrial reporter tools can be utilized to examine mitochondrial flux in ARPE-19 cells. During oxidative stress in ARPE-19 cells, levels of mitochondrial protein oxidation and synthesis are elevated. We hypothesize that an increase in mitochondrial flux can be protective against oxidative damage. Our future goal is to use these tools in animal models of oxidative stress in the RPE to determine if mitochondrial flux is altered under these conditions. We also aim to identify whether an increase in mitochondrial flux is protective and if treatment with agonists, such as metformin, results in a shift in mitochondrial dynamics in these models.
Commercial Relationships: Emily Brown, None; **Casey Keuthan**, None; **John Ash**
Support: Funding support to JDA include NIH R01EY016459-10, Foundation Fighting Blindness, and an unrestricted departmental grant from Research to Prevent Blindness, Inc.

Program Number: 6554 **Poster Board Number:** D0303
Presentation Time: 11:00 AM–12:45 PM
Monomeric C-reactive Protein and Altered Human Endothelial Cell Function in Age-related Macular Degeneration
Kathleen Chirco[1, 3], S Scott Whitmore[1, 3], Shemin Zeng[1, 3], Grefachew Workalemahu[1, 3], Lawrence A. Potempa[2], Sara E. Miller[1, 3], Edwin M. Stone[1, 3], Budd A. Tucker[1, 3], Robert F. Mullins[1, 3].
[1]Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA; [2]Roosevelt University, Schaumburg, IL; [3]Wynn Institute for Vision Research, Iowa City, IA.
Purpose: C-reactive protein (CRP) is an acute phase molecule that primarily exists in two functionally independent forms: net anti-inflammatory pentameric CRP (pCRP) and pro-inflammatory monomeric CRP (mCRP). Previous studies have suggested a link between elevated total CRP in the choroid, *CFH* genotype, and age-related macular degeneration (AMD) status; however, the exact form and functional consequences of CRP on choroidal cells remains unknown. In the current study, we employed immunohistochemical, biochemical, and functional assays to evaluate the role of mCRP on choroidal endothelial cells in the context of AMD.
Methods: Genotyped human donor macula sections (n=42) were labeled using antibodies specific to mCRP (3H12) and pCRP (1D6), and the labeling patterns were compared, masked to donor genotype, to those of sections incubated with secondary antibody only. To quantitate these differences in CRP levels based on AMD risk, ELISA (n=10) and semi-native Western blotting (n=14) was performed using RPE-choroid tissue from genotyped donors. RNA-Seq (n=3 per group) and Western blotting (n=18) of human RPE-choroid organ cultures treated with mCRP (20μg/mL) or media alone were utilized to assess gene expression and protein level changes, respectively.
Results: Donors homozygous for the high-risk *CFH* (Y402H) allele had elevated mCRP immunolabeling within the choriocapillaris and Bruch's membrane compared to those with the low-risk genotype, which is in agreement with semi-native Western blotting analysis. Additionally, the ELISA data show that, on average, total CRP levels in HH donor choroids are 189% higher than the levels in YY donor choroids. Organ cultures treated with mCRP (20μg/mL) exhibit a dramatic increase in expression of inflammatory genes including ICAM-1 (fold change=2.1; $p<0.05$), with a corresponding increase in ICAM-1 protein levels ($p<0.05$).
Conclusions: Our data indicate that mCRP is the more abundant form of the protein in human choroid, and that mCRP levels are elevated in individuals with the high-risk *CFH* genotype. Moreover, pro-inflammatory mCRP significantly affects endothelial cell phenotypes *in vitro* and *ex vivo*, suggesting a potential role for mCRP in choroidal vascular dysfunction in the pathogenesis of AMD.

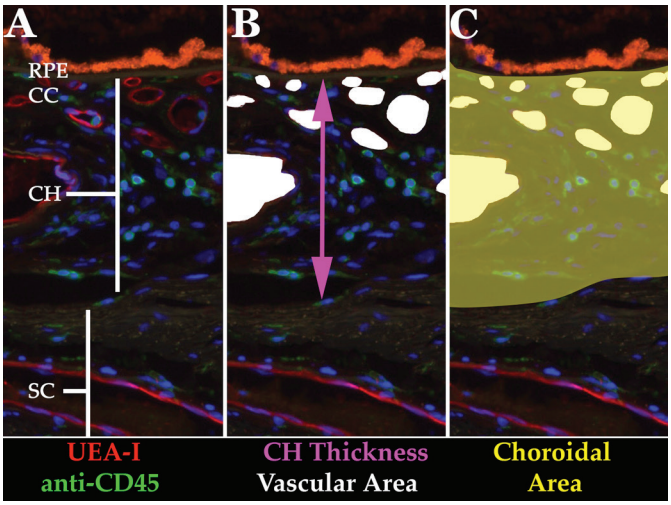


Human donors homozygous for the high-risk *CFH* allele have increased mCRP immunoreactivity (B) in the choroid compared to low-risk eyes (E).
Commercial Relationships: Kathleen Chirco, None; **S Scott Whitmore**, None; **Shemin Zeng**, None; **Grefachew Workalemahu**, None; **Lawrence A. Potempa**, None; **Sara E. Miller**, None; **Edwin M. Stone**, None; **Budd A. Tucker**, None; **Robert F. Mullins**, None
Support: Elmer and Sylvia Sramek Charitable Foundation, NIH R01 EY024605, Wynn Institute Endowment for Vision Research

Program Number: 6555 **Poster Board Number:** D0304
Presentation Time: 11:00 AM–12:45 PM
Pathophysiology of Geographic Atrophy in AMD: Role of Choroidal Vascular Dropout
Robert F. Mullins[1], Elliott H. Sohn[1], Kathleen Chirco[1], Xiuying Liu[1], Kai Wang[2], Alexander Marneros[3], Li Zhang[1], Michael D. Abramoff[1], Budd A. Tucker[1], Edwin M. Stone[1]. [1]Ophthalmology and Visual Sciences, Wynn Institute for Vision Research, University of Iowa, Iowa City, IA; [2]Biostatistics, Wynn Institute for Vision Research, University of Iowa, Iowa City, IA; [3]Harvard Medical School, Boston, MA.
Purpose: Geographic atrophy (GA) is a severe, blinding complication of age-related macular degeneration. The pathogenesis of GA is incompletely understood. While photoreceptor cells, RPE and choriocapillaris all degenerate in GA, the health of the choroid in GA eyes has not been comprehensively examined. In this report we evaluated the vascular density of the choroid in both human donor eyes and in patients with GA, and assessed a marker of hypoxia in the retina as a function of choroidal vascularity.
Methods: Macular sections were collected from human donor eyes and were labeled with the UEA-I lectin to quantify the vascular endothelium of the choroid. Eyes with GA (n = 11 eyes from 11 donors) or non-GA eyes (n = 25 eyes from 20 donors) were assessed. Choriocapillaris density, choroidal area, and vascular lumen cross sectional areas were determined, and the ratio of lumen to total area was determined as a single value for each eye. Five living patients with GA and 4 controls were similarly evaluated using three-dimensional SD OCT analysis, in which the areas of non-reflective choroidal large vessel volumes were measured and divided by the entire choroidal volume to generate the lumen to stroma ratio. Eyes with varying choriocapillaris densities were employed for retinal VEGF ELISA analysis.
Results: The lumen to stroma ratio did not differ significantly in healthy eyes with the thickest and thinnest choroids ($p=0.49$). GA eyes, however, showed significantly reduced lumen to stroma ratios

compared to controls (0.19 vs. 0.28, corrected p<0.05). The lumen to stroma ratio was also lower in living GA patients (0.13, 95%CI 0.12–0.14) than controls (0.27, 95%CI, 0.25–0.29). Histologically, GA eyes also showed significant loss of choriocapillaris (corrected p<0.01) especially in regions of RPE degeneration (p<0.05). In ELISA analyses, retinas overlying choroids with degenerative choriocapillaris showed upregulated VEGF protein (r2 for trend = 0.66).

Conclusions: The choroid undergoes thinning during normal aging with consistent ratios of choroidal vasculature to total area. In GA, vascular loss occurs in both large vessels and in the choriocapillaris. This vascular dropout is associated with increased VEGF synthesis in the overlying retina, consistent with ischemic injury. Protecting the choroid from vascular loss may be an important avenue for treatment and prevention of GA.



Example of vascular quantification.

Commercial Relationships: Robert F. Mullins, None; **Elliott H. Sohn**, None; **Kathleen Chirco**, None; **Xiuying Liu**, None; **Kai Wang**, None; **Alexander Marneros**, None; **Li Zhang**; **Michael D. Abramoff**, IDX LLC (C), IDX LLC (I), University of Iowa (P); **Budd A. Tucker**, None; **Edwin M. Stone**, None
Support: NIH EY-024605, the Elmer and Sylvia Sramek Charitable Foundation, the Wynn Institute Endowment for Vision Research

Program Number: 6556 **Poster Board Number:** D0305
Presentation Time: 11:00 AM–12:45 PM
CD36-mediated uptake of oxidized LDL induces retinal pigment epithelial cell death through NLRP3 inflammasome activation
Gopalan Gnanaguru[1], Ariel Choi[2], Dhanesh Amarnani[1], Patricia A. D'Amore[1]. [1]Schepens Eye Research Institute, MEEI, HMS, Boston, MA; [2]Program in Liberal Medical Education, Brown University, Providence, RI.

Purpose: Accumulation of lipids that become oxidized has been suggested to contribute to the pathogenesis of AMD. We have previously shown that oxidized LDL (ox-LDL), but not native LDL, are cytotoxic to human retinal pigment epithelial (RPE) cells. We therefore have examined the mechanism by which ox-LDL leads to RPE cell death.

Methods: Primary human fetal RPE (hf-RPE) cells were treated with 500 µg/ml of LDL or ox-LDL, and ARPE-19 cells were treated with 100 µg/ml of LDL or ox-LDL. CD36, NLRP3, and caspase-1 expressions were analyzed by RT-PCR, and western blot. Fluorescent probe assay was performed to confirm caspase-1 activation. The role of CD36 in ox-LDL uptake was studied using DiI-labeled-ox-LDL uptake and CD36 function blocking antisera. Lysosomal integrity was examined by labeling with Lysotracker DND-99. The role of inflammasome activation was assessed by measuring RPE cell death (LDH release) in the presence or absence of the NLRP3 inflammasome inhibitor isoliquiritigenin (10 µM/ml).

Results: Treatment of hf-RPE and ARPE-19 cells with ox-LDL, but not with LDL, led to a significant increase (more than 2-fold, P<0.01) in CD36 mRNA at 24 hr and CD36 protein at 48-72 hr. Incubation of RPE cells for 30 min with 40 µg/ml of CD36-IgA antibody significantly (P<0.01) reduced DiI-labeled-ox-LDL uptake by 75% when compared to 40 µg/ml of control-IgA treated cells. Blocking of CD36 receptor for 48 hr reduced ox-LDL induced RPE cell death by 40%. Further analysis show that DND-99-positive lysosomes were reduced in RPE cells incubated with ox-LDL for 48 hr. The treatment of RPE cells with ox-LDL but not LDL induced NLRP3 mRNA levels over 6 fold and activated caspase-1. Incubation of ARPE-19 cells with isoliquiritigenin in the presence of ox-LDL reduced cell death by 95%.

Conclusions: Uptake of ox-LDL by RPE cells induces NLRP3 inflammasome activation, which can lead to cell death and/or the release or pro-inflammatory cytokines, and may contribute to the initiation and/or progression of AMD. Anyone of a number of steps along this pathway may represent novel targets for the prevention or attenuation of AMD.

Commercial Relationships: Gopalan Gnanaguru, None; **Ariel Choi**, None; **Dhanesh Amarnani**, None; **Patricia A. D'Amore**, Eleven Biotherapeutics (S), AGTC (C)
Support: EY015435, Grimshaw Foundation

Program Number: 6557 **Poster Board Number:** D0306
Presentation Time: 11:00 AM–12:45 PM
Expression of A Disintegrin and Metalloproteinase 17 (ADAM 17) in RPE cell culture is associated with apoptosis
Chen Liang[1, 2], Jing Z. Cui[2], Luba Kojic[3], William Jia[3, 4], Joanne A. Matsubara[2]. [1]Dept. of Ophthalmology, West China Hospital, Sichuan University, Chengdu, China; [2]Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada; [3]Brain Research Centre, University of British Columbia, Vancouver, BC, Canada; [4]Department of Surgery, University of British Columbia, Vancouver, BC, Canada.

Purpose: ADAM17 has been reported to be an indispensible regulator of almost every cellular event from proliferation to death. However, the role of ADAM17 in eye diseases is unknown. The purpose of this study is to investigate the expression and the function of ADAM17 in RPE cells *in vitro* and in postmortem eyes of AMD and non-AMD donors.

Methods: ARPE19 cells were stimulated with IL-1β, H_2O_2 or TNF-α to trigger ADAM17 activation for 24 hrs. Cells were then lysed, and samples extracted for total, cytoplasmic or nuclear protein. ADAM17 protein expression was assessed by western blot. Nuclear localization of ADAM17 was further evaluated by immunocytochemistry on culture slides. Apoptosis in stimulated ARPE19 cells was studied using a pan-caspase inhibitor, flow cytometry and annexin V/PI labeling. Results were analyzed by one-way ANOVA, $p < 0.05$.

Results: Total protein analysis revealed three forms of ADAM17: proform (130kD), mature form (100kD) and cytoplasmic tail form (40kD). Significant fold increase of the mature form was observed for IL-1β (1.8±0.45), H_2O_2 (1.5±0.04), TNF-α (2.0±0.28) stimulation; no significant changes were observed in the cytoplasmic tail form in any of the stimulation regimes. However, the cytoplasmic tail form was significantly increased in the nuclear protein material in all treatment groups (1.9 -4.0 fold). Cell fluorescence staining further confirmed ADAM17 signal in RPE nuclei. Nuclear cytoplasmic tail form was

reduced with pretreatment of pan-caspase inhibitor, which blocks caspase-dependent apoptosis. Corroborative immunohistochemistry data were observed from sections of human AMD eyes, in which increased ADAM17 levels were found in the AMD eyes (N=13) and in the nuclear compartment when compared to age-matched controls (N=7).
Conclusions: This is the first study to demonstrate a nuclear localization of ADAM17 in RPE cell under stress *in vitro* and its role in RPE apoptosis. Additionally, the presence of ADAM17 in RPE nuclei of postmortem eyes is suggestive of a possible association with AMD disease processes.
Commercial Relationships: Chen Liang, None; **Jing Z. Cui**, None; **Luba Kojic**; **William Jia**, None; **Joanne A. Matsubara**, None
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Program Number: 6558 **Poster Board Number:** D0307
Presentation Time: 11:00 AM–12:45 PM
Interplay of zinc, complement, and retinal pigment epithelial cells
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Purpose: Compared to the rest of the body, retina and retinal pigment epithelial (RPE) cells hold strikingly high zinc concentrations that are decreased in age-related macular degeneration (AMD). Genetic alterations point to an over-activation of the complement system in AMD patients and previous studies showed functional impact of complement on RPE cells that undergo cell death during AMD. Therefore, in this *in vitro* study, the influence of complement on zinc-supplemented RPE cells was examined.
Methods: Human ARPE-19 cells as RPE cell model were incubated with increasing concentrations of zinc sulphate and zinc chloride alone and in combination with human complement serum (HCS). Controls included heat-inactivated HCS, C7-deficient control sera, or bovine serum albumin (BSA). Viability was monitored by conversion of thiazolylblue, uptake of propidiumiodide, and Hoechst staining. In the cell culture supernatants the content of reactive oxygen species (ROS) were measured. Further, production of interleukin (IL)-6, IL-8, monocyte chemoattractant protein (MCP)-1, and vascular endothelial growth factor (VEGF) was assayed by sandwich ELISA.
Results: RPE cells incubated with >150 µM of both zinc compounds showed decreased viability after single administration for 24 hours. No obvious toxicity was demonstrated by 25-50 µM zinc for several weeks. All serum formulations including HCS attenuated the toxicity of high dose and short term-added zinc, while BSA was less effective. After 48 hours, the HCS group showed impaired morphology comparable to BSA-treated cells. In contrast, the cells treated with HI-HCS and especially those with C7-deficient serum displayed a better appearance. There was a tendency of higher ROS amounts by zinc-treatment and by HCS-treatment. The production of cytokines and VEGF was enhanced by HCS and unaffected by zinc.
Conclusions: The degeneration of zinc-rich RPE cells may poison the retinal microenvironment during AMD. Unspecific binding of potentially toxic free zinc ions by serum proteins like albumin may provide in part the initially observed protection by HCS. On the other hand negative side-effects like zinc-induced complement-activation may predominate after longer incubation period. Increased ROS suggest a stress response towards zinc and complement.
Commercial Relationships: Susanne Wasmuth, None; **Martin Busch**, None; **Albrecht Lommatzsch**, None; **Daniel Pauleikhoff**
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Program Number: 6559 **Poster Board Number:** D0308
Presentation Time: 11:00 AM–12:45 PM
Age-related accumulation of metal ions in Bruch's membrane: implications for AMD
Anthony J. Day[1], Alex Langford-Smith[1], Viranga Tilakaratna[1], Larisa Logunova[1], Paul Lythgoe[2], Simon J. Clark[3], Paul N. Bishop[3, 4]. [1]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom; [2]Environmental Sciences, University of Manchester, Manchester, United Kingdom; [3]Faculty of Medical and Human Sciences, University of Manchester, Manchester, United Kingdom; [4]Manchester Royal Eye Hospital, Manchester, United Kingdom.
Purpose: A major hallmark of age-related macular degeneration (AMD) is the accumulation of extracellular debris (including drusen) around the interface between Bruch's membrane (BM) and the retinal pigment epithelium (RPE). Multiple genetic and environmental risk factors have been identified, whereas, the role of normal ageing is less well characterized. Here we have investigated the age-related accumulation of metal ions in the BM and how this affects the function of the adjacent RPE.
Methods: We analysed eyes from human donors without known AMD (aged 11-88 years) and quantified the level of 14 metal ions in BM preparations by inductively coupled plasma mass spectrometry (ICPMS) (n=131), determined gene expression changes in the adjacent RPE cells by quantitative PCR (n=81) and transcriptomics (n=24), and performed histological analysis on the macula regions from the contralateral eyes (n=45).
Results: ICPMS revealed a significant linear increase in cadmium and cobalt ions, and a decrease in zinc ions, with age. Furthermore we identified a population of older donors with high levels of aluminium; histological Walton staining localised this metal ion to the BM and RPE (it was also present in drusen in 5 AMD donors). The amounts of metal ions in the BM (e.g. Al^{3+}, Cd^{2+}, Co^{2+}, Zn^{2+}) correlated with RPE gene expression changes in components of the oxidative stress and complement pathways and of the major AMD risk factor, HTRA1; multivariate analysis revealed that aluminium was one of the most important factors influencing gene expression, more so than age alone. However, age and Cd^{2+} and Co^{2+} correlated with histological changes in carboxymethyl lysine (an oxidative stress marker) and the terminal complement complex in the BM of the contralateral eye. Analysis of the transcriptomics data further demonstrated that pathways associated with oxidative stress and complement correlated with certain metal ions in the adjacent BM, and additionally identified changes in eumelanin biosynthesis, mitochondrial dysfunction, autophagy and proteasomal activity with age; in the case of Al^{3+}, this was also highly associated with vasculogenesis.
Conclusions: Overall this study has identified that several metal ions accumulate in human BM during normal ageing, and are associated with gene expression changes in multiple pathways implicated in AMD initiation and progression.
Commercial Relationships: Anthony J. Day, None; **Alex Langford-Smith**, None; **Viranga Tilakaratna**, None; **Larisa Logunova**, None; **Paul Lythgoe**, None; **Simon J. Clark**, None; **Paul N. Bishop**, None
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Program Number: 6560 **Poster Board Number:** D0309
Presentation Time: 11:00 AM–12:45 PM
Circulating autoantibodies in age-related macular degeneration (AMD) recognize human macular tissue antigens implicated in immunomodulation, protection from oxidative stress and apoptosis, and autophagy
Alessandro Iannaccone[1], Marko Z. Radic[2], Sarka Beranova-Giorgianni[3], Nataliya Lenchik[4], TJ Hollingsworth[1], Ivan Gerling[4], Francesco Giorgianni[3]. [1]Ophthalmology/Hamilton Eye Institute, University of Tennessee Health Science Center, Memphis, TN; [2]Microbiology, Immunology and Biochemistry, Univ Tennessee Health Sci Ctr, Memphis, TN; [3]Pharmaceutical Sciences, Univ Tennessee Health Sci Ctr, Memphis, TN; [4]Internal Medicine/ Endocrinology, Univ Tennessee Health Sci Ctr, Memphis, TN.
Purpose: To report on two new antigens recognized by serum auto-antibodies (AAbs) found in the serum of 131 AMD participants (compared to 245 unaffected subjects), to present evidence that these biomarkers discriminate well AMD from control sera, and to illustrate a mechanistic hypothesis for how the specific AAbs identified in our studies may play a role in AMD pathogenesis.
Methods: Protein lysates obtained from human macular full-thickness retina/ retinal pigment epithelium (RPE)/Bruch's membrane/choroid punches were immunoprecipitated with a representative selection of AMD sera positive (n=28, 18 with advanced and 10 with early to mid-stage AMD) to WB testing to identify antigens targeted by AAbs using 2D gel electrophoresis (GE) and mass spectrometry (MS). MS methods have been previously reported (Lenchik et al. ARVO 2013, Abs. 4103). Two candidate antigens were confirmed by immunoprecipitation-WB (IP-WB) and by direct ELISA against recombinant proteins on 18 AMD and 16 control sera.
Results: Novel targets recognized by serum AAbs from AMD participants were shown to be Annexin A5 (ANXA5) and Protein S100-A9 (S100A9). Anti-ANXA5 autoreactivity was 0.44±0.03 in AMD samples and 0.24±0.01 in controls (p=0.0000001), and anti-S100A9 autoreactivity was 0.52±0.06 in AMD samples and 0.26±0.02 in controls (p=0.001). In 2x2 table analyses, ELISA reactivity against ANXA5 ≥0.3 [χ^2=30.68, p=0.00003 by Fisher exact test; odds ratio (OR) = 48.00 (5.89–391.21, 95%CI)] and against anti-S100A9 ≥0.3 [χ^2=6.51, p=0.026 by Fisher exact test; OR=5.42 (1.14–25.83, 95%CI)] was associated with much higher likelihood of having AMD. All these autoantigens share implications in immunomodulation, protection from oxidative stress and apoptosis, and in particular autophagy.
Conclusions: With the identification of anti-ANXA5 and anti-S100A9 AAbs, we have now characterized an array of six autoimmune biomarkers in AMD which include CD5L/AIM [New et al. *IOVS* 2014; 55: E-Abstract 65] and three heath shock proteins (HSPs), HSPA8 and HSPA9, two members of the HSP70 family, and HSPB4/CRYAA [Iannaccone et al. *PLOS One* 2015, in press]. We propose that these AAbs could contribute to AMD biogenesis and progression particularly (yet not only) by compromising autophagy and leading to activation of the inflammasome.
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Presentation Time: 11:00 AM–12:45 PM
Loss of P2X7 receptor function increases the risk of age related macular degeneration: Investigation of human genetics and mouse models
Kirstan A. Vessey[1], Ben J. Gu[2], James S. Wiley[2], Paul N. Baird[3, 4], Robyn H. Guymer[3, 4], Erica L. Fletcher[1]. [1]Anatomy and Neuroscience, The University of Melbourne, Melbourne, VIC, Australia; [2]Florey Department of Neuroscience and Mental Health, Melbourne, VIC, Australia; [3]Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia; [4]Ophthalmology, Department of Surgery,The University of Melbourne, Melbourne, VIC, Australia.
Purpose: Age related macular degeneration (AMD) is a leading cause of blindness in Western countries. Recently a novel pathway, involving the scavenger receptor function of the P2X7 receptors (P2RX7) expressed on microglia/macrophages was identified as a risk factor for advanced AMD. Our aim was to determine whether single nucleotide polymorphisms (SNPs) in P2RX7 may be correlated with early AMD. In addition, the ocular phenotype of P2RX7null mice was characterised to determine whether they displayed phenotypic characteristics of early AMD with age.
Methods: Patients with early and intermediate drusen (n=738, early AMD) and age-matched controls (n=349) were genotyped for 12 functional SNPs in the P2RX7 gene and analysed by PLINK. P2RX7null mice and C57blk6J-mice (n>6 at each age/outcome) were investigated at 4, 12 and 18 months of age. Retinal appearance of the mice was assessed *in vivo* using a Micron fundus camera and *ex vivo* using histology at the confocal and transmission electron microscope. The function of the rod and cone pathways was assessed using the electroretinogram (ERG).
Results: Inheritance of the P2RX7 SNPs, R270H/R307Q haplotype together with E496A was found to be a risk factor for patients in the early AMD cohort when compared with age-matched control patients (OR=1.21, p<0.041). These SNPs are known to impart loss of function to the P2X7 receptor. Homozygous loss of P2RX7 function in mice induced abnormal fundus lesions, accompanied by loss of rod photoreceptor function (~35%) at 18 months when compared with age-matched C57blk6J-mice (p<0.01). Histological evaluation indicated no loss of photoreceptor nuclei but a significant increase in Bruch's membrane thickness (~90%) and RPE cell loss in P2RX7null versus C57blk6J-mice at 18 months (p<0.05 for BM and RPE changes). In addition, P2RX7null mice had a significant increase in microglia/macrophage numbers in the subretinal space at 18 months (p<0.05) and subtle changes in intraretinal microglial morphology and function from 4 months of age.
Conclusions: In humans, inheritance of loss of function P2RX7 SNPs is a risk factor for the early stages of AMD and in aged mice, loss of this receptor induces ocular changes characteristic of early AMD providing a valuable model in which to investigate the role of scavenger receptor function and the innate immune system in the development of this disease.

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