281 Bruch's membrane and choroid in macular disease
Monday, May 08, 2017 3:45 PM–5:30 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 2257–2272/B0211–B0226
Organizing Section: Retinal Cell Biology

Program Number: 2257 **Poster Board Number:** B0211
Presentation Time: 3:45 PM–5:30 PM
Superior cervical gangliectomy induces geographic atrophy in mice
Damian Dorfman[1], Hernan Hugo Dieguez[1], Maria Florencia Gonzalez Fleitas[1], Marcos Luis Aranda[1], Pablo Sande[1], Horacio Romeo[2], Ruth E. Rosenstein[1]. [1]Human Biochem/Sch of Med, University of Buenos Aires, Buenos Aires, Argentina; [2]Instituto de Investigaciones Biomédicas UCA-CONICET, Pontificia Universidad Católica Argentina, Buenos Aires, Argentina.
Purpose: Age related macular degeneration (AMD) is a main cause of irreversible blindness. AMD is classified in two forms: wet or neovascular, and dry or geographic atrophy (GA). GA is characterized by hypopigmentation of the retinal pigment epithelium (RPE) and photoreceptor (FR) loss in a localized area of the retina. Alterations in choroid blood flow have been related in the pathogenesis of GA. Sympathetic terminals from the superior cervical sympathetic ganglion (SCG) innervate choroid vessels and modulate their flux. Superior cervical gangliectomy (SCGx) induces complete and irreversible ipsilateral choroid denervation. The aim of this work was to analyze the effect of SCGx on the retina/RPE in mice.
Methods: Unilateral SCGx was induced in adult male C57BL/6J mice, while the contralateral side was submitted to a sham procedure, without excision of SCG (control). At 4, 6, and 10 weeks after SCGx, retinal function (electroretinography, ERG), RPE melanin content (histology), all-trans retinyl ester binding protein (RPE65) levels (immunohistochemistry), retinal histology (toluidine blue), FR and RPE apoptosis (TUNEL assay), and choroid, Bruch′s membrane, RPE and FR ultrastructure (electron microscopy) were assessed.
Results: At 4 weeks post-SCGx, a significant decrease in scotopic ERG a-wave amplitude, which was even higher after 6 and 10 weeks of SCGx was found, without changes in ERG b-wave or oscillatory potentials. SCGx induced a loss of the temporal (but not nasal) RPE melanin content and RPE65 levels at 4 weeks post-SCGx, which was greater at 6 and 10 weeks post-SCGx. At 10 weeks after SCGx, TUNEL(+) cells were found in the temporal retinal outer nuclear layer and temporal RPE, while no TUNEL(+) cells were observed in the nasal side at all time points. After 4 weeks, SGCx induced a significant increase in choriocapillaris thickness both in the nasal and temporal side, which persisted after 10 weeks of surgery, while no changes in total choroid thickness was found neither in the nasal nor in the temporal side in all experimental groups. After 6 and 10 weeks of SGCx, we found a significant increase Bruch′s membrane thickness and RPE and FR outer segment morphological alterations in the temporal (but not nasal) side.
Conclusions: These results show that SCGx induced alterations which are compatible with GA in the retina of the mice.
Commercial Relationships: Damian Dorfman, None; **Hernan Hugo Dieguez**, None; **Maria Florencia Gonzalez Fleitas**, None; **Marcos Luis Aranda**, None; **Pablo Sande**, None; **Horacio Romeo**, None; **Ruth E. Rosenstein**, None

Program Number: 2258 **Poster Board Number:** B0212
Presentation Time: 3:45 PM–5:30 PM
Cytokine and angiogenesis factor changes in the eye following laser induction of choroidal neovascularization in nonhuman primates
Matthew S. Lawrence[1], Shaun Cote[2], Michael Goldstein[2], Eric Furfine[2], Anish Kurian[1], Wenzheng Hu[1], Robin J. Goody[1]. [1]Research, RxGen, Hamden, CT; [2]Eleven Biotherapeutics, Cambridge, MA.
Purpose: Disruption of Bruch's membrane by laser photocoagulation is a validated model of choroidal neovascularization (CNV). In this study early changes in cytokine and angiogenesis factors were profiled in St. Kitts green monkey (*Chlorocebus sabaeus*) eyes subjected to laser treatment to define potential mediators of CNV complex development.
Methods: CNV was induced in the perimacular region. Six laser spots were symmetrically placed around the fovea with an Iridex Oculight TX 532 nm laser with a duration of 100 ms, spot size 50 µm, power 750 mW by methods previously established to trigger CNV. Color fundus photography, OCT and slit lamp exams were performed immediately after laser treatment to document the lesions, and again prior to sacrifice at either 24 (n=4 eyes) or 72 hours (n = 4 eyes). After collection of vitreous, the neural retina and RPE/choroid were subdissected from 5 mm punch biopsies of the macula, and the temporal and nasal periphery, and the remaining fundus. Samples were analyzed using NHP cytokine and human angiogenesis panels and compared to control eyes receiving no laser treatment (n = 8 eyes).
Results: Laser disruption of Bruch's membrane was associated with significant differences in select assayed protein concentrations at 24 and 72 post-laser. The putative angiogenesis factor MCP-1 was significantly elevated ($P<0.01$) in the macular retina while vitreous levels of Flt-1 were also markedly increased. IL-7, IL-8, IL-12 and IFN-g were elevated in both macular and peripheral retina. IFN-g was additionally elevated in the vitreous. Generally, though not consistently, changes were progressive from 24 to 72 hours, and most pronounced in the macula and within the retina rather than RPE/choroid or vitreous.
Conclusions: Multiplex cytokine and angiogenesis factor analysis of retina, RPE/choroid, and vitreous samples revealed early temporal changes in abundance of select proteins following perimacular photocoagulation, suggestive of their involvement in insult recovery and CNV development in a multi-pathway process. Tissues in the closest proximity to disrupted Bruch's membrane revealed the greatest change, as well as certain soluble factors, further supporting the involvement of the defined proteins in pathogenesis.
Commercial Relationships: Matthew S. Lawrence, RxGen (E); **Shaun Cote**, Eleven Biotherapeutics (E); **Michael Goldstein**, Eleven Biotherapeutics (E); **Eric Furfine**, Eleven Biotherapeutics (I); **Anish Kurian**, RxGen (E); **Wenzheng Hu**, RxGen (E); **Robin J. Goody**, RxGen (E)

Program Number: 2259 **Poster Board Number:** B0213
Presentation Time: 3:45 PM–5:30 PM
Inhibition of the chemokine receptor CXCR4 reduces pathology in a laser induced mouse model of choroidal neovascularization
xilun A. wang[1], Michael Foley[1, 3], Gene Venables[2], Erica L. Fletcher[2]. [1]Biochemistry and Genetics, La Trobe Institute For Molecular Science, Melbourne, VIC, Australia; [2]Anatomy and Neuroscience, The University of Melbourne, Melbourne, VIC, Australia; [3]AdAlta Limited, Melbourne, VIC, Australia.
Purpose: Age related macular degeneration (AMD) is treated with a range of anti-VEGF inhibitors. Although these treatments have had

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a profound effect on the acute pathology, in the longer term, vision loss continues for many patients. New drugs are needed to effectively treat wet AMD. One approach is to target cytokine signalling, which has been implicated in the development of neovascularization pathology. The central aim of this project was to evaluate the role of the chemokine receptor CXCR4 in a mouse model of choroidal neovascularization (CNV).
Methods: CNV lesions were induced in 8 week old female BL6 mice (n= 10 eyes/group) using laser photocoagulation (Micron III, 532nm, 350 mW). Animals were intravitreal injected with either a single domain like antibody known as an i-body targeting CXCR4 (AD-114 12μg/ml), a negative control i-body (AD-21H5 12μg/mL) or vehicle (PBS). Leakage was assessed using fluorescence angiography and lesion size was quantified using image J at 7 days and the eyes were removed, fixed and stained using Masson's trichrome stain. The CNV lesion height/choroid height ratio was measured in image J. mRNA expression and a gene ontology (GO) study was also undertaken. RNA was extracted from the retina and RPE, 7 days after laser. mRNA expression levels were compared using qPCR arrays (84 fibrosis-associated genes). Significantly expressed genes relative to control ($p< 0.05$, fold change ±1.5) underwent overrepresentation testing on the Panther GO platform to identify any potential gene networks modified by CXCR4 inhibiting i-bodies.
Results: The size of the lasered lesions was reduced with the addition of the anti-CXCR4 i-body AD-114 relative to the negative control i-body or PBS treated mice. The total leakage area (μm^2; 1.82 vs. 5.06, $p<0.0001$) and the CNV lesion size (B/C ratio; 2.0 vs. 4.3 $p<0.0001$) were significantly reduced at 7 days. Gene expression changes showed that AD-114 significantly altered ($p<0.05$) the mRNA expression of cytokine genes (IL13, IL1b) and pro-fibrotic genes. Gene ontological analysis confirmed these findings, with the cytokine signaling pathways found to be overrepresented.
Conclusions: Inhibition of CXCR4 reduced neovascularization pathology following laser photocoagulation. These results suggest an alternative treatment mechanism targeting the chemokine receptor CXCR4 using i-body AD-114 may reduce ocular pathology of CNV.
Commercial Relationships: xilun A. wang, None; **Michael Foley**, AdAlta (E); **Gene Venables**, None; **Erica L. Fletcher**, None
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Program Number: 2260 **Poster Board Number:** B0214
Presentation Time: 3:45 PM–5:30 PM
Analysis of choriocapillaris ultrastructure in macular regions of eyes obtained from patients with neovascular AMD, geographic atrophy, and pure one and ten chromosomal abnormalities
Rhonda Grebe[1], Irum Mughal[1], William Bryden[1], Jing Tian[1], Malia M. Edwards[1], Scott McLeod[1], Gregory S. Hageman[2], Gerard A. Lutty[1]. [1]Ophthalmology, The Wilmer Eye Institute, Baltimore, MD; [2]Ophthalmology, Moran Eye Center, Steele Center for Transitional Medicine, University of Utah, Salt Lake, UT.
Purpose: Few quantitative studies have analyzed the ultrastructure of the human choriocapillaris (CC) and compared the structural variations in disease states to that of age matched controls. This study concentrates on the CC endothelial (EC) cell differences.
Methods: Thirty donor eyes (74±7yrs) were obtained from Dr. G. Hageman (Moran Eye Institute, UT) and from Dr. R. Green's repositories (Wilmer, MD). One eye of each donor was prepared for electron microscopy (TEM). TEM images of the choroidal capillaries (CC) were taken at 4K and 25K. The length of Bruchs membrane (BM) and area of EC soma were measured as well as the area of caveolae and transcytotic vesicles within the EC body. Fenestrations per millimeter of BM were counted. Collages were prepared with a minimum of 3 capillaries of the 25K images for each subject and analyzed with Image J. Patients were divided into five groups: controls (n=6), no chromosomal disorder with no history of AMD; chromosome1-directed AMD (n=4) and chromosome10-directed (n=4); neovascular (NV) AMD(n=8); and geographic atrophy (GA) (n=6). The NV and GA groups were subdivided into border regions in which images were taken adjacent to choroidal neovascularization (CNV) and within or just outside of the atrophic regions in GA.
Results: Based on the Wilcoxon test, significant decreases were found in the number of fenestrations counted from areas under CNV ($P=0.039$) and in atrophic areas of GA eyes ($P=0.020$). A significant decrease ($P=0.039$) was found in the percent of caveole area within the EC bodies of CC beneath CNV. Loss of CC vessels was evident in areas of atrophy and CNV. Capillaries in areas bordering CNV did not have significant loss of fenestrations but appeared to have a slightly increased EC area and with a larger area of caveolae compared to controls. In CC regions of controls and in border areas less affected by disease, fenestrations were observed on both the BM and scleral sides of vessels. CC EC's in patients with chromosomal disorders exhibited degenerative changes. CC loss was evident in areas of GA and CNV.
Conclusions: CC EC's show significant changes in the localized areas of pathology in CNV and GA. Subjects with chromosome1 and 10 directed AMD demonstrate marked CC changes and EC degradation. We conclude that CC EC transport systems are severely affected by AMD.
Commercial Relationships: Rhonda Grebe, None; **Irum Mughal**, None; **William Bryden**, None; **Jing Tian**, None; **Malia M. Edwards**, None; **Scott McLeod**, None; **Gregory S. Hageman**, None; **Gerard A. Lutty**, None
Support: NIH grants EY016151 (GL), EY01765 (Wilmer)

Program Number: 2261 **Poster Board Number:** B0215
Presentation Time: 3:45 PM–5:30 PM
Tissue-specific expression of alpha-2-macroglobulin by choroid endothelial cells in response to RPE-secreted factors: implications for AMD
Ignacio Benedicto[1], Guillermo Lehmann-Mantaras[1], Olivier Elemento[2], Arvydas Maminishkis[3], Sheldon S. Miller[3], Shahin Rafii[4], Enrique J. Rodriguez-Boulan[1]. [1]Ophtalmology, Weill Cornell Medical College, New York, NY; [2]Physiology and Biophysics, Weill Cornell Medical College, New York, NY; [3]Section of Epithelial and Retinal Physiology and Disease, NEI, NIH, Bethesda, MD; [4]Genetic Medicine, Weill Cornell Medical College, New York, NY.
Purpose: The onset and progression of AMD correlates with the dysfunction and atrophy of choroid blood vessels, as well as with structural and functional alterations of Bruch's membrane and choroid extracellular matrix (ECM). However, whether and how these processes are connected remains unknown. Recent research suggests that, beyond their generic role as blood conduits, endothelial cells (ECs) secrete tissue-specific angiocrine factors that regulate organ differentiation and regeneration in response to local cues (Rafii et al., Nature 2016). Extension of this concept to the eye led us to test the hypothesis that choroid ECs perform unique retinal homeostasis roles in response to local signals (e.g. from RPE).
Methods: We isolated to high purity adult mouse choroid, neural retina, lung, liver and heart ECs and determined their transcriptome by RNAseq. We carried out qPCR and western blot assays to test *in vitro* whether incubation of ECs with human fetal RPE-conditioned medium induces the expression of choroid EC-enriched genes. We conducted chemical inhibition, gelatin zymography and lentiviral-mediated knockdown experiments to investigate molecular mechanisms underlying RPE-EC crosstalk. We assessed by qPCR the

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in vivo expression of mouse choroid EC-enriched genes after sodium iodate treatment, an established procedure to damage RPE.
Results: Choroid ECs display high expression levels of genes encoding various secretory proteins including alpha-2-macroglobulin (A2M), a proteinase inhibitor that regulates ECM stability and turnover. RPE-conditioned medium induced A2M transcription specifically in ECs in a VEGF-dependent manner, which resulted in increased levels of secreted A2M. RPE-conditioned medium reduced the gelatinase activity of EC culture supernatants, which was partially restored after A2M knockdown in ECs. Induction of RPE damage *in vivo* by sodium iodate treatment resulted in decreased levels of A2M expression exclusively in the central part of the RPE/choroid tissue, where RPE damage is most severe.
Conclusions: Our results suggest that RPE-secreted factors (e.g. VEGF) promote increased expression of choroid EC-specific angiocrine factors (e.g. A2M) essential for normal tissue homeostasis. Disruption of this crosstalk likely perturbs choroid ECM turnover and Bruch's membrane stability, contributing to the onset and progression of AMD.
Commercial Relationships: Ignacio Benedicto, None; **Guillermo Lehmann-Mantaras**, None; **Olivier Elemento**, None; **Arvydas Maminishkis**, None; **Sheldon S. Miller**, None; **Shahin Rafii**, Angiocrine Bioscience (I); **Enrique J. Rodriguez-Boulan**, None
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Program Number: 2262 **Poster Board Number:** B0216
Presentation Time: 3:45 PM–5:30 PM
Constitutive erythropoietin receptor signaling exacerbates pathologic choroidal neovascularization in an animal model
Eric Kunz[1], Colin A. Bretz[1], Vladimir Divoky[2], M Elizabeth Hartnett[1]. [1]Ophthalmology, University of Utah, Salt Lake City, UT; [2]Medicine and Dentistry Palacky University, Olomouc, Czech Republic.
Purpose: Erythropoietin (EPO) is increasingly recognized for neuroprotective and angiogenic effects in addition to its well established role in hematopoiesis. What remains less clear is the role of EPO receptor (EPOR) signaling in the context of physiologic and pathologic neovascularization. We tested the hypothesis that EPOR signaling contributes to the development of pathologic angiogenesis using a laser-induced choroidal neovascularization (LCNV) model.
Methods: This study utilized 6-week old knock-in mice in which the mouse EPOR (mWtEPOR) gene was replaced with either the human EPOR (hWtEPOR) gene, resulting in decreased EPOR signaling, or a constitutively active human mutant EPOR (hMutEPOR) gene, resulting in increased EPOR signaling. The Phoenix micron IV laser module was used to create three laser-burns (450mW, 100ms) in the Bruch's membrane of each eye roughly two disk diameters from the optic nerve head. Seven days post-laser treatment, choroids were dissected and stained with isolectin-B4 to visualize choroidal neovascularization. Z-stacks of each individual lesion were captured using a confocal microscope, and the lesion volume was calculated using IMARIS software as well as by hand. Each group contained an n of ≥ 40 lesions, data was normalized to the control mWtEPOR mice and analyzed using a one-way ANOVA.
Results: Analysis of lesion volume 7 days after laser, revealed that the average lesion volume of hWtEPOR mice was 33.8 % smaller than that of control mWTEPOR mice ($p = 0.0466$), and that the average lesion in hMutEPOR mice was 30% larger compared to mWtEPOR ($p = 0.0002$).
Conclusions: In this study, transgenic hWtEPOR mice that have constitutively decreased EPOR signaling develop smaller CNV after laser-induced damage, whereas hMutEPOR mice that have constitutively active EPOR signaling develop larger CNV. These results support the hypothesis that EPOR signaling is important in the development of pathologic angiogenesis, and support previous studies by us and others that defined a role for EPO and EPOR signaling in retinal angiogenesis associated with oxygen induced retinopathy. Additional studies are indicated to understand the cell specific contributions of EPOR signaling in this context.
Commercial Relationships: Eric Kunz, None; **Colin A. Bretz**, None; **Vladimir Divoky**, None; **M Elizabeth Hartnett**, None
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Program Number: 2263 **Poster Board Number:** B0217
Presentation Time: 3:45 PM–5:30 PM
Geographic Atrophy (GA): Correlation Between Confocal Scanning Laser Ophthalmoscopy (SLO), Histology and Genotypic Analysis in the Region of Expanding Lesions
Vera L. Bonilha, Brent A. Bell, Mary E. Rayborn, Joe G. Hollyfield, Stephanie A. Hagstrom, Gayle Pauer. Ophthalmology, Cole Eye Inst/ Cleveland Clin Lerner Ctr, Cleveland, OH.
Purpose: Dry AMD occurs when the retinal pigment epithelium (RPE) cells begin to thin and degenerate followed by photoreceptor loss. Geographic atrophy (GA) represents the atrophic late-stage of dry AMD. AMD pathology affects the RPE physiology and results in several noticeable morphological features in these cells. Visible light SLO autofluorescence (VAF-SLO) imaging visualizes the accumulation of the autofluorescent lipids composing lipofuscin at the level of the RPE cell monolayer *in vivo* in patients; whereas near-infrared SLO autofluorescence (IRAF-SLO) visualizes the distribution of melanin. Here, we characterized the GA from several donors eyes by VAF- and IRAF-SLO and correlated it to the morphology of RPE/photoreceptors.
Methods: SLO, and fundus macrography (FM) were used to identify and measure the extent of GA lesions from 22 fixed donor eyes. Adjacent areas of the retina/RPE/choroid from the margins of the GA border were cut to generate two smaller fragments: one was processed for epon embedding while the other was processed for cryosectioning and immunofluorescence in the green-red and near-IR (NIR) channels that closely matched VAF- and IRAF-SLO ranges. DNA was obtained from blood samples or fixed eye tissue of the donors and genotyped for single nucleotide polymorphisms (SNPs) previously shown to be associated with the development and progression of AMD (rs800292 and rs1061170 (*CFH),* rs10490924 *(ARMS2),* rs11200638 *(HTRA1),* and rs2230199 *(C3)*).
Results: The majority of the GA donors were homozygous for the risk alleles at the CFH I62V site. GA tissue displayed high and low levels of hyper-fluorescent rings that circumscribed the effected region. The cytoplasm of RPE cells at the GA margins contained fluorescent granules visible in both NIR and green-red channels. Very few NIR fluorescent melanin granules were still present in these RPE cells. Histologically, few photoreceptor nuclei were observed in the margin between GA abnormal and normal regions. Dissociated RPE cells lying on basal laminar deposit (BLamD), with or without dissociated RPE migrating towards the INL, were observed at the margins with high IRAF-SLO. Sloughed RPE was present at the lesion margins with low IRAF-SLO.
Conclusions: GA can be sub-grouped based on the IRAF-SLO and RPE morphology.
Commercial Relationships: Vera L. Bonilha, None; **Brent A. Bell**, None; **Mary E. Rayborn**, None; **Joe G. Hollyfield**, None; **Stephanie A. Hagstrom**, None; **Gayle Pauer**, None

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Program Number: 2264 **Poster Board Number:** B0218
Presentation Time: 3:45 PM–5:30 PM
Hypoxia and VEGF overexpression induce the formation of labyrinth capillaries, which are described to be responsible for leakage in wet AMD

Antje K. Biesemeier[1], Shan Liu[1], Marina Tikhonovich[1], Guido Hartmann[2], Ulrich Schraermeyer[1]. [1]Sect of Experimental Vitreoret Surg, Center for Ophthalmology, Tuebingen, Germany; [2]Roche Pharma Research and Early Development, Neuroscience Ophthalmology and Rare Disease DTA, Basel, Switzerland.

Purpose: Our previous work described so called labyrinth capillaries (LC) as a possible source of leakage in surgically excised choroidal neovascular (CNV) membranes: the leakage was caused by capillaries with open cellular junctions, but more importantly, these vessels also showed microvillar projections into the vessel lumen which blocked cellular perfusion while allowing unhamperered flow of plasma (Schraermeyer et al., 2015, doi: 10.1007/s00417-014-2733-0). The LCs were proposed to be responsible for the permanent leakage of fluid in AMD and therefore merit awareness. Early stages of LCs, like microvillar processes of endothelial cells into the vessel lumen were coincidently observed in our neovascular disease models after VEGF overload or induction of hypoxia. They are described for the first time in this work.

Methods: Endothelial alterations of the choriocapillaris and CNV vessels were ultrastructurally investigated in plastic sections of surgically excised CNV membranes and compared to the following models: CNV induced by viral VEGF overexpression in 1) rats and 2) rabbits, 3) CNV induced by subretinal VEGF protein injection in rats, 4) rat eyes after exposure to hypoxia.

Results: Early signs of LC formation were found in all groups investigated and showed the following features: irregular structure of endothelial cells leading to formation of microvillar processes into the vessel lumen. The processes regularly grew into bridging connections to neighbouring endothelial cells forming new internal lumina. If choriocapillaris was involved, those bridges also contained fenestrations. Already after VEGF challenge for one day, endothelial cells were highly activated and showed luminal microvillar projections and endothelial gaps. Only in the human excised CNV membranes and the rabbit VEGF overexpression model, fully grown labyrinth capillaries were observed.

Conclusions: Here we show that experimental challenge with VEGF or hypoxia can lead to formation of labyrinth capillaries in the choroid in four different model systems. These models can be used to study new drugs to inhibit the formation of leaky capillaries in wet AMD.

Commercial Relationships: Antje K. Biesemeier; **Shan Liu**, None; **Marina Tikhonovich**, None; **Guido Hartmann**, Roche (E); **Ulrich Schraermeyer**, Roche (F)

Support: Roche research grant

Program Number: 2265 **Poster Board Number:** B0219
Presentation Time: 3:45 PM–5:30 PM
Extracellular matrix nitration alters growth factor release and activates bioactive complement in human retinal pigment epithelium

Mark Fields[1], Hannah Bowrey[2], Jie Gong[1], Ernesto F. Moreira[3], Hui Cai[1], Lucian Del Priore[1]. [1]Ophthalmology and Visual Science, Yale School of Medicine, New Haven, CT; [2]Rutgers Brain Health Institute, The State University of New Jersey, Piscataway, NJ; [3]Ophthalmology, Medical University of South Carolina, Charleston, SC.

Purpose: We have shown previously that non-enzymatic nitration (NEN) of extracellular matrix, which serves as a model Bruch's membrane (BM) aging, has a profound effect on the behavior of retinal pigment epithelium (RPE), including altered phagocytic ability, reduced cell adhesion, and inhibition of proliferation. We know that transplanted RPE will encountered a hostile subretinal environment, including BM alterations that may compromise cell function and survival. Here we use our previous model of BM aging (NEN) to determine the effects of NEN on growth factor release and complement activation in RPE.

Methods: Human induced pluripotent stem cells (iPSC) were differentiated into RPE, and analyzed by immunohistochemistry, confocal microscopy, and polymerase chain reaction (PCR). IPSC-derived RPE were plated onto RPE-derived extracellular matrix (ECM) conditions (untreated or nitrite-modified). Cells were cultured for 7 days and barrier function measured by transepithelial resistance (TER) and vascular endothelial growth factor (VEGF), pigment epithelium-derived factor (PEDF), and C3a were measured using enzyme linked immunosorbent assay (ELISA).

Results: Nitrite-modified ECM increased VEGF release both apically and basally by 0.14 ng/mL (p <0.001) and 0.27 ng/mL (p <0.001), respectively, in iPSC-derived RPE. Nitrite-modified ECM increased PEDF release in iPSC-derived RPE apically by 0.26 ng/mL (p <0.001). Nitrite-modified ECM increased production of C3a both apically and basally in iPSC-derived RPE by 0.19 ng/mL (p <0.001) and 0.15 ng/mL (p <0.001), respectively.

Conclusions:
Nitrite-modified ECM increase VEGF, PEDF release, and C3a production in human iPSC-derived RPE. This model demonstrates changes seen in the pathophysiology of age-related macular degeneration.

Commercial Relationships: Mark Fields; **Hannah Bowrey**, None; **Jie Gong**, None; **Ernesto F. Moreira**, None; **Hui Cai**, None; **Lucian Del Priore**, None

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Program Number: 2266 **Poster Board Number:** B0220
Presentation Time: 3:45 PM–5:30 PM

A mineralomic study of the retinal pigment epithelium-Bruch's membrane complex in human eyes with age-related macular degeneration

Matthew Pilgrim[1, 2], Anna C. Tan[3, 4], Sarah Fearn[5], Elena Tsolaki[6], Sergio Bertazzo[6], Imre Lengyel[7, 1], Christine Curcio[8]. [1]UCL Institute of Ophthalmology, University College London, London, United Kingdom; [2]Biomaterials and Tissue Engineering, UCL Eastman Dental Institute, London, United Kingdom; [3]Singapore National Eye Centre, Singapore, Singapore; [4]Singapore Eye Research Institute, Singapore, Singapore; [5]Department of Materials, Imperial College London, London, United Kingdom; [6]Department of Medical Physics and Biomedical Engineering, University College London, London, United Kingdom; [7]Centre for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom; [8]Department of Ophthalmology, University of Alabama at Birmingham, Birmingham, AL.

Purpose: Clinical and histological imaging has suggested the existence of 3 distinct, potentially mineralized deposits in the retinal pigment epithelium (RPE)-Bruch's membrane (BrM) complex in human eyes with age-related macular degeneration (AMD): spherules, BrM plaques and large nodules. Whilst the elemental composition and mineral form of spherules (PMID 25605911), and to a lesser extent plaques (PMID 1543459), has been investigated, the elemental composition and mineral content of nodules remains undetermined (PMID 9620067). This study aims to identify the mineral components of plaques and nodules and compare them to the calcium (Ca) and phosphate (P) spherules previously observed in human drusen.

Methods: Human cadaveric eyes meeting histologic criteria for AMD were dissected and embedded in epoxy resin or paraffin wax, sectioned at 2 μm or 4 to 8 μm respectively, and mounted on to glass slides or on to Ultralene when appropriate. The elemental composition of BrM plaques and nodules was investigated using density dependent-scanning electron microscopy (DDC-SEM), energy dispersive x-ray spectroscopy (EDX), secondary ion mass spectroscopy (SIMS), and synchrotron micro-focus x-ray fluorescence (μXRF). Mineral components were determined using transmission electron microscopy-selected area electron diffraction (TEM-SAED).

Results: Using DDC-SEM, spherules, BrM plaques, and nodules were shown to contain dense material suggestive of mineralization. All 3 of these features were shown to contain Ca and P by EDX and SIMS: spherules (eyes, n=3), BrM plaques (eyes, n=3) and nodules (eyes, n=3). TEM-SAED confirmed that all types of mineralized deposits were composed of apatite.

Conclusions: Ca and P were detected in all types of mineralized deposit, spherules, BrM plaques and, importantly, for the first time in large nodules. Identification of Ca and P and the mineral forms in each deposit type, along with our prior demonstration of apatite in RPE cell cultures (ARVO 2016), highlights the need for further research on Ca and P homeostasis in the RPE-BrM complex. Better understanding of this aspect of RPE physiology can aid the development of novel therapeutic interventions for AMD. Furthermore, mineralization is readily observed using multimodal clinical imaging; therefore these data can also help improve clinical image interpretation.

Commercial Relationships: Matthew Pilgrim; **Anna C. Tan**, Zeiss (R), Bayer (R); **Sarah Fearn**, None; **Elena Tsolaki**, None; **Sergio Bertazzo**, None; **Imre Lengyel**, University College London (P), Optos (F); **Christine Curcio**, Novartis (C), Hoffman La Roche (F)

Program Number: 2267 **Poster Board Number:** B0221
Presentation Time: 3:45 PM–5:30 PM

A Possible Role for Mast Cell-Derived Tryptase in the Pathogenesis of Geographic Atrophy

Scott McLeod, Imran A. Bhutto, Malia M. Edwards, Manasee Gedam, Raj Baldeosingh, Gerard A. Lutty. Ophthalmology, Johns Hopkins School of Medicine, Washington, DC.

Purpose: We have previously shown that significant numbers of degranulating mast cells are found in choroids of eyes with age-related macular degeneration compared to aged controls. In this study, we examined the immunolocalization of tryptase, the most abundant mast cell secretory granule-derived serine protease, in aged control eyes and eyes with geographic atrophy (GA).

Methods: Postmortem human eyes with and without GA were obtained from the National Disease Research Interchange (NDRI). Tissue was fixed, cryopreserved, sectioned and immunostained with antibodies against tryptase, UEA lectin and DAPI. Additional sections were immunolabeled with antibodies against chymase, c-Kit, histamine and IgE. Sections were examined and imaged on a Zeiss 710 Confocal Microscope.

Results: In the posterior pole region of all aged control eyes, tryptase immunoreactivity was confined to choroidal mast cells (MCs), which were located primarily in Sattlers layer. In eyes with GA, many MCs were located in the inner choroid near the choriocapillaris and Bruch's membrane (BM). tryptase was found not only in MCs but also diffusely around them in choroidal stroma, suggesting they had degranulated. In sharp contrast to aged control eyes, eyes with GA also had strong tryptase staining in BM. Tryptase was observed within BM in the regions of retinal pigment epithelial atrophy, at the border of atrophy and extending well into the nonatrophic region. cKit and IgE were localized in all mast cells while chymase was present only in 2% of the MCs.

Conclusions: Our results demonstrate that tryptase, released during degranulation of choroidal mast cells, binds to BM in GA in advance of RPE atrophy. Tryptase activates MMPs which can degrade extracellular matrix (ECM) and basement membrane components found in BM. ECM modifications are likely to have a profound effect on the function, health and transport of RPE in GA.

Commercial Relationships: Scott McLeod, None; **Imran A. Bhutto**, None; **Malia M. Edwards**, None; **Manasee Gedam**, None; **Raj Baldeosingh**, None; **Gerard A. Lutty**, None

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Program Number: 2268 **Poster Board Number:** B0222
Presentation Time: 3:45 PM–5:30 PM
CFH and HTRA1 genes associated with AMD in Mexican population
Antonieta Martínez-Velasco[1], Andric C. Perez-Ortiz[6], Juan C. Zenteno[2, 3], ALEXA B. LUNA-ANGULO[5], Antonio R. Villa-Romero[7], Lourdes Martínez-Villaseñor[1], Alvaro Rendon[4], Francisco J. Estrada[7]. [1]Facultad de Ingeniería, Universidad Panamericana, MEXICO, Mexico; [2]Department of Biochemistry, Faculty of Medicine, UNAM., MEXICO, Mexico; [3]Department of Genetics and Research Unit, Institute of Ophthalmology Conde de Valenciana, MEXICO, Mexico; [4]Centre de Recherche, Institut de la Vision, PARIS, France; [5]Department of Neuroscience, Instituto Nacional de Rehabilitación, Mexico, Mexico; [6]School of Public Health, Yale University, New Haven, CT; [7]Escuela de Medicina, Universidad Panamericana, Mexico, Mexico.
Purpose: Genetic contribution of age-related macular degeneration (AMD) is well known from association studies. CFH and HTRA1 are relevant genes reported in Caucasian populations. Here, we performed a hospital-based case-control study to evaluate the effects of these genes on Mexican population.
Methods: 119 cases and 137 controls were genotyped using Taqman probes. Experienced ophthalmologists defined AMD phenotypes following the American Association of Ophthalmology (AAO) guidelines. Cases were 60 years or older with CARMS grade 4 or 5, with no other retinal disease or history of vitreous-retinal surgery. Controls were outpatients aged 60 or older, with no drusen of RPE changes on fundus exam and negative family history of AMD. 30 clinical variables were recorded from electronic medical records. We looked for the following single-nucleotide polymorphisms (SNPs) in these genes: rs1329428 and rs203687 in CFH, and rs11200638 in HTRA1. Genotyping quality check (QC) and univariate analyses were performed with plink v. 1.9. Furthermore, logistic regression models and machine learning based methods were done in R v. 3.3.2 and corroborated with SAS v. 9.4.
Results: Assuming a dominant mode of inheritance, CFH and HTRA1 alleles are significantly associated with AMD (Table 1). By means of machine learning based methods, we identified 10 important variables to include in a logistic regression model. HTRA1 significantly decreases the odds of disease [OR 0.10, 95CI 0.02 – 0.46, $p = 3.5e^{-4}$] even after holding variables such as age and hypertension status constant. Conversely, CFH (rs203687) significantly increases the odds of AMD [OR 11.8, 95CI 2.72 – 51.22, $p = 4.8 \times 10^{-5}$].
Conclusions: This work shows a strong association between CFH and HTRA1 genetic variants with AMD in Mexican population. We determined the odds of disease which are remarkable if we compare those OR values with other studies. Models generated with machine learning techniques support the diagnosis of AMD disease.
Commercial Relationships: Antonieta Martínez-Velasco, None; **Andric C. Perez-Ortiz**, None; **Juan C. Zenteno**, None; **ALEXA B. LUNA-ANGULO**, None; **Antonio R. Villa-Romero**, None; **Lourdes Martínez-Villaseñor**, None; **Alvaro Rendon**, None; **Francisco J. Estrada**, None

Program Number: 2269 **Poster Board Number:** B0223
Presentation Time: 3:45 PM–5:30 PM
A valid ultrastructural rat choroidal neovascularization model developed by overexpression of VEGF
Shan Liu, Alexander V. Tschulakow, Sylvie Julien-Schraermeyer, Ulrich Schraermeyer, Antje K. Biesemeier. Center for Ophthalmology, University Tuebingen, Tuebingen, Germany.
Purpose: Vascular endothelial growth factor (VEGF) promotes choroidal neovascularization (CNV), an important component of subsequent vision loss in age related macular degeneration (AMD). Our aims were to develop a new efficient and reliable CNV model induced by overexpression of VEGF, to test its effects on extracellular matrix formation and use the model to facilitate the study of antiangiogenic and antiproliferative therapies for ocular diseases.
Methods: We developed a CNV model by subretinal injection of a high-capacity adenovirus vector encoding for human $VEGF\text{-}A^{165}$ (HC Ad.VEGF) into 8 rats (Long Evans). Two rats injected with HC Ad.EGFP and five untreated rats were used as controls. Scanning laser ophthalmoscopy (SLO), fluorescein angiography (FA), indocyanine green angiography (ICG) and optical coherence tomography (OCT) were performed in all rats at postoperative 2 and 4 weeks. The eyes were excised 4 weeks after the injection and fixed for paraffin and EPON embedding. Sections were observed by light and electron microscopy (EM). HE staining and immunohistochemistry (IHC) were performed. Masson trichrome staining showed the distribution of extracellular matrix components (collagen, elastin and fibrin). Loss of retinal pigment epithelium (RPE) and choriocapillaris (CC) was tested by t-test (significance level: P=0.05).
Results: The hyperfluorescent areas, shown in FA and ICG of 81.25% of the VEGF treated eyes, were possibly caused by alterations or leakages of the CC. OCT showed a marked subretinal edema-like change in most eyes. In the EM, we found newly formed blood vessels with perivascular cells and fenestrations between Bruch's membrane (BM) and RPE or between RPE cells, multi-layered RPE, loss of photoreceptors, loss of RPE and CC compared with controls ($P<0.05$), thickened BM and collagen accumulation in CNV areas, resembling human CNV. We are not able to confirm the existence of leakage in the model, because of the lack of fibrin around newly formed blood vessels. IHC verified human VEGF expression, high proliferation (Ki67), occurrence of pericytes (alpha SMA) and absence of macrophages (CD68) in CNV areas.
Conclusions: Based on the results of examinations, especially EM, this rat model resembles human CNV. Running experiments using additional angiopoietin1, 2 and PDGF-B vectors will provide a tool to modulate CNV severity and might yield new therapeutic options for the future.
Commercial Relationships: Shan Liu, None; **Alexander V. Tschulakow**, None; **Sylvie Julien-Schraermeyer**, Katairo (P); **Ulrich Schraermeyer**, Katairo (P), Roche (F); **Antje K. Biesemeier**, Roche (F)
Support: China Scholarship Council

Program Number: 2270 **Poster Board Number:** B0224
Presentation Time: 3:45 PM–5:30 PM
Choroidal Pericytes contribute to Subretinal Fibrosis afterLaser-Induced Photocoagulation
Xueting Luo, Xiaodong Sun. Ophthalmology, Shanghai Jiao-Tong University, Shanghai, China.
Purpose: To determine the role of choroidal pericytes in subretinal fibrotic scar formation during pathogenesis of wet AMD.
Methods: Collagen1α1-GFP reporter mice were used to lineage-trace choroidal pericytes with GFP expression. After laser-induced photocoagulation, a widely used model for wet AMD, antigenic profiles of infiltrating pericytes and other cells were obtained. Patterns of extracellular matrix (ECM) components were also determined by immunostaining.
Results: Pericytes associated with choroidal microvasculature are the major contributors to subretinal lesion after photocoagulation.

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Activated pericytes proliferate, migrate into the subretinal space and demarcate the distribution of ECM deposits characteristic of fibrotic scar. Systemic inhibition of PDGFR signaling attenuates pericyte infiltration and deposition of ECM components.
Conclusions: Our results definitively identify choroidal pericytes as a novel and significant source of subretinal fibrotic scar after photocoagulation. We suggest that PDGFR signaling blockade may be a potential therapeutic strategy to prevent pericyte infiltration and subretinal fibrosis.
Commercial Relationships: Xueting Luo, None; **Xiaodong Sun**, None
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Program Number: 2271 **Poster Board Number:** B0225
Presentation Time: 3:45 PM–5:30 PM
Function analysis of *HTRA1* regulatory element in Patients with Exudative Age-Related Macular Degeneration
Daisuke Iejima, Takeshi Iwata. National Inst of Sensory Organs, Tokyo Medical Center, Meguro-ku, Japan.
Purpose: Age-related macular degeneration (AMD) is a leading cause of vision loss and blindness in the elderly. *ARMS2/HTRA1* mutation is known to major risk factor for AMD (De Wan et al., Science 2006). But, AMD pathogenic mechanism which derived from *ARMS2/HTRA1* gene mutation is still unclear. Our previous studies suggest that the promoter sequence experiment showed that a great number of AMD patients had specific insertion/deletion (indel) mutation in 3.8 kb upstream of HTRA1 gene. 2-3-fold increase of promoter activity was observed in indel *HTRA1* promoter compared to control sequence (Iejima et al., JBC 2015). Furthermore, we created transgenic mice ubiquitously overexpressing mouse HtrA1 using the chicken actin promoter, of HtrA1 in vivo was shown to lead to choroidal neovascularization (CNV), similar to wet AMD patients (Nakayama, Iejima et al., IOVS 2014). So, we think that human HTRA1 expression is enhanced by AMD specific indel mutation in the promoter region of HTRA1 gene, and this enhanced HTRA1 may be concerned with induce retinal neovasucularization. But, this indel function is unclear. So, our study aim is to elucidate *HTRA1* gene expression mechanism in indel mutation.
Methods: To elucidate the indel sequence binding protein, we designed both normal and indel mutant complementary DNA probes. Double strand DNA probe was designed based on the normal and mutant sequence and Electrophoresis Mobility Shift Assay (EMSA) was performed. The same probe was used to isolate binding transcription factors and to determine the peptide sequence using liquid chromatography-mass spectrometry (LC-MS/MS).
Results: We detected indel specific binding protein using LC-MS/MS, and obtained candidate protein list. Especially, we focused on top-hit candidate transcription factor protein in this protein list, and this protein is General Transcription Factor IIi (GTF2I). Furthermore, we detected indel sequence binding GTF2I for western blot analysis using anti-GTF2I antibody.
Conclusions: Human HtrA1 expression is enhanced by AMD specific indel mutation in the promoter region of HtrA1 gene. Specific transcription factor, which likely to be involved in this enhancement was isolated and peptide sequence determined. Furthermore, we determined indel specific transcription factor: GTF2I.
Commercial Relationships: Daisuke Iejima; **Takeshi Iwata**, None
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Program Number: 2272 **Poster Board Number:** B0226
Presentation Time: 3:45 PM–5:30 PM
Hydroxyapatite induced experimental retinal degeneration in a murine model
Elena Pipi[1], Paul Ibbett[1], Andrew J. Lotery[1], Imre Lengyel[3, 2], V. Hugh Perry[1], Jessica Teeling[1]. [1]University of Southampton, Southampton, United Kingdom; [2]Centre for Experimental Medicine, Belfast, United Kingdom; [3]UCL, London, United Kingdom.
Purpose: Current models of experimental age-related macular degeneration fail to recapitulate the hallmarks of human AMD pathology, including drusen formation and deposition. Recent human studies using post-mortem aged tissue indicate that hydroxyapatite (HAP) spherules are present in all types of sub-RPE deposits. These spherules act as a scaffold for proteins to initiate drusen formation under the RPE. We believe that these spherules could come into direct contact with the neurosensory retina in geographic atrophy (GA), leading to retinal damage. To test the consequences of this contact, we developed a murine model using intraocular injections of HAP to form drusen-like deposits and then examined retinal function and cellular pathology.
Methods: Female C57BL/6J (5 weeks, n=8) received an intravitreal injection of ~500 HAP chromatography beads (20 µm diameter, BioRad) in the right eye, while the untreated left eye served as a control. Retinal structure and function was examined at 4- and 8-weeks post-injection with electroretinography (ERG) and optical coherence tomography (OCT). At 8-weeks post injection eyes were enucleated, embedded in OCT and snap frozen for immunohistochemical analysis for indicators of pathology (CD11b, FcγRI and GFAP).
Results: ERG (b-wave) was significantly reduced in HAP injected eyes compared to control eyes. These ERG deficits were progressive from 4- to 8-weeks in 5 out of 8 mice. Both the number of retinal deposits seen in OCT and the ERG (b-wave) deficits (right to left eye) correlated strongly with reduced thickness of the ONL, as determined by OCT segmentation. Histology revealed that HAP spherules deposit at the outer retina and acquire autofluorescent properties. Finally, CD11b, FcγRI and GFAP expression was significantly increased following HAP injection compared to control, suggesting that HAP induced retinal inflammation and gliosis.
Conclusions: Our results support the hypothesis that HAP deposition is directly involved in retinal degeneration in the mouse retina. These findings might be relevant for human AMD pathology, particularly in atrophic AMD where the neurosensory retina is likely to be directly exposed to the calcifications in and around Bruch's membrane. The full characterisation of this HAP-induced retinopathy may prove helpful in unravelling the molecular steps leading to retinal degeneration.
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