549 Retinal detachment: Basic research
Thursday, May 11, 2017 11:30 AM–1:15 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 5960–5979/B0760–B0779
Organizing Section: Retina

Program Number: 5960 **Poster Board Number:** B0760
Presentation Time: 11:30 AM–1:15 PM
iTRAQ proteomic analysis of proliferative vitreoretinopathy vitreous
Brett L. Smith[1], Marcus Colyer[1], Dal Chun[2], Bongsu Kim[3], Maria del Carmen Piqueras[4], Sanjoy K. Bhattacharya[4], Colleen M. Cebulla[3]. [1]Department of Ophthalmology, Walter Reed National Military Medical Center, Bethesda, MD; [2]The Retina Group of Washington, Vienna, VA; [3]Department of Ophthalmology and Visual Science, Havener Eye Institue, The Ohio State University, Columbus, OH; [4]Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL.
Purpose: Proliferative vitreoretinopathy (PVR) is a challenging pathologic process that contributes to suboptimal visual outcomes in patients with retinal detachment. The purpose of this study was: (1) to assess whether quantitative protein analysis of vitreous fluid in human eyes of patients with PVR with retinal re-detachment was feasible, and (2) whether significant changes in protein levels occur in vitreous from eyes with PVR compared with that of controls.
Methods: Undiluted vitreous samples were obtained during routine pars plana vitrectomy surgery under an IRB-approved protocol from the eyes of two patients (age 30 and 48; PVR grade B and C, respectively) who developed recurrent retinal detachment with PVR. These were compared to undiluted vitreous samples of two eye bank specimens without prior retinal detachment or other vitreoretinal disease. Vitreous samples were labeled with iTRAQ reagent and analyzed by liquid chromatography and tandem mass spectrometry on an Orbitrap type device. Bioinformatics analyses were performed using the Proteome Discoverer software suite.
Results: A total of 107 proteins were identified in the vitreous of eyes with PVR compared to controls. We further classified the proteins according to biological process activity, revealing that proteins were active in regulation of biological processes, response to stimulus, and cell organization and biogenesis (44, 44, and 25 proteins respectively). The protein functions included protein binding, catalytic activity, metal ion binding, and enzyme regulator activity (87, 39, 25 and 20 proteins respectively). The most frequent up-regulated proteins (with respect to presence in donors) in PVR vitreous compared to control with the highest peptide number and sequence coverage were EFEMP1, TF, IGHG4, APOE, and IGHM. Similarly, the most frequent down-regulated PVR vitreous proteins were FABP1, CRYBA1, ENO1, CRYBB2, and PARK7.
Conclusions: This study serves as a basis for future investigation of PVR with iTRAQ labeled mass spectrometric analyses. Our current study has identified candidate proteins for investigation into their potential role in PVR. Further sample collection and multidisciplinary biomedical research in the future will point out their role in PVR.
Commercial Relationships: Brett L. Smith, None; **Marcus Colyer**, None; **Dal Chun**, None; **Bongsu Kim**, None; **Maria del Carmen Piqueras**, None; **Sanjoy K. Bhattacharya**, None; **Colleen M. Cebulla**, None
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Program Number: 5961 **Poster Board Number:** B0761
Presentation Time: 11:30 AM–1:15 PM
Perfluoro-n-octane cytotoxicity in human retinal pigment epithelial cell culture
Girish K. Srivastava[1, 2], Ivan Fernandez-Bueno[1, 3], M L. Alonso-Alonso[1], Maria Teresa Garcia-Gutierrez[1], Manuel Gayoso[4], Rosa M. Coco[1], Jose-Carlos Pastor[1, 5]. [1]Instituto Universitario de Oftalmobiologia Aplicada (IOBA), Universidad de Valladolid, Valladolid, Spain; [2]Centro en Red de Medicina Regenerativa y Terapia Celular, Junta de Castilla y León, Spain; [3]Red Temática de Investigación Cooperativa Sanitaria (RETICS), Oftared, Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, Madrid, Spain; [4]Departmento de Histología, Biología Celular y Farmacología, Universidad de Valladolid, Valladolid, Spain; [5]Departmento de Oftalmología, Hospital Clinico Universitario, Valladolid, Spain.
Purpose: Several cases of acute blindness post non – complicated retinal detachment surgery produce recently a sanitary alert in Spain attributed to several lots of perfluoro-n-octane (Alaocta® PFO) from Ala Medics®. These lots were certified non-toxic by performing cytotoxicity test of extracts of PFO on fibroblasts cultures following UNE EN ISO 10993 - 5 & 12 : 2009 norms by a certified sanitary product testing company. This study proposes to compare this extract method to a new direct exposure method (in patent consideration).
Methods: The cultures of ARPE19 (retinal pigment epithelial cells) and L929 cell line (fibroblasts) were grown at a seeding density of 1×10^5 to reach an OD ≥ 0.2. The extracts were prepared by stirring 0.2 g/ml of PFO samples in DMEM culture medium for 24 hours at 37 ^{0}C. Cell cultures were incubated with these extracts for 72 hours in standard culture conditions followed by XTT assay to detect cytototoxicity. Cultures were also exposed directly to PFO, blocking its evaporation by cell culture medium, for 30 and 60 minutes. Cultures were grown for 24 and 72 hours and cytototoxicity were detected by MTT assay. Experiments were performed fulfilling the requirements of the ISO 10993 - 5 & 12 : 2009 norms and repeated three times in triplicate (n=9).
Results: Both cultures (ARPE19 and L929) grew well and achieved standards required in ISO norms. The experiments performed for extract method showed non-toxic nature of PFO (> 70% viability; non-toxic; < 70%; toxic according to ISO norms). However, the new proposed direct method is capable to differentiate between non toxic and toxic PFO lots. Some lots were more toxic and showed severe damages in cell morphology and cell cultures and related to the permanence time of PFO in storage.
Conclusions: Ala Medics® PFO time-dependent cytotoxicity has been confirmed in ARPE19 cell cultures. This study shows that extract preparation, and testing extract with cultures does not guarantee to detect a toxic component if it is non leachable. Parameters of a test are critical to certify a sanitary product.
Commercial Relationships: Girish K. Srivastava, None; **Ivan Fernandez-Bueno**, None; **M L. Alonso-Alonso**, None; **Maria Teresa Garcia-Gutierrez**, None; **Manuel Gayoso**, None; **Rosa M. Coco**, None; **Jose-Carlos Pastor**, None
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Program Number: 5962 **Poster Board Number:** B0762
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Detached outer retina surface tension is dictated by pericellular interphotoreceptor matrix
Federico Gonzalez-Fernandez[1, 2], Mary Alice Garlipp[6], Priscilla Gonzalez-Fernandez[1, 3], Dongjin (Luke) Sung[4], Anne Meyer[5], Robert Baier[5]. [1]Medical Research Service, G.V. (Sonny) Montgomery Veterans Affairs Med Center, Jackson, MS; [2]Ophthalmology & Pathology, University of Mississippi Medical Center, Jackson, MS; [3]Research, PathRD Inc., Jackson, MS; [4]Ophthalmology & Pathology, SUNY Buffalo, Ross Eye Institute, Buffalo, NY; [5]Center for Biosurfaces, SUNY, Buffalo, NY; [6]Neuroscience, SUNY, Buffalo, NY.
Purpose: Little is known about the nature of adhesive interactions between the retina and retinal-pigmented epithelium (RPE). Such interactions may play an important role in maintaining normal retinal attachment, and the pathophysiology of detachment. Here, we ask what structures contribute to the critical surface tension of the outer retinal surface.
Methods: We used a material sciences approach to examine the physical and biochemical characteristics of the outer retinal surface of detached bovine retina. Contact angle goniometry determined the critical surface tension of the outer retinal surface, isolated insoluble interphotoreceptor matrix (IPM) and purified interphotoreceptor retinoid binding protein (IRBP). Mid-infrared attenuated total reflectance (MIR-ATR) spectroscopy was used to characterize the molecular composition of the retinal surface. This data was correlated with light microscopy, immunohistochemistry and electronmicroscopy study of the detached retinal surface. MIR-ATR depth penetration was established through ellipsometric measurement of barium-sterate films. This data was correlated to MIR-ATR spectra of docosahexaenoic acid, hyaluronan, chondroitin-6-sulfate and IRBP.
Results: We found that the retinal surface tension is 24 mN/m similar to isolated insoluble IPM and lower than IRBP. Barium-sterate calibration studies established that the MIR-ATA spectroscopy penetration depth to be only 0.3 μm. Correlation with electronmicroscopy and immunohistochemical studies indicate that a layer of IPM coating the outer retinal surface is responsible for these properties.
Conclusions: The surface tension of detached bovine retina is dictated not by the outer segments, but by a thin film of IPM coating its outer surface. These observations may help us to understand the molecular pathophysiology of retina adhesion and detachment.
Commercial Relationships: Federico Gonzalez-Fernandez, PathRD Inc. (I); **Mary Alice Garlipp**, None; **Priscilla Gonzalez-Fernandez**, None; **Dongjin (Luke) Sung**, None; **Anne Meyer**, None; **Robert Baier**, None
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Program Number: 5963 **Poster Board Number:** B0763
Presentation Time: 11:30 AM–1:15 PM
Optimization of retinal detachment vitreous sample preparation methods for protein analysis
Bongsu Kim[1], Bernard Wen[1], Rania Kusibati[1], Tyler N. Heisler-Taylor[1], Frederick Davidorf[1], Matthew P. Ohr[1], Michael B. Wells[1], Dino D. Klisovoc[2, 1], John B. Allen[1], Sanjoy K. Bhattacharya[3], Colleen M. Cebulla[1]. [1]Havener Eye Institute, Ophthalmology and Visual Science, The Ohio State Universty, Columbus, OH; [2]Midwest Retina, Dublin, OH; [3]Bascom Palmer Eye Institute, Department of Ophthalmology, University of Miami Miller School of Medicine, Miami, FL.
Purpose: Vitreous structure can hinder the measurement of consistent protein concentration levels over subsequent assays for research or clinical testing in retinal detachment. The purpose of this study is to compare multiple methods of vitreous sample preparation for the consistency of measured protein levels between multiple assays.
Methods: Patient vitreous samples (n=4 /group) were randomly selected from sample collection under an IRB-approved protocol during retinal detachment surgical procedure. BCA assay with 96 well plates was performed for the comparison of five groups of vitreous preparation: 1) vortex (S3200, BioExpress), 2) hand held-homogenization (Disposable pellet mixer and cordless motor, VWR), 3) sonication (Dismembrator-500, Fisher Scientific), 4) the use of lysis buffer (9803S, Cell signaling), and 5) no treatment as a control. Three independent assays were run with each treatment and all 4 subjects. Samples in each assay were run in triplicate accounting for pipetting error. The coefficient of variation (CV %) from the three assays was averaged between the 4 subjects and compared between the 5 methods. Student's t-test was used for statistical analysis.
Results: The mean CV for the no treatment control was 13.48% ± 9.21. The mean CV of lysis buffer treatment and sonication are significantly lower than that of control (4.34% ± 1.79, P=0.0417 and 4.49% ± 3.14, p=0.0449, respectively). The mean CV of vortex treatment and hand-held homogenization were 17.05% ± 7.73 and 18.43% ± 3.28, respectively. These were not statistically significant compared to control (p=0.3988 and p=0.2464, respectively).
Conclusions: Vitreous sample sonication or lysis buffer extraction significantly improves the consistency of protein measurement of samples. Future study will evaluate the quality of protein detection with ELISA assays after using these methods
Commercial Relationships: Bongsu Kim, None; **Bernard Wen**, None; **Rania Kusibati**, None; **Tyler N. Heisler-Taylor**, None; **Frederick Davidorf**, None; **Matthew P. Ohr**, None; **Michael B. Wells**, None; **Dino D. Klisovoc**, None; **John B. Allen**, None; **Sanjoy K. Bhattacharya**, None; **Colleen M. Cebulla**, None
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Program Number: 5964 **Poster Board Number:** B0764
Presentation Time: 11:30 AM–1:15 PM
The *MDM2* T309G mutation in primary human retinal epithelial cells enhances experimental proliferative vitreoretinopathy
wenyi wu[1], yajiang duan[1], gaoen ma[1], Yewlin Chee[1], Arif Samad[2], Jing Z. Cui[2], Joanne A Matsubara[2], Shizuo Mukai[1], Patricia A. D'Amore[1], Hetian Lei[1]. [1]schepens eye research insititution, Boston, MA; [2]Department of Ophthalmology and Visual Sciences, Vancouver, BC, Canada.

Purpose: The murine double minute 2(MDM2), whose human homologue is also called HDM2, is a critical negative regulator of the p53 tumor suppressor and the G309 allele of singe nucleotide polymorphisms (SNPs) in the *MDM2* promoter locus is associated with a higher risk of proliferative vitreoretinopathy (PVR). We have shown that the *MDM2* T309G mutation created using clustered regularly interspaced short palindromic repeats (CRISPR)/associated endonuclease (Cas)9 enhances vitreous-induced expression of MDM2 and survival of primary human primary retinal pigment epithelial (hPRPE) cells. The goal of this project is to determine whether this MDM2 mutation contributes to the development of experimental PVR.
Methods: Human vitreous from PVR patients (HV) was used to treat the hPRPE cells with *MDM2* T309T or T309G, and the expression of MDM2 and p53 in the treated cells was examined by western blot. The vitreous-induced cellular responses such as contraction were assessed, PVR was evaluated by intravitreal injection of the hPRPE cells with *MDM2* T309T or T309G into rabbit eyes.
Results: Western blot analysis indicated that HV enhanced a increase (1.7 ± 0.2 fold) in MDM2 and a significant decrease in p53 in the PRPE cells with the *MDM2* T309G compared to those with *MDM2* T309T. In addition, we found that HV promoted cell contraction in the PRPE cells with the cells expressing *MDM2* T309G contracting more than cells with *MDM2* T309T. Furthermore, *MDM2* T309G enhanced the development of PVR in the rabbit model.
Conclusions: The *MDM2* SNP G309 contributes to the development of experimental PVR.
Commercial Relationships: wenyi wu; **yajiang duan**, None; **gaoen ma**, None; **Yewlin Chee**, None; **Arif Samad**, None; **Jing Z. Cui**, None; **Joanne A Matsubara**, None; **Shizuo Mukai**, None; **Patricia A. D'Amore**, None; **Hetian Lei**, None
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Program Number: 5965 **Poster Board Number:** B0765
Presentation Time: 11:30 AM–1:15 PM
Fasudil, a clinically used ROCK inhibitor, stabilizes photoreceptor synapses after retinal detachment
Ellen Townes-Anderson[1], Jianfeng Wang[1], Eva Halasz[1], Ilene Sugino[2], Amy Pitler[3], Marco A. Zarbin[2]. [1]Pharmacology, Physiology & Neuroscience, Rutgers New Jersey Medical School, Newark, NJ; [2]Institute of Ophthalmology and Visual Science, Rutgers New Jersey Medical School, Newark, NJ; [3]Microbiology, Biochemistry and Medical Genetics, Rutgers New Jersey Medical School, Newark, NJ.
Purpose: Retinal detachment causes the disjunction of the rod-bipolar synapse in the outer plexiform layer (OPL) by retraction of rod axons. In animal models, reattachment does not restore broken synapses fully. We have determined that breakage is due to activation of the RhoA pathway and that inhibition of RhoA signaling, using the Rho kinase (ROCK) inhibitor Y27632, reduces retinal disruption (Wang et al. IOVS 2016). Here we test whether the ROCK inhibitor fasudil, used for other clinical applications, can prevent synaptic injury after detachment.
Methods: Retinal explants, detached from the retinal pigmented epithelium, were maintained in culture for 2 hrs and then subjected to western blotting. In vivo, detachments were made in young Yorkshire pigs by subretinal injection of BSS in the inferior nasal retina. Fasudil (1,10mM) was injected either subretinally or intravitreally at the time of detachment. After 2 hrs, retinas were fixed for examination by immunocytochemistry and confocal microscopy. Retraction of rod terminals was quantified by imaging the amount of synaptic vesicle label in the outer nuclear layer (ONL); normally label remains exclusively in the OPL. Additionally, retinas were analyzed for apoptosis in the ONL using propidium iodide staining.
Results: Phosphorylated cofilin, a downstream effector of ROCK, was decreased by 26% with 30μM fasudil in vitro (N=5 explants, $p<0.05$); total cofilin remained unchanged. In vivo, subretinal injection of fasudil (10mM) reduced retraction of rod spherules by 59% compared to untreated control detachments (N=3 pigs, $p<0.001$, paired t-test). This compares well with previous results with Y27632 (Wang et al.). Intravitreal injection of 10mM fasudil, potentially a more clinically relevant route of administration, also reduced retraction of rod axons by 34% (N=5, $p<0.05$). Controls had no evidence of photoreceptor degeneration at 2 hrs, but by 4 hrs apoptosis was evident. With 10mM fasudil the number of apoptotic nuclei was substantially reduced at 4 hrs (51% reduction, N=4, $p<0.05$).
Conclusions: Fasudil reduced photoreceptor degeneration and preserved the rod-bipolar synapse after retinal detachment. These results add additional support to the possibility, previously tested with Y27632, that ROCK inhibition may increase visual restoration after detachment when combined with surgical reattachment.
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Presentation Time: 11:30 AM–1:15 PM
Expression of pro-fibrotic genes in an *in vitro* model of Proliferative Vitreoretinopathy
Heuy-Ching H. Wang, Teresa A. Burke, Ramesh Kaini, Whitney Greene. Ocular Trauma, US Army Institute of Surgical Research, Fort Sam Houston, TX.
Purpose: Proliferative vitreoretinopathy (PVR) is the result of aberrant wound healing and fibrosis following a retinal tear or detachment. Expression of genes that promote TGF-β/CTGF signaling, epithelial-mesenchymal transition, extracellular matrix remodeling and cell adhesion, inflammation, and angiogenesis is increased during fibrosis and may lead to the development of PVR. The purpose of this study is to examine the expression of 84 pro-fibrotic genes using an *in vitro* model of PVR.
Methods: Retinal pigment epithelium derived from induced pluripotent stem cells (iPS-RPE) was cultured on matrigel-coated transwells for 60 days until confluent and pigmented. The cell monolayers were then scratched to create a wound. To recapitulate the *in vivo* conditions that lead to the development of PVR, one set of cells was treated with 5% fresh vitreous immediately after wounding. Unscratched cells and fresh vitreous alone were included as controls. Cells were lysed on Days 0, 3, and 12 post wounding. Total RNA was extracted and reverse transcribed to prepare cDNA. A commercially-available human fibrosis PCR array was used to analyze expression of 84 genes associated with fibrosis. Data was analyzed to identify pro-fibrotic genes that were differentially expressed during wound healing 4-fold or greater compared to controls.
Results: Analysis of eleven sample sets revealed a distinctive pattern of pro-fibrotic gene expression. Wounded cells had increased expression of 10 pro-fibrotic genes (Day 3), and 29 pro-fibrotic genes (Day 12) compared to control cells. The most highly expressed pro-fibrotic genes included TIMP1, TIMP3, STAT1, and COL1A2 (Day 3); and COL1A2, CTGF, TIMP1, TIMP2, TIMP3, and MMP2 (Day 12). Cells treated with vitreous immediately after wounding had increased expression of 20 pro-fibrotic genes (Day 3) and 17 pro-fibrotic genes (Day 12) compared to unscratched cells also treated with vitreous. The most highly expressed genes during wound healing with vitreous treatment included COL1A2, CTGF, and CAV1 in both Day 3 and Day 12 samples.

Conclusions: The results of this study reveal distinct patterns of pro-fibrotic gene expression during wound healing. The most significantly increased genes are involved in tissue remodeling, collagen production, cell proliferation, and caveolar-mediated endocytosis. These results may provide novel therapeutic targets for the treatment of this blinding disorder.
Commercial Relationships: Heuy-Ching H. Wang; **Teresa A. Burke**, None; **Ramesh Kaini**, None; **Whitney Greene**, None
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Program Number: 5967 **Poster Board Number:** B0767
Presentation Time: 11:30 AM–1:15 PM
miR-155 regulates microglia activation in a murine model of retinal detachment
Yoko Okunuki, Garrett Klokman, Kip M. Connor. Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA.
Purpose: Degeneration of photoreceptors, a primary cause of vision loss worldwide, is caused by a wide range of retinal pathologies, including retinal detachment (RD). Understanding the underlying mechanisms surrounding photoreceptor cell death is critical to developing new treatment strategies. Since photoreceptors are non-dividing cells, their loss leads to irreversible visual impairment, even after successful retinal reattachment surgery. Currently, our knowledge of the processes for which photoreceptors degenerate is very poorly understood. Retinal microglia become activated during RD however, the role of microglia in the pathophysiology of photoreceptor cell death is not well understood. The association of pro-inflammatory microRNA-155 (miR-155), abundantly expressed in microglia, is suggested to be associated with worsening of neurodegenerative brain diseases. In this study, we examined the role of miR-155 on photoreceptor cell death in RD.
Methods: RD was induced by subretinal injection of 4 µl of sodium hyaluronate in miR-155 $^{-/-}$ mice and C57BL/6J mice. RD-induced photoreceptor cell death was compared between miR-155 $^{-/-}$ mice and C57BL/6J mice by TUNEL staining. Microglia were isolated from intact retinas and 24hr post RD retinas of C57BL/6J mice by fluorescence-activated cell sorting, and miR-155 expression in microglia was measured by RT-PCR.
Results: The number of TUNEL positive cells in miR-155 $^{-/-}$ retinas was significantly decreased 24hrs after RD ($p=0.008$, $n=8$). Microglial miR-155 expression was significantly increased in 24hr post RD retinas compared to intact retinas ($p<0.001$).
Conclusions: Loss of miR-155 suppressed photoreceptor cell death in response to RD. Moreover, miR-155 expression was significantly induced in retinal microglia in response to RD. Preliminary data suggests that miR-155 loss protects photoreceptor cells from cell death, although the exact role of microglial expression of miR-155 with retinal damage still needs further elucidation.
Commercial Relationships: Yoko Okunuki, None; **Garrett Klokman**, None; **Kip M. Connor**, Achillion (F), OMEICOS (F), OMEICOS (C), MEEI (P)
Support: GOAP research award 2016

Program Number: 5968 **Poster Board Number:** B0768
Presentation Time: 11:30 AM–1:15 PM
Vitreous and subretinal fluid ATP concentrations in rhegmatogenous retinal detachment
Takashi Tachibana, Toshio Hisatomi, Shoji Notomi, Shunji Nakatake, Kohta Fujiwara, Jun Funatsu, Yusuke Murakami, Yasuhiro Ikeda, Shigeo Yoshida, Tatsuro Ishibashi, Koh-hei Sonoda. Ophthalmology, Kyushu University, Fukuoka, Japan.
Purpose: Adenosine triphosphate (ATP) plays an important role in cell energy metabolism and intercellular signaling processes. During the course of apoptosis, dying cells release ATP into extracellular space and extracellular ATP accelerates photoreceptor apoptosis. Then, extracellular ATP degrades into adenosine diphosphate (ADP). However, physiology of ATP in vitreous is not fully understood. The purpose of this study is to determine the vitreous levels of ATP and ADP in rhegmatogenous retinal detachment (RRD) with photoreceptor apoptosis.
Methods: Vitreous samples were obtained from 96 eyes of 96 patients with RRD (n = 54), macular hole (MH; n=15), and epiretinal membrane (ERM; n =27) during pars plana vitrectomy. In RRD patients, subretinal fluids were also collected from retinal tear during vitrectomy. We measured ATP and ADP concentration using luciferin-luciferase assay. Clinical data were analyzed by retrospective chart review. RRD patients were divided into two groups according to duration of symptoms. ATP concentration was compared between two groups.
Results: Compared with MH and ERM patient, RRD patient have higher vitreous ATP concentration (RRD: 1.74ng/ml, MH: 0.11ng/ml, ERM: 0.065ng/ml, $p < 0.005$, respectively) and higher ADP concentration ($p < 0.01$). No significant difference was observed in vitreous ATP concentration between short-duration group and long-duration group ($p= 0.07$, Mann-Whitney U test). However, short-duration group have significantly higher subretinal fluid ATP concentration compared with long-duration group ($p<0.001$). Duration of symptoms showed negative correlation with subretinal fluid ATP concentration ($r= -0.61$m $p < 0.001$, Spearman's rank correlation test), but no significant correlation with vitreous ATP concentration ($r= -0.32$, $p= 0.06$).
Conclusions: RRD patients have high ATP and ADP concentration in vitreous and subretinal fluid. These data suggest physiological and pathological metabolic pathways of ATP and ADP in the retina and vitreous.
Commercial Relationships: Takashi Tachibana; **Toshio Hisatomi**, None; **Shoji Notomi**, None; **Shunji Nakatake**, None; **Kohta Fujiwara**, None; **Jun Funatsu**, None; **Yusuke Murakami**, None; **Yasuhiro Ikeda**, None; **Shigeo Yoshida**, None; **Tatsuro Ishibashi**, None; **Koh-hei Sonoda**, None

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Presentation Time: 11:30 AM–1:15 PM
Neuroprotective and anti-gliotic effects of MIF Inhibitor ISO-1 in experimental murine retinal detachment
Colleen M. Cebulla[1], Bongsu Kim[1], Rania Kusibati[1], Tyler Heisler-Taylor[1], Dimosthenis Mantopoulos[1], Jiaxi Ding[1], Abhay Satoskar[2], Jonathan P. Godbout[3], Sanjoy K. Bhattacharya[4], Mohamed H. Abdel-Rahman[1, 5]. [1]Havener Eye Institute, Ophthalmology and Visual Science, The Ohio State University, Columbus, OH; [2]Division of Experimental Pathology, The Ohio State University, Columbus, OH; [3]Department of Neuroscience, The Ohio State University, Columbus, OH; [4]Bascom Palmer Eye Institute, Department of Ophthalmology, University of Miami Miller School of Medicine, Miami, FL; [5]Division Human Genetics, The Ohio State University, Columbus, OH.
Purpose: Pro-inflammatory factors promote retinal gliosis and photoreceptor death that underlie vision-threatening complications of retinal detachment (RD). The purpose of this study is to test the hypothesis that inhibition of the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) blocks photoreceptor apoptosis and retinal gliosis in RD.
Methods: RDs were induced by subretinal injection of hyaluronic acid into left eyes of (16-24 week-old) C57BL/6 female mice

(n=6-18/group) or BALB/c Mif knockout mice and background controls (n=10/group) under an IACUC-approved protocol. Fellow eyes served as controls. Eyes were enucleated and processed for immunofluorescence and TUNEL analysis. Retinas were isolated for RNA or protein extraction. 8-plex iTRAQ labeled proteomics was performed to evaluated up- and down-regulated proteins in RD compared to controls. ELISA evaluated MIF expression. C57BL/6 mice were treated with daily intraperitoneal injection of MIF inhibitor ISO-1 or vehicle and retinas were evaluated for apoptosis, inflammatory infiltrate, and gliosis.
Results: MIF was up-regulated 1.9-fold in RDs on iTRAQ analysis, which was further corroborated by ELISA (1.7 ± 0.079 ng MIF/µg total protein in controls vs. 1.87±0.138 in RD, *p≤0.089). Upregulated MIF was also detected in subretinal macrophages in RD. ISO-1 (40mg/kg) significantly decreased TUNEL positive photoreceptors in RD ((4125.89 ± 849.5 cells/mm^2 retina vehicle vs 588.68 ± 409.5 cells/mm^2 retina ISO-1, p=0.003). The density and polarization of macrophages were not altered. Retinal gliosis GFAP expression was reduced by ISO-1 treatment and MIF genetic depletion. Retinal phosphorylated ERK levels were increased in ISO-1 and MIF genetic depletion.
Conclusions: MIF inhibition should be explored as a potential therapeutic adjunct for RD.
Commercial Relationships: Colleen M. Cebulla, None; **Bongsu Kim**, None; **Rania Kusibati**, None; **Tyler Heisler-Taylor**, None; **Dimosthenis Mantopoulos**, None; **Jiaxi Ding**, None; **Abhay Satoskar**, None; **Jonathan P. Godbout**, None; **Sanjoy K. Bhattacharya**, None; **Mohamed H. Abdel-Rahman**, None
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Program Number: 5970 **Poster Board Number:** B0770
Presentation Time: 11:30 AM–1:15 PM
Expression of pro-fibrotic miRNAs in an *in vitro* model of Proliferative Vitreoretinopathy
Whitney Greene, Teresa A. Burke, Ramesh Kaini, Heuy-Ching H. Wang. Ocular Trauma, United States Army Inst of Surgical Rsrch, San Antonio, TX.
Purpose: Proliferative vitreoretinopathy (PVR) is the result of aberrant wound healing and fibrosis following a retinal tear or detachment. MicroRNAs (miRNAs) may promote the pathogenesis of fibrosis by regulating the expression of genes involved in TGF-β signaling, epithelial-mesenchymal transition, extracellular matrix remodeling and cell adhesion, and inflammation. An *in vitro* model of PVR that uses retinal pigment epithelium derived from induced pluripotent stem cells (iPS-RPE) was used to investigate the expression of pro-fibrotic miRNAs.
Methods: iPS-RPE was cultured on matrigel-coated transwells for 60 days until fully confluent and pigmented. Cell monolayers were scratched to create a wound. To recapitulate the *in vivo* conditions that lead to the development of PVR, one set of cells was treated with 5% fresh vitreous immediately after wounding. Unscratched cells and fresh vitreous alone were included as controls. Cells were lysed on Days 0, 3, and 12 post wounding. Total RNA including small RNA was extracted. RNA was reverse transcribed to prepare cDNA. A commercially-available human fibrosis miRNA PCR array was used to analyze expression of 84 distinct miRNAs associated with fibrosis. Data was analyzed to identify pro-fibrotic miRNAs that were differentially expressed during wound healing 4-fold or greater compared to controls.
Results: Analysis of eleven sample sets revealed a distinctive pattern of miRNA expression. Wounded cells had increased expression of 43 pro-fibrotic miRNAs (Day 3), and 30 pro-fibrotic miRNAs (Day 12) compared to control cells. The most highly expressed miRNAs during wound healing included miR-122-5p (Day 3); and miR-101-3p, miR-150-5p, miR-223-3p (Day 12). Cells treated with vitreous immediately after wounding had increased expression of 12 pro-fibrotic miRNAs (Day 3) and 36 pro-fibrotic miRNAs (Day 12) compared to unscratched cells also treated with vitreous. The most highly expressed miRNAs during wound healing with vitreous treatment included miR-10a-5p (Day 3); and miR-10a-5p and miR-133a-3p (Day 12).
Conclusions: The results of this study reveal distinct patterns of miRNA expression during wound healing. The expression of pro-fibrotic miRNAs was segregated according to wounding versus control, time post-wound, and exposure to vitreous. The profiling results from this array may yield insights into the molecular mechanisms that underlie the pathogenesis of PVR.
Commercial Relationships: Whitney Greene, None; **Teresa A. Burke**, None; **Ramesh Kaini**, None; **Heuy-Ching H. Wang**, None

Program Number: 5971 **Poster Board Number:** B0771
Presentation Time: 11:30 AM–1:15 PM
Protein Kinase A inhibitor H89 attenuated experimental proliferative vitreoretinopathy
Yali Lyu[1, 2], Junsheng Fan[1, 2], Lian Cui[1, 2], Zhongzhu Deng[1, 2], Chao Wang[1, 2], Mengwen Li[1, 2], Nan Yang[1, 2], Jieping Zhang[1, 2], Juan Wang[1, 2], Qingjian Ou[1, 2], Lixia Lyu[1, 2], Guotong Xu[1, 2].
[1]Department of Ophthalmology of Shanghai Tenth People's Hospital, Tongji Eye Institute, Tongji University School of Medicine (TUSM), Shanghai, China; [2]Department of Regenerative Medicine and Stem Cell Research Center, Tongji University School of Medicine (TUSM), Shanghai, China.
Purpose: Proliferative vitreoretinopathy (PVR) is the major cause of failure in surgery for rhegmatogenous retinal detachment. Epithelial-to-mesenchymal transition (EMT) of reitnal pigment epithelium(RPE) mediates the main pathogenesis of PVR. Currently there is no effective treatment available, so this study aimed to explore the role of Protein Kinase A(PKA) inhibitor H89 in experimental PVR and thus develop a novel therapeutic approach for PVR.
Methods: In vivo PVR model was made by intravitreal injection of ARPE19 and Platelet rich plasma (PRP) in SD rats. The rats were divided into 3 groups: PBS, ARPE19 +PRP and ARPE19+PRP+H89 respectively. Electroretinogram (ERG) was recorded at indicated times, EMT related markers were evaluated by immunofluorescence (IF) for dissected eyes. In vitro PVR model was prepared by TGFβ treatment. Q-PCR, Western Blot and IF were performed. Scratch assay, cell migration by trans well assay and PKA kinase activity were also examined.
Results: In vivo PVR model, the intervention of H-89 prevented the decrease of b-wave amplitude of ERG in PVR group. H89 attenuated the formation of epiretinal membrane and downregulated the expression of a-SMA and FN1. In vitro, H89 treatment inhibited EMT related genes expression at both protein and mRNA level in RPE cells. In addition, TGFβ1-induced cell migration and proliferation were blocked by H89 treatment, as shown in scratch assay and transwell experiments. It was also found that TGFβ1

treatment increased PKA activity in dose- and time dependent manner.

Conclusions: This study demonstrated that activation of PKA pathway was involved in PVR, H89 can effectively inhibit the EMT of RPE cells in vivo and in vitro model. These data will provide a novel insight into the pathogenesis of PVR. H89 intervention merits further clinical investigation.

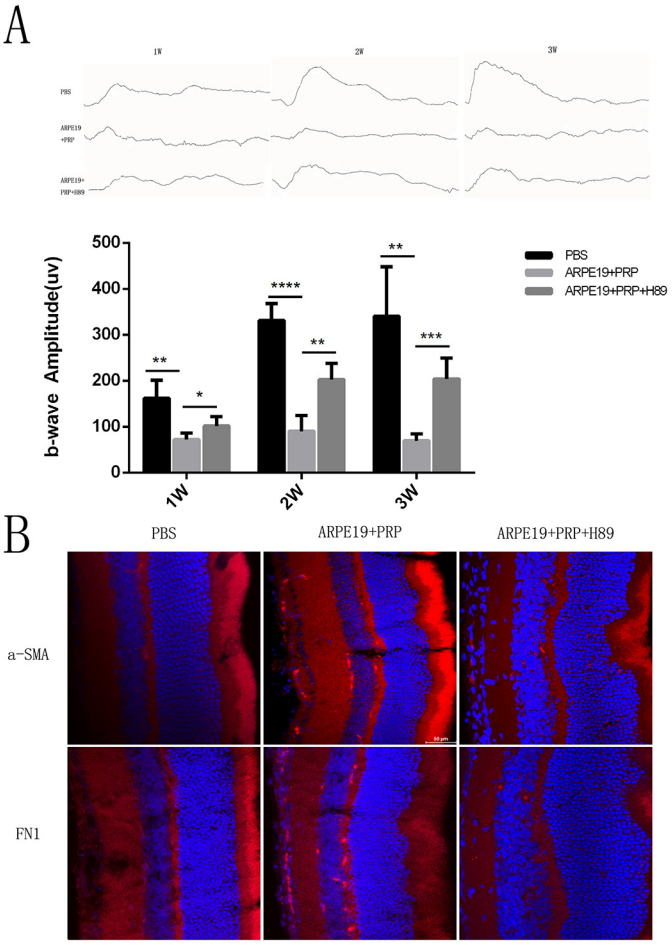

Figure 1. H89 inhibited PVR in vivo. A: Treatment of H-89 can rescue the decrease of the b-wave amplitude(There were 6 eyes for each group). B: Treatment of H89 attenuated the formation of epiretinal membrane and downregulated the expression of a-SMA and FN1.

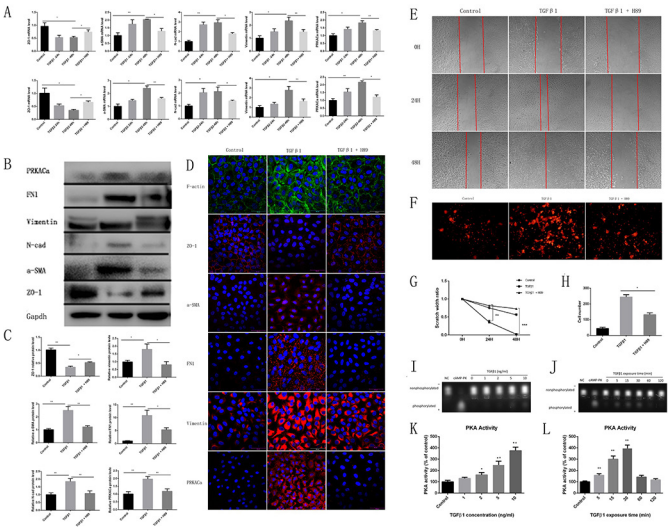

Figure 2. H89 inhibited PVR in vitro. A-D: H89 treatment inhibited EMT related genes expression at both mRNA and protein level (q-PCR, WB and IF). E-H: H89 treatment bloked TGFβ1 induced cell migration and proliferation (scratch and trans well experiments.) I-L:TGFβ1 increased PKA activity in dose- and time dependent manner.

Commercial Relationships: Yali Lyu, None; **Junsheng Fan**, None; **Lian Cui**, None; **Zhongzhu Deng**, None; **Chao Wang**, None; **Mengwen Li**, None; **Nan Yang**, None; **Jieping Zhang**, None; **Juan Wang**, None; **Qingjian Ou**, None; **Lixia Lyu**, None; **Guotong Xu**, None

Program Number: 5972 **Poster Board Number:** B0772
Presentation Time: 11:30 AM–1:15 PM

Development of an experimental cell preparation and delivery protocol to the murine subretinal space using 27-gauge vitreoretinal surgery instruments

Karl A. Hudspith[1], Ying Liu[1], Simrat Sodhi[1], Diana Ottulich[1], Thomas Hilton[1, 2], Mandeep S. Singh[1]. [1]Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; [2]University of Cambridge, Cambridge, United Kingdom.

Purpose: Transplantation of stem-cell derived retinal tissue is a promising potential therapy for inherited retinal degenerations, which are currently incurable. The recent development of three-dimensional cultured retina in vivo has provided a new donor substrate for evaluation. However, experimental transplantation protocols would require dissection of the stem-cell derived retinal micro-organs into micro-sheets. We investigated the use of 27-gauge vitreoretinal surgery micro-instruments to prepare micro-sheets of photoreceptor cells for transplantation to the subretinal space of mice.

Methods: Primary donor mouse neuroretinal sheets were used to model the stem cell derived three-dimensional retinal micro-organs for transplantation. The donor retinas were dissected into micro-sheets using commercially-available 27-gauge sharp-tipped vertical and horizontal scissors (Vitreq USA Inc., NH), loaded into a micro needle, and injected into the subretinal space of a mouse eye. Post-surgery retinas were imaged *in vivo*, before the mice were sacrificed, and the eyes extracted, fixed, sectioned and stained for immunofluorescent microscopy.

Results: Using the 27-gauge micro-scissors, the donor retinal tissue was successfully dissected into micro-sheets measuring approximately 2 by 3 mm. The tested instruments achieved clean cutting of the tissue, perpendicular to the retina, and avoided tearing

the edges of the micro-sheets. The micro-sheets could be aspirated up into the 21-gauge transplantation needle and successfully injected into the subretinal space of murine eyes. *In vivo* Spectralis imaging and fluorescent microscopy of stained sections of extracted eyes confirmed that the micro-sheets had survived following injection into the subretinal space.
Conclusions: The use of 27-gauge micro-scissors in human vitreoretinal surgery, based on their ability to safely and efficiently dissect retina and resect membranes *in vivo*, is already commonplace. Here we have demonstrated that these surgical tools are also useful for preparation of micro-sheets of dissected retinal tissue for delivery into the eye of a mouse model organism, achieving clean perpendicular cutting and avoiding tearing of tissue edges. Commercially-available fine-gauge scissors could be useful in the preparation of biosynthetic retinal tissue for human clinical trials of photoreceptor transplantation.
Commercial Relationships: Karl A. Hudspith; **Ying Liu**, None; **Simrat Sodhi**, None; **Diana Ottulich**, None; **Thomas Hilton**, None; **Mandeep S. Singh**, None
Support: Juliette RP Vision Foundation Young Scientist Award

Program Number: 5973 **Poster Board Number:** B0773
Presentation Time: 11:30 AM–1:15 PM
Optical density of subretinal fluid in patients with chronic central serous chorioretinopathy treated with eplerenone: a potential imaging biomarker
Nisarg Joshi[1], Murtaza K. Adam[1], Wasim Samara[1], Abtin Shahlaee[1], Ehsan Rahimy[2], Jason Hsu[1], Mitchell S. Fineman[1], Sunir Garg[1].
[1]Retina, Wills Eye Hospital, Philadelphia, PA; [2]Palo Alto Medical Foundation, Palo Alto, CA.
Purpose: Optical density (OD) of subretinal fluid (SRF) assessed by optical coherence tomography (OCT) in eyes with central serous retinopathy (CSR) has been shown to be a potentially unique biomarker to distinguish it from other macular pathology. We investigated whether optical density of SRF in patients with chronic CSR treated with eplerenone correlates with presenting visual acuity (VA) and treatment response.
Methods: A retrospective, observational study of patients with chronic CSR who were treated with eplerenone between January 2014 and March 2016 with at minimum 1.4 months of follow up (range 1.4 - 11.0) and had spectral domain OCT was conducted. OD measurements of the vitreous and SRF were obtained using ImageJ (National Institutes of Health) and a SRF to vitreous OD ratio (ODR) was calculated. Patients with OCTs that did not have enough SRF for analysis were excluded. VA, central macular thickness (CMT), peak SRF height, and foveal SRF height were measured. The Mann-Whitney U was used to compare continuous variables and the Pearson's correlation coefficient was used to determine statistical significance for linear regressions.
Results: Twenty-seven eyes of 21 patients with a mean age of 56 years (range 32-75) met the inclusion criteria. Seven eyes (28%) responded to eplerenone treatment with complete resolution of SRF. Mean logMAR VA at baseline was unchanged 2 months following initiation of treatment (0.39 ±0.31 versus 0.36 ±0.34; P=0.89). There was no significant difference between the ODR of eyes that had SRF resolution with eplerenone versus eyes that did not respond to eplerenone and had persistent SRF (1.32 ±1.04 versus 2.09 ±2.52; P=0.58). A linear correlation was not found between ODR and changes in CMT (P=0.26), peak SRF (P=0.19), and foveal SRF (P=0.29). There was an inverse correlation between ODR and presenting logMAR VA with linear regression (P=0.02).
Conclusions: Presenting OD of SRF on OCT in eyes with chronic CSR treated with eplerenone did not correlate with degree of macular SRF change. There was also no difference in ODR between eyes that had complete anatomic resolution with eplerenone compared to eyes that did not respond. Presenting visual acuity was correlated with ODR, however, this relationship is potentially confounded by the degree of foveal involvement.
Commercial Relationships: Nisarg Joshi; **Murtaza K. Adam**, None; **Wasim Samara**, None; **Abtin Shahlaee**, None; **Ehsan Rahimy**, Google (C), Allergan (C); **Jason Hsu**, None; **Mitchell S. Fineman**, None; **Sunir Garg**, J and J/Centcor (F), Deciphera (C), Genentech (C), Eyegate (F), Allergan (F), Allergan (C)

Program Number: 5974 **Poster Board Number:** B0774
Presentation Time: 11:30 AM–1:15 PM
Change in choroidal blood flow and morphology after segmental scleral buckling for rhegmatogenous retinal detachment?
Takeshi Iwase, Misato Kobayashi, Kentaro Yamamoto, Hiroko Terasaki. Ophthalmology, Nagoya University Hospital, Nagoya, Japan.
Purpose: Although scleral buckling is a well-established, anatomically successful surgical treatment for rhegmatogenous retinal detachment (RRD), the procedure can reportedly cause problems in the ocular circulation. We evaluated choroidal blood flow determined by laser speckle flowgraphy (LSFG) and morphological changes following segmental scleral buckling in eyes affected by RRD.
Methods: Segmental scleral bucking without a concomitant encircling procedure was performed on 46 eyes of 46 patients with successfully reattached macula-on RRD. Macular choroidal blood flow was assessed with LSFG using mean blur rate (MBR). Spectral-domain optical coherence tomography was used to image macular regions, to measure the subfoveal choroidal thickness (SFCT), and to calculate the luminal and the stromal areas by the binarisation method preoperatively and 1, 4, 8 and 12 weeks postoperatively.
Results: In eyes affected by RRD, the mean macular choroidal MBR was 9.4 ± 3.2 arbitrary units (AU) before surgery, 10.1±3.7 AU at 1 week, 9.2±3.7 AU at 4 weeks, 9.0±3.1 AU at 8 weeks and 9.3±2.6 AU at 12 weeks following surgery. There was no significant change in macular choroidal MBR over time in the affected or unaffected opposite eyes. The mean SFCT in the operated eyes was 237.3±71.1 μm before surgery, 263.5±83.5 μm at 1 week, 240.1±70.3 μm at 4 weeks, 232.6± 55.9 μm at 8 weeks and 232.1±65.9 μm at 12 weeks following surgery. The mean SFCT at 1 week after surgery was significantly thicker than that before surgery ($P < 0.001$). The mean choroidal, luminal and stromal areas increased only at one week following surgery ($P<0.001$, $P<0.001$, $P=0.025$, respectively), while there was no significant difference in those areas in the fellow eyes throughout the follow-up period. The trend of SFCT with time coincided with that of the luminal area ($P<0.001$). Multiple regression analysis demonstrated that the SFCT postoperative week 1:preoperative ratio was positively correlated with that of the luminal area ($P = 0.018$).
Conclusions: Venous drainage obstruction induced by compression force of scleral buckling leads to SFCT thickening in the acute postoperative phase. The macular choroidal blood flow might be less susceptible to the partial compression force, because the blood flow does not change following segmental scleral buckling.
Commercial Relationships: Takeshi Iwase, None; **Misato Kobayashi**, None; **Kentaro Yamamoto**, None; **Hiroko Terasaki**, None

Program Number: 5975 **Poster Board Number:** B0775
Presentation Time: 11:30 AM–1:15 PM
The effect of Dasatinib in PVR model
Kazuhiko Umazume[1, 2], Ryousuke Matsushima[1], Rintaro Tsukahara[1, 2], Naoyuki Yamakawa[1], Shigeo Tamiya[2], Hiroshi Goto[1]. [1]Ophthalmology & Visual Sciences, Tokyo Medical University, Tokyo, Japan; [2]Ophthalmology & Visual Sciences, University of Louisville, Louisville, KY.
Purpose: To investigate the effect of Dasatinib, a tyrosine kinase inhibitor currently used in the treatment of chronic myeloid leukemia, on experimental in vitro proliferative vitreoretinopathy (PVR) models.
Methods: Freshly isolated porcine retinal pigment epithelium (RPE) sheets were cultured in 25% vitreous supplemented DMEM in the presence or absence of Dasatinib. Dasatinib was used at concentrations of 0.1, 0.3 and 1.0 μM. RPE sheet enlargement was measured, and immunohistochemical staining of epithelial mesenchymal transition (EMT) marker S100A4 was performed. Cultured RPE cells were seeded on collagen gels to evaluate proliferative membrane contractions by measuring the shrinkage of peeled gel after 6 days in culture (collagen gel contraction assay). Dasatinib at varying concentration was either added on days 0, 3 and 5, designated groups 1, 2, and 3, respectively.
Results: Dasatinib significantly prevented the enlargement of RPE sheet in a concentration dependent manner. Reduced expression of S100A4 by immunohistochemical staining on cultured RPE sheets confirmed suppression of EMT by Dasatinib. Proliferative membrane in group 3 and in the absence of Dasatinib strongly expressed α-SMA compared to groups 1 and 2 at day 6. Dasatinib inhibited collagen gel contraction in groups 1 and 2(p<0.05). On the other hand, shrinkage of collagen gel was not suppressed in group 3 that had dense fibrotic membranes, despite the addition of Dasatinib.
Conclusions: Dasatinib significantly inhibited PVR associated cellular changes of RPE cells at early stages. However, relatively short exposure (24hrs) of dasatinib failed to prevent contraction of well-established fibrotic membranes.
Commercial Relationships: Kazuhiko Umazume, None; **Ryousuke Matsushima**, None; **Rintaro Tsukahara**, None; **Naoyuki Yamakawa**, None; **Shigeo Tamiya**, None; **Hiroshi Goto**, None
Support: JSPS KAKENHI Grant Number 16K20335 (K Umazume)

Program Number: 5976 **Poster Board Number:** B0776
Presentation Time: 11:30 AM–1:15 PM
Fucoidan Inhibits Epithelial-Mesenchymal Transition of Retinal Pigment Epithelium in Proliferative Vitreoretinopathy
Yao Zhang, Hui Li, Shuai Yang, Haipei Yao, Jingfa Zhang, Guo-Tong Xu, Fang Wang. Department of ophthalmology, Shanghai Tenth People's Hospital, Tongji Eye Institute, Tongji University School of Medicine, Shanghai, China.
Purpose: Proliferative vitreoretinopathy (PVR) is a severe blinding complication of rhegmatogenous retinal detachment. Epithelial-mesenchymal transition (EMT) of retinal pigment epithelium (RPE) is thought to play a pivotal role in the pathogenesis of PVR. In this study, we investigated the potential effect of Fucoidan on EMT in PVR animal model as well as in cultured human RPE cells line ARPE-19.
Methods: Pigmented rabbits and ARPE-19 cells were employed in this study. PVR animal model was induced in pigmented rabbits on Day 1 with intravitreal injection of primary rabbit RPE cells immediately after removal of 0.1ml of aqueous humor. Fucoidan was administered intravitreally on Day 1 and Day 7; the animal with intravitreal PBS injection served as control. Two weeks later, the fundus was examined. TGF-β1-treated ARPE-19 cells were incubated with or without Fucoidan; 48 hours later, the cells were harvested and the expressions of Fibronectin, α-SMA, E-cadherin and ZO-1 were studied with immunoblotting.
Results: PVR progression was suppressed significantly by Fucoidan compared to that injected with PBS. *In vitro*, TGF-β1-induced the decrease of E-cadherin and the increase of α-SMA and fibronectin could be reversed by Fucoidan.
Conclusions: The preliminary data showed Fucoidan could ameliorate PVR development via inhibiting EMT of RPE. The detailed mechanisms need further exploration.
Commercial Relationships: Yao Zhang, None; **Hui Li**, None; **Shuai Yang**, None; **Haipei Yao**, None; **Jingfa Zhang**, None; **Guo-Tong Xu**, None; **Fang Wang**, None
Support: This work was supported by National Natural Science Foundation of China (81300772,81271029)

Program Number: 5977 **Poster Board Number:** B0777
Presentation Time: 11:30 AM–1:15 PM
The effects of Pigment Epithelium-Derived Factor on Epithelial-to-mesenchymal transition of the retinal pigment epithelium induced by TGF-β
Sha Ouyang[1, 2], Shikun He[1], Chirstine Spee[1], David R. Hinton[1, 2]. [1]University of South California Keck Medicine, Los Angeles, CA; [2]Ophthalmology, Central South University, Changsha, China.
Purpose: Proliferative vitreoretinopathy (PVR) may lead to irreversible vision loss. Epithelial-to-mesenchymal transition (EMT) of retinal pigment epithelium (RPE) is a critical event in the pathogenesis of PVR. Downregulation of Pigment Epithelium-Derived Factor (PEDF) is demonstrated to be associated with increased EMT in some cancers. The aim of the current study was to investigate the effects of PEDF on EMT induced by TGF-β in RPE cells.
Methods: Sub-confluent human RPE cells were cultured in DMEM and pretreated with PEDF (10, 50,100, 200ng/ml) for 24hs and then stimulated with recombinant TGF-β2 (10ng/ml) for additional 24, 48 and 72 hrs with or without PEDF. The expressions of α-smooth muscle actin (α-SMA, an EMT marker) and fibronectin (FN) were examined using immunofluorescent staining, qRT-PCR and Western blotting respectively. The sub-confluent human RPE cells were cultured in DMEM and pretreated with PEDF (10, 50,100ng/ml) for 24hs and then stimulated with 20ng/ml PDGF-BB. RPE cell proliferation and migration were examined using MTT assay and Boyden chamber migration assay.
Results: PEDF pretreatment caused a significant inhibition of RPE cell migration induced by PDGF-BB in a dose-dependent manner compared with controls (p<0.05). The maximal reduction of migration was seen with pretreatment of 100ng/ml of PEDF for 24 hours (p<0.01). However, PDGF-BB didn't significantly stimulate the proliferation of the RPE cells. Furthermore, TGF-β2 increased expression of SMA and FN at both mRNA and protein levels compared with controls (p<0.05), but pre-treatment with PEDF did not significantly alter this effect. The result of immunofluorescent staining showed that the increased α-SMA and FN induced by TGF-β2 were not inhibited by treatment with PEDF.
Conclusions: PEDF inhibits RPE cell migration induced by PDGF-BB. PEDF does not inhibit TGF-β2 induced EMT in RPE cells. Further experiment will be performed to analyze the mechanism of PEDF's inhibitory effect on PDGF-BB induced RPE cell migration.
Commercial Relationships: Sha Ouyang; **Shikun He**, None; **Chirstine Spee**, None; **David R. Hinton**, None

Program Number: 5978 **Poster Board Number:** B0778
Presentation Time: 11:30 AM–1:15 PM
Murine Vitreous Tap (MurViTap): a novel technique to extract uncontaminated mouse vitreous humor, quantify retinal vascular permeability, and compare proteins secreted by diseased and normal retina
Seth D. Fortmann[1, 2], Valeria E. Lorenc[1, 2], Sean Hackett[1, 2], Peter A. Campochiaro[1, 2]. [1]Ophthalmology, Johns Hopkins Medical Institute, Baltimore, MD; [2]Neuroscience, Johns Hopkins Medical Institute, Baltimore, MD.
Purpose: To obtain uncontaminated mouse vitreous for precise measurement of blood-retinal barrier breakdown in disease states and to compare secreted protein levels in diseased versus normal retina
Methods: MurViTap was done using the Harvard Pump Microinjection System and pulled glass micropipettes. The micropipette tip was cleaved at an inner diameter of 80-100 microns and was inserted into the vitreous cavity 1-2 mm posterior to the limbus. Gentle aspiration was used to obtain 2-4 µL of undiluted vitreous per eye. To quantify vascular permeability, vitreous was collected from P17 normoxic (n=6) and oxygen-induced ischemic retinopathy (OIR, n=5) mouse eyes, adult wild type mice (n=6), P30 Rho/VEGF transgenic mice (n=5), Tet/Opsin/VEGF transgenic mice 2 days post-doxycycline induction (n=6), and mice with choroidal neovascularization (CNV) 7 days after laser induced rupture of Bruch's membrane in 5 locations (n=5). A mouse albumin ELISA was used to measure albumin levels in 1 µL of diluted vitreous. To compare the retinal secretome of OIR mice to age-matched controls, vitreous was collected from P15 OIR mice (n=40 eyes) and P15 wild type mice (n=40 eyes). A mouse angiogenesis protein array was used to compare the vitreous levels of 53 angiogenesis-related proteins.
Results: Mean vitreous albumin concentration was significantly greater in OIR versus age-matched control mice (106± 10.3 µg/mL vs. 7.37±1.13 µg/mL; $p<0.01$). Compared to adult wild type mice (2.58±0.35 µg/mL), mean vitreous albumin concentration was significantly greater in Rho/VEGF mice (15.9±3.18 µg/mL; $p<0.05$), doxycycline-treated Tet/Opsin/VEGF mice (56.5±3.18 µg/mL; $p<0.01$), and mice with CNV in 5 locations (13.8±3.01 µg/mL; $p<0.05$). Eighteen secreted angiogenesis-related proteins were found to be differentially concentrated in OIR vitreous: PAI-1, IGFBP-3, IGFBP-1, IGFBP-2, NOV/CCN3, OPN, CXCL4, Endostatin, PTX-3, PIGF-2, CXCL16, DPPIV, MMP-3, MMP-9, PEDF, TIMP-1, SDF-1, and MCP-1.
Conclusions: Obtaining uncontaminated vitreous combined with albumin ELISA provides a precise quantitative measurement of the extent of blood-retinal barrier breakdown and combined with protein arrays allows the comparison of proteins secreted by the retina under disease versus normal conditions.
Commercial Relationships: Seth D. Fortmann; **Valeria E. Lorenc**, None; **Sean Hackett**, None; **Peter A. Campochiaro**, None

Program Number: 5979 **Poster Board Number:** B0779
Presentation Time: 11:30 AM–1:15 PM
Transforming growth factor beta 2 as a biomarker for detection of disease progression of proliferative vitreoretinopathy
Ghazala Begum[1], Jenna O'Neill[1], Karen Blachford[2], Saaeha Rauz[3], Robert A H Scott[4], Ann Logan[1], Richard J. Blanch[1]. [1]Immunology & Immunotherapy, Inflammation & Ageing, Microbiology & Infection, University of Birmingham, Birmingham, United Kingdom; [2]Academic Unit of Ophthalmology - Sandwell and West Birmingham Hospitals NHS Trust/Birmingham and Midland Eye Centre/City Hospital Birmingham), Birmingham, United Kingdom; [3]Birmingham Midland Eye Centre, Birmingham, United Kingdom; [4]Moorfields, Dubai, United Kingdom.
Purpose: Proliferative vitreoretinopathy (PVR) is a debilitating disease which affects 5-10% of patients with retinal detachment. PVR development is in part caused by epithelial mesenchymal transition (EMT) of retinal pigment epithelial cells (RPE). Transforming growth factor beta 2 (TGFB2) promotes RPE EMT. We hypothesised that vitreous TGFB2 levels would predict and cause the development of PVR, modulated by Decorin and that *in vitro;* Decorin would sequester TGF-beta and prevent EMT in human RPE cells.
Methods: We collected Vitreous from patients undergoing vitrectomy for retinal detachment or macular holes. Levels of TGFB2 and Decorin were measured by TGFB2 and Decorin ELISA. Data was compared to PVR development. To assess the effects of Decorin and TGFB2 on EMT, human ARPE-19 cells were treated with TGFB2 and different concentrations of Decorin. Markers of EMT and fibrosis were assessed by qPCR.
Results: Vitreous TGFB2 levels, were similar between patients presenting with macular holes, retinal detachment, those that went onto develop PVR and those that initially presented with PVR. Patients who subsequently developed PVR had a trend towards increased Decorin expression compared to those that initially presented with retinal detachment. Treatment of ARPE-19 cells with TGFB2 had increased Alpha-SMA ($p<0.01$), Collagen type 1 ($p<0.01$) and Fibronectin expression ($p<0.05$). Addition of increasing concentrations of Decorin caused decreased Alpha-SMA ($p<0.05$), Collagen type 1 ($p<0.05$), Vimentin ($p<0.005$)and Fibronectin ($p<0.05$) mRNA expression.
Conclusions: TGFB2 levels in the vitreous at the time of vitrectomy are not associated with PVR disease progression. TGFB2 treatment in human RPE cells causes EMT. Decorin reduces EMT in a dose-dependent fashion and could therefore provide a potential therapeutic strategy to block EMT inducing factors, preventing RPE EMT and subsequent fibrosis characteristic of PVR.
Commercial Relationships: Ghazala Begum, None; **Jenna O'Neill**, None; **Karen Blachford**, None; **Saaeha Rauz**, None; **Robert A H Scott**, None; **Ann Logan**, None; **Richard J. Blanch**, None
Support: SRMRC grant