422 Diabetic Retinopathy: New Targets and Emerging Therapies

Wednesday, May 10, 2017 8:30 AM–10:15 AM
Exhibit/Poster Hall Poster Session

Program #/Board # Range: 4028–4051/B0001–B0024
Organizing Section: Retinal Cell Biology

Program Number: 4028 Poster Board Number: B0001
Presentation Time: 8:30 AM–10:15 AM

Runt-related transcription factor 1 (Runx1) has been implicated in endothelial cell function. We recently identified Runx1 expression to be enhanced in endothelial cells isolated from fibrovascular membranes of patients with proliferative diabetic retinopathy. Runx1-neutralizing mAb ameliorates DR leakage with high efficacy, implicating its potential for DME therapy. These results also demonstrate the validity and utility of the comparative ligandomics for systematic mapping of therapeutic ligands. This study discovers Scg3 as a novel and highly disease-associated VPF and angiogenic factor. Scg3-neutalizing mAb ameliorates DR leakage with high efficacy, implicating its potential for DME therapy. These results also demonstrate the validity and utility of the comparative ligandomics for systematic mapping of therapeutic ligands.

Commercial Relationships: Wei Li, Michelle LeBlanc, Inventor - University of Miami (P); Weiven Wang, Inventor - University of Miami (P); Xiuping Chen, None; Nora B. Caberoyn, None; Chen Shen, None; Yanli Ji, None; Hong Tian, Co-founder - Everglades Biopharma (S); Spouse - Everglades Biopharma (S); Hui Wang, None; Rui Chen, None
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**Program Number:** 4030 Poster Board Number: B0003  
**Presentation Time:** 8:30 AM–10:15 AM  
**Administration of a gap junction coupler reduces retinal vascular cell loss associated with diabetic retinopathy**

**Dongjoon Kim**, **Dayeun Lee**, **Ulrik Mouritzen**, **Bjarne D. Larsen**, **Sayon Roy**, **1** Medicine and Ophthalmology, Boston University School of Medicine, Boston, MA; **2** Department of Clinical Development, Zealand Pharma A/S, Glostrup, Denmark; **3** Department of Medicinal Chemistry, Zealand Pharma A/S, Glostrup, Denmark.  

**Purpose:** In diabetic retinopathy (DR), cell-cell coupling appears to be compromised, leading to retinal vascular cell death and the development of retinal vascular lesions. The aim of this study is to evaluate whether administration of a gap junction coupler may be protective against retinal vascular cell death in the retinas of diabetic rats.  

**Methods:** To determine the optimal concentration of danegaptide (DG), a Cx43 gap junction coupler, two concentrations (200 nM or 1000 nM) were tested via intravitreal injections in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced via STZ in 6 weeks and at the end of the study, eyes were enucleated, and retinas subjected to retinal trypsin digest (RTD) for isolation of capillary networks and stained with Hematoxylin and Periodic Acid Schiff (PAS) to analyze the number of acellular capillaries (AC) and pericyte loss (PL). Based on this pilot study, 1000 nM DG was found to be optimal and therefore injected intravitreally in the follow-up study to determine the effects of improved cell coupling on retinal vascular cell death. Additionally, DG was administered systemically via osmotic pumps. The number of rats used in this study was at least 6 per group. Overall, rats were divided into 6 groups: wild type (WT) control rats, STZ-induced diabetic rats, diabetic rats intravitreally injected with DG, diabetic rats intravitreally injected with water, diabetic rats systemically delivered with DG, and diabetic rats systemically delivered with water. Diabetes was induced for 15 weeks and at the end of the study all animals were sacrificed and their retinas were isolated and subjected to RTD for analyses of AC and PL.  

**Results:** The retinal vasculature showed a significant decrease in the development of AC in diabetic rats treated either via intravitreal injections or systemically with DG (134±28% of control, p<0.01 and 133±37% of control, p<0.001, respectively) compared to those of diabetic rats (296±26% of control, p<0.001). Likewise, retinal capillaries of diabetic rats treated intravitreally or systemically with DG exhibited a significant decrease in the number of PL (112±62% of control, p<0.01 and 103±65% of control, p<0.005, respectively) compared to those of diabetic rats (335±40%, p<0.005).  

**Conclusions:** Findings from this study indicate that improved cell-cell coupling may be protective against retinal vascular cell death associated with DR.  

**Commercial Relationships:** Dongjoon Kim, Zealand Pharma A/S (F); Dayeun Lee, Zealand Pharma A/S (F); Ulrik Mouritzen, Zealand Pharma A/S (E); Bjarne D. Larsen, Zealand Pharma A/S (E); Sayon Roy, Zealand Pharma A/S (F)  

**Support:** Zealand Pharma A/S

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**Program Number:** 4031 Poster Board Number: B0004  
**Presentation Time:** 8:30 AM–10:15 AM  
**A novel regulator of cytosolic oxidative stress in the development of Diabetic retinopathy**

**Manish Mishra, Renu A. Kowluru**, Ophthalmology, Kresge Eye Institute, Detroit, MI.  

**Purpose:** In diabetes, increased retinal activity of Rac1, a small molecular weight GTPase, increases NADPH-oxidase 2 mediated cytosolic reactive oxygen species (ROS) that damages retinal mitochondria and accelerates capillary cell death. Activity of Rac1, in turn, is stimulated by p66Shc, an adapter protein that promotes oxidative stress. Regulation of p66Shc is controlled by histone acetylation, which facilitates the binding of p53 transcription factor at its promoter. In diabetes, expression of p66Shc and p53 are increased in the retina, activity of Sirt1, a histone deacetylase, is decreased, and induction of Sirt1 prevents increase in cellular ROS. Our aim is to examine the role of p66Shc in Rac1 activation and its regulation in the development of diabetic retinopathy.  

**Methods:** To investigate the interrelationship between p66Shc and the Rac1, human retinal endothelial cells, incubated in high glucose for 24-96 hours were employed and tested for p66Shc expression and Rac1 activity using qPCR and G-LISA colorimetric assay respectively. Cellular localization of p66Shc and Rac1 was determined by immunofluorescence imaging. Role of p53 and the effect of histone acetylation on its binding at p66Shc promoter was determined in the cells overexpressing Sirt1 using chromatin immunoprecipitation assay.  

**Results:** High glucose increased p66Shc expression as early as 24 hours of its exposure, and it continued with the increased duration of glucose exposure. P66Shc expression and Rac1 activation presented a strong correlation, and this was confirmed by cytosolic co-localization of Rac1 and p66Shc. The binding of p53 at p66Shc promoter was increased by ~40%, which was prevented in the cells overexpressing Sirt1. In the same Sirt1 overexpressing cells, glucose-induced Rac1 activation was also ameliorated.  

**Conclusions:** In diabetes, p66Shc plays an important role in retinal Rac1 activation, and Sirt1 regulates p66Shc via modulating p53 binding at p66Shc promoter. Thus, understanding the relationship between Rac1, p66Shc and Sirt1 will provide a novel mechanism of regulating early oxidative response to maintain cellular homeostasis and prevent the development of diabetic retinopathy.  

**Commercial Relationships:** Manish Mishra, None; Renu A. Kowluru, None  

**Support:** EY014370, EY017313, EY022230

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**Program Number:** 4032 Poster Board Number: B0005  
**Presentation Time:** 8:30 AM–10:15 AM  
**Role of activated monocytes in lysyl oxidase-mediated retinal vascular cell stiffening and inflammation associated with diabetes**

**Andrea P. Cabrera, Xiao Yang, Kaustabh Ghosh**, Bioengineering, University of California, Riverside, Riverside, CA.  

**Purpose:** Leukocyte adhesion to ICAM-1-expressing retinal vascular endothelial cells (EC) is a critical early step in diabetic retinopathy (DR). The adherent (activated) leukocytes release various factors to further amplify inflammation. Since we have recently shown that high glucose-induced vascular stiffening promotes leukocyte-EC adhesion, here we tested the hypothesis that adherent leukocytes also contribute to progressive stiffening of retinal vessels in diabetes, thereby amplifying retinal vascular inflammation associated with DR.  

**Methods:** Human U937 monocytes were activated with high glucose and PMA (200nM). Monocyte activation was confirmed by staining with DCF, a reactive oxygen species (ROS) indicator. Conditioned medium (CM) from activated monocytes was then added to human
Heterozygous male Ins2Akita mice were intravitreally injected with CD140b+ ASC (1000 cells/eye), conditioned media (CM, 20x, 1mL/eye) or saline at 24 weeks of age. Age matched C57BL/6J mice with saline injections served as controls. The treated eyes also received an intravitreal injection of hELOVL4 packaged in adenovector particles. 

**Results:**
- CD140b+ASC injection demonstrated robust increase in b-wave amplitude; visual acuity and decreased need for contrast sensitivity compared to CD140b-ASC.
- Overexpression of hELOVL4 in BREC significantly reduced vascular permeability through the decrease in VLC ceramides.
- Ceramide colocalization with tight junction complex was determined in confluent monolayers by RITC dextran. Tight junction proteins were assayed by Western blot and immunostaining.

**Conclusions:**
- Overexpression of ELOVL4 stabilizes tight junctions and prevents VEGF-induced vascular permeability in diabetic retina and retinal cells.
- Overexpression of hELOVL4 in BREC significantly reduced vascular permeability through the decrease in VLC ceramides.
- Tight junction proteins were assayed by Western blot and immunostaining.
- Ceramide colocalization with tight junction complex was determined in confluent monolayers by RITC dextran. Tight junction proteins were assayed by Western blot and immunostaining.
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revealed the presence of VLC ceramides. Intravitreal delivery of hELOVL4-AAA2 in STZ mice reduced diabetes-induced increase in vascular permeability by 75% after 8 weeks of diabetes (n=8-12 per group, p<0.01).

Conclusions: Normalization of retinal ELOVL4 expression could prevent blood-retina barrier dysregulation in DR through increase in VLC ceramides and stabilization of tight junctions.

Commercial Relationships: Nermin Kady, None; Xuen Liu, None; Todd Lydic, None; Seger Sereg, None; Andrea Amalfitano, None; Vince A. Chiody, None; Sanford L. Boye, None; William Hauswirth, Bionic Sight (I), AGTC (I); Maria B. Grant, None; David Antonetti, None; Julia V. Busik, None

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Program Number: 4035 Poster Board Number: B0008
Presentation Time: 8:30 AM–10:15 AM

The miRNA451a/ATF2 signal pathway regulates mitochondrial function of RPE in diabetic conditions

yan shao1,2, Lijie Dong, Yusuke Takahashi, Qian Chen, Xiaorong Li, Jian-Xing (Jay) Ma1, 2, 3.

Purpose: Mitochondrial dysfunction plays a pathogenic role in diabetic retinopathy. The purpose of this study was to explore the role of miRNA451a (miR-451a) in mitochondrial dysfunction induced by diabetes.

Methods: Vitreous and epiretinal membrane samples were collected from 14 patients with proliferative diabetic retinopathy (PDR). The membranes were used for immunolabeling of ki-67 protein and RPE65 protein to identify retinal pigment epithelial cells. The vitreous samples were divided into two groups according to Ki-67 expression. Vitreous miRNA profiles were analyzed using miRNA-specific qPCR microarray. The expression of miR-451a was measured by RT-PCR in DB/DB mice. The expression of miR-451a was quantified by q-PCR in ARPE-19 cells under diabetic conditions. The potential downstream targets of the miRNA were predicted and confirmed by dual luciferase assay, qRT-PCR and Western blotting. Mitochondrial function changes were measured by Seahorse after transfection of miR-451a mimics and inhibitor into ARPE19 cells.

Results: In proliferative vitreoretinopathy membrane, RPE cells account for 2.57%±3.19%(1.61%-11.11%). Proliferative index (PI) = 2.57%±3.19%(1.61%-11.11%). Some RPE cells were Ki-67-positive cells. MiRNA-specific qPCR microarray showed that the level of miR-451a was substantially higher from the eye with Ki-67-positive membrane than the eye with Ki-67-negative membrane. The expression of miR-451 in the retina was increased by 4-fold in 6-month-old DB/DB mice, compared to age-matched non-diabetic controls (P=0.03). In ARPE19 cells, high glucose up-regulated miR-451a expression. HNE down-regulated miR-451a expression. Bioinformatic analysis and luciferase assay identified activating transcription factor 2 (ATF2) as a potential target of miR-451a. RT-PCR and Western blotting revealed that overexpression of miR-451a down-regulated the expression of ATF2 (P<0.05). The mitochondrial function was enhanced by miR-451a mimics, while suppressed by inhibitor. The basal oxygen consumption rate (OCR) and maximal OCR in miR-451a overexpression group were significantly higher than control group (P=0.014, 0.015). Under high glucose condition, overexpression of miR-451a enhanced the mitochondrial function.

Conclusions: MiR-451a/ATF2 plays a role in regulation of the mitochondrial function in the RPE, which open new perspectives for the development of effective therapies of PDR.

Commercial Relationships: yan shao; Lijie Dong, None; Yusuke Takahashi, None; Qian Chen, None; Xiaorong Li, None; Jian-Xing (Jay) Ma, None

Support: Natural science foundation of Tianjin, China 15JCQN11400

Program Number: 4036 Poster Board Number: B0009
Presentation Time: 8:30 AM–10:15 AM

Novel Mechanism which Promotes Diabetic Complications in Renal and Ocular Systems

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Purpose: To determine the roles of TGFβ1 and TGFβ2 receptors, as well as Tranforming Growth Factor Beta-Induced (TGFβ1 or TGFβ3), in the deaths of renal and ocular cells in culture.

Methods: BIGH3 was introduced in to cell medium of cultured RPTEC, REC, and HRPs. Macrophages and macrophage derived TGF-B1/TGFβ2 receptor blockers were also added at various concentrations. Cell viability was determined via TUNEL assay manufacturer’s instructions.

Conclusions: Renal (RPTEC) and Human Retinal Pericytes (HRP’s) were purchased from Clonetics/Lonza (Walkersville, MD). Retinal Endothelial Cells (REC) were purchased from ATCC (Manassas, VA). Monocyte-derived Macrophage (Mφ) were prepared in accordance with established methodology (Wittergerst et al., 1998). Polyclonal anti-BIG-H3 was generated in house.

Identification of the Mφ source of BIGH3 in the deaths of renal and ocular cells in culture. The membranes were used for immunolabeling of ki-67 protein to identify retinal pigment epithelial cells. The vitreous samples were divided into two groups according to Ki-67 expression. Vitreous miRNA profiles were analyzed using miRNA-specific qPCR microarray. The expression of miR-451a was measured by RT-PCR in DB/DB mice. The expression of miR-451a was quantified by q-PCR in ARPE-19 cells under diabetic conditions. The potential downstream targets of the miRNA were predicted and confirmed by dual luciferase assay, qRT-PCR and Western blotting. Mitochondrial function changes were measured by Seahorse after transfection of miR-451a mimics and inhibitor into ARPE19 cells.

Results: In proliferative vitreoretinopathy membrane, RPE cells account for 2.57%±3.19%(1.61%-11.11%). Proliferative index (PI) = 2.57%±3.19%(1.61%-11.11%). Some RPE cells were Ki-67-positive cells. MiRNA-specific qPCR microarray showed that the level of miR-451a was substantially higher from the eye with Ki-67-positive membrane than the eye with Ki-67-negative membrane. The expression of miR-451 in the retina was increased by 4-fold in 6-month-old DB/DB mice, compared to age-matched non-diabetic controls (P=0.03). In ARPE19 cells, high glucose up-regulated miR-451a expression. HNE down-regulated miR-451a expression. Bioinformatic analysis and luciferase assay identified activating transcription factor 2 (ATF2) as a potential target of miR-451a. RT-PCR and Western blotting revealed that overexpression of miR-451a down-regulated the expression of ATF2 (P<0.05). The mitochondrial function was enhanced by miR-451a mimics, while suppressed by inhibitor. The basal oxygen consumption rate (OCR) and maximal OCR in miR-451a overexpression group were significantly higher than control group (P=0.014, 0.015). Under high glucose condition, overexpression of miR-451a enhanced the mitochondrial function.

Conclusions: Identification of the Mφ source of BIGH3 in diabetic conditions and the sequence of the integrin-ligand peptides derived from BIGH3, as well as the integrins involved in BMA, is expected to offer novel therapeutic targets for interventions to block development and progression of diabetic complications.

Commercial Relationships: Andrew T. Tsin; Brandi S. Betts-Obregon, None; Robert Mortiz, None; Richard LeBaron, None

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Program Number: 4037 Poster Board Number: B0010
Presentation Time: 8:30 AM–10:15 AM

Endoplasmic reticulum stress at the intersection of inflammation and hyperglycemia in mediating tight junction alterations in diabetic retinopathy
RAJI RAJESH LENIN, Rajashekhar Gangaraju. Department of Ophthalmology, University of Tennessee, Memphis, TN.

Purpose: Endoplasmic Reticulum (ER) stress plays a major role in several metabolic disorders including diabetes. We hypothesized that ER stress pathways interact with inflammatory signaling leading to retinal barrier dysfunction in diabetic retinopathy (DR). Using a well characterized chronic proinflammatory tie2-TNF mouse model and retinal endothelial cells we assessed the connection between ER stress and hyperglycemic stress in mediating vascular permeability.

Methods: Tie2-TNF and age matched wildtype mice were made diabetic with intraperitoneal injections of Streptozotocin and evaluated for retinal vascular permeability by fluorescein labeled-BSA leakage assay. Human retinal microvascular endothelial cells (HREC) were treated with 10 ng/mL TNF-α and 30 mmol/L glucose for 3 hours with and without ER stress inhibitor (10μM TUDCA). Gene and protein expression of ER stress markers were analyzed by Taqman assay and Western blot analysis respectively. Alterations in endothelial junction proteins were evaluated by western blot.

Results: Frozen sections of retina from tie2-TNF transgenic mice demonstrated increased vascular leakage compared to wildtype mice which was further exacerbated with diabetes. Interestingly, whole mount retinal preparation from diabetic tie2-TNF mice demonstrated hyper permeable vessels with strong extravasation of FITC-BSA and extravasation of red blood cells. Retinal extracts from 6-month old tie2-TNF mice exhibited increased mRNA levels of ER stress markers (GRP78, PERK, IRE1α and CHOP) compared to age matched wild type mice (n=3 each, p<0.05). In HREC cells, ER stress was significantly upregulated with TNF and high glucose combination as evidenced by increased gene expression of GRP78, PERK, IRE-1α, ATF-6, XBP-1, CHOP and increased protein expression of GRP78 and a decreased ZO-1 expression (p<0.05 compared to control). On the other hand, TUDCA treated cells reversed ER stress markers.

Conclusions: ER stress is a key mediator of vascular damage in the setting of increased inflammatory burden in retinal endothelial cells. Understanding the underlying ER stress mechanisms may shed new insights into novel therapeutic targets for DR.

Commercial Relationships: RAJI RAJESH LENIN, None; Rajashekhar Gangaraju, None
Support: VA Merit Award

Program Number: 4038 Poster Board Number: B0011
Presentation Time: 8:30 AM–10:15 AM

Role of Cathepsin-D in alteration of Endothelium-Pericyte interaction in Diabetic Retinopathy
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Purpose: Previously, we have demonstrated the effect of Cathepsin D (CD) on the mechanical disruption of retinal endothelial cell junctions and increased vaso-permeability, and increased levels of cathepsin D in retinas of diabetic mice. Here we have further examined the effect of CD on the endothelial-pericyte interaction, and the effect of Dipeptidyl peptidase-4 (DPP-4) inhibitor on CD in the endothelial-pericyte interaction. The DPP-4 inhibitor has been shown to alter the function of the IGF2 receptor, which is the presumed binding site for CD on endothelial cells.

Methods: Human retinal endothelial cells (HRECs) and human retinal pericytes (HRPs) were co-cultured and treated overnight with 25μg/ml of recombinant pro-CD along with 250nM DPP-4 inhibitor. Western blots were performed to check the levels of PDGFR-β, N-Cad, PKC-α and phosphor-PKC-α. Real-time PCR was used to measure angiopoietin-2 (Ang-2) levels in the treated co-cultured cells. GFP-HRP cells were co-cultured with HRECs to check the binding efficiency in treated conditions.

Results: Co-cultured cells treated with pro-CD showed a significant decrease in the expression of PDGFR-β, a tyrosine kinase receptor required for pericyte cell survival and N-Cad, the key adherens junction protein between endothelium and pericytes, and also increase in the vessel destabilizing agent, Ang-2. The effect was reversed in the cells treated with DPP-4 inhibitor along with pro-CD. With pro-CD treatment, there was a significant increase in the downstream signaling protein PKC-α, which disrupts tight junction structure and function, and this was significantly reduced with DPP-4 inhibitor treatment. We observed significantly increased binding of endothelial cells and pericytes when cells were treated with the DPP-4 inhibitor and pro-CD.

Conclusions: The cathepsin D decreases N-cad and PDGFR-β in the endothelium-pericyte alteration in the blood-retinal barrier, and the drug DPP-4 inhibitor can significantly diminish this effect. Thus, the DPP-4 inhibitor may be used as a potential adjuvant therapeutic strategy for treating diabetic macular edema.

Commercial Relationships: Finny Monickaraj, None; Paul McGuire, None; Arup Das, None
Support: VA Merit Award

Program Number: 4039 Poster Board Number: B0012
Presentation Time: 8:30 AM–10:15 AM

ACTIVATION OF THE SIRT1-LXR SIGNALLING PATHWAY IN RETINAL PIGMENTED EPITHELIAL CELLS PROMOTES CHOLESTEROL METABOLISM
Kiana Wood1, Sandra Hammen2, Elahe Crockett1, Maria B. Grant2, Julia V. Busik1. 1Michigan State University, East Lansing, MI; 2Indiana University, Indianapolis, IN.

Purpose: Diabetic Retinopathy (DR) is a sight threatening disease with few treatment options. Dyslipidemia has shown to play a significant role in the progression of DR. Liver X Receptors (LXRs) are known cholesterol metabolism regulators that also play a role in preventing pro-inflammatory genes upregulation. Retinal Pigmented Epithelial Cells (RPEs) are important in retinal cholesterol uptake and elimination. Additionally, RPEs are one of the retinal cells heavily affected by DR. Knockdown of LXR in animals leads to increased retinal cholesterol levels when compared to controls. SIRT1 has recently been shown to activate LXR in non-retinal studies but the role of the SIRT1-LXR signaling axis in DR has not yet been studied.

Methods: Bovine retinal pigmented cells (BRPE) were treated with TNFα (10μg/ml), and the role of SIRT1-LXR signaling axis was examined using SIRT1 activator SIRT1720 (1μM) and LXR activator DMHCA (1μg/ml), LXRα/β, the ATP binding cassette transporters (ABCA1 and ABCG1), and cholesterol metabolizing enzymes (CYP27A1, CYP46A1, and CYP11A1) were analyzed using qRT-PCR. SIRT1-directed siRNA was used to inhibit SIRT1 expression levels.

Results: LXRα, ABCA1 and ABCG1 mRNA levels decreased when BRPEs were treated with TNFα for 24hrs (p<0.01, n=6). The cholesterol metabolizing enzymes (CYP27A1, CYP46A1, and CYP11A1) were decreased when treated with TNFα (p=0.01, n=6).
Furthermore, treatment with SIRT1 activator, SRT1720, for 24hrs prevented TNFα-induced ABCA1 and ABCG1 downregulation (p<0.001, n=9). LXR activator, DMHCA, prevented TNFα-induced ABCA1 and ABCG1 downregulation (p<0.01, n=3). Additionally, SIRT1 inhibition it decreased cholesterol metabolizing enzyme CYP11A1 and ABCA1 (p<0.05, n=3) while having no statistical effect on LXRα expression.

Conclusions: Inflammatory cytokine stimulation caused a decrease in retinal cholesterol metabolizing enzymes and transporter proteins. Activation of the SIRT1-LXR signaling axis prevented TNFα-induced downregulation of cholesterol metabolism in retinal cells. Taken together this work suggest that activation of the SIRT1-LXR pathway can help restore normal cholesterol metabolism in diabetic retina and prevent dyslipidemia-induced retinal pathology.

Commercial Relationships: Kiana Wood, None; Sandra Hammer, None; Elaehe Crockett, None; Maria B. Grant, None; Julia V. Busik, None

Support: NH Grant EY025383

Program Number: 4040 Poster Board Number: B0013
Presentation Time: 8:30 AM–10:15 AM
Role of high glucose-induced lysyl oxidase overexpression on Ras activity in rat retinal endothelial cells
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Purpose: In diabetic retinopathy, cells in the retina undergo significant apoptosis resulting in the development of acellular capillaries and pericyte ghosts. In this study, we determined whether high glucose (HG)-induced lysyl oxidase (LOX) overexpression plays a role in contributing to increased apoptosis by lowering Ras activity, which is known to promote cell survival via the Ras-Raf-ERK pathway.

Methods: Rat retinal endothelial cells were grown in normal (N; 5 mM) or HG (30 mM) medium for 7 days, and in parallel, cells grown in HG were transfected with LOX siRNA, or scrambled siRNA as control. Total protein isolated from the cells was assessed for Ras activity, which is known to promote cell survival via the Ras-Raf-ERK pathway.

Results: WB analysis showed significant LOX upregulation in cells grown in HG medium compared to those grown in N medium (132±11% of control, P<0.05). Ras pulldown assay and WB analysis revealed significant decreased Ras activity in cells grown in HG medium compared to those grown in N medium (59±18% of control, P=0.005). When HG-induced LOX overexpression was reduced by approximately 40% via LOX siRNA, Ras activity increased by approximately 20%, compared to those grown in HG medium, and those grown in HG medium transfected with scrambled siRNA. As expected, a significant increase in the number of apoptotic cells was observed under HG medium compared to those grown in N medium (210±37% of control). Interestingly, the number of apoptotic cells was significantly decreased when cells grown in HG exhibited increased Ras activity via LOX siRNA transfection (154±48% of control, P<0.05).

Conclusions: Findings from this study indicate that HG-induced LOX overexpression promotes apoptosis, at least in part, by inhibiting Ras signaling. Therefore, reducing LOX overexpression may protect retinal vascular cells from undergoing HG-induced apoptosis associated with the pathogenesis of diabetic retinopathy.

Commercial Relationships: Brian Chirn; Dongjoon Kim, None; Philip C. Trackman, None; Sayon Roy, None
Support: NEI, NIH grant EY025528

Program Number: 4041 Poster Board Number: B0014
Presentation Time: 8:30 AM–10:15 AM
Effect of a single injection of anti-VEGF agent on retinal edema in non-obese diabetic mice
Tamar Leibovitch1, moshe ben hemo1, 4, Myles Brookman1, Orit Barinfeld1, 4, Orkun Muhsingolgu1, Shalom Michowiz2, Nitza Goldenberg-Cohen1, 4.
1Tel Aviv University, Tel Aviv, Israel; 2Department of Neurosurgery, Rabin Medical Center – Beilinson Hospital, PetachTikva, Israel; 3Department of Ophthalmology, Bnai Zion Medical Center of Israel, Haifa, Israel; 4Krieger Eye Research Laboratory Felsenstein Medical Research Center, Beilinson Hospital, PetachTikva, Israel.

Purpose: The aims of the study were to characterize the retinal changes in a mouse model of diabetic retinopathy and to evaluate the response of the retinal edema to a single injection of anti-VEGF-A agent.

Methods: 96 transgenic NOD mice were monitored. Diabetes type 1 (blood glucose levels>250mg/dl) developed spontaneously in 30 (DMT1-NOD group) and was chemically induced in 28 (STZ-NOD group); the remainder served as controls. Mice were examined after the appearance of uncontrolled high glucose levels (>500mg/dl). Retinal blood flow and leakage were evaluated with fluorescein angiography (FA); vascular perfusion, with retinal flat mounts following infusion of India ink or fluorescent gelatin; gliosis and vasculopathy, with Vimentin and GFAP immunostaining; retinal thickness with light microscopy; and expression of genes involved in ischemia (SOD-1, HO-1), angiogenesis (VEGF-A, VEGFR-1, -2), gliosis (GFAP, Vimentin), and diabetes (RAGE, IGF-1, EPO), with RT-PCR. To test the effect of anti-VEGF treatment, the right eyes of STZ-NOD mice were evaluated histologically one week after injection of bevacizumab, ranibizumab, or saline or no treatment.

Results: In the first part of the study, the diabetic mice showed no neovascularization (NVE) on FA. Flat mount show microaneurysms and attenuated retinal vessels, and apparent tufts of NVE in a single retina. Immunostaining revealed reactive gliosis and prominent Muller cells. Mean retinal thickness was significantly higher in the DMT1-NOD mice than the non-diabetic mice. Of all genes evaluated, only GFAP decreased significantly (0.5-fold, p=0.04). Anti-VEGF treatment led to complete suppression of gliosis with retinal thinning. Bevacizumab (retinal thickness 260±53mm right eye Vs. 241±34mm left eye) appeared to be more effective than ranibizumab (267.5±47mm right eye Vs 223+39mm left eye).

Conclusions: In conclusion, the retinas of DMT1-NOD mice are characterized by vasculopathy and edema, reactive gliosis, and good response to a single injection of anti-VEGF agent. The short survival time of the diabetic mice may have limited the development of NVE. Accordingly, discrepancies were noted between the increase in GFAP histologically and the decrease in GFAP gene expression and between the retinal response to anti-VEGF agents and the lack of change in VEGF gene expression. The reason for the higher effect of bevacizumab than ranibizumab is unclear.

Commercial Relationships: Tamar Leibovitch, moshe ben hemo, None; Myles Brookman, None; Orit Barinfeld, None; Orkun Muhsingolgu, None; Shalom Michowiz, None; Nitza Goldenberg-Cohen, None

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**Purpose:** Increased oxidative/nitrative stress and chronic inflammatory responses have been implicated in the pathogenesis of diabetic retinopathy (DR). Xanthine oxidoreductase (XOD) is an enzyme involved in purine metabolism which generates superoxide anions and, as catabolic by-product, uric acid (UA). Recently we have shown that XOD is activated in the diabetic retina leading to increased production of UA. The mechanisms by which hyperglycemia stimulates XOD expression and activity as well as its pathogenic consequences in DR are poorly understood. Here, we have investigated the effects of in vivo blockade of XOD in the diabetic retina and the contribution of different toll-like receptors (TLRs) to hyperglycemia-induced up-regulation of XOD in human retinal endothelial cells (HuREC).

**Methods:** Male streptozotocin-induced diabetic rats (STZ-rats) were kept diabetic for 8 weeks. Some of the STZ-rats received a dose (55 mg/kg) of the XOD inhibitor allopurinol (ALL) administrated orally every other day. At the time of sacrifice, the retinas were excised and analyzed for oxidative/nitrative stress parameters (4-hydroxynonenal (4-HNE) and nitrotyrosine (NY)) and expression of inflammatory markers such as ICAM-1, IL-1beta and IL-6. TLR4, 2 and 7/8 were silenced in HuREC by transfection with specific siRNAs. XOD expression at the mRNA and protein level was measured by qPCR and Western blotting analyses, respectively. XOD activity was assessed by spectrophotometric enzymatic analysis measuring UA production.

**Results:** Treatments with ALL resulted in significant decreases of 4-HNE and NY and in blunted expression of inflammatory markers, such as IL-1beta, IL-6 and ICAM-1, in the diabetic rat retina. XOD expression and activity in HuREC exposed to glycidic stress (HG = 25 mM D-glucose for 48 hours) were significantly up-regulated in comparison to cells cultured in normal glucose levels (NG = 5 mM D-glucose) or with the osmotic control (LG = 25 mM L-glucose). Transfection of the cells with siRNA specific for TLR4, 2 or 7/8 significantly reduced HG-induced up-regulation of XOD expression and activity and showed highest inhibitory effects by TLR7/8 signaling.

**Conclusions:** The data obtained suggest that TLR-mediated up-regulation of XOD plays a central role in mediating pro-oxidative/nitrative and pro-inflammatory challenges in the diabetic retina.

**Commercial Relationships:** Prerana Mallà; Menaka Thounaojam; Diana Gutsaeva; Amany Tawfik; Manuela Bartoli.

**Support:** D-CURE, foundation, ministry of health & Krieger Eye Research Laboratory Felsenstein Medical Research Center, Petach Tikva, Israel.

**Program Number:** 4042 Poster Board Number: B0015
**Presentation Time:** 8:30 AM–10:15 AM
**Poster Board Number:** 2

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**Program Number:** 4043 Poster Board Number: B0016
**Presentation Time:** 8:30 AM–10:15 AM
**Title:** Hydrogen sulfide as a novel therapeutic for diabetic retinopathy

**Authors:**
Claire Allen1, Jacqueline L. Whatmore2, Mark E. Wood3, Matthew Whiteman2, David O. Bates3, 1Cancer Biology & Stem Cells, The University of Nottingham, Nottingham, United Kingdom; 2Medical School, University of Exeter, Exeter, United Kingdom; 3Biosciences, University of Exeter, Exeter, United Kingdom.

**Purpose:** Diabetic retinopathy (DR) is a major complication of diabetes and a leading cause of blindness worldwide. Current treatments for DR are limited, expensive and non-specific. The anti-inflammatory gaseous transmitter hydrogen sulfide (H2S) is significantly reduced in type 2 diabetic patients and correlates with microvascular dysfunction. H2S is thought to contribute to the healthy functioning of the microvasculature by preventing loss of the retinal endothelial glycocalyx and subsequent breakdown of the blood retinal barrier.

**Methods:** Fundus Fluorescein Angiography (FFA) was performed in isoflurane anaesthetised Norway Brown rats on day 0 and 7 using the Micron IV retinal imaging microscope (Phoenix Research Labs). Animals received an intravenous injection of the slow release H2S donor sodium 4-methoxyphenyl(morpholino)-phosphinodithioate (NaGYY4137; 1µM) on day 0 (Prevention) or on day 6 (Treatment). On day 1 some animals received a single dose of streptozotocin (50mg/kg, i.p.) to induce diabetes and blood glucose levels measured. Angiograms were imported into Imagej software and fluorescence was measured in the interstitium and vessel. The ratio of interstitial to vascular fluorescence was adjusted for background and plotted against time and the slope used to determine an estimate of permeability.

**Results:** NaGYY4137 significantly (p <0.05) reduced retinal permeability in non- and diabetic rats on day 7 when given as a single intravenous preventative dose on day 0. In addition NaGYY4137 significantly reduced (p <0.05) retinal permeability in non-diabetic rats when administered therapeutically (day 6) and stabilised retinal permeability in rats with pre-existing diabetes. This was further supported by fundus images showing increased capillary leakage and microaneurysm formation in the retina of diabetic animals.

**Conclusions:** NaGYY4137 protected the retinal endothelial permeability barrier from diabetes-associated loss of integrity and reduced the progression of DR. Slow release H2S donors may therefore be a potential alternative and more specific therapeutic candidate for DR.

**Commercial Relationships:** Claire Allen, NuVision (P); Jacqueline L. Whatmore, None; Mark E. Wood, None; Matthew Whiteman, None; David O. Bates, None

**Program Number:** 4044 Poster Board Number: B0017
**Presentation Time:** 8:30 AM–10:15 AM
**Title:** NFAT-dependency of IL-1β-induced diabetes-relevant behaviors in human retinal microvascular endothelial cells and Müller cells

**Authors:**
Meredith J. Giblin1, Megan E. Capozzi2, Gary W. McCollum2, John S. Penn3, 1Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 3Department of Ophthalmology and Visual Sciences, Vanderbilt University, Nashville, TN.

**Purpose:** Early diabetic retinopathy (DR) involves chronic low-grade inflammation, characterized by elevated vitreous cytokines, such as IL-1β. This inflammation promotes many pathologic consequences, including the hallmark vascular changes for which DR is best known. Nuclear factor of activated T cells (NFAT) is involved in the regulation of inflammatory mediators, extracellular matrix proteins, and adhesion molecules and thus may control multiple pathogenic

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steps early in DR. We have examined NFAT’s role in IL-1β auto-amplification and explored the NFAT-dependency of endothelial cell responses to IL-1β, including monolayer permeability and expression of targets involved in leukocyte adhesion and basement membrane thickening.

**Methods:** For IL-1β production, human Müller cells (HMC) were treated with 50pg/mL IL-1β. Inhibitor of NFAT-Calcinurine Association-6 (INCA, 2.5μM) and proper vehicles for 8 hrs before collection for qRT-PCR. For ICAM expression, human retinal microvascular endothelial cells (HRMEC) were treated with 1ng/mL IL-1β and 2.5μM INCA for 2 hrs. For collagen IV expression, HRMEC were treated with 10ng/mL IL-1β and 1μM INCA for 48 hrs. In permeability experiments, HRMEC were pre-treated with vehicle or 2.5μM INCA for 16 hrs before 24 hrs of exposure to 0.5ng/mL IL-1β with/out INCA. Transendothelial electrical resistance values were measured using the EVom™.

**Results:** IL-1β increased HMC IL-1β expression by 82-fold (p<0.01); INCA inhibited this induction by 28% (p<0.05). HRMEC treatment with IL-1β caused a 328-fold induction of ICAM expression (p<0.01); INCA inhibited this induction by 20% (p<0.01). HRMEC treatment with IL-1β caused a 2-fold induction of collagen IV expression (p<0.01); INCA inhibited this induction by 50% (p<0.01). Treatment with IL-1β decreased HRMEC monolayer resistance by 25% (measured vs resistance at time=0, p<0.01), which was partially rescued (44%, p<0.01) by INCA treatment.

**Conclusions:** These data demonstrate the potential of NFAT as a multi-targeted therapy for retinal inflammation secondary to diabetes. NFAT inhibition not only prevented HMC IL-1β auto-amplification, but also inhibited the pathogenic response of HRMEC to IL-1β, including the expression of adhesion proteins, excessive extracellular matrix deposition and increased permeability. Future work will investigate the therapeutic potential of in vivo NFAT inhibition.

**Commercial Relationships:** Meredith J. Giblin, None; Megan E. Capozzi, None; Gary W. McCollum, None; John S. Penn, None

**Support:** T32EY021453, R01-EY007533, R01-EY023639, and P30-EY008126; Research To Prevent Blindness and The Reeves Foundation.

**Program Number:** 4045 **Poster Board Number:** B0018

**Presentation Time:** 8:30 AM–10:15 AM

**Program Number:** 4046 **Poster Board Number:** B0019

**Presentation Time:** 8:30 AM–10:15 AM

**TLR4 phosphorylation at tyrosine 674 is necessary for NF-kB and inflammasome activation in the diabetic retina and in retinal endothelial cells exposed to glucidic stress**

**Manuela Bartoli¹, Menaka Thounaojam¹, Diana Gutsaeva¹, Wan Jin Jahng².**

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**Purpose:** Up-regulation of Toll-like receptor 4 (TLR4) expression and signaling has been implicated in the pathogenesis of diabetic retinopathy (DR), however the molecular mechanisms of TLR4 activation in the diseased retina are poorly understood and often referring to promiscuous downstream signaling events. We have conducted studies assessing the occurrence and biological significance of TLR4 phosphorylation at tyrosine 674, which is located in the highly biologically active toll-IL-1 resistance (TIR) domain of this molecule.

**Methods:** Western blotting analysis was conducted to measure phospho-Tyr (674)-TLR4 in diabetic and non-diabetic human postmortem retinas and in retinas of streptozotocin-induced diabetic rats (STZ-rats) as compared to their respective normoglycemic controls. The occurrence of TLR4 phosphorylation at Tyr674 was also measured by immunoblotting in human retinal endothelial cells exposed to glucidic stress (HG=25mM D-glucose) or to normal glucose levels (NG=5mM D-glucose) or to osmotic control (LG=25mM L-glucose). HuREC were transfected with TLR4-Tyr(674)-Ala mutants and exposed to the different glucose conditions for 48 hours. Downstream TLR4-mediated signaling events were measured by assessing NF-kB and p38MAPK phosphorylation/activation and expression of IL-1beta in cells exposed to the different treatments.

**Results:** TLR4 expression and phosphorylation at Tyr 674 was significantly up-regulated in the diabetic rat retinas as well as in human postmortem diabetic retinas as compared to their respective normoglycemic controls. Importantly, HG stimulated TLR4 expression and phosphorylation at Tyr674 and consequent phosphorylation/activation of NF-kB and p38MAPK as well as production of mature IL-1beta. In addition blockade of TLR4 phosphorylation at Tyr674, achieved by transfection of HuREC with TLR4-Tyr(674)-Ala mutants, resulted in significantly decreased phosphorylation/activation of NF-kB and p38MAPK in these cells as well as decreased levels of mature IL-1beta.

**Conclusions:** Our data suggest that monitoring and study of TLR4 phosphorylation at Tyr 674 in response to hyperglycemia may offer a more specific diagnostic and therapeutic target for the prevention and treatment of DR and, potentially, of other ischemic retinopathies involving TLR4 activation.

**Commercial Relationships:** Manuela Bartoli, None; Menaka Thounaojam, None; Diana Gutsaeva, None; Wan Jin Jahng, None

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Role of N-methyl-D-aspartate receptor activation in Blood- Retinal barrier dysfunction: Potential role in Diabetic retinopathy

Riyaz Mohamed, Ahmed Ibrahim, Nehal M. El-Sherbiny, Mohamed A. Al-Shabrawey, Amany M. Tawfik

Purpose: Homocysteine (Hcy) is a risk factor for neurodegenerative and cardiovascular diseases and has been reported to be elevated in patients with diabetic retinopathy (DR). Our recent studies showed that Hcy induced retinal ischemia, neovascularization and Blood- Retinal Barrier dysfunction (BRB). The current study is aiming to investigate the role of N-methyl-D-aspartate receptor (NMDAr) in Hcy induced BRB dysfunction.

Methods: We confirmed the expression of NMDAr in Human Retinal Endothelial Cells (HRECs) by real-time polymerase chain reaction (qPCR) in comparison with Retinoblastoma cell line as a positive control. In addition, HRECs treated with or without different concentrations of Hcy (20, 50 and 100µM) were subjected to Evaluation of NMDAr activation in by Western blot (WB) and immunofluorescence (IF). FITC–Dextran flux permeability assay in presence and absence an NMDAr antagonist (MK801, 25µM). Furthermore, mice with increased level of Hcy (che+/−) and different mouse models of diabetes (Streptozotocin (STZ), db/db and Akita) mice were evaluated for NMDAr activation in retinal cryosections by Immunofluorescence (IF).

Results: NMDAr is expressed in HRECs and WB and IF analysis showed that Hcy significantly increased NMDAr expression in HRECs in a dose-dependent manner, increased FITC-dextran leakage in HRECs which was rescued by NMDAr antagonist MK801. Furthermore, NMDAr expression was increased in che+/− mice retina as well as the diabetic mice retinal sections in comparison to control mice.

Conclusions: Homocysteine disturbs the BRB function via activation of NMDAr receptor in HRECs. Targeting NMDAr may propose new therapeutic avenue for patients with DR.

Commercial Relationships: Riyaz Mohamed, None; Ahmed Ibrahim, None; Nehal M. El-Sherbiny, None; Mohamed A. Al-Shabrawey, None; Amany M. Tawfik, None

Support: AHA Grant SDG

Program Number: 4047 Poster Board Number: B0020

Presentation Time: 8:30 AM–10:15 AM

Rap1 activation promotes vascular blood-retinal barrier

Carla Jhoaana Ramos, Xuwien Liu, Chengmao Lin, David Antonetti

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Purpose: Purpose: Activation of Rap1 through the cAMP-dependent guanine nucleotide exchange factor EPAC was reported to enhance barrier function in human umbilical endothelial cells. However, the effect of EPAC-Rap1 signaling on retinal endothelial barrier, tight junctions, and the relationship to vascular endothelial growth factor (VEGF)-induced permeability are unknown. We hypothesize that EPAC-Rap1 activation inhibits VEGF-induced permeability in retinal endothelial cells.

Methods: Methods: Primary, bovine retinal endothelial cells (BREC) were used to model the blood retinal barrier. The EPAC-specific activator cAMP analog 8-pCPT-2-O-Me-cAMP-AM (8CPT) was used. Cell monolayer permeability was measured by electrical resistance using the ECIS-Zθ system or by measuring 70kDa FITC dextran flux across transwell filters. Rap1 activation was determined using a Rap1 capture assay with GST-RalGDS followed by Western blot. EPAC specific antagonist (ESI-09) or Rap1B siRNA (bovine) were used to inhibit EPAC or silence Rap1 expression in BREC. Confocal microscopy assessed junctional protein organization. Male Sprague-Dawley rats were used to test retinal vascular permeability in vivo.

Results: Results: Treatment of BREC monolayers with 8CPT and activation of Rap1 prevented both VEGF or VEGF and tumor necrosis factor (TNF)-induced permeability to 70kDa dextran (p<0.0001) and reduction in electrical resistance (p<0.0001). 8CPT treatment reversed VEGF-induced permeability to solute and ion flux. EPAC inhibition or Rap1 silencing led to an increase in basal permeability and VEGF had no additional permeability increase. Pre-treatment with 8CPT decreased VEGF signaling in the Erk pathway but Rap1 knockdown revealed no effect on VEGF signaling. Immunofluorescence staining showed disruption of tight junction organization in VEGF treated cells and 8CPT blocked VEGF junctional disruption (p<0.0001). Permeability studies in vivo showed that VEGF/TNF increased permeability and 8CPT blocked VEGF/ TNF permeability.

Conclusions: Conclusions: These data demonstrate that activation of Rap1 through EPAC in retinal endothelial cells promotes barrier properties, inhibits and reverses VEGF and VEGF/TNF-induced permeability, and protects tight junction organization. Collectively, these data suggest activation of Rap1 through cAMP analogs may provide a therapeutic means to restore barrier properties in diseases of increased retinal permeability.

Commercial Relationships: Carla Jhoaana Ramos, None; Xuwien Liu, None; Chengmao Lin, None; David Antonetti, None

Support: 5T32EY013934-14

Program Number: 4049 Poster Board Number: B0022

Presentation Time: 8:30 AM–10:15 AM

Genetically modified probiotics for oral delivery of Angiotensin-(1-7) confers protection against diabetic complications

Qiuhong Li, Zhibing Liang, Kang Xu, Tao Du, Ping Zhu, Tuhina Prasad, Shengquan Liao, Manoj Kulkarni, Amrisha Verma, Mohan Raizada

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Purpose: Genetically modified probiotics for oral delivery of Angiotensin-(1-7) confers protection against diabetic complications

Methods: Methods: We genetically modified the commensal bacterium Lactobacillus paracasei (LP) to serve as a live vector for the oral delivery of Ang-(1-7) and investigated its therapeutic potential in attenuating diabetes and associated complications. Ang-(1-7) was expressed as a secreted fusion protein with a trans-epithelial carrier to allow uptake into circulation. The vector pTRKH3-ldh-GFP or pTRKH3-ldh-GFP were introduced by electroporation into LP. Two-month old adult diabetic eNOS−/− mice induced by intraperitoneal injection of streptozotocin and Akita mice were orally gavaged every other day with 1×106 CFU of LP secreting Ang-(1-7) (LP-A), LP secreting GFP (LP-GFP), or LP alone for eight and twelve weeks respectively.

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Results: Compared to age-matched untreated diabetic control animals, oral feeding of LP and LP-A significantly improved STZ-induced damage to insulin producing beta cells in pancreas in diabetic eNOS-/- mice. LP-A treatment also enhanced glucose tolerance, improved structure and morphology of islets and kidney, increased insulin expression in both diabetic eNOS-/- mice and Akita mice; and are associated with reduced retinal gliosis, inflammation, apoptotic neuronal cell death and loss of retinal vascular capillaries.

Conclusions: Oral administration of a genetically modified commensal bacterium that can secrete Ang-(1-7) provides protection against diabetes-induced tissue damage and diabetic retinopathy. Thus, LP-based delivery of Ang-(1-7) may hold important therapeutic potential for the treatment of diabetic complication.

Commercial Relationships: Qihong Li, None; Zhibing Liang, None; Kang Xu, None; Tao Du, None; Ping Zhu, None; Tuhina Prasad, None; Shenuan Liao, None; Manoj Kulkarni, None; Amrisha Verma, None; Mohan Raizada, None

Support: Supported in part by NIH grants EY021752, EY024564, American Diabetes Association, BrightFocus Foundation (QL) and NIH grant R01 HL102033 (MKR). Core facilities were supported by NEI grant P30 EY02172 and Research to Prevent Blindness to University of Florida.

Program Number: 4050 Poster Board Number: B0023
Presentation Time: 8:30 AM–10:15 AM

Hypoxia-Inducible Factor 1-Regulated Angiogenic Cytokines in Proliferative Diabetic Retinopathy

Haley Megarity1, Monika Deshpande1, Savalan Babapoor-Farrokhran2, Brooks Puchner1, Gregg Semenza1, Silvia Montaner2, Akrit Sodhi2

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Supported in part by NIH grants EY021752, EY024564, American Diabetes Association, BrightFocus Foundation (QL) and NIH grant R01 HL102033 (MKR). Core facilities were supported by NEI grant P30 EY02172 and Research to Prevent Blindness to University of Florida.

Program Number: 4050 Poster Board Number: B0023
Presentation Time: 8:30 AM–10:15 AM

Purpose: Recent studies have demonstrated that monthly intravitreal injections with therapies targeting VEGF delays the development of PDR in some, but not all, treated patients. This suggests that additional factor(s) may contribute to the development of retinal neovascularization in diabetic patients. In addition to VEGF, hypoxia inducible factor (HIF)-1 regulates the expression of other angiogenic factors in ischemic retinal disease, and plays a central role in the promotion of retinal neovascularization in PDR. Here we set out to examine the potential contribution of three other HIF-1-regulated angiogenic cytokines, angiopoietin 2 (ANGPT2), erythropoietin (EPO), and angiopoietin-like 4 (ANGPTL4), all previously implicated in the promotion of diabetic eye disease, to the development of PDR.

Methods: HIF-1-regulation of VEGF, AGPT2, EPO, and ANGPTL4 mRNA and protein expression was assessed in vitro by qPCR and ELSIAs in primary and immortalized (MIO-M1) retinal Müller cells in vitro or the OIR model in vivo. The angiogenic potential was assessed using the tubule formation assay. Expression of these factors in vitreous biopsies from patients with active NV due to PDR was determined by ELISA. Complementary IHC studies were performed in a second group of eyes from PDR patients. Mann-Whitney U test and Spearman correlation statistical models were used to compare protein concentrations and to identify factors that independently correlate with PDR.

Results: VEGF, AGPT2, EPO and ANGPTL4 mRNA levels were elevated in hypoxic Müller cells in vitro and in the OIR model in vivo; inhibition of HIF-1 blocked their expression. However, protein levels of EPO and AGPT2 were not significantly increased in retinal Müller cells, suggesting that these proteins may be expressed by a different cell type, or their local (intraocular) expression may be increased in diabetic eyes due to increased vascular permeability.

Conclusions: These results suggest that ANGPT2, EPO, and ANGPTL4 – or HIF-1 to target all four – may be potential therapeutic targets for the prevention and/or treatment of PDR.

Commercial Relationships: Haley Megarity, None; Monika Deshpande, None; Savalan Babapoor-Farrokhran, None; Brooks Puchner, None; Gregg Semenza, None; Silvia Montaner, None; Akrit Sodhi, None

Program Number: 4051 Poster Board Number: B0024
Presentation Time: 8:30 AM–10:15 AM

Cell Therapy Model of Diabetic Retinopathy Using Vascular Progenitor Cells Derived from Human iPSC

Tea Soon Park1, 4, Imran A. Bhutto, Ludovic Zimmerlin1, 3, Il Minn1, Nensi Ruzgar1, 4, Martin Pomper1, Mary A. Johnson1, Gerard A. Lutty1, Elias Zambidis1, 2

Pediatric Oncology, Johns Hopkins University, Baltimore, MD; 1Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; 2Department of Radiology, Johns Hopkins University, Baltimore, MD; 3Department of Ophthalmology, University of Maryland, Baltimore, MD; 4Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD; 1Health Sciences, McMaster University, Toronto, ON, Canada

Purpose: Successful cellular therapy of diabetic vascular complications (DVC) will require novel sources of angiogenic progenitors that can sustain long-term functional recovery in a clinical setting. Since adult stem cell sources such as diabetic endothelial progenitor cells (D-EPC) have proven to be scarce or have impaired migration into the injury sites, human induced pluripotent stem cell (hiPSC) technology may offer unlimited amounts of embryonic vascular progenitor (VP) to repair ischemic diabetic tissues with clinically relevant and efficient protocols for reprogramming and differentiation.

Methods: To test this potential, we generated diabetic iPSC (D-iPSC) from skin fibroblasts of type-1 diabetic patient using non-integrative episomal reprogramming system. Short exposure to a GSK3β inhibitor and ascorbic acid dramatically facilitated the reprogramming kinetics. Additionally, we recently developed novel chemical methods [LIF with GSK3β, ERK and tankyrase inhibitors (L3i)] that stably revert hPSC to a mouse ESC-like naïve state. We demonstrated that LIF-3i-reverted hPSC acquired transcriptomic, epigenetic, and signaling signatures of naïve-pluripotency, and significantly improved multi-lineage differentiation efficiencies with reduced lineage bias.

Results: This L3i reversion method was successfully applied to generating naïve D-iPSC to obtain higher quality of therapeutic VP. Primed- (traditional) and naïve-D-iPSC were compared for differentiation potential, vascular functional assays in vitro and in vivo. To investigate the regenerative capacity of iPSC-VP in a DVC-relevant model, we employed a streptozotocin (STZ) injected diabetic athymic nude rat model that exhibited hyperglycemia over 24 weeks and decreased retinal function as measured by electroretinography. Our preliminary results showed that injection of healthy hiPSC-VP into this diabetic rat model validated human cell capacity to migrate and engraft to injured sites. We are currently examining the engraftment of D-iPSC-VP, naïve-D-iPSC-VP, healthy cord blood- and fibroblast-iPSC-VP. Furthermore, intravitreal injection of hiPSC-VP expressing a luciferase transgene allowed detection of human cell migration using noninvasive live animal bioluminescent imaging.

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**Conclusions:** This STZ-induced diabetic rat model provides a translational opportunity to evaluate the use of patient specific iPSC-VP for treatment of DVC.

**Commercial Relationships:** Tea Soon Park, None; Imran A. Bhutto, None; Ludovic Zimmerlin, None; Il Minn, JHU (P); Nensi Ruzgar, None; Martin Pomper, JHU (P); Mary A. Johnson, None; Gerard A. Lutty, None; Elias Zambidis, JHU (P), Thermo Fisher Scientific (R)

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