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Venue

Sonesta Fort Lauderdale Beach
999 N Fort Lauderdale Beach Blvd
Fort Lauderdale, FL

Exhibitor

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Keynote Talks

Vascular Basement Membrane Thickening in Diabetic Retinopathy

Sayon Roy
Boston University School of Medicine, Boston, MA

Biography

Dr. Sayon Roy is a Professor of Medicine and Ophthalmology in Department Ophthalmology, Boston University. He completed his B.S. and M.S. from University of Kalyani, India. He received his PhD from Boston University. Dr. Roy's seminal work has identified several genes in the retina that are abnormally expressed in diabetic retinopathy. His pioneering work has led to novel gene modulatory techniques in retinal vascular cells using antisense oligonucleotides via intravitreal injection. Dr. Roy has received numerous awards including the American Diabetes Association Research Award for the commitment and dedication towards the fight against diabetes, the 2006 Mentor of the Year Award from Boston University, and the 2008 Innovative Award from the Juvenile Diabetes Research Foundation

Does Genomics Play a Role in Diabetic Retinopathy?

Arup Das[1], Sampath Kumar Rangasamy[2], Finny Monickaraj[1], David Duggan[2], Nicholas Schork[2] and Paul McGuire[1]
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Abstract

Genetic risk factors play an important role in the development and progression of diabetic retinopathy (DR). Using a well-defined, clinical phenotype, we have examined the role of rare genetic variants in DR progression, or protection by undertaking whole exome sequencing (WES). We performed WES analysis on two cohorts of patients selected from Diabetic Retinopathy Genomics (DRGen) study population. Group 1 had "extreme" phenotype (No DR in spite of >25 years of diabetes) (n=6), while Group 2 had "advanced" DR (PDR within 15 years of diabetes) (n=6). DNA was isolated from white blood cells and the WES was performed using SureSelect All Human XT v5 exome kits, on Illumina NovaSeq platform. This was followed by an in-house downstream analysis pipeline to align sequence reads and complete variant calling and annotation. Analysis of rare coding variants using MAF cutoff revealed novel genetic variants with heterozygous missense mutations. Among the variants predicted to be disease-causing, variants in five genes (KLF17, COL18A1, CD33, PLEKHG5, ZNF395) with MAF<0.5% were enriched in the Group 2 (PDR), while variant in a gene (NKX2-3) with MAF<0.5% was shared among the cohort 1 (extreme phenotype). Thus, we identified coding sequence variants in a novel set of genes involved in the angiogenesis/inflammatory pathway that contributes to DR progression, or protection. Future functional validation of the identified variants in disease pathogenesis could potentially lead to the identification of novel biomarkers and molecular targets for treating DR.

Biography

Dr. Arup Das is Regents' Professor of Ophthalmology at University of New Mexico School of Medicine, Albuquerque, USA. After his medical education from Medical College of Calcutta, he did his PhD in Physiology at Medical College of Ohio, and then a postdoctoral fellowship in diabetic retinopathy research at Wayne State University in Detroit. Subsequently, he did his residency in Ophthalmology there, and then a retinal fellowship at Doheny Eye Institute, University of Southern California. Currently, Dr. Das maintains an active research lab funded by NIH and VA. His research interests include angiogenesis, retinal vascular biology, and genetics of diabetic retinopathy.

Endothelial Progenitor Cells and Retinal Vascular Regeneration

Alan W. Stitt
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Abstract

There is growing evidence that many vascular beds possess resident progenitor cells that have important roles in

homeostasis and normal repair processes. In disease contexts, such as diabetes, these progenitors often become dysfunctional and this may make a significant contribution to the vasodegenerative pathology that is characteristic of many diabetic complications. This presentation will examine progenitor cell types with myeloid, mesenchymal or endothelial profiles and their role in regenerative medicine. The molecular profiles and mechanism of action of these progenitors will be discussed and examples shown of how exposure to the diabetic milieu can alter their vasoreparative function. Particular emphasis will be given to progenitors that regenerate damaged endothelium and a sub-type known as endothelial colony forming cells (ECFCs). Data will be shown that demonstrates how ECFCs have recently emerged as the principal endothelial progenitor both in vivo. The presentation will discuss the possibilities of treating vasodegenerative disease by harnessing vessel-resident regenerative cells and also using them for to regenerate ischaemic tissues.

Biography

Professor Stitt is the McCauley Chair of Experimental Ophthalmology with a research focus on pathogenesis and new therapeutic avenues for diabetic retinopathy and age-related retinal disease. He also contributes to the international academic community by serving on advisory boards, grant panels and as editor for Current Eye Research and Associate Editor for Diabetologia. He has received a Royal Society Merit Award, the Sir Jules Thorn Biomedical Science Award, and the 5th Fincham Medal. He is a member of the Royal Irish Academy (MRIA) and a Fellow of the Association for Research in Vision & Ophthalmology (FARVO).

Diabetic Retinopathy: Mitochondria and a Web of Epigenetics

Renu A. Kowluru
Wayne State University, Detroit, MI

Abstract

In the pathogenesis of diabetic retinopathy, mitochondria are damaged in the retina and its vasculature, and the damaged mitochondria continues to fuel into the vicious cycle of free radicals. Mitochondrial dysfunction activates the apoptotic machinery and accelerated capillary cell apoptosis precedes the development of histopathology characteristic of diabetic retinopathy. Expression of many genes associated with mitochondrial homeostasis is also altered in diabetes. Recent evidence has clearly documented the role of epigenetics in gene expression, and diabetes is shown to alter the activities of many enzymes implicated in epigenetic modifications including DNA methylation and hydromethylation. This presentation will discuss how epigenetic modifications affect mitochondrial structural and genomic stability, and their role in the development of diabetic retinopathy.

Biography

Dr. Kowluru is a Professor and Director of Translational Research in the Ophthalmology and Visual and Anatomical Sciences Department at Wayne State University, Detroit. Her research is focused on understanding the molecular mechanism of diabetic retinopathy, especially the role of mitochondrial dysfunction and epigenetics. She has published over 160 peer-reviewed articles, and is on the editorial board of leading Vision and Diabetes journal. Dr. Kowluru serves as an expert reviewer on many NIH, Department of Defense and Veterans Administration panels. The research is currently funded by R01 awards from the National Eye Institute, and from a Private Foundation.

The UPR Signaling in Bone Marrow Stem Cells and Diabetic Retinopathy

Sarah X. Zhang
Ira G. Ross Eye Institute, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, the State University of New York, NY

Abstract

Dysfunction of the bone marrow (BM)-derived angiogenic stem and progenitor cells is an important pathogenic factor contributing to the persistent vascular injury and degeneration in diabetic tissues. In this study, we investigate the role of unfolded protein response (UPR) signaling in diabetic BM stem cell dysfunction and their implication in diabetic retinopathy. In type 1 and type 2 diabetic mice, ER stress markers and inflammatory gene expression in BM mononuclear cells and hematopoietic progenitor cells increase dynamically with disease progression. Inhibition of ER stress by ex vivo or in vivo chemical chaperone treatment significantly improves the viability of BM stem cells and enhances their angiogenic function. ER stress inhibition also increases the number of circulating angiogenic cells, alleviates BM pathology, and promotes retinal vascular repair after ischemic injury in diabetic mice. Furthermore, manipulation of the UPR signaling mediated by the IRE/XBP1 or the CHOP pathways significantly altered bone marrow progenitor cell survival and function under normal and diabetic conditions. These findings suggest that targeting the UPR signaling in the BM and angiogenic stem cells may provide a novel and promising approach to the prevention and treatment for diabetic retinopathy.

Plenary Session

Activation of Classical Complement Pathway by Exosomes in Diabetic Retinopathy

Julia Busik*, Chao Huang, Kiera Fisher, Sandra Hammer and Denis Proshlyakov
Michigan State University, East Lansing, MI

Abstract

Diabetic retinopathy (DR) is a sight threatening microvascular complication of diabetes. Activation of complement system was recently implicated in the development of vascular damage and progression of DR. The mechanism(s) involved in complement system activation in DR are not fully understood. Exosomes, small vesicles that are secreted into the extracellular environment, have a cargo of complement proteins in plasma, suggesting that they can participate in causing the vascular damage associated with DR. We demonstrate that IgG-laden exosomes in plasma activate the classical complement pathway and that the quantity of these exosomes is increased in diabetes. Moreover, a lack of IgG in exosomes in diabetic mice results in a reduction in retinal vascular damage. In conclusion, complement activation by IgG-laden plasma exosomes could contribute to the development of DR.

Biography

Julia Busik, PhD, is Professor of Physiology at Michigan State University. She received her B.S. and M.S. degrees from Novosibirsk State University, USSR, and PhD from the Graduate University for Advanced Studies, Yokohama, Japan. Dr. Busik started research in the area of diabetic complications after joining Michigan State University as a post-doctoral fellow under the guidance of Dr. Douglas Henry. She published over 60 papers in high profile journals, and she is a well-recognized expert on the role of dyslipidemia in the development of diabetic complications. Research in Busik lab is supported by NIH NEI, JDRF and ADA awards.

Phosphoinositide Signaling in Eye Development and Disease

Philipp P. Prosseda[1], Jorge A. Alvarado[1], Na Luo[2], Biao Wang[1], Tia J. Kowal[1], Ke Ning[1], Yang Hu[1], and Yang Sun[1,3]
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[2] Eugene and Marilyn Glick Eye Institute, Indiana University School of Medicine, Indianapolis, IN
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Abstract

Phosphoinositides (PIs) are lipid signaling molecules involved in wide-ranging cellular functions including vesicular trafficking, membrane dynamics, and primary cilia signaling. Inositol polyphosphate 5-phosphatases are critical enzymes that regulate PI levels in cellular subcompartments. Mutations in the 5-phosphatase gene INPP5E cause ciliopathies such as Joubert and MORM syndrome with retinal degeneration. Mutations in OCRL result in Lowe syndrome which presents with congenital glaucoma and cataracts. Recently INPP5K has been implicated in syndromic forms of congenital cataracts, muscular hypotonia, and mental retardation. The mechanisms of 5-phosphatase signaling in eye diseases will be explored.

Biography

Dr. Sun is a clinician-scientist and an Associate Professor of Ophthalmology at Stanford University, Byers Eye Institute. Dr. Sun received his BA in Biophysics from Johns Hopkins University, followed by a MD., PhD. degree from Washington University School of Medicine. He completed Ophthalmology residency at Stanford University and a prestigious Heed fellowship at University of Michigan, Ann Arbor. Currently the Laurie Kraus Lacob Faculty Scholar at Stanford Child Health Institute, Dr. Sun holds several U.S. patents on novel regulators of eye pressure and is the primary investigator on a number of glaucoma clinical trials.

Ranibizumab Alters Levels of Soluble Cytokine Receptors in Patients with Diabetic Macular Edema

Susanne Mohr[1*], Brandon A. Coughlin[1], Pratim Guha-Niyogi[1], Alla Sikorskii[1] and Louis C. Glazer[1]
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[2]Vitreo-Retinal Associates Grand Rapids, MI

Abstract

Purpose: Ranibizumab is a well-established treatment for diabetic patients with macular edema. However, very little is known about the effect of ranibizumab on regulation of pro- and anti-inflammatory signaling pathways and their regulation of VEGF family members, which was the aim of this study.

Materials and Methods: Diabetic patients (n=10) aged ≥18 years with central diabetic macular edema, BCVA >24 and <78, and central macular thickness (CMT) greater than 250 µm was enrolled in this study. Following a full eye exam, imaging, and an aqueous tab, patients received ranibizumab (0.3mg/0.05mL) injections at day one and weeks four and eight. At week 12, a full eye exam, imaging, and a second aqueous tab was obtained prior to the last injection of ranibizumab. Pre- and post-treatment aqueous humor samples were then analyzed using Milliplex MAP magnetic bead assays.

Results: Ranibizumab lowered levels of VEGF-A, decreased CMT, and improved VA (visual acuity). Changes in levels of VEGF-A and VEGF-C strongly correlated with changes in soluble receptors, sgp130 and sIL-6R, associated with IL-6 signaling. In contrast, changes in VEGF-D correlated with sIL-1R1 and sIL-1R2, soluble receptors participating in IL-1 signaling. Changes in CMT and VA did not correlate with changes in levels of VEGF family members. However, post-treatment values of CMT correlated with post-treatment levels of VEGF-C. Post-treatment VA values correlated with a wide variety of potential biomarkers linked to inflammation.

Conclusions: Ranibizumab treatment had strong effects on regulating levels of soluble receptors closely linked to IL-1 and IL-6 signaling pathways.

Biography

Dr. Mohr is Associate Professor with tenure (2009-present) in the Department of Physiology at Michigan State University, USA. She graduated from the University of Konstanz, Germany, with a PhD in Biological Chemistry (magna cum laude) in 1996. After completion of a postdoctoral fellowship (awarded by German Research Foundation/DFG) at Case Western Reserve University, Molecular Cardiovascular Research Center, Dr. Mohr was appointed Assistant Professor in the Division of Clinical and Molecular Endocrinology in 1999. Her research background is in the field of acute and chronic inflammation and regulation of cell death which she is translating into the field of diabetic retinopathy.

VEGF is a Master Regulator for Neuronal Integrity in Diabetic Retinopathy and Hypoxic Retinal Diseases

Yun-Zheng Le[1*], Shuhua Fu[1,2], Fangfang Qiu[1], Ana J. Chucair-Elliott[1] and Meili Zhu[1]
[1]University of Oklahoma Health Sciences Center, OK
[2]The Second Affiliated Hospital of Nanchang University, China

Abstract

Purpose: To determine the role of Müller glia (MG) in diabetic retinopathy and age-related macular degeneration (AMD), we investigated the mechanism of vascular endothelial growth factor receptor-2 (VEGFR2)-mediated MG and neuronal integrity and trophic factor production by MG.

Methods: Neuronal and MG integrity was analyzed with MG-specific VEGFR2 knockout (KO) mice. Mechanistic analysis was performed in cells with RNAi or KO.

Results: Diabetic/hypoxic MG-specific VEGFR2 KO mice demonstrated reduction in MG density, accelerated neuronal loss in all retinal layers, and significant decrease of retinal glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF). Mechanistic analysis indicated that VEGFR2 disruption affected Müller cell viability through the reduction of AKT survival signal. VEGF was capable of upregulating BDNF and GDNF production and stimulating Müller cell viability with VEGF synergistically. Detailed mechanistic analysis for BDNF- and GDNF-mediated MG viability and treatment of retinal degeneration with trophic factors in diabetic/hypoxic conditional VEGFR2 KO mice are in progress.

Conclusions: VEGF is a master regulator of MG health and trophic factor production under diabetic/hypoxic stress. As our diabetic/hypoxic MG-specific VEGFR2 KO mice have very thin retina, which bears striking resemblance to that in a substantial portion of wet-AMD patients treated with long-term anti-VEGF drugs, it is most likely that anti-VEGF drugs may interfere with VEGF signaling in MG in these patients. Therefore, supporting MG viability with neurotrophins may be a feasible strategy for neuroprotection during anti-VEGF treatment. We are actively examining this possibility with our animal models.

Biography

Dr. Le is the Harold Hamm Chair in Diabetes Research and Professor of Medicine, Cell Biology, and Ophthalmology at the University of Oklahoma Health Sciences Center. He received BS in Biochemistry at Fudan University in china, PhD in Microbial Biochemistry/Genetics at Dalhousie University in Canada, and postdoctoral training in genome biology at Dalhousie and in cellular and molecular biology and gene targeting with inducible and Cre/lox technology at the National Institutes of Health in the United States. His current research is focused on the mechanisms and therapeutic strategies for diabetic retinopathy and hypoxic retinal diseases using animal models.

CTGF Regulates Vascular Morphogenesis and Integrity by Buffering Extracellular Matrix Stiffness

Brahim Chaqour
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Abstract

The formation of a functional vascular network in the retina involves coordinated signaling between vascular cells and the extracellular matrix (ECM), a network of proteins and glycans providing not only anchorage points to the cells but also chemical and mechanical cues for directional migration, morphogenesis, and stability. Dysregulation of these signals plays an important role in the pathogenesis of ocular vascular diseases. Connective tissue growth factor (CTGF) is a secreted ECM protein essential for skeletal development because global CTGF deficiency produces skeletal dysplasia and perinatal lethality in mice. In adulthood, aberrant CTGF expression is associated with fibrosis, scarring and malignancies of virtually every organ. Despite its prominent expression in vascular cells, the role of CTGF in vessel formation and regeneration is unknown. Herein, we used mouse genetics to study the regulation and function of CTGF in the retinal vasculature. Using CTGF-GFP BAC transgenic mouse, as a proxy marker for endogenous CTGF, we found a heterogeneous spatiotemporal expression pattern of CTGF in the retina. CTGF is predominantly produced by endothelial and pericytes of sprouting and fully formed blood vessels. CTGF expression is also dynamic within subsets of retinal glial and microglial cells as well as and differentiating retinal ganglion neurons. Global and endothelial-specific deletion of CTGF using a Cre-lox system greatly reduced vessel area, micro vessel density, and vascular branching. While arteriovenous differentiation and vascular expansion to the retinal edge is not affected, the vascular network was partially shaped into rudimentary arterioles, venules, and capillaries. Importantly, CTGF mutant mice exhibited a diffuse and patchy hyper fluorescence in the extravascular space following systemic injection of fluorescently labeled albumin indicating blood retinal barrier breakdown. Genome-wide analysis of the retina transcriptome of wild-type and CTGF mutant mice identified numerous CTGF target genes that include cytoskeletal, extracellular matrix, integrin and inflammatory genes whose expression is commonly regulated by ECM matrix stiffness. Ex vivo measurements of blood vessel mechanical properties showed that retinal vessels from wild-type mice are softer than those of CTGF mutant mice. Thus, CTGF and/or CTGF "regulome" promotes normal expansion, cell-cell junctional organization and mechanical stability of retinal vessels by buffering the mechanical properties of the ECM. Our study broadens the therapeutic options for targeting this important cue in retinal vascular pathogenesis.

The Role of Mitochondrial Ceramide in Diabetes-Induced Retinal Endothelial Cells Damage

Denis A. Proshlyakov*, Yan Levitsky, David Pegouske, Sandra S. Hammer, Anand Saripalli, Todd Lydic and Julia V. Busik
Michigan State University, East Lansing, MI

Abstract

Mitochondrial damage precedes histopathological abnormalities in diabetic retinopathy (DR). Retinal endothelial cells (REC) have the highest level of Acid Sphingomyelinase (ASM) expression in the retina and the increase in ASM activity and ceramide production were shown to promote diabetes-induced pro-inflammatory and pro-apoptotic changes in the retina and REC. We investigate the role of diabetes-induced increase in ASM and ceramide production in mitochondrial damage in the retina and REC using a combination of traditional molecular biological techniques and the novel microfluidic respirometry on adherent cells and isolated mitochondria. We demonstrate decrease in mitochondrial sphingomyelin (mSM)

and increase in mitochondrial ceramide (mCer) in 7 wks diabetic rat retina. ASM-/- mouse model showed increased mSM and decreased mCer compared to wildtype, confirming that ASM is an essential contributor to mitochondrial ceramide regulation. Pre-treatment of BREC with an ASM inhibitor Desipramine revealed a 1.75-fold increase in maximal respiratory capacity of whole adherent cells. Inhibition of ASM in BREC results in increased maximal, but not basal, respiratory rates. Diabetes-induced changes in mSM and mCer are consistent with ASM-dependent ceramide production and support the hypothesis that ASM activation leads to mitochondrial ceramide accumulation and inhibition of oxidative phosphorylation and REC damage.

Biography

Dr. Denis A. Proshlyakov is an Assistant Professor of Chemistry at the Michigan State University. He received his B.S. and M.S degrees from the Pirogov State Medical Institute (RNRMU) and the Institute for Biomedical Chemistry, USSR. Dr. Proshlyakov received Ph.D. from the Graduate Institute for Advanced Studies and the Institute for Molecular Sciences, Okazaki, Japan, investigating molecular mechanisms of respiration using laser spectroscopy. At the Michigan State University, he continued investigation of transient mechanisms of metalloenzymes' reactions with oxygen. He is now using chemical approaches for the development of novel precision tools for biomedical studies.

Choroidal Vascular Network in Eye Diseases

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[2]Department of Ophthalmology, Asan Medical Center, Korea

Abstract

The choroid is a highly vascularized tissue of the eye. Choriocapillaris, the innermost choroidal layer, provides oxygen and nourishment to the retina, which is crucial for maintaining visual function. Choriocapillaris has larger luminal diameter, and it is fenestrated with circular openings covered by a diaphragm. Unidirectional fenestrations toward retina allow easy movement of macromolecules between the choroid and retina. On the other hand, perivascular cells in choroidal vascular system regulate vascular stability and blood flow. Changes at the level of the choroid feature in the pathology of vision-threatening retinal diseases, including age-related macular degeneration (AMD) and diabetic retinopathy. This presentation will cover our recent data on age- or diabetes-associated changes in choroidal blood vessels. We have demonstrated that trans-endothelial transport through the choriocapillaris is compromised in aging and diabetes. In addition, choroidal vascular network is covered with several subtypes of pericytes, which changes dramatically with aging. Transcriptome analysis revealed expression differences in the genes associated with pericyte-endothelial interactions, depending on age. Diabetic animal models and pericyte-deficient mice presented prominent choroidal vascular phenotypes. In summary, polarized distributions of choroidal pericyte and fenestration are important for vascular stability and molecular transport in choroidal vascular network. Age- or diabetes-associated changes in choriocapillaris limit the choroidal function to provide metabolic and nutrient support to the retina, which gives rise to vision-threatening diseases including AMD and diabetic retinopathy.

Biography

Dr. Junyeop Lee is a vitreoretinal surgeon whose laboratory studies vascular biology. He is an assistant professor in Yeungnam University. He received MD at Yeungnam University in 2005, Ph.D. from Graduate School of Medical Science and Engineering, KAIST in 2014. He finished a clinical fellowship at division of vitreoretina, at Asan medical center in 2016. He received many academic awards from the basic and clinical societies. He aims to identify the regulatory mechanisms of vascular remodeling in the chorioretinal blood vessels, and to translate these basic insights into the pathogenesis of the retinal diseases including AMD and diabetic retinopathy.

Sphingolipid Metabolism Contributes to Mitochondrial Dysfunction Induced by Diabetogenic Condition in Retinal Epithelial Cells

Kiera P. Fisher*, Yan Levitsky, Sandra S. Hammer, Todd Lydic, David Pegouske, Denis A. Proshlyakov and Julia V. Busik
Michigan State University, East Lansing, MI

Abstract

Purpose: Mitochondrial damage is accepted to precede histopathological abnormalities in diabetic retinopathy (DR) and significantly contribute to DR pathogenesis. Recent studies identified sphingolipid metabolism and mitochondrial ceramide content as factors in mitochondrial damage. This study sought to determine the role of acid sphingomyelinase (ASMase) activation and ceramide accumulation in diabetes-induced mitochondrial damage in retinal pigment epithelial cells (RPEs).

Methods: Human RPEs were isolated from control and diabetic donors; ARPE-19 cells were treated with high-glucose (HG) or HG with ASM inhibitor. Mitochondria isolated using differential centrifugation and gradient ultracentrifugation. Mitochondrial sphingolipid composition was assessed by electrospray ionization high resolution mass spectrometry (nESI-MS). Purity of mitochondria assessed by western blot analysis. Fragmentation assessed by morphometric analysis of fluorescently labeled mitochondria. Citrate synthase activity determined with commercially available kit. Mitochondrial respiration measured using custom designed microrespirometer.

Results: Retinal mitochondria demonstrated diabetes-induced short chain ceramide accumulation. Purified mitochondria from HG-treated ARPE-19 cells demonstrated significant increase in short-chain ceramides, this increase abrogated by ASM inhibition (*$p<0.05$). Mitochondria from diabetic donors showed a decrease in the average mitochondrial length of 1.2 ± 0.57 μm compared to 3.4 ± 0.78 μm in control donors. Citrate synthase activity is altered in HG-treated ARPE-19 cells but not in presence of ASM inhibitor. RPEs obtained from diabetic donor showed a decreased RCR of 3.9 (95% CI 2.81 to 4.99) compared to 11.1 (95% CI 9.91 to 12.3) from control donor.

Conclusions: These results suggest that diabetes-induced mitochondrial functional changes may form part of the diabetic sequelae leading to DR pathogenesis.

Control of Retinal Cholesterol Levels by Fasting-Induced Activation of SIRT1-LXR Pathway in Diabetic Retinopathy

Delaney McFarland*, Sandra Hammer, Maria B. Grant and Julia V. Busik
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Abstract

Purpose: Diabetic retinopathy (DR) is a growing health concern with limited treatment options. Previous research demonstrated dyslipidemia and cholesterol level dysregulation play significant roles in DR development. Strategies to normalize retinal cholesterol regulation are limited. Major regulators of cholesterol are Liver X-receptors-α/β (LXR). LXR signaling has been shown to activate reverse cholesterol transport (RCT). SIRT1 is a nutrient-sensing deacetylase activated during fasting, and a regulator of LXR activity. Both SIRT1 and LXR levels are decreased in diabetic retina. We hypothesize that fasting-induced increase in SIRT1 followed by LXR deacetylation and activation, leads to increased RCT and lowered cellular cholesterol levels.

Methods: Bovine retinal endothelial cells (BRECs) were isolated/validated according to a previous protocol. BRECs were treated with diabetic relevant stimuli TNFα (10ng/ml) for 24hrs. To model calorie restriction BRECs were serum starved (0% FBS) for 24hrs. ABCA1 and ABCG1 were analyzed by qRT-PCR. Cholesterol concentrations were measured via cholesterol assay. Cell death was measured via trypan blue exclusion assay.

Results: Serum starvation increased SIRT1 mRNA levels (n=3; $p=0.001$), as well as ABCA1 (n=3; $p<0.01$), and ABCG1 (n=3; $p<0.01$) mRNA levels. TNFα treatment increased cholesterol levels (n=6, $p=.0233$); serum deprivation for 24hrs decreased cholesterol levels in BRECs (2% FBS; $p=0.0025$, 0% FBS; $p<0.001$, n=6). No significant difference in cell death among cells cultured in 10% FBS, 2% FBS or 0% FBS after 24hrs.

Conclusion: The results suggest that serum starvation promotes activation of the SIRT1-LXR pathway. Targeting this pathway has dual benefits of preventing low-grade retinal inflammation and promoting metabolic reprograming.

Effect of Serum Starvation on the SIRT1-LXR Signaling Pathway in Retinal Endothelial Cells

Basma Baccouche[1*], Maximilian Sandler[1], Sandra S. Hammer[1], Elahè Crockett[2] and Julia V. Busik[1]
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[2]Department of Medicine, Michigan State University, East Lansing, MI

Abstract

Chronic inflammation is accepted as a leading cause of Diabetic Retinopathy (DR) pathogenesis, however factors leading to inflammatory changes in diabetic retina are not fully understood. Liver X Receptors α/β (LXR) are well accepted anti-inflammatory regulators that can be activated through deacetylation by nutrient-sensing deacetylase SIRT1. TNFα has been shown to play an important role in the initiation of inflammation and to be responsible for the expression of adhesion molecules. We hypothesize that serum starvation will lead to increased levels of SIRT1, resulting in decreased levels of inflammation in retinal endothelial cells.

Bovine Retinal Endothelial cells (BRECs) were isolated and cultured according to published protocols. Cells were treated with pro-inflammatory stimulus, TNFα (10ng/ml) and/or serum starved (0% Fetal Bovine Serum) for 24hrs. mRNA expression levels were analyzed via qRT-PCR. LXR total protein levels were analyzed via western blot analysis.

Treatment with TNFα, caused a significant decrease in SIRT1 expression levels (n=6, p=0.0126). Serum starvation for 24hrs caused a significant increase in SIRT1 mRNA levels (n=6, p=0.0031) and LXRα protein levels (n=3, p=0.0069). Additionally, treatment with TNFα caused a significant increase in ICAM1 levels (n=6, p<0.001) while serum starvation led to a significant decrease in inflammation markers including ICAM1 (n=6, p<0.001).

TNFα treatment upregulated SIRT1 levels and lead to increased levels of inflammation in BRECs. Activation of SIRT1, via serum starvation, lead to decreased levels of the vascular adhesion molecule, ICAM1. This data suggests that amplified SIRT1 expression has the potential to reduce DR pathogenesis brought on by retinal inflammatory changes.

Ceramide-Induced Mitochondrial Changes in Retinal Endothelial Cells

Yan Levitsky[*], Sandra S. Hammer, Todd Lydic, David Pegouske, Kiera P. Fisher, Denis A. Proshlyakov and Julia V. Busik
Michigan State University, East Lansing, MI

Abstract

Diabetic retinopathy (DR) is a sight threatening complication of diabetes. Pathogenic mechanisms involve hyperglycemia, dyslipidemia, and chronic inflammation causing break down of the blood retinal barrier and DR progression. Acid sphingomyelinase (ASMase) induced ceramide accretion is a potent mediator of retinal endothelial cell (REC) apoptosis but detailed mechanistic insights are lacking. This study aims to elucidate the role of mitochondrial ceramide (mCer) accretion in REC apoptosis. Mitochondria prepared by differential centrifugation showed lysosomal membranes which were undetectable after magnetic assisted cell sorting (MACS) isolation. High resolution/accurate mass spectrometry analysis of retinal mitochondria from 7-week diabetic rats revealed a 1.6-fold increase in sphingomyelin to ceramide ratio compared to control ($n = 3$; $p < 0.05$). Retinal mitochondria from 36-week diabetic rats ($n = 4$) showed a 1.4-fold increase in short chain ceramides compared to control ($n = 3$; $p < 0.01$). Bio-mimetic microrespirometry of BREC revealed robust respiratory activity unaffected by media flow. Pre-treatment of BREC with an ASMase inhibitor revealed a 1.75-fold increase in maximal respiratory capacity compared to control cells whereas changes to basal respiration were not detectable with on-chip inhibition. MACS mitochondrial isolation results in pure mitochondrial preparations amenable to "-omic" analyses. Short- and long-term diabetes results in increased mitochondrial ceramide to sphingomyelin ratio, consistent with ASMase dependent mCer accretion. Inhibition of ASMase in REC results in increased maximal, but not basal, respiratory activity. These results support the hypothesis that ASMase-dependent mitochondrial ceramide accumulation leads to inhibition of oxidative phosphorylation and REC cell damage in diabetic environment.

Acid Sphingomyelinase Upregulation Alters Membrane Fluidity and Migration of Circulating Angiogenic Cells in Diabetic Retinopathy

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[1]Michigan State University, Department of Physiology, East Lansing, MI
[2]University of Alabama, Birmingham, AL

Abstract

Diabetic retinopathy (DR) is a sight-threatening complication of diabetes. Diabetes leads to retinal endothelial cell damage and inadequate vascular repair. The inability to repair retinal vasculature is due, in part, to the compromised function of circulating angiogenic cells (CAC). Previously, we demonstrated that diabetes-induced acid sphingomyelinase (ASM) upregulation causes CAC dysfunction by altering membrane fluidity and migration. In this study, we isolated CD34+ CAC from the blood of non-diabetic control, type 1 and type 2 diabetic patients with varying degrees of retinopathy. CAC isolated from type 1 diabetic patients had decreased migration and altered fluidity that was sensitive to the ASM inhibitor desipramine. However, type 2 diabetic patients showed no changes in fluidity and migration compared to non-diabetic control; and were not sensitive to desipramine. These results suggest that lipid lowering medications used for the management of type 2 diabetes may exert their affects by altering membrane dynamics of CAC. These findings indicate that correcting membrane fluidity through modulation of sphingolipid metabolism, in diabetic CAC, could be explored as a novel therapeutic strategy for treating DR.

Transcriptomics of Retinal Pericytes shows Candidate Genes and Pathways pertaining to Alteration of Blood-Retinal Barrier in Diabetic Retinopathy

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[2]Cell Biology and Physiology, University of New Mexico, Albuquerque, NM
[3]NMVA Health Care System, Albuquerque, NM
[4]Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ

Abstract

Purpose: Selective pericyte loss, the histological hallmark of early diabetic retinopathy (DR), enhances the breakdown of the blood-retinal barrier (BRB) in diabetes. In this study, using RNA-sequencing, we define the transcriptomic profile of pericytes isolated from retinas of diabetic animals and provide insight into the role of pericytes in the regulation of vascular cell interaction and function in DR.

Methods: Retinal tissue from diabetic (3 months duration) and non-diabetic mice (n = 10 in each group) were digested with collagenase D to obtain single cell suspension. Purification of pericytes was done through fluorescent activated cell sorting (FACS) using pericyte specific fluorescent antibodies, PDGFRb-APC. For RNA sequencing and qPCR analysis, a cDNA library was generated using template switching oligo and the resulting libraries were sequenced using paired-end Illumina sequencing. Molecular functional pathways were analyzed using differentially expressed genes (DEGs) through gene set enrichment analysis (GSEA).

Results: Differential expression analysis revealed that 246 genes were significantly down- and 124 genes were up-regulated in diabetic pericytes. Downregulation of genes like Notch3, and AKT3 related to pro-survival of pericytes was observed in pericytes of diabetic animals. Also, upregulation of genes like PTEN, EXT2, GPC6 related in immune cell infiltration, apoptosis, ECM modulation and cell migration were observed in pericytes of diabetic animals.

Conclusions: Using next generation sequencing and bioinformatic approach, we have precisely defined the transcriptomic profile of "dysfunctional pericytes" in diabetes that leads to BRB breakdown, and the molecules thus identified can be targeted for development of novel biomarkers and therapies for DR.

Biography

Dr. Finny Monickaraj is a Research Assistant Professor at the University of New Mexico School of Medicine in Albuquerque, NM. Dr. Monickaraj primary focus of research is to study the implications of inflammation in diabetic macular edema with special reference to proinflammatory cell infiltration, cytokines, chemokines, cell junctions and pericyte-endothelial cell interaction and dynamics. They use in vitro and in vivo models, molecular biology tools and next generation sequencing technology. Their future goal is to find effective novel biomarkers and drug targets to screen and treat patients with diabetic retinopathy.

Transcriptomic Profiling of Retinal Endothelial Cells Reveals Novel Signature of Blood Retinal Barrier (BRB) Alteration in Diabetic Retinopathy

Sampath Kumar Rangasamy[1*], Finny Monickaraj[2, 3], Christopher Legendre[1], Ignazio Piras[1], Andrea P Cabrera[2], Paul McGuire[2, 3] and Arup Das[2,3,4]

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Abstract

Purpose: The molecular mechanisms of retinal endothelial cell dysfunction that contributes to the blood-retinal barrier (BRB) alteration in diabetic retinopathy (DR) are not clearly understood. In this study, we performed comprehensive transcriptomic profiling of retinal endothelial population isolated from diabetic animals to provide better understanding of the molecular underpinnings of vascular barrier alteration in diabetic retinopathy.

Methods: Retinas from diabetic (3 months duration) and non-diabetic mice (n = 10 in each group) were digested with collagenase D to obtain single cell suspension. Endothelial cells were obtained through fluorescent activated cell sorting (FACS) using CD31-FITC (ebioscience) staining. RNA sequencing of the samples was sequenced using paired-end Illumina sequencing (NovaSeq 6000 System). Gene expression signature in the retinal endothelial cells was identified using differentially expressed genes (DEGs) set through gene set enrichment analysis (GSEA).

Results: Gene expression analysis of isolated retinal endothelial cells from diabetic animals recognized distinct transcriptional subgroups of genes related to endothelial dysfunction. The transcriptional clusters enriched in the diabetic retinal endothelia cells were significantly associated with the activators of angiogenesis and inflammation. In addition, endothelial cells from diabetic animals showed down regulation of Trap1, Tsr2, Sav1 and Pkig genes, which are involved in the endothelial prosurvival and barrier maintenance.

Conclusion: Using genome wide transcriptomic profiling through state-of-art next generation sequencing (NGS) and bioinformatics technique, we have identified novel transcriptomic signature of retinal endothelial cells linked to vascular barrier function. Characterization of these new markers may lead to the development of novel and effective therapies for DR.

Biography

Dr. Sampath Kumar Rangasamy currently working as an Assistant Professor at Translational Genomic Research Institute (TGen), Phoenix, AZ. The primary focus of the lab is to investigate the cellular and molecular mechanisms underlying diabetic retinopathy and neurogenetics disorders. Advances in next generation sequencing (NGS) capabilities have provided new tools to discover novel drug targets in the treatment of rare and complex disease such as diabetic retinopathy. In collaboration with Dr. Arup Das from University of New Mexico, he utilizes NGS to address the molecular complexity of diabetic retinopathy and apply functional systems genomics approaches to identify novel therapies for diabetic retinopathy.

ER Stress Contributes to O-GlcNAcylation of VE-Cadherin Induced Endothelial Permeability

Raji Rajesh Lenin[*], Peter G Nagy, Kumar Abhiram Jha and Rajashekhar Gangaraju
University of Tennessee Health Science Center, Memphis, TN

Abstract

Excess O-glycosylation has been linked to the pathogenesis of several diseases including diabetes and the pathogenesis of DR. Previously we have shown that endothelial activation induced by inflammation and hyperglycemia results in the endoplasmic reticulum (ER) stress-mediated intercellular junction alterations accompanied by visual deficits in a tie2-TNF-α transgenic mouse model. In this study, we tested the hypothesis that increased ER stress under chronic inflammation and hyperglycemia via O-GlcNAcylation of VE-Cadherin likely contribute to endothelial permeability. Human retinal microvascular endothelial cells (HREC) were treated with 10 ng/mL TNF-α and 30 mmol/L glucose for 24 h with and without ER stress inhibitor (Tauroursodeoxycholic acid: TUDCA 10 μM). HREC exposed to TNF-α, and high glucose demonstrated increased ER stress as evidenced by elevated gene and protein expression of GRP78, p-IRE, sXBP1, CHOP, and translocation of GRP78 to the plasma membrane. Furthermore, a decreased trans-endothelial resistance in HRECs accompanied by decreased VE-Cadherin, ZO-1 & JAM-A expression. Glycosylation Antibody Array used for glycoproteome analysis demonstrated about 70 proteins were significantly upregulated (>1.5 fold vs. con) or downregulated (0.65 fold vs.

con). Interestingly O-glycosylation of VE-Cadherin leads to barrier loss accompanied by trans-endothelial migration of monocytes across HRECs. Finally, TUDCA partially mitigated all these effects. Our findings suggest an essential role for ER stress and O-GlcNAcylation in altering the endothelial barrier function and reveal a potential therapeutic target in the treatment of DR. Understanding the underlying ER stress mechanisms may shed new insights into novel therapeutic targets.

Biography

Dr. Raji Rajesh Lenin is a third-year postdoctoral fellow under the preceptorship of Dr. Rajashekhar Gangaraju at the University of Tennessee Health Science Center in Memphis, TN. Dr. Lenin's research program addresses the central hypothesis that Endoplasmic reticulum (ER) stress signaling in diabetic retinopathy (DR) regulates the junction protein alterations in endothelial cells, thus contributing to endothelial permeability. They use *in vitro* and *in vivo* models of endothelial activation to address our hypothesis. Their long-term goal is to develop novel therapeutic drugs that address retinal ER stress mechanisms for prevention, as well as treatment strategies related to diabetes complications.

Effect of High Glucose on Ocular Surface Tight Junction Proteins and Barrier Function

Saleh Alfuraih*, Ashley Barbarino, Kiumars Shamloo, and Ajay Sharma
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Abstract

Ocular surface epithelial cells express tight junction proteins which play a critical role in maintaining the ocular surface barrier functions. Multiple clinical studies have shown that ocular surface barrier function is significantly impaired in patients with diabetes mellitus. The present study was designed to investigate the effect of high glucose exposure on the ocular surface barrier function, and expression of occludins and claudins. Immortalized human corneal and conjunctival epithelial cells were exposed to high glucose (15 mM and 30 mM) for 24 hours and 72 hours. A separate group of cells was exposed to mannitol (15 mM and 30 mM) as a control for osmotic effects. The mRNA was isolated, and reverse transcribed to cDNA for quantification of claudin1,2,3 and occludin1,2,3 gene expression using real-time PCR. The protein lysates were used for quantification of claudins and occludins protein expression using western blotting. The transepithelial electrical resistance (TEER) was measured using chopstick method. Exposure of corneal and conjunctival epithelial cells to 15 mM and 30 mM glucose caused a significant decrease in TEER. High glucose exposure caused a significant increase in the gene expression of claudins and occludins. On the other hand, a decrease in protein expression of tight junction proteins was noted. In conclusion, our data demonstrates that high glucose can significantly impact ocular surface barrier function and expression of tight junction proteins.

Biography

Saleh Alfuraih is currently a Masters in Pharmaceutical Sciences student at Chapman University School of Pharmacy, Irvine California. Saleh is funded by a scholarship from Northern Border University, Saudi Arabia. Saleh obtained his Bachelors in Pharmaceutical Sciences in 2013 from Buraydah Private Colleges, AL Qassim, Saudi Arabia. Saleh has more than five years of experience working as a pharmacist and a teaching assistance at Northern Border University, Arar Saudi Arabia.

Quantitative Assessment of Mitochondrial Outer Membrane Permeability and Cytochrome c Release using Spectroscopic and Electrochemical Approaches

Kylee Voorhis*, Nathan Frantz, Yan Levitsky, Julia V. Busik and Denis A. Proshlyakov
Michigan State University, East Lansing, MI

Abstract

Diabetic retinopathy (DR) is a major complication associated with diabetes and is the leading cause of blindness in adults. Mitochondrial damage is recognized to play an important role in DR pathogenesis; however, the mechanistic details of diabetes-induced retinal mitochondrial damage are not well understood. Increase in Mitochondrial Outer Membrane (MOM) permeability, followed by the release of cytochrome *c,* is a well-known initiator of apoptotic cells death. Current techniques for analyzing mitochondrial activity rely on measurement of the final step in the oxidative phosphorylation (OXPHOS), the oxygen consumption rate (OCR) by complex IV (CmpIV). While it is possible to assess catalytic states of other mitochondrial complexes by measuring OCRs, the decrease in activity due to the loss of cytochrome *c* is hard to decipher. Cytochrome *c* cannot cross intact MOM but can interact with CmpIV when MOM is damaged. In this study

UV-Vis spectroscopy and electrochemical techniques were used to compare the activity of OXPHOS complexes in intact mitochondria vs. the mitochondria with permeabilized or removed MOM. Exogenous cytochrome *c* was used to assess the permeability of the MOM in two assays: by measuring the rate of oxidation of exogenous reduced cytochrome *c* by CmpIV; and electrochemically as a mediator of an electron transfer between an electrode and mitochondria. We demonstrate that the combined measurements of cytochrome *c* reduction and electrochemical activity of the mitochondria provide a specific and sensitive approach that can be used to access the intactness of MOM and cytochrome *c* release in retinal cells.

Complex-specific Electrochemistry to Access the Role of Mitochondrial Damage in Diabetic Retinopathy

Nathan Frantz*, Yan Levitsky, Gabrielle Brakoniecki, Julia Busik and Denis A. Proshlyakov
Michigan State University, East Lansing, MI

Abstract

Diabetic retinopathy (DR) is the leading cause of blindness among working age adults in the United States. Mitochondrial-dependent oxidative stress is a well-accepted feature underlying retinal microvascular dysfunction and is known to precede histopathological abnormalities in DR. Detailed studies of oxidative phosphorylation have not been undertaken due to technical limitations with currently available methodology. To fill this gap, this work describes the use of microfluidic electrochemistry and mediators to facilitate electron transfer between an electrode and the mitochondria to study complex-specific turnover of the mitochondrial electron transport chain (mETC). We show that electrochemical current correlates well with oxygen consumption in cell-free systems and mitochondria using N,N,N',N'-tetramethyl-p-phenylenediamine (TPMD) as an electrochemical mediator. When sodium azide was utilized to partially inhibit mitochondria, a 79 ± 19% or 81 ± 6% reduction in activity was observed when using oxygen consumption rate (OCR) or electrochemical current as measurements respectively. By supplying substrates to mitochondria, electrons can be withdrawn from complex III electrochemically, allowing for direct assessment of complex III independent of OCR measurements. These results demonstrate proof-of-concept *in situ* assessment of complex III and IV activities that are obtained independent from measuring OCR, directly from the complex of interest. By using electrochemical detection of complex-specific activity within mitochondria in microfluidics-based instruments, further studies can be carried out using small amounts of tissue such as the retina. This work will allow for the relationship between DR pathogenesis and mETC dysfunction to be more clearly resolved.

Role of X-box Binding Protein 1 in Regulation of Retinal Müller Glia Metabolism

Joshua J. Wang, Kristen Kelly and Sarah X. Zhang
Ross Eye Institute, University at Buffalo, State University of New York, Buffalo, NY

Abstract

Müller glia plays a critical role in maintaining retinal homeostasis through shuttling metabolic substrates to and from retinal neurons, releasing trophic factors, regulating neurotransmission, providing structural support, and regulating the extracellular environment. Understanding the regulation of Müller glia metabolism could provide helpful insights into the mechanisms of retinal dysfunction and degeneration in chronic sight-threatening diseases such as diabetic retinopathy. In this study, we investigate the role of X-box binding protein 1(XBP1), a stress-inducible transcription factor, in regulation of retinal Müller glial metabolism. Previous studies have shown that XBP1 is also involved in glucogenesis and glucose metabolism in the liver and pancreatic cells. We hypothesize that deletion of XBP1 in Müller glia could alter the glycolytic capacity of these cells and, in turn, affect retinal metabolism. To test this hypothesis, we generated XBP1 conditional knockout (cKO) mice that lack XBP1 specifically in Müller glia using the Cre/LoxP system. Using a Seahorse extracellular flux metabolic analyzer, we measured mitochondrial respiration and glycolysis in isolated Müller cells from XBP1 cKO mice. Our results demonstrate an increased rate of glycolysis in XBP1-deficient Müller cells. These changes are associated with elevated glucose uptake and upregulation of glucose transporters GLUT1 and GLUT2. Taken together, our data suggest that XBP1 plays an important role in regulation of glucose metabolism in retinal Müller glia, which, although consisting of a relatively small population of retinal cells, could consequently influence retinal metabolic profile and function.

Keynote Talks

Dysregulation of Wnt Signaling in Retinal Neovascularization

Jian-xing Ma, Qian Chen and Yusuke Takahashi
University of Oklahoma Health Sciences Center, Oklahoma City, OK

Abstract

The canonical Wnt signaling pathway plays a key role in the regulation of inflammation, angiogenesis and fibrosis. Our previous studies have shown that Wnt signaling is over-activated in the retinas of patients with diabetic retinopathy (DR) and age-related macular degeneration (AMD), and in the retinas of DR, oxygen-induced retinopathy (OIR) and laser-induced choroidal neovascularization (CNV) models. Blockade of Wnt signaling alleviated retinal inflammation vascular leakage and neovascularization in DR, OIR and CNV models. Further, we demonstrated that multiple endogenous angiogenic inhibitors such as pigment epithelium-derived factor (PEDF) and kallistatin confer anti-inflammatory and anti-angiogenic effects through binding to a Wnt co-receptor and blocking Wnt signaling. We also identified that very low-density lipoprotein receptor (VLDLR), originally known to mediate lipid transport and metabolism, is an endogenous inhibitor of Wnt signaling in the retina. Ablation of VLDLR resulted in retinal inflammation, vascular leakage and sub-retinal neovascularization. Unlike other tissues, the retina expresses exclusively VLDLR2, an isoform of VLDLR with a low affinity for lipoprotein binding, while a higher rate of extracellular domain shedding. We found that the soluble extracellular domain of VLDLR functions as an extracellular inhibitor of Wnt signaling via dimerizing with and destabilizing a Wnt co-receptor. The VLDLR extracellular domain shedding rate is decreased under diabetes stress, contributing to the aberrant activation of Wnt signaling in DR. These findings suggest that dysregulation of Wnt signaling plays a key pathogenic role in retinal inflammation, neovascularization and fibrosis and represents a potential therapeutic target for DR and AMD.

Biography

Dr. Jian-xing (Jay) Ma completed his training in clinical medicine from Jiangxi Medical College and then received his M.S. degree in Pharmacology in Chinese Academy of Medical Sciences. He received his Ph.D. degree of Biochemistry and Molecular Biology from Medical University of South Carolina. He joined the faculty at Storm Eye Institute. Later, he moved to University of Oklahoma Health Sciences Center as Laureate Professor. In 2007, he became Director of Center of Biological Research Excellence funded by NIH. He was appointed Chairman of Department of Physiology in 2010 and Director of Research of Harold Hamm Diabetes Center in 2015.

Super Resolution Microscopy and Mutational Analysis Reveal Novel Role of Occludin in VEGF-driven Neovascularization

David A. Antonetti*, Steven Lentz and Xuwen Liu
University of Michigan, Kellogg Eye Center, Ann Arbor, MI

Abstract

Vascular endothelial growth factor (VEGF) induction of endothelial permeability requires occludin phosphorylation on Ser490 and alanine point mutants preventing this phosphorylation reduce or prevent VEGF-driven permeability both in cell culture and in transgenic mouse models. However, previous studies also demonstrated that the same Ser490 phosphorylation of occludin is required for VEGF-driven endothelial cell proliferation and that occludin could be found in the centrosomes of dividing cells. These studies revealed that occludin contributes essential functions to both vascular permeability and proliferation in response to VEGF. The current research explored the hypothesis that VEGF-driven phosphorylation of occludin and centrosomal localization regulates cell proliferation. Transgenic expression was used to demonstrate that occludin S490A mutant acts in a dominant manner to inhibit neovascularization but not developmental angiogenesis. Localization to centrosomes requires both the C-terminal coiled-coil domain with Ser490 phosphorylation and the fourth transmembrane domain of occludin. A truncation mutant lacking all external loops, that mimics a naturally occurring splice variant, localized to centrosomes but an S490A point mutant inhibited VEGF-driven proliferation, strongly suggesting a specific centrosomal role for occludin in proliferation. Super-resolution microscopy was used to identify the location of occludin relative to centrioles and the gamma tubulin ring complex through mitosis. Occludin can be observed in multiple vesicles at the start of mitosis that condense to a single vesicle by telophase. These studies demonstrate novel mechanistic insight into VEGF control of neovascularization through occludin phosphorylation and regulation of mitosis.

Elucidating Molecular Mechanisms and Developing Therapeutics for Retinal Diseases Through International Collaborations

Takeshi Iwata
National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

Abstract

Common retinal diseases such as age-related macular degeneration (AMD) and normal tension glaucoma (NTG) or rare inherited retinal diseases (IRD) are multilaterally associated with genome sequence. Recent advancement in genome sequencing, genome editing and iPS cells technologies has accelerated discoveries of disease-causing genes and elucidation of disease onset mechanisms. We have focused on ARMS2/HTRA1 genes, the highest AMD associated locus, to study haplotype to animal model and develop potential therapy for AMD. We also focused on optineurin (OPTN) gene, which is responsible for inherited NTG. We discovered that interaction of mutant OPTN E50K with TANK Binding Kinase-1 (TBK1) lead to the aggregation in endoplasmic reticulum of neural cells differentiated from NTG patient iPS cells. The FDA approved TBK1 inhibitor "Amlexanox" suppressed retinal ganglion cells degeneration in Optn E50K knock-in mouse by disassociation of OPTN-TBK1 complex. In 2011, we established the Japan Eye Genetics Consortium (JEGC, http://jegc.org) to collect patient phenotype and genotype information. We have collected 2500 DNA samples from over 1300 families with IRD. Mutations in over 70% of the Japanese IRD pedigrees resulted with novel mutations or novel genes. These results led us to establish Asian Eye Genetics Consortium in 2014 and further expanded in 2018 as Global Eye Genetics Consortium (GEGC, http://gegc.org) to target Asia, Africa and South America. GEGC has now 200 scientists and ophthalmologists as members from more than 30 counties. GEGC is now jointly working with the International Council of Ophthalmology (ICO, http://icoph.org) to explore unique population around the globe with eye diseases and to collect phenotype and genotype information.

Biography

Dr. Iwata is director of the Molecular and Cellular Biology Division at the National Institute of Sensory Organs. He received his Ph.D. at Meijo University in Japan and spent his postdoctoral fellowship at National Eye Institute, NIH and at Bascom Palmer Eye Institute, University of Miami. In 2000, he returned to Japan to start his lab in Tokyo. He has focused on genes associated with various retinal diseases including age-related macular degeneration, normal tension glaucoma and inherited retinal diseases. He has worked on the molecular mechanisms of ARMS2/HTRA1, optineurin and discovered novel genes RP1L1, CTT2, C21orf2, LRRTM4 etc. for inherited retinal diseases. He is currently the head of Japan Eye Genetics Consortium (JEGC, http://jegc.org) and the president of Global Eye Genetics Consortium (GEGC, http://gegc.org).

Thioredoxin-Interacting Protein and Pathogenesis of Diabetic Retinopathy: Potentials of Gene Therapy Approaches

susan Singh Pukhrambam*, Thangal Yumnamcha, Fayi Yao and Takhellambam S Devi
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Abstract

Thioredoxin-interacting protein (TXNIP) is strongly induced by diabetes and high glucose in retinal cells. TXNIP binds to and inhibits the antioxidant and thiol reducing capacity of thioredoxin (Trx) and causes oxidative stress, inflammation, and premature cell death. TXNIP is present in all cellular compartments while Trx is present in the cytosol and nucleus (Trx1 isoform) and Trx2 in mitochondria. TXNIP mediates dysregulation of the mitophagy-lysosomal axis and NLRP3 inflammasome activation. TXNIP knockdown by siRNA in vivo or by shRNA in vitro normalizes various deleterious effects of high glucose (HG, 25 mM vs. 5 mM, LG) on neuroglia and microvascular abnormalities, which include RGC injury, Müller cell gliosis and capillary basement membrane thickening. In addition, HG induces TXNIP expression in RPE and photoreceptors causing mitochondrial dysfunction and cell death. Therefore, we propose that reducing TXNIP level will prevent DR progression. We published that TXNIP gene promoter exists in an opened and poised configuration that HG activates TXNIP transcription immediately and significantly. Hence, TXNIP promoter can drive the expression of a therapeutic gene that reduces oxidative stress and cell death. Our in vitro results indicate that nucleic acid constructs bearing the proximal TXNIP promoter linked with Trx1-cDNA is induced by HG in Müller cell and RPE by HG and normalizes autophagic/mitophagic flux. Furthermore, this TXNIP promoter Trx1 construct reduces TXNIP expression itself, providing dual benefits from a single gene construct. Further animal studies will be conducted to fully understand the benefit of the TXNIP promoter Trx1 gene therapy in DR.

Biography

Dr. Lalit Singh Pukhrambam (aka, Lalit P. Singh) is an Associate Professor in the Department of Ophthalmology, Visual and Anatomical Sciences (OVAS) at Wayne State University School of Medicine, Detroit, Michigan, USA. He obtained his Ph.D. degree in Biochemistry from the Indian Institute of Science (IISc), Bangalore, India. His research interests include understanding cellular and molecular mechanisms of the pathogenesis of diabetic retinopathy and targeted gene and drug combination therapies. Particularly, Dr. Pukhrambam is interested on the role of thioredoxin-interacting protein (TXNIP) in mitochondrial-lysosomal axis dysregulation and retinal cell death mechanisms in diabetes.

Protective Effects of Reduced Lysyl Oxidase Level in Retinas of Diabetic Mice: Implications for Diabetic Retinopathy

Dongjoon Kim and Sayon Roy
Boston University School of Medicine, Boston, MA

Abstract

Purpose: Retinal capillary basement membrane (BM) thickening is closely associated with the development of vascular lesions in diabetic retinopathy. Thickened capillary BM can compromise blood-retinal-barrier characteristics and contribute to retinal vascular permeability, a significant clinical manifestation of diabetic retinopathy. We have previously shown that high glucose increases the expression and activity of lysyl oxidase (LOX), a cross-linking enzyme, in retinal endothelial cells. Additionally, concomitant with overexpression of LOX, increased vascular permeability was observed in diabetic rat retinas. However, it is unknown whether decreasing LOX overexpression may have protective effects against development of retinal vascular lesions in diabetes.

Methods: To investigate whether reduced LOX level protects against diabetes-induced development of retinal vascular lesions characteristic of diabetic retinopathy, four groups of mice: wild type (WT) control mice, streptozotocin (STZ)-induced diabetic mice, LOX +/- mice, and STZ-induced diabetic LOX +/- mice were used for this study. Diabetes was maintained for 16 weeks; at the end of the study, retinas were assessed for LOX protein level by Western Blot (WB) analysis, and retinal capillary networks were isolated using retinal trypsin digestion and stained with hematoxylin and periodic acid Schiff to identify the number of acellular capillaries (AC) and pericyte loss (PL). In parallel, TUNEL assay was performed on retinal trypsin digests (RTDs) to detect cells undergoing apoptosis in the retinal capillary networks. Retinal vascular permeability was analyzed following FITC-dextran injection in retinal whole mounts.

Results: A significant increase in LOX expression was detected in diabetic retinas compared to those of WT control retinas. As expected, a significant decrease in LOX expression in diabetic LOX +/- retinas was observed compared to those of diabetic retinas. Retinas of diabetic mice exhibited a significantly increased number of AC and PL, increased number of TUNEL-positive cells as well as elevated vascular permeability in the retinas of diabetic mice compared to those of the WT control mice. Importantly, the number of AC and PL was significantly decreased, as was the number of TUNEL-positive cells and vascular permeability in the retinas of diabetic LOX +/- mice compared to those of diabetic mice.

Conclusion: Findings from this study suggest that reducing diabetes-induced LOX overexpression may have protective effects against the development of vascular lesions characteristic of diabetic retinopathy. Therefore, LOX overexpression may be a potential target in preventing retinal vascular cell loss and excess permeability associated with diabetic retinopathy.

Diabetes Impact on the Cornea

Alexander V. Ljubimov
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Abstract

As a systemic disease, diabetes mellitus alters all tissues and organs in the body including cornea. Diabetic corneal abnormalities include slow epithelial wound healing, edema, recurrent erosions, functional impairment of epithelial stem cells and neuropathy with loss of corneal sensitivity. They are seen in up to 70% of patients with both types of diabetes but remain underdiagnosed complications due to lesser severity than diabetic retinopathy. Diabetes affects corneal epithelium, nerves, stroma, tear film, and to a lesser extent, endothelium, and also conjunctiva. These abnormalities frequently manifest following trauma, vitrectomy for nonclearing hemorrhage or corneal refractive surgery. Recent studies identified a number of diabetic markers with altered expression in diabetic cornea, which was shown to have functional significance. These

markers include basement membrane and integrin proteins, various proteinases, advanced glycation end products (AGEs), specific growth and motility factors (including opioid, epidermal, and hepatocyte growth factors) as well as regulatory microRNA. Functional impairment of epithelial stem cells may be related to abnormal communication to the stromal niche via exosomes. Another mechanism of diabetic alterations affecting the cornea as well is related to epigenetic changes that modify gene expression. Currently, diabetic corneal disease is treated only symptomatically. However, new experimental therapies have emerged including topical naltrexone, insulin, gene therapy, nanomedicine, and cell transplantation. Such novel therapeutics may hold promise for improved management of diabetic corneal disease in the near future.

Biography

Dr. Alexander V. Ljubimov is Professor and Director of Eye Program at Cedars-Sinai Medical Center, and UCLA Professor of Medicine. He authored over 120 papers and is on Editorial boards of 14 journals. He is a member of Royal Society of Medicine (London) and Gold Fellow of ARVO. Dr. Ljubimov's interests include diabetic retinopathy, diabetic keratopathy, and cancer nanotechnology. He pioneered diabetic corneal gene therapy, uncovered diabetic epithelial stem cell dysfunction, and discovered angiogenic role of protein kinase CK2. He is developing cell-targeted polymeric nanodrugs to treat cancer and corneal diabetes. He is using iPSC technology to normalize diabetic cells.

The Effects of Vitamin D on Diabetic Corneal Epithelial Wound Healing, Nerve Density, and Keratocyte Function

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[1]Medical College of Georgia at Augusta University, Augusta, GA
[2]The Graduate School, Augusta University, Augusta, GA

Abstract

Our lab has found that vitamin D can be produced and activated locally within the cornea and that vitamin D affects many aspects of corneal function. While vitamin D deficiency is typically not associated with primary ophthalmic pathologies, we hypothesize that it exacerbates pre-existing pathologies, choosing diabetes as a model pre-existing pathology. Diabetic keratopathy occurs in approximately 70% of diabetics. We examined epithelial wound healing, nerve density, and keratocyte calcium waves in mouse and human cells. We found that vitamin D receptor knockout and vitamin D deficient diabetic mice have slower healing rates and decreased nerve densities than wildtype diabetic mice. These effects were not related to hypocalcemia.

We also examined intracellular calcium following a repairable plasma membrane tear known as a transient plasma membrane disruption (TPMD) for the first time in keratocytes or in any tissue. TPMDs were produced using a multiphoton microscope in mouse and human corneal cells, and mouse and human corneas. A single TPMD resulted in calcium waves in neighboring cells of all preparations. There was no difference between normal vs. diabetic mouse calcium waves.

We conclude that VDR KO and VDD significantly reduce corneal epithelial wound healing and nerve density in diabetic mice. These results support the hypothesis that low vitamin D exacerbates pre-existing ophthalmic conditions. Diabetic keratocyte TPMDs are not altered in diabetic corneas, we demonstrate that keratocytes have a robust TPMD-induced calcium wave response. TPMDs are likely routine events happening in otherwise quiescent keratocytes and are thus a normal aspect of corneal function.

Biography

Dr. Mitchell Watsky, PhD, FARVO, is Dean of The Graduate School at Augusta University, Associate Dean of Graduate Studies for the Medical College of Georgia, and Professor of Cellular Biology & Anatomy at the Medical College of Georgia. He has a long-standing research interest in corneal physiology and wound healing. His research focuses on the interaction of corneal wound healing and corneal cell junctions, ion channels, cell signaling, and the influence of vitamin D on the cornea, as well as development of an artificial cornea. His work encompasses both basic science and translational research.

A Peptide-Based Approach to Prevent Ganglion Cell Death in Retinal Diseases

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[1]Sue Anschutz-Rodgers Eye Center, University of Colorado Aurora, CO
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[3]North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX

Abstract

Retinal ganglion cell (RGC) loss is a frequent occurrence in diseases such as glaucoma, age-related macular degeneration and diabetic retinopathy. We tested a novel cell permeable peptide (Peptain-1) against RGC death in animal models. Intraperitoneally injected Peptain-1 was detected in the retina in mice, indicating that it is permeable to the blood retinal barrier. For the ischemia/reperfusion (I/R) model, the right eye of C57BL/6J mice was cannulated into the anterior chamber connected to an elevated saline reservoir resulting in elevation of the intraocular pressure (IOP) to 70 mmHg for 60 min, the needle was removed to induce I/R injury. Mice were injected with Peptain-1 i.p. at 50 µg twice daily. Following 14 days of injury, Brn3a staining was used to determine RGC survival. Peptain-1 treatment significantly inhibited RGC death and improved retinal functions following I/R injury. IOP was elevated in Brown Norway rats by injecting a hypertonic saline solution through the episcleral vein, while the contralateral eye served as control. The rats were i.p. injected with 10 µg of Peptain-1 three times per week for five weeks. Fluorogold labelled RGCs were counted in retinal flat mounts and automated axon count was performed following p-phenylenediamine staining. Peptain-1 significantly inhibited RGC death and reduced axonal loss. In addition, Peptain-1 significantly decreased hypoxia-induced RGC death and reduced hypoxia-mediated RGC loss in retinal explants. Together, our results demonstrate that Peptain-1 is permeable to the blood retinal barrier and offers neuroprotection through its axoprotective properties. Peptain-1 could be developed for neuroprotection in retinal diseases.

Biography

Dr. Ram Nagaraj received his PhD in Biochemistry from Mysore University and postdoctoral training at Case Western Reserve University (CWRU). He was appointed as an Assistant Professor of Ophthalmology at CWRU in 1994 and as a Professor in 2004. He held the Carl F. Asseff Endowed Professorship in Ophthalmology until 2014 when he moved to his current position of Professor of Ophthalmology and Pharmacy at University of Colorado in Denver. His research involves identification of signal transduction mechanisms during cell interaction with basement membrane, identification of chemical modifications in aging lens, mechanisms of capillary death in diabetic retinopathy and neuroprotection in retina.

Transcriptomic Profiling of Retinal Endothelial Cells Reveals Novel Signature of Blood Retinal Barrier (BRB) Alteration in Diabetic Retinopathy

Sampath Kumar Rangasamy[1], Finny Monickaraj[2, 3], Christopher Legendre[1], Ignazio Piras[1], Andrea P Cabrera[2], Paul McGuire[2, 3] and Arup Das[2,3,4]

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[4]NMVA Health Care System, Albuquerque, NM

Abstract

Purpose: The molecular mechanisms of retinal endothelial cell dysfunction that contributes to the blood-retinal barrier (BRB) alteration in diabetic retinopathy (DR) are not clearly understood. In this study, we performed comprehensive transcriptomic profiling of retinal endothelial population isolated from diabetic animals to provide better understanding of the molecular underpinnings of vascular barrier alteration in diabetic retinopathy.

Methods: Retinas from diabetic (3 months duration) and non-diabetic mice (n = 10 in each group) were digested with collagenase D to obtain single cell suspension. Endothelial cells were obtained through fluorescent activated cell sorting (FACS) using CD31-FITC (ebioscience) staining. RNA sequencing of the samples was sequenced using paired-end Illumina sequencing (NovaSeq 6000 System). Gene expression signature in the retinal endothelial cells was identified using differentially expressed genes (DEGs) set through gene set enrichment analysis (GSEA).

Results: Gene expression analysis of isolated retinal endothelial cells from diabetic animals recognized distinct transcriptional subgroups of genes related to endothelial dysfunction. The transcriptional clusters enriched in the diabetic retinal endothelia cells were significantly associated with the activators of angiogenesis and inflammation. In addition, endothelial cells from diabetic animals showed down regulation of Trap1, Tsr2, Sav1 and Pkig genes, which are involved in the endothelial prosurvival and barrier maintenance.

Conclusion: Using genome wide transcriptomic profiling through state-of-art next generation sequencing (NGS) and bioinformatics technique, we have identified novel transcriptomic signature of retinal endothelial cells linked to vascular barrier function. Characterization of these new markers may lead to the development of novel and effective therapies for DR.

Biography

Dr. Sampath Kumar Rangasamy currently working as an Assistant Professor at Translational Genomic Research Institute (TGen), Phoenix, AZ. The primary focus of the lab is to investigate the cellular and molecular mechanisms underlying diabetic retinopathy and neurogenetics disorders. Advances in next generation sequencing (NGS) capabilities have provided new tools to discover novel drug targets in the treatment of rare and complex disease such as diabetic retinopathy. In collaboration with Dr. Arup Das from University of New Mexico, he utilizes NGS to address the molecular complexity of diabetic retinopathy and apply functional systems genomics approaches to identify novel therapies for diabetic retinopathy.

The Role of the SIRT1/LXR Signaling Axis in Retinal Endothelial Cell Inflammation and Metabolism

Sandra S. Hammer*, Tim F. Dorweiler, Maria B. Grant and Julia V. Busik
Michigan State University, East Lansing, MI

Abstract

Purpose: Liver x receptors (LXRs) are hypothesized to serve as a link between lipid metabolism and inflammation by promoting cholesterol efflux as well as exhibiting anti-inflammatory properties. Fasting activated, NAD^+-dependent deacetylase SIRT1, is known to play a critical role in regulating inflammation and promoting LXR activation. The purpose of this study was to investigate the role of SIRT1/LXR activation in control of inflammation and subsequent metabolic changes in retinal endothelial cells.

Methods: Bovine retinal endothelial cells (BRECs) were treated TNFα (10ng/ml); LXR activator, DMHCA (1uM); or SIRT1 activator, SRT1720 (1uM). BRECs were serum starved to model calorie restriction. SIRT1, IL1β, ABCA1 and ABCG1 were analyzed by qRT-PCR. Sirt1 activity was measured via HDAC assay. Mice were fasted overnight, and retinal and liver samples were collected.

Results: Treatment with TNFα (10ng/ml) significantly increased cholesterol levels ($p=0.0233$, $n=6$), IL1β expression ($p=0.334$, $n=8$), IL6 ($p<0.001$, $n=3$) expression and resulted in decreased levels of HDAC activity ($p=0.0123$, $n=3$). Activation of LXR ($p=0.0178$, $n=8$) or SIRT1 ($p=0.0084$, $n=6$) prevented TNFα-induced inflammation. Serum starvation resulted in a significant increase in HDAC activity ($p=0.0005$, $n=6$) and SIRT1 ($p=0.0063$, $n=3$) expression levels. Serum starvation caused a decrease in LXR acetylated levels. Lastly, overnight fasting increased mouse retinal SIRT1 levels.

Conclusion: The results of this study demonstrate that serum starvation promotes activation of the SIRT1/LXR pathway metabolism in retinal endothelial cells.

Therefore, this study suggests that therapeutic fasting may serve to activate the SIRT1/LXR pathway providing the dual benefits of decreasing inflammation and promoting cholesterol metabolism in the retina.

MicroRNAs in Pathological Ocular Angiogenesis

Jing Chen
Harvard Medical School, Boston, MA

Abstract

Pathological ocular angiogenesis is a leading cause of blindness in various eye diseases. MicroRNAs (miRNAs), a group of small non-coding RNAs, regulate both physiological and pathological angiogenesis through post-transcriptional regulation of target gene expression. To study the function and therapeutic potential of miRNAs in retinopathy, we assessed the expression profile of miRNAs in a mouse model of oxygen-induced retinopathy (OIR) with pathological proliferation of neovessels and identified a group of differentially expressed miRNAs in OIR. Specifically, we found that miR-150, a miRNA enriched in normal quiescent retinal blood vessels, is suppressed in OIR, and functions as a vascular endothelium specific endogenous inhibitor of pathologic ocular neovascularization. On the other hand, miR-145, a miRNA substantially upregulated in OIR, alters endothelial cytoskeletal architecture dynamics and thereby promotes pathological ocular angiogenesis. These findings identify multiple miRNAs as potential targets to develop treatments for neovascular eye disorders and to prevent blindness in children and adults.

Biography

Dr. Jing Chen is an Associate Professor of Ophthalmology at Boston Children's Hospital (BCH) and Harvard Medical School (HMS). She received her PhD from Boston University and completed a postdoctoral research fellowship in Ophthalmology at BCH/HMS, and afterwards she was recruited as a faculty member at BCH/HMS. Her current research investigates the cellular and molecular mechanisms controlling pathological angiogenesis and blood-retinal barrier breakdown in experimental models of vascular eye diseases, with a focus on transcriptional and post-transcriptional regulatory mechanisms by miRNAs, nuclear receptors and transcription factors. These studies are funded by NIH/NEI and other foundations.

Leveraging Human Donor Eye Tissue to Elucidate Disease Mechanism in AMD

Margaret DeAngelis
University of Utah, Salt Lake City, UT

Abstract

Since the precise mechanisms leading to age-related macular degeneration (AMD) are unclear, we focused the present study on tissues specifically affected by the disease, the retina pigment epithelium/choroid (RPE) and the neural retina, in an effort to ascertain differential gene expression (DEG) between the clinical stages of AMD. We employed a standardized phenotyping protocol of both donor eyes recovered within 6 hours post mortem, the maximum interval to achieve minimal RNA half-life variability and enhanced RNA quality. In addition, motivated by previous studies showing evidence of allele-specific expression (ASE) in genes associated with risk of autism, Alzheimer disease and cancer, we interrogated the DNA of each donor at a genome wide level to determine whether an imbalance of expression between alleles may underlie phenotypic variation and hence pathophysiology of AMD.

Nuclear Receptors: New Therapeutic Targets for AMD?

Goldis Malek
Duke University, Albert Eye Research Institute, Durham, NC

Abstract

Nuclear receptors are transcription factors that control a myriad of biological and disease processes. A subset of these receptors is activated by lipids and have been shown to play a vital role in chronic diseases such as diabetes, atherosclerosis, coronary heart disease, inflammatory skin disorders and obesity. These diseases share common pathogenic mechanisms with retinal diseases including age-related macular degeneration (AMD) and diabetic retinopathy. Recently we completed a nuclear receptor atlas of human retinal pigment epithelial cells, cells vulnerable in all clinical sub-types of AMD. We identified several candidate receptors that may be important in disease initiation and progression. In this presentation, we will review the impact of these signaling pathways on retinal function, morphology and AMD-related pathogenic pathways such as lipid metabolism, inflammation, angiogenesis and fibrosis. Furthermore, we will discuss the therapeutic potential of targeting these signaling pathways on pathobiology associated with the different clinical sub-types of AMD.

Biography

Dr. Malek is an Associate Professor with Tenure in the Departments of Ophthalmology and Pathology at Duke University. She has a strong background in cell biology a broad understanding of retinal and retinal pigment epithelial cell function during aging, and the pathology and pathogenic mechanisms involved in age-related macular degeneration (AMD), the leading cause of vision loss in the elderly Her research focuses on identifying and defining the mechanisms of action as well as the therapeutic potential of targeting nuclear receptors, a large superfamily of transcription factors, in aging and AMD. Her work is funded by the National Eye Institute. Her laboratory research efforts have received a number of recognitions including an Alcon Research Institute Young Investigator Award, Edward & Della Thome Memorial Foundation AMD Research Award, Carl and Mildred Reeves Foundation Award, and Research to Prevent Blindness Sybil B. Harrington Scholar Award. She is active in the vision science community and serves as an Editorial Board Member for several journals and is a scientific grant review member for NIH. She has served on several committees within ARVO and ISER including the Annual Meeting Program Committee, Animals in Research Committee, Communications Committee and WEAVR.

A Novel Role of Autophagy in Regulating Fibrogenesis In Trabecular Meshwork Cells: Implications for Glaucoma

Paloma B. Liton*, April Nettesheim and Myoung Sup Shim
Duke University, Albert Eye Research Institute, Durham, NC

Abstract

The trabecular meshwork (TM) is a specialized ocular tissue responsible for maintaining appropriate levels of intraocular pressure. Dysfunction of this tissue leads to ocular hypertension and increases the risk for developing glaucoma. Previous work by our laboratory revealed dysregulated autophagy in aging and in glaucomatous TM cells. In order to gain more insight in the role of autophagy in the TM pathophysiology, we have conducted transcriptome and functional network analyses of TM primary cells with silenced expression of the autophagy genes Atg5 and Atg7. Atg5/7-deficient TM cells showed changes in transcript levels of several fibrotic genes, including TGFβ2, BAMBI, and SMA. Furthermore, genetic and pharmacological inhibition of autophagy was associated with a parallel reduction in TGFβ-induced fibrosis, caused by reduced activation of Smad2/3 signaling in autophagy-deficient cells mediated by BAMBI. Intriguingly, at the same time, TGFβ treatment led to Smad2/3-dependent dysregulation of autophagy in TM cells. Altogether, our data shows an intricate interplay between autophagy and TGFβ signaling, and a role of autophagy in regulating fibrogenesis via BAMBI and Smad2/3 signaling in TM cells. The implication of autophagy in the induction of the fibrotic response opens a novel area for investigation of therapeutic targets for amelioration of fibrosis in the TM.

Biography

Paloma B. Liton, PhD is an Associate Professor at the Departments of Ophthalmology & Pathology at Duke University. Dr. Liton received her PhD in Molecular Biology at the Universidad Autonoma of Madrid (Spain). After completing her postdoctoral studies at the laboratory of the late Dr. David Epstein, Dr. Liton joined the Department of Ophthalmology at Duke University as a faculty member in 2006. Dr. Liton's lab focuses on understanding the molecular mechanisms underlying glaucoma disease. More in particular, she is interested in investigating the role of autophagy in the physiology and pathophysiology of the outflow pathway, and neurodegeneration in glaucoma.

Genetics and Biomarkers of Primary Open-Angle Glaucoma: Novel Data

Marilita M. Moschos
Department of Ophthalmology, University of Athens, Greece

Abstract

Glaucoma is a heterogenous group of optic neuropathies leading to progressive degeneration of the optic nerve and vision loss.

Primary open-angle glaucoma (POAG) is one of the most prevalent causes of irreversible blindness and is considered as a group of ocular diseases characterized by progressive thinning of the neuroretinal rim of the optic nerve head and loss of the retinal nerve fiber layer.

The cause of glaucoma is still unclear today. Over the past decades, disease-causing genes have been identified and multigenic inheritance theory has-beens investigated for many cases of glaucoma. Also, mechanical and vascular causes have been proposed. The mechanical theory suggests that elevated intraocular pressure (IOP) is the most important risk factor for developing glaucomatous optic neuropathy (GON). So far, IOP reduction remains the only available intervention for treating glaucoma. The vascular theory considers glaucomatous inflammation is a causative mechanism of endothelial dysfunction and arterial stiffness in this population and specific anti-inflammatory treatment may be beneficial in POAG patients.

Biography

Dr. Marilita M. Moschos graduated first the Pharmacy School and then the Medical School of the University of Athens in 2000 with the highest grade amongst all students. Dr. Moschos is working as Associate Professor of the Department of Ophthalmology of Athens University where she has the clinical and scientific co-responsibility of the Laboratory of Electrophysiology of Vision and the department of Glaucoma. Marilita was honorary lecturer in the University of Cambridge in 2009 in the University Glaucoma Department of Addenbrookes Hospital in Cambridge and an official collaborator of Biomedical Research Foundation of the Academy of Athens. Marilita authored or co-authored over 190 scientific papers in pubmed reviewed journals and presented over 120 at international conferences, in many of them as invited speaker. Also co-authored 4 international ophthalmological books and two greek ones. Marilita M. Moschos is a member of many international ophthalmological societies and reviewer in several ophthalmological journals.

Plenary Session

Diagnosis & Management of Diabetic Tractional Detachments

Siva S.R. Iyer
University of Florida College of Medicine, Gainesville, FL

Abstract

This talk with focus on the diagnosis and management of diabetic tractional retinal detachments. The following are objectives of this talk:

- Demonstrate a basic understanding of the pathophysiology of diabetic tractional retinal detachments (TRDs).
- Demonstrate comprehension of the necessary history, examination, and diagnostic testing for patients with this condition.
- Understand the available vitreoretinal treatments for TRDs.
- Provide general counsel (at the level of a comprehensive ophthalmologist) for affected patients.

Biography

Dr. Siva S.R. Iyer, MD is an Assistant Professor in the Department of Ophthalmology at the University of Florida College of Medicine. He is a board-certified ophthalmologist and vitreoretinal surgeon with particular expertise in the surgical management of advanced proliferative diabetic retinopathy and complex retinal detachments. His research focuses on surgical advances in diabetic retinopathy, vitreous biology, and retinal imaging.

Eye to Brain: The Association of Eye Proteins to Cognition

Manju L. Subramanian
Boston University School of Medicine/Boston Medical Center, Boston, MA

Abstract

Objective: Beta amyloid (Aβ) and Tau in the cerebrospinal fluid are known biomarkers of Alzheimer's Disease (AD). An early diagnostic test is needed for AD, because therapeutic efforts are most successful in the pre-symptomatic phase. The purpose was to determine if the levels of AD biomarkers in the vitreous humor correlate with cognitive status in a non-AD cohort of patients undergoing vitrectomy for eye disease.

Methods: Undiluted vitreous fluid was prospectively collected during surgery from 80 eyes of 80 patients over the age of 18. All patients underwent a mini-mental status examination (MMSE) prior to surgery. Demographic information, education history, clinical data, and medical history were obtained. Undiluted vitreous samples were quantitatively measured for $A\beta_{40}$, $A\beta_{42}$, phosphorylated Tau (pTau) and total Tau (tTau). Linear regression was used to test the association between MMSE score and biomarker levels, adjusting for age, sex, education level, and eye disease.

Results: Nine patients (5 males, 4 females) exhibited mild to severe cognitive impairment. Higher vitreous levels of $A\beta_{40}$ (p=0.015), $A\beta_{42}$ (p=0.0066), and tTau (p=0.0085) were significantly associated with better MMSE scores while lower vitreous levels were associated with lower MMSE scores. Levels of pTau were not significantly associated with MMSE scores (p=0.40).

Conclusion: These results suggest patients with poorer cognitive function have significantly lower vitreous levels of $A\beta_{40}$ and $A\beta_{42}$. These findings are consistent with the abnormally low levels of CSF Aβ observed in AD patients compared with cognitively normal people. The study indicates that vitreous biomarkers may be an early diagnostic tool for AD.

Biography

Dr. Subramanian is a Vitreoretinal Specialist and has an academic clinical practice at Boston University Medical Center. She is the Vice-Chairman of Faculty Affairs at Boston University, where she works in a supportive role in the professional and career development and engagement of the faculty. Her research interests include the study of eye-based

protein biomarkers for Alzheimer's Disease. Additionally, she was Principal Investigator for the first head to head clinical trial comparing bevacizumab and ranibizumab in the treatment of AMD, and she is currently the PI for a comparative effectiveness study assessing the role of oral versus intravenous sedation in eye surgery.

Bench to Bedside: Development of the First Broad Spectrum Therapy for Inherited Retinal Disease.

Neena B. Haider

Harvard Medical School, Boston, MA

Abstract

Retinitis pigmentosa (RP) is a group of heterogenic inherited retinal diseases associated with over 150 gene mutations, affecting over 1.5 million individuals worldwide. RP varies in age of onset, severity, and rate of progression. Further, approximately 40% of RP patients cannot be genetically diagnosed, which confounds the ability to develop personalized therapies for each specific form of RP. The Haider lab has evaluated the role of the nuclear hormone receptor gene Nr2e3 as a genetic modifier and broad-spectrum therapy with the potential to attenuate early to intermediate stage of RP disease. Multiple mouse RP models were treated with AAV-Nr2e3 before disease onset and then evaluated at one and three months post treatment. Remarkably, treatment with Nr2e3 at early stage of disease progression showed improvement in all models. Molecular analysis demonstrated Nr2e3 delivery allows for a transcriptome reset of key genes and biological networks, improving retinal homeostasis in diseased tissue. Our results provide evidence that Nr2e3 can serve as a broad-spectrum therapy to treat multiple forms of RP.

Simultanagnosia as the Presenting Symptom in Neuro-ophthalmology

Grant Hopping[1*], Subhan Tabba[1], Bayan Al Othman[2], Ashwini Kini[2], and Andrew G. Lee[2,3,4,5,6,7,8]
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[4]Houston Methodist Hospital Research Institute, Houston Methodist Hospital, Houston, TX
[5]Weill Cornell Medicine, New York, NY
[6]University of Texas Medical Branch, Houston, TX
[7]University of Texas MD Anderson Cancer Center, Houston, TX
[8]The University of Iowa Hospitals and Clinics, Lowa City, IA

Abstract

A 57-year old male presented with difficulty seeing and identifying objects that were crowded and gait difficulty. Computed tomography (CT) showed ventriculomegaly due to aqueductal stenosis and a ventriculoperitoneal shunt (VPS) was placed. Humphrey visual field (HVF 24-2) showed left homonymous hemianopsia and a juxtaposed right paracentral homonymous hemianopic field defect was found. Ishihara color plates testing was 0/14 OU. Neuropsychological testing showed impairment across multiple domains, primarily of memory, attention, and visuospatial dysfunction which initially improved after VPS placement. 11 months after first presentation, the patient complained of sudden worsening of symptoms, and subsequently underwent VPS revision. Repeat CT of the head showed ventricular decompression, and there was reported subjective improvement of simultanagnosia symptoms.

A 30-year-old, previously healthy Caucasian male suffered a witnessed cardiac arrest and underwent subsequent cardiopulmonary resuscitation with cardioversion for ventricular fibrillation. MRI of the brain showed symmetric and restricted diffusion on diffusion weighted images (DWI) in the frontal, parietal, and occipital lobes with corresponding hypointensities on apparent diffusion coefficient (ADC) images consistent with cytotoxic edema secondary to ischemic infarction. The patient complained of bilateral visual loss described as difficulty interpreting complex information. On examination, visual acuity was 20/80 OU. HVF 24-2 showed juxtaposed, denser inferiorly homonymous hemianopsia with macular sparing on the right and macular splitting on the left. The patient tested positive for the SCN5A mutation (Brugada syndrome) and underwent automatic implantable cardioverter defibrillator (AICD) implantation. Family history was negative for cardiac disease, but genetic testing confirmed the SCN5A mutation in his daughter.

Simultagnosia, Prosopagnosia, and Other Visuospatial Symptoms as the Presenting Manifestation of Posterior Cortical Atrophy

Yi-Hsien Yeh[1*], Subhan Tabba,[2], Ashwini Kini,[3], Bayan Al Othman[3] and Andrew G. Lee[1,3,4,5,6,7,8]
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[7]The University of Texas MD Anderson Cancer Center, Houston, TX
[8]The University of Iowa Hospitals and Clinics, Iowa City, Iowa, IA

Abstract

Patient is a 55-year-old woman with painless, progressive, bilateral loss of peripheral vision for the past two years. Of note, patient also have multiple strange visual complaints including difficulty with depth perception, facial recognition, and synthesizing information from pictures with multiple small objects. In addition, patient also reported inability to perform her job as an elementary school teacher because she could not recognize her students and took the wrong class out during a fire drill. Past medical history includes rheumatoid arthritis and depression. Surgical history was noncontributory. Current medication includes diclofenac, duloxetine, etanercept, folate, methotrexate and sulfasalazine. Past ocular history includes age-related dry form of macular degeneration. Ocular examination does not explain the functional and visual field defect. Clock draw was abnormal. Patient was unable to identify a theme but can distinguish individual elements of the Boston Cookie Theft Test. Magnetic Resonance Imaging shows supratentorial ventriculomegaly and chronic vascular ischemic changes. PET brain metabolism showed hypometabolism in occipital cortex and visual association cortex. Discussion: PCA is an atypical variant of Alzheimer disease. However, typical Alzheimer disease is associated with deterioration in memory, language, and perception while patients with PCA tend to have well preserved memory and language until late during the disease and instead exhibit early loss of visuospatial cues. The insidious onset of vague symptoms, younger age of presentation than typical Alzheimer disease, and less memory/executive functional loss makes diagnosing PCA difficult. Thus, when seeing patients with multiple unexplained visual disturbances, clinicians must keep PCA in the differential.

Transcriptomics of Retinal Pericytes shows Candidate Genes and Pathways pertaining to Alteration of Blood-Retinal Barrier in Diabetic Retinopathy

Finny Monickaraj[1,3*], Sampath Kumar Rangasamy[4], Ignazio Piras[4], Andrea P Cabrera[1], Paul McGuire[1,2] and Arup Das[1,3]
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[2]Cell Biology and Physiology, University of New Mexico, Albuquerque, NM
[3]NMVA Health Care System, Albuquerque, NM
[4]Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ

Abstract

Purpose: Selective pericyte loss, the histological hallmark of early diabetic retinopathy (DR), enhances the breakdown of the blood-retinal barrier (BRB) in diabetes. In this study, using RNA-sequencing, we define the transcriptomic profile of pericytes isolated from retinas of diabetic animals and provide insight into the role of pericytes in the regulation of vascular cell interaction and function in DR.

Methods: Retinal tissue from diabetic (3 months duration) and non-diabetic mice (n = 10 in each group) were digested with collagenase D to obtain single cell suspension. Purification of pericytes was done through fluorescent activated cell sorting (FACS) using pericyte specific fluorescent antibodies, PDGFRb-APC. For RNA sequencing and qPCR analysis, a cDNA library was generated using template switching oligo and the resulting libraries were sequenced using paired-end Illumina sequencing. Molecular functional pathways were analyzed using differentially expressed genes (DEGs) through gene set enrichment analysis (GSEA).

Results: Differential expression analysis revealed that 246 genes were significantly down- and 124 genes were up-regulated in diabetic pericytes. Downregulation of genes like Notch3, and AKT3 related to pro-survival of pericytes was observed in pericytes of diabetic animals. Also, upregulation of genes like PTEN, EXT2, GPC6 related in immune cell infiltration, apoptosis, ECM modulation and cell migration were observed in pericytes of diabetic animals.

Conclusions: Using next generation sequencing and bioinformatic approach, we have precisely defined the transcriptomic profile of "dysfunctional pericytes" in diabetes that leads to BRB breakdown, and the molecules thus identified can be targeted for development of novel biomarkers and therapies for DR.

Biography

Dr. Finny Monickaraj is a Research Assistant Professor at the University of New Mexico School of Medicine in Albuquerque, NM. Dr. Monickaraj primary focus of research is to study the implications of inflammation in diabetic macular edema with special reference to proinflammatory cell infiltration, cytokines, chemokines, cell junctions and pericyte-endothelial cell interaction and dynamics. They use in vitro and in vivo models, molecular biology tools and next generation sequencing technology. Their future goal is to find effective novel biomarkers and drug targets to screen and treat patients with diabetic retinopathy.

Downregulation of Lysyl Oxidase in Diabetic Retinal Endothelial Cells Activates Ras Pro-Survival Pathway

Vyoma Shah* and Sayon Roy
Boston University School of Medicine, Boston, MA

Abstract

Purpose: Diabetic retinopathy is the leading cause of blindness in the working age population. Previous studies in our lab have suggested that downregulation of lysyl oxidase (LOX) protects rat retinal endothelial cells (RRECs) from high-glucose (HG) induced apoptosis. However, the mechanism by which this occurs is unclear. Ras is a GTPase that is involved in cell survival, cell differentiation, and cell growth pathways. In this project, we hypothesized that reducing HG induced LOX overexpression will activate the Ras pro-survival pathway.

Methods: To test whether LOX overexpression induces apoptosis via the Ras pathway, RRECs were grown in normal or HG medium (30 mM glucose). In parallel, cells grown in HG were transfected after 5 days in culture with LOX small interfering RNA (siRNA) or scrambled siRNA as control. Cells were harvested on day 7 and subjected to Ras immunoprecipitation followed by Western blot analysis. Differential dye staining was also performed to directly visualize effects on apoptosis.

Results: Cells grown in HG medium showed a significant reduction in Ras activity compared to that of cells grown in normal medium. Interestingly, Ras activity was significantly increased in cells grown in HG and transfected with LOX siRNA. As expected, the scrambled siRNA had no effect on the Ras activity, and showed similar results to the HG samples.

Conclusions: Our results are consistent with the hypothesis that HG reduces Ras activity, and thereby promotes apoptosis. Reducing the expression of LOX in HG cells may be beneficial for cell survival in a Ras-dependent manner.

A Clinical Neuro-ophthalmologic Spectrum: The Hiemann-Bielschowsky Phenomenon and Ocular Neuromyotonia

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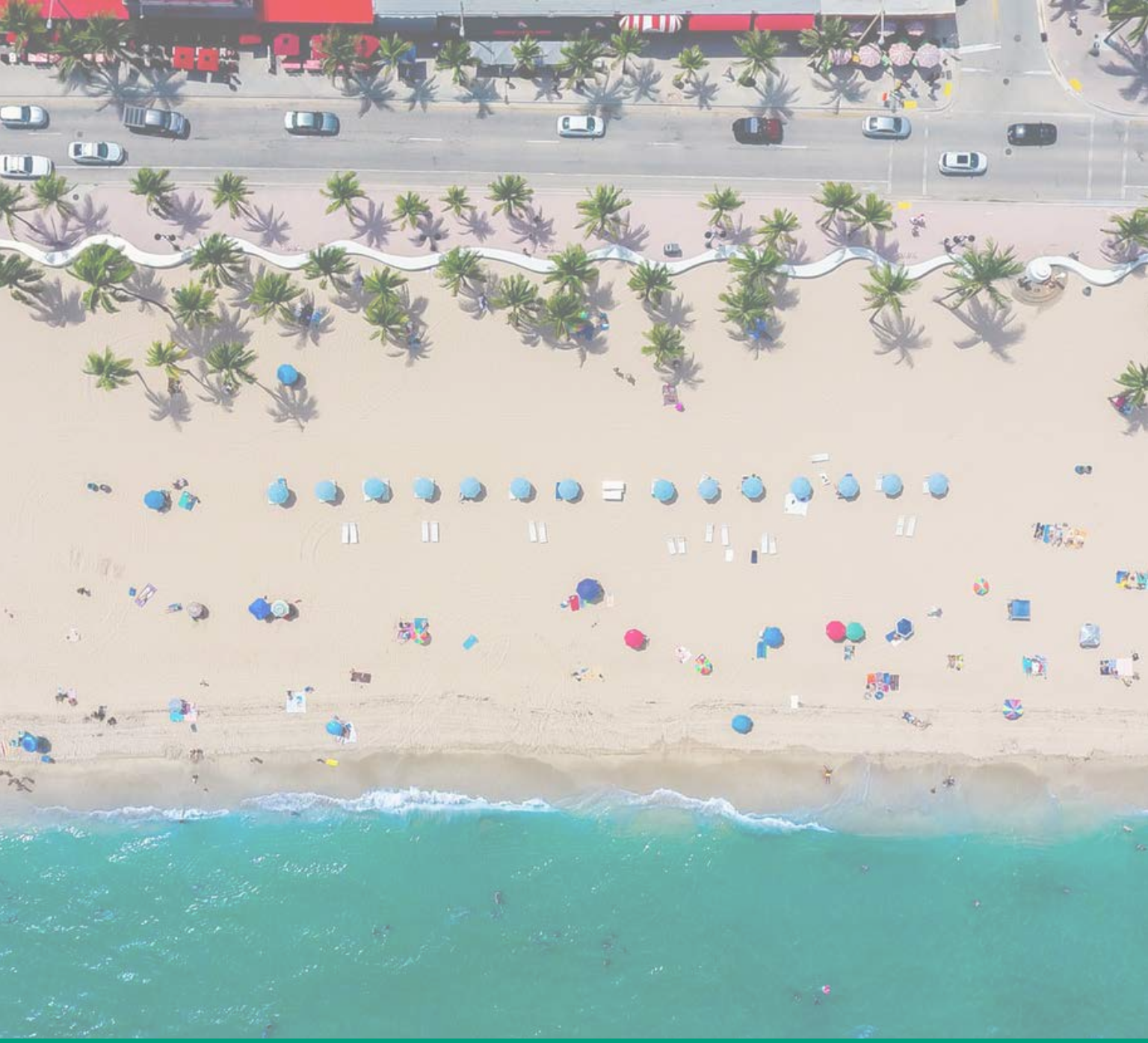
[7]The University of Iowa Hospitals and Clinics, Iowa City, Iowa, IA

Abstract

A 57-year-old white female, with no significant past ocular, medical or surgical history, presented with a recent diagnosis of benign meningioma compressing the optic nerve on the right eye (OD) resulting in best corrected 20/40 vision. Her vision in her left eye (OS) was 20/20, pupils were 4mm in the dark and 2 mm in the light with right relative afferent pupillary defect. Extraocular motility was within normal limits. External and anterior segment examination was normal in both eyes. Fundus exam in OD showed a diffuse optic atrophy and OS was normal. A Humphrey Visual Field showed mean deviation of -5.45 with inferior arcuate defect in the right eye while the left eye was normal. The patient elected to forego radiation therapy for surgical excision of the meningioma. After the excision surgery, the patient could no longer see from her right eye. The vision did not recover in the following eight months, but there was recurrence of the meningioma for which the patient underwent fractionated external beam radiation therapy. Serial imaging was stable for the following seven years. The patient subsequently presented to clinic with 25 degree vertically pendular oscillations that had a rate of 2 cycles per second in the right eye which resembled Hiemann-Bielschowsky oscillations; however, the oscillations were transient, lasting approximately 2 minutes, like ocular neuromyotonia.



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