c-fos promotes retinal angiogenesis in a mouse model of neovascular AMD
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Purpose: Abnormal proliferative angiogenesis outside the normal vascular boundaries of the retina in diseases like age-related macular degeneration (AMD) and proliferative diabetic retinopathy, can cause irreversible blindness. The photoreceptor layer is a privileged site, which is normally completely avascular. Any abnormal blood vessels extending into the photoreceptor layer from retinal vessels or choroid, may disrupt the retinal function as is seen in neovascular AMD. However, the mechanisms of maintaining photoreceptor avascularity are not well understood. We identified a novel mechanism by which c-fos controls angiogenesis in the photoreceptor layer mediated through retinal inflammation.

Methods: Very low-density lipoprotein receptor knockout (Vldlr−/−) mice which show pathologic retinal neovascularization in the normally avascular photoreceptor layer, are a valuable model to study the mechanisms of maintaining avascularity in the photoreceptor layer. Laser-capture microdissection, RNA Isolation, real-time PCR, western blot and immunohistochemistry were used to analyze gene expression and protein localization. Confocal imaging, fundus fluorescein angiography and HE staining were used to identify phenotypes. Adeno associated virus (AAV) was used to modulate gene expression.

Results: In Vldlr−/− retinas, c-fos mRNA and protein levels were markedly increased during retinal neovascularization development (n=6, P<0.001). Increased c-fos expression was mainly localized to the photoreceptor cell layer where Vldlr is highly expressed. The proinflammatory markers Il6 and Tnf were seen in the same layers as Vldlr and c-fos consistent with a role of Vldlr/c-fos in mediating angiogenic privilege in the retinal photoreceptor cell layer through suppression of proinflammatory cytokines. Suppression of c-fos with AAV expressing shc-fos in the subretinal space and photoreceptor layer prevented neovascularization (reduced 83% compared with control, n=8–10, p<0.001) and leakage. Pharmacologic treatment with a c-fos inhibitor, SR11302 suppressed retinal neovascularization in Vldlr−/− mice.

Conclusions: These data suggest that c-fos mediates neovascularization through control of inflammation in Vldlr−/− retinas indicating synergistic functions of c-FOS and VLDLR in maintaining an avascular photoreceptor layer. Together these findings confirm that inhibition of c-FOS protected against neovascularization.

Commercial Relationships: Ye Sun, None; zhiqiang Lin; Chi-Hsiu Liu, None; Yan Gong, None; Raffael Liegl, None; Thomas Fredrick, None; Steven Meng, None; Nicholas Saba, None; Samuel Burnim, None; Jing Chen, None; Lois E. Smith, None

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Role of PIGF in cellular immune response in CNV
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Purpose: Age-related macular degeneration (AMD) is a major reason for vision loss mainly due to choroidal neovascularization (CNV). A cellular immune response, driven by mononuclear phagocytes (MP), seems to play a pivotal role in the pathogenesis of AMD. In clinical practice it has been shown that a switch from anti-VEGF-A to a combined anti-VEGF-A anti Placenta growth factor (PIGF) treatment is beneficial for some patients. Since MP carry VEGFR1 (Flt-1), a receptor specific for both VEGF-A and PIGF, we hypothesize that a regulation of MP activity by PIGF contributes to the pathophysiology of CNV.

Methods: Laser-induced CNV was used in MacGreen (Csf1r-GFP) mice, creating 5 laser spots around the optic nerve (argon laser, 120mW, 50µm, 100ms). MP were visualized in vivo by SLO angiofluorescence (AF) and quantified ex vivo in whole-mounts using GFP and Iba-1. Differential expression of angiogenic factors and M1/M2 macrophage polarization markers were analyzed by qPCR. Protein expression of PIGF and VEGF-A was detected both in sagittal sections and whole-mounts of the retina.

One day after laser (D1), intravitreal injection of anti-VEGF-A+anti-PIGF (aflibercept) or anti-PIGF was performed and macrophage recruitment was analyzed. Unspecific IgG served as control.

Results: After CNV, PIGF mRNA expression increases at D1 and declines to normal levels at D4 whereas VEGF-A expression did not increase during the early phase (D1) and even decreased at D4. At D14, in sagittal sections or retina whole-mounts, we observed that up-regulation of VEGF-A expression in response to laser impact is limited to the scar area, while PIGF shows a more homogenous distribution. Additionally, comparable to PIGF expression, activated MP were also present in distant areas from the laser-spots. Among macrophages, we found an increase of M1 markers (CD68 and CD86) up to D4, whereas the M2 marker IL4R did not show significant changes.

Intravitreal Injection of aflibercept significantly decreased the amount of activated MP and minimized the size of the laser scar at D7 and D14 respectively, whereas anti PIGF could not suppress the macrophage recruitment effectively. Laser scars in PIGF injected eyes were similar to controls.

Conclusions: Thus we show first hints that the interplay of PIGF and microglia plays an important role in the initial phase of CNV after laser. However, anti-PIGF treatment alone is not sufficient to suppress MP recruitment.

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Support: Bayer Healthcare
Purpose: To investigate a correlation with Muller cells and sirtuin 1 (sirt1) in a development of choroidal neovascularization (CNV).

Methods: We developed laser induced choroidal neovascularization (CNV) in C57/BL6 mice. And then the eyes were applied intravitreal injection of sirt1 activator, Resveratrol (RSV; 0 and 30μM) at same day. After 1, 3, 5 and 7 days from the application, the eyes were sampled and used for immunohistochemistry of glial fibriillary acidic protein (GFAP: marker of activated Muller cell) (n=4 each).

Furthermore, we made whole mount samples of the eyes and immunostained with isoelectin B4 (IB4) at day 7 from the application (n=10). Finally, we analyzed the volume of CNV using confocal microscopy and Imaris software.

Results: The GFAP(+) cells were observed around the experimental CNV area. We revealed that intravitreal injection of RSV promoted the GFAP(+) cells migration into CNV region from day 5. However, the GFAP(+) cells migration was not observed until day 7 in control eyes. And the intravitreal injection of RSV significantly decreased the CNV volume by 33% comparing to control at day 7 (p<0.05).

Conclusions: Our results showed sirt1 activation could modulate Muller cells activation, which may have an inhibitory effect on CNV development. Muller cells may become a new therapeutic target for treatment of CNV.

Commercial Relationships: Takeshi Yoshida, None; Tomoka Ishida, None; Kosei Shinohara, None; Ikuo Morita, None; Kyoko Ohno-Matsui, None; Takeshi Yoshida, None; Katrin Brockhaus, None; Stephanie Hummel, None; Stefan Schlatt, None; Harutyun Melkonyan, None; Solon Thanos, None

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Presentation Time: 11:00 AM–12:45 PM

Hypoxia induce endothelial invasion by using 3-dimensional choroidal neovascularization model

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Purpose: Aged-related macular degeneration, the biggest cause of blindness in the world, were connected angiogenesis between retinal pigment epithelial (RPE) cells and choroid. However, there was no an appropriate 3D model, 2D in vitro model has a limitation reflecting the disease. We developed 3D retina-vascular mimetic system reflecting angiogenesis in AMD by stacking each cell layer.

Methods: Human RPE cells and peripheral micro-vascular endothelial (PMVE) cells were co-cultured in normoxia and hypoxia condition for 24 hours. The hypoxia conditions were induced chemically using CoCl2. We examined the invasion assay of endothelial cells under 3D co-culture system and the expression of VEGF and HIF-1α in retinal pigment epithelial cells.

Results: HI15C0001 and VEGF mRNA level were increased in RPE cells under the hypoxia condition. ELISA assay data showed that higher level of the secretion of VEGF than a normoxia a CoCl2 dose-dependent manner.

In the co-culture system combined RPE cells and PMVE cells, we observed PMVE cell invasion (35 ± 3.08 cells/unit area) into RPE cells in hypoxia condition. In the 50 ng/ml VEGF treated cells, also invaded toward RPE cells (13.3 ± 0.81 cells/unit area). Invaded cells of endothelial cells were detected by a CoCl2 concentration-dependent manner.

Conclusions: In this study, data showed hypoxia induced VEGF expression and then PMVE cell invasion in 3D co-culture model. We established new 3D model, an angiogenesis mimetic model related with AMD, would be able to detect invasion of endothelial cells under a disease condition. This model would be to employ for a candidate selection before a pre-clinical trial.

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The correlation between sirt1 and Muller cells in the development of CNV

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Purpose: To investigate a correlation with Muller cells and sirtuin 1 (sirt1) in a development of choroidal neovascularization (CNV).

Methods: We developed laser induced choroidal neovascularization (CNV) in C57/BL6 mice. And then the eyes were applied intravitreal injection of sirt1 activator, Resveratrol (RSV; 0 and 30μM) at same day. After 1, 3, 5 and 7 days from the application, the eyes were sampled and used for immunohistochemistry of glial fibriillary acidic protein (GFAP: marker of activated Muller cell) (n=4 each).

Furthermore, we made whole mount samples of the eyes and immunostained with isoelectin B4 (IB4) at day 7 from the application (n=10). Finally, we analyzed the volume of CNV using confocal microscopy and Imaris software.

Results: The GFAP(+) cells were observed around the experimental CNV area. We revealed that intravitreal injection of RSV promoted the GFAP(+) cells migration into CNV region from day 5. However, the GFAP(+) cells migration was not observed until day 7 in control eyes. And the intravitreal injection of RSV significantly decreased the CNV volume by 33% comparing to control at day 7 (p<0.05).

Conclusions: Our results showed sirt1 activation could modulate Muller cells activation, which may have an inhibitory effect on CNV development. Muller cells may become a new therapeutic target for treatment of CNV.

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**Involvement of long noncoding RNAs in ocular angiogenesis**

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**Purpose:** The role of long noncoding RNAs (IncRNAs) in ocular angiogenesis is largely unknown. The purpose of the project is to identify human endothelial cell (EC)-specific IncRNAs and test their involvement in ocular angiogenesis.

**Methods:** IncRNA profiling using ocular EC lines and non EC lines was performed to identify EC-specific IncRNAs. Quantitative RT-PCR and bioinformatics analysis were used to confirm the microarray results, and test their involvement in angiogenesis. Various angiogenesis assays, including Matrigel assays and EC-fibroblast co-culture assays were used to study the candidate IncRNAs in angiogenesis.

**Results:** We identified ~500 IncRNAs that are enriched more than 2 folds in primary endothelial cells (ECs) compared to non-EC cells. A list of the IncRNAs show a correlated expression profile with nearby coding mRNAs that are implicated in vascular development by functional enrichment analysis. For many of them, the EC-specific expression is more robust than their neighboring genes. To study the function of IncRNAs in ECs, we focused on one novel IncRNA, named as Inc-Angio1, which is enriched in highly vascularized human tissues, including lung, placenta and heart. Silencing of Inc-Angio1 represses EC proliferation and migration, and impairs vascular tube formation in both Matrigel and EC/fibroblast co-culture angiogenesis assays.

**Conclusions:** Our study established the IncRNA expression profile in ocular ECs and identified Inc-Angio1 as an EC-enriched IncRNA that is critical for angiogenesis.

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**Targeting the tight junction protein, zonula occludens-1, with the connexin 43 mimetic peptide, αCT1, reduces VEGF-dependent RPE pathophysiology**

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1. Medical University of South Carolina, Charleston, SC; 2. Virginia Tech Carilion Research Institute, Roanoke, VA; 3. First String Therapeutics, Mount Pleasant, SC; 4. Ralph H. Johnson VA Medical Center, Charleston, SC.

**Purpose:** A critical target tissue in age-related macular degeneration (AMD) is the retinal pigment epithelium (RPE), which together with Bruch's membrane forms the outer blood-retina barrier (BRB). RPE-barrier dysfunction in AMD might result from attenuation and disruption of intercellular tight junctions. Zonula occludens-1 (ZO-1) is a major structural protein of intercellular junctions. A connexin-based peptide mimetic, αCT1 (Alpha Connexin carboxy-Terminal 1), was developed which competitively inhibits ZO-1 interaction with its binding partners. We hypothesized that targeting ZO-1 signaling using αCT1 would maintain BRB integrity and reduce RPE pathophysiology by stabilizing gap- and/or tight-junctions.

**Methods:** Choroidal neovascularization (CNV) was induced using laser-photocoagulation; RPE-cell barrier loss was triggered by bright light exposure. Both models lead to VEGF-dependent cell damage. αCT1 was delivered via eyedrops. CNV size and fluid leakage were determined using optical coherence tomography. RPE flatmounts were stained for ZO-1 and occludin, and tiling patterns analyzed (CellProfiler). ARPE-19 monolayers were used to evaluate αCT1's mechanism of action in response to VEGF exposure.

**Results:** αCT1 treatment reduced CNV development and fluid leakage, and damage was correlated with disruption in cellular integrity of the surrounding RPE cells. Light-damage significantly disrupted RPE cell morphology, which was prevented by αCT1 pre-treatment. *In vitro* experiments using ARPE-19 cell monolayers suggest that αCT1 stabilizes intercellular tight junctions.

**Conclusions:** Taken together, stabilization of cellular junctions with αCT1 was effective in ameliorating RPE dysfunction in AMD models of photo-coagulation-induced CNV and bright-light exposure RPE-cell barrier loss. Future research will include additional investigation into the peptide's mechanism of action.

**Commercial Relationships:** Elisabeth Obert, Robert Gourdie, First String Therapeutics (P); Gautam Ghatnekar, First String Therapeutics (P); First String Therapeutics (P); Baerbel Rohrer, First String Therapeutics (P)

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Presentation Time: 11:00 AM–12:45 PM

**Effect of Isolation and Aging on VEGF Expression in ARPE-19 Cells**

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**Purpose:** VEGF is a well-established role in neovascularization of age-related macular degeneration (AMD). However, the causes of VEGF over-expression in AMD are not clearly understood yet. This work aims to test the effect of isolation and aging of human retinal pigment epithelial (RPE) cells on VEGF expression.

**Methods:** Isolation of RPE cells was achieved by containing cells on circular micro-patterns of different sizes (100, 200 and 300 μm in diameter) to replicate the atrophy of RPE cells during early to late stages of retinal degeneration in AMD. Micro-patterned substrates were made by using polydimethylsiloxane (PDMS) membranes with holes of desired sizes, manufactured in-house. The effect of aging was tested by growing micro-patterned and unpatterned RPE cultures over 1-day, 6-day and 21-day time periods. The amount of VEGF secreted to the media was measured 24 hours after patterning and/or after the end of each time period.

**Results:** Higher levels of VEGF expression per cell (specific VEGF expression) were observed for cells grown on smaller pattern sizes for all time periods. In addition, aging of RPE cells grown on smaller pattern sizes significantly elevated the levels of specific VEGF expression. Particularly, the specific VEGF expression for cells grown on 100 μm circles for 21 days was over 3-fold higher than that for cells grown on the same pattern size for 24 hours.

**Conclusions:** Our results indicate that increased isolation of RPE cells increases VEGF expression. Moreover, we showed that *in vitro* aging of RPE cells amplifies the effect of isolation. These results can help to better understand the mechanisms contributing to VEGF overexpression at different stages of AMD.
Inhibition of VEGF and IL-6 expression by beta2-Adrenergic receptor antagonism attenuates CNV through adrenoreceptor signaling on the expression of VEGF and interleukin-6 (IL-6) was investigated in primary mouse choroidal endothelial cells, norepinephrine and the beta2-specific adrenoreceptor antagonist (ICI 118,551). After 14 days, neovascularization was measured on choroidal-scleral flatmounts using intercellular adhesion molecule-2 immunofluorescence staining. The effect of beta2-adrenoreceptor signaling, and extended these results into primary human cells.

Methods: Mice were subjected to laser burns, inducing CNV, and were treated with a single intravitreal dose of beta2-adrenoreceptor antagonist (ICI 118,551). After 14 days, neovascularization was measured on choroidal-scleral flatmounts using intercellular adhesion molecule-2 immunofluorescence staining. The effect of beta2-adrenoreceptor signaling on the expression of VEGF and interleukin 6 (IL-6) was investigated in primary mouse choroidal endothelial cells, retinal pigment epithelial (RPE) cells, microglia cells, and human fetal RPE cells using specific beta-adrenoreceptor agonists and antagonists.

Results: Intravitreal injection of ICI 118,551 reduced CNV by 40% compared to vehicle control (N=30, p<0.01). In primary mouse microglia cells, norepinephrine and the beta2-specific adrenoreceptor agonist formoterol increased Vegf mRNA expression 4-fold, while propranolol and ICI 118,551 prevented norepinephrine-stimulated Vegf mRNA expression. In primary mouse microglia, RPE, and choroidal endothelial cells, norepinephrine elevated IL-6 mRNA expression 9-fold, 24-fold, and 12-fold, respectively. This effect was completely inhibited by both propranolol and ICI 118,551 in all cell types. In primary human fetal RPE cells, norepinephrine and formoterol increased Vegf mRNA expression 2-fold.

Conclusions: beta2-adrenergic receptor antagonism reduces laser-induced CNV in vivo and decreases Vegf and IL-6 mRNA expression in vitro. Anti-VEGF therapy for CNV is effective for most patients; however, some patients are resistant to therapy while other patients undergo a significant burden of repeated treatments and high cost. The beta2-adrenoreceptor is a potential therapeutic target for CNV lesions because of its combined anti-angiogenic and anti-inflammatory properties.

Commercial Relationships: None; SOESIAWATI DARJATMOKO, None; Lynda S. Wright, None; David M. Gamm, None; Michael S. Ip, None; Omeros (C); Thrombogenics (C), Boehringer Ingelheim (C); Nader Sheibani, None

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Inhibition of VEGF and IL-6 expression by beta2-Adrenergic receptor antagonism attenuates CNV


Purpose: The role of beta-adrenergic signaling in neovascular retinal and choroidal disease has recently emerged. We previously reported that propranolol inhibits choroidal neovascularization (CNV) in vivo and beta2-adrenoreceptor blockade reduces vascular endothelial growth factor (VEGF) expression in vitro. Here we tested the hypothesis that beta2-adrenergic receptors regulate CNV in vivo, investigated the role of beta2-adrenoreceptors in inflammatory signaling, and extended these results into primary human cells.

Methods: Mice were subjected to laser burns, inducing CNV, and were treated with a single intravitreal dose of beta2-adrenoreceptor antagonist (ICI 118,551). After 14 days, neovascularization was measured on choroidal-scleral flatmounts using intercellular adhesion molecule-2 immunofluorescence staining. The effect of beta2-adrenoreceptor signaling on the expression of VEGF and interleukin 6 (IL-6) was investigated in primary mouse choroidal endothelial cells, retinal pigment epithelial (RPE) cells, microglia cells, and human fetal RPE cells using specific beta-adrenoreceptor agonists and antagonists.

Results: Intravitreal injection of ICI 118,551 reduced CNV by 40% compared to vehicle control (N=30, p<0.01). In primary mouse microglia cells, norepinephrine and the beta2-specific adrenoreceptor agonist formoterol increased Vegf mRNA expression 4-fold, while propranolol and ICI 118,551 prevented norepinephrine-stimulated Vegf mRNA expression. In primary mouse microglia, RPE, and choroidal endothelial cells, norepinephrine elevated IL-6 mRNA expression 9-fold, 24-fold, and 12-fold, respectively. This effect was completely inhibited by both propranolol and ICI 118,551 in all cell types. In primary human fetal RPE cells, norepinephrine and formoterol increased Vegf mRNA expression 2-fold.

Conclusions: beta2-adrenergic receptor antagonism reduces laser-induced CNV in vivo and decreases Vegf and IL-6 mRNA expression in vitro. Anti-VEGF therapy for CNV is effective for most patients; however, some patients are resistant to therapy while other patients undergo a significant burden of repeated treatments and high cost. The beta2-adrenoreceptor is a potential therapeutic target for CNV lesions because of its combined anti-angiogenic and anti-inflammatory properties.

Commercial Relationships: Jeremy Lavine; Shoujian Wang, None; SOESIAWATI DARJATMOKO, None; Lynda S. Wright, None; David M. Gamm, None; Michael S. Ip, Omeros (C); Thrombogenics (C), Boehringer Ingelheim (C); Nader Sheibani, None

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Intraocular and systemic pharmacokinetics of brolucizumab (RTH258) in nonhuman primates

Erik L. Nimz, Clinton W. Van’t Land1, Jaime A. Yañez, James E. Chastain, Ocular PKD, Alcon Research, Ltd., Fort Worth, TX; ‘Merial Limited, North Brunswick, NJ.

Purpose: Brolucizumab, (RTH258, Alcon Research Ltd., a Novartis Company, Fort Worth, TX) is a 26.3 kDa humanized monoclonal single-chain variable domain antibody fragment consisting of 252 amino acids that inhibits human vascular endothelial growth factor A (VEGF-A). Brolucizumab is in clinical development for the treatment of neovascular age-related macular degeneration (nAMD). This study investigated the intraocular and systemic pharmacokinetics of brolucizumab after intravitreal or intravenous injection in nonhuman primates.

Methods: A total of 29 cynomolgus monkeys received brolucizumab as either a single bilateral intravitreal dose (1.0 or 6.0 mg/eye; n=9/group) or a single intravenous injection (2.06±0.05 mg/kg; n=11). Brolucizumab concentrations were determined in the vitreous, aqueous humor, retina, retinal pigment epithelium (RPE)/choroid, and serum following intravitreal injection, and in serum alone following intravenous injection using an enzyme-linked immunosorbent assay. Pharmacokinetic parameters were determined by compartmental and noncompartmental methods.

Results: After intravitreal injection, brolucizumab was cleared in parallel from all ocular compartments with a mean terminal half-life of 56.8±7.6 h. It distributed to the retina and RPE/choroid with maximal concentration in the central retina and RPE/choroid being 42% and 18% of that observed in the vitreous, respectively. Maximal serum concentrations were very low (<6000-fold less than those observed in vitreous) and also cleared in parallel with the ocular compartments with a serum half-life of 46.5 h. After intravenous administration, brolucizumab had a terminal half-life in serum of 5.6±1.5 h. The difference in serum half-life following intravitreal and intravenous administrations suggests that clearance from the ocular compartments is the rate-limiting step in the systemic clearance of brolucizumab when administered via intravitreal injection.

Conclusions: This study demonstrates that in nonhuman primates following intravitreal injection, brolucizumab is cleared in parallel from all ocular compartments with a mean terminal half-life of 56.8±7.6 h, while the systemic half-life following intravenous injection is 5.6±1.5 h. Brolucizumab readily penetrates through the retina to reach the RPE/choroid with minimal subsequent systemic exposure, supporting its clinical development for nAMD.

Commercial Relationships: Erik L. Nimz, Alcon Research, Ltd., Novartis; Clinton W. Van’t Land, Alcon Research, Ltd., Novartis;

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Choriocapillaris Loss of Endothelial Polarity and Fenestration May Be Contributing Factors in AMD

Christopher P. Ardeljan, Mones S. Abu-Asab, Maria M. Campos, National Eye Institute, National Institutes of Health, Bethesda, MD.

Purpose: This study aims to uncover and interpret the ultrastructural abnormalities in the choriocapillaris in AMD. Published ultrastructural reports of AMD are scarce and have not addressed abnormalities in the choriocapillaris and their pathological implications. This study will examine the choriocapillaris of the macular and peripheral regions in AMD patients using electron microscopy, since whether changes exist between the two regions has not been addressed before.

Methods: An ultrastructural examination was performed on the choriocapillaries of the macular and temporal regions of 7 AMD donors. All of the donors were over 80 years old. The eyes where fixed in formalin, and macular and temporal regions were dissected out separately. The specimens were prepared for transmission electron microscopy as follows: tissues were embedded in epoxy resin, sectioned at 90nm thickness, stained with uranyl acetate and lead citrate, and viewed with a JOEL JM-1010 TEM. Diameters of the choriocapillaries’ vessels adjacent to Bruch’s membrane were measured in the macular and temporal regions.

Results: Endothelial cells of the choriocapillaris showed loss of fenestration, and anterior hypertrophic or complete lack of cytoplasm. Nuclei of endothelial cells were mostly hypertrophic and majority were anteriorly displaced toward Bruch’s membrane side rather than the usual lateral or posterior positions (i.e., loss of normal polarity). Pericytes were degenerate and the basement membranes were scant. Cellular debris was found between the choriocapillaris and Bruch’s membrane. The average diameter of capillaries was within the normal range in both the macula and temporal regions (6.1-7.8 μm).

Conclusions: AMD choriocapillaris showed ultrastructural aberrations that have not been reported before; most significant are endothelial abnormalities such as the loss of polarity of the endothelial cells. Nuclei of normal endothelial cells are usually posteriorly located at the scleral side of the capillary and not anteriorly at the side of Bruch’s membrane. The confluence of a number of ultrastructural aberrations within the choriocapillaris and the accumulation of cellular debris below Bruch’s membrane indicate perfusion abnormalities in AMD. No significant differences in these ultrastructural aberrations or in vessel size were found between the macular and peripheral choriocapillaria.

Commercial Relationships: Christopher P. Ardeljan, None; Mones S. Abu-Asab, None; Maria M. Campos, None

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Role of Mineralocorticoid Receptor in laser-induced choroidal neovascularization

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Purpose: Neovascular age-related macular degeneration (AMD) accounts for 90% of severe vision loss in AMD patients. The current monthly anti-VEGF treatments are effective in reducing the leakage from choroidal neovascularisation (CNV), but do not induce a total regression of the CNV. Identification of additional therapies is required to reduce patient visits and injections, and to improve outcomes by targeting additional pathways. We aimed to investigate the contribution of mineralocorticoid receptor in laser-induced CNV.

Methods: CNV was induced by Argon laser (532 nm) in eyes of Long-Evans rats. Spironolactone (25mg/kg) was injected subcutaneously daily from day 0 to day 14 after laser. Rats received intravitreal injection of rat anti-VEGF (1.5μg/μl) at day 0 served as positive controls. Fluorescein angiography was performed at day 14 to estimate CNV leakage. The volume of CNV was quantified on choroidal flat mounts. Animals were also killed at day 3 after laser induction; eyes were used for IBA-1 immunolabelling of macrophages/microglia. Expression of MR, pro-inflammatory and pro-angiogenic factors was also assessed using quantitative PCR.

Results: Spironolactone significantly suppressed laser-induced CNV formation by 36% and reduced vascular leakage. MR expression was up-regulated in the neuroretina and retinal pigment epithelium (RPE)-choroidal at day 3. MR antagonist inhibited microglial/macrophage activation and migration towards the CNV lesions, and down-regulated MCP1, IL-1β, TNF-α and iNOS in both neuroretina and RPE-choroid.

Conclusions: Our results suggest that MR activation promotes early inflammation and thus contributes to CNV formation. MR antagonist could be an alternative or complementary treatment of anti-VEGF for CNV.

Commercial Relationships: Min Zhao, None; Xinxin Li, None; Manon Le Normand, None; Alejandro Arboleda, None; Francisco Halili, None; Rinath Levy, None; Francine Behar-Cohen, None

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The regulatory role of hepatoma-derived growth factor as an angiogenic factor in the eye

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Purpose: Hepatoma-derived growth factor (HDGF) is a mitogen previously reported to promote endothelial proliferation and neuronal survival. However, the role of HDGF and its expression in the eye have not been described. We hypothesized that HDGF is expressed in the eye and functions as a retinal angiogenic factor.

Methods: HDGF expression in the mouse retina was characterized using immunohistochemistry and Western blot analyses. We analyzed the proliferation (HDGF 100 ng/ml, n=7), migration (HDGF 500 ng/ml, n=3) and permeability (HDGF 100 ng/ml, n=3) of human retinal microvascular endothelial cells (HRMECs) to determine the angiogenic activity of HDGF in vitro. Corneal pocket assay independently verified HDGF (1 µg/µl) as an angiogenic factor in vivo (n=5). Evans blue dye was used to assess acute retinal leakage in vivo induced by intravitreal injection of HDGF (0.5 µg/eye, n=4).

To determine whether HDGF expression was altered in disease conditions, we generated animal models of laser-induced choroid neovascularization (LCNV) and oxygen-induced retinopathy (OIR). Western blot was used to quantify HDGF expression in the retina and vitreous fluid of diseased or healthy mice.

Results: Immunohistochemistry showed HDGF was predominantly expressed in the inner nuclear layer, outer nuclear layer and photoreceptor outer segments. Minor HDGF signals were found in retinal ganglion cells and photoreceptor inner segments. Few
signals were detected in the inner and outer plexiform layers. In vitro functional assays showed HDGF induced the proliferation (p<0.05), migration (p<0.05) and permeability (p<0.05) of HRMVECs. Corneal pocket assay indicated HDGF is sufficient to stimulate angiogenesis (p<0.01; 22.0+/−3.82 versus 2.0+/−1.4). Intravitreal injection of HDGF significantly promoted retinal vascular leakage (p<0.01; 5.11+/−1.09 versus 0.8+/−0.14). HDGF expression was not significantly altered in the retina or vitreous fluid of LCNV (n=3) or OIR mice (n=6-7).

Conclusions: These results suggest that HDGF without a classical signal peptide can be secreted through an unconventional pathway and regulate angiogenesis in the retinal vasculature. The results of corneal pocket assay suggest that the functional activity of HDGF is not limited to retinal vasculature. The ability of HDGF to induce retinal permeability in vivo implicates that this factor may play a role in retinal vascular diseases.

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