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Sunday, May 07, 2017 3:15 PM–5:00 PM
Ballroom 1 Paper Session
Program #/Board # Range: 802–808
Organizing Section: Retinal Cell Biology

Program Number: 802
Presentation Time: 3:15 PM–3:30 PM
Retinoic acid signaling promotes proliferation and neuronal differentiation from Muller glia-derived progenitor cells in the avian retina
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Purpose: Understanding the signaling pathways that regulate the transition of Muller glia into neurogenic Muller glia-derived progenitor cells (MGPCs) is imperative to promoting retinal regeneration. This study investigates how Retinoic acid (RA) signaling impacts the proliferative and neurogenic potential of MGPCs in the chick retina *in vivo*.
Methods: Experiments were performed on posthatch chicks (7-21 days old). Compounds were delivered via intraocular injections. An analog of retinoic acid (TTNPB) and a RA-receptor inhibitor (BMS 493) were used to modulate RA-signaling. Retinas were damaged by an injection of N-Methyl-D-aspartate (NMDA). FGF2 was used to stimulate MGPC formation in undamaged retinas. EdU was applied to label proliferating cells. Following experimental paradigms, retinas were processed for immunoflourescence and digital photomicroscopy. Significance of difference was determined using a paired, two-tailed t-test.
Results: We found that stimulating RA-signaling in NMDA damaged retinas significantly increased Muller glia proliferation (control 16±5.5 vs. TTNPB treated 52±7.4, n=5, p<0.01). Conversely, inhibition of the RA-receptor decreased Muller glia proliferation in damaged retinas (control 87±55.9 vs. BMS 493 treated - 20±13.7, n=6, p<.05). We found that Pax6 expression was increased in Muller glia by RA-signaling and decreased by inhibition of the RA-receptor. FGF2 is the only known factor that can stimulate MGPC formation in the uninjured chick retina. We found that TTNPB combined with FGF2 increased MGPC proliferation (FGF2 14.4±6.3 vs. FGF2+TTNPB-33.2±6.8, n=5, p<.01). Furthermore, RA-signaling promoted the expression of cFOS and Pax6 in Muller glia in FGF2 treated retinas. Inhibition of RA-receptor in FGF2-treated retinas suppressed the proliferation of MGPCs (FGF2 68.5±22.6 vs. FGF2+BMS493 27.4±19.9, n=7,p<.003). Lastly, we found that stimulating RA-signaling in damaged retinas significantly increased neurogenesis from MGPCs (+37.9%±25.9, n=5, p=0.01).
Conclusions: Our data indicate that RA-signaling plays a significant role in promoting the proliferation and neurogenic capacity of MGPCs. Furthermore, we find that RA-signaling coordinates with MAPK-signaling to stimulate MGPC-formation in the absence of damage. This work provides novel data suggesting that RA-signaling is a promising target to promote retinal regeneration.
Commercial Relationships: Levi Todd, None; **Lilianna Suarez**, None; **Colin Quinn**, None; **Andrew J. Fischer**, None
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Program Number: 803
Presentation Time: 3:30 PM–3:45 PM
The effect of Serine/Glycine metabolism on Müller cells mitochondrial function, redox homeostasis and cells' survival under oxidative stress
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Purpose: There has been a recent surge of interest in the role of serine/glycine metabolism in cancer, but its role in the pathogenesis of retinal diseases remains unclear. Serine/glycine metabolism plays an important role in cellular anti-oxidative capacity through regulating Glutathione (GSH) synthesis, a key factor that maintains cell redox homeostasis. We aim to explore the effects of serine/glycine pathway on the function of Müller cell mitochondria and the redox environment that maintains cell survival during oxidative stress.
Methods: We induced oxidative stress in MIO-M1 and hPMCs (human primary Müller cells) by exposing them to low concentrations of hydrogen peroxide (H_2O_2). We used CBR-5884, a specific inhibitor of phosphoglycerate dehydrogenase (PHGDH), a rate limiting enzyme in serine/glycine metabolism, to inhibit serine/glycine metabolism. Alamarblue assay and LDH assay were performed to evaluate cells survival. Seahorse experiments were conducted to determine the mitochondrial function of MIO-M1 and hPMCs treated with CBR-5884 and/or supplementation with exogenous serine and glycine. Reactive oxygen species (ROS), GSH levels and apoptosis (Annexin V/Propidium Iodide assay) were detected by flow cytometry on MIO and hPMCs. Hoechst, PI and Calcium AM immune-fluorescence staining were used to identify viable, apoptotic and dead cells.
Results: There was a significant reduction of MIO and hPMCs cell viability as well as an increased cytotoxicity after the serine/glycine synthesis pathway was inhibited under conditions of oxidative stress. Cell viability was improved by exogenous application of serine/glycine. MIO and hPMCs exhibited decreased mitochondrial ATP production in response to serine/glycine pathway inhibition. GSH levels were reduced, and ROS levels were significantly increased after inhibiting serine/glycine metabolism under oxidative stress compared with untreated and controls that were rescued with exogenous application of serine/glycine. Meanwhile, rates of apoptosis and cell death significantly increased in the MIO and hPMCs when they were exposed to H_2O_2 and the Serine/Glycine pathway was inhibited.
Conclusions: Serine/glycine metabolism appears to be important for Müller cells survival under oxidative stress, probably through maintaining mitochondrial function and generating GSH to counteract ROS.
Commercial Relationships: Ting Zhang; **Ling Zhu**, None; **Weiyong Shen**, None; **Mark C. Gillies**, None

Program Number: 804
Presentation Time: 3:45 PM–4:00 PM
The insulin receptor substrate-1 (IRS-1) regulates Kir4.1 expression in retinal Müller cells
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Purpose: The Müller cells, principal glia of the retina, maintain water homeostasis and K^+ concentration via the specialized inwardly rectifying K^+ (Kir) channels. Diabetes leads to a decrease in Kir4.1 expression. Our studies suggest that the diurnal rhythm of Kir4.1 is

dampened in diabetes. The insulin receptor substrate-1 (IRS-1) is a critical regulator of insulin signaling in Müller cells, however, the potential role of signaling mediated by IRS-1 in regulating the Kir4.1 expression remains unknown.
Methods: To determine the importance of insulin in Kir4.1 regulation, the rat Müller (rMC-1) cells were treated with insulin and Kir4.1 expression was examined by western blot. To evaluate the role of IRS-1 in Kir4.1 regulation, the rMC-1 cells were treated with an IRS-1 inhibitor Sectin-H3. In parallel, the type 2 diabetic mice (db/db) mice maintained under a regular light-dark condition or a constant dark (circadian dysrhythmia) were euthanized at different time intervals and the retinal expression of IRS-1 was determined using western blot.
Results: The treatment of rMC-1 cells with insulin resulted in an increase in Kir4.1 expression. The pharmacological inhibition of IRS-1 with Secin-H3 resulted in a 1.5-fold decrease in a *Kcnj10* expression (gene for Kir4.1). The IRS-1 exhibited a diurnal rhythm with a peak increase at 4 am and at the lowest levels at 4 pm. The circadian dysrhythmia led to a loss of diurnal rhythm Kir4.1 along with a change in the characteristic pattern of IRS-1 rhythm.
Conclusions: Our studies suggest that IRS-1 is a critical regulator of Kir4.1 expression in retinal Müller cells. In diabetes, the dysfunctional signaling mediated by IRS-1 is detrimental to Kir4.1 expression.
Commercial Relationships: Qianyi Luo, None; **Kayla Thompson**, None; **Ashay D. Bhatwadekar**, None

Program Number: 805
Presentation Time: 4:00 PM–4:15 PM
microRNAs are required for maintenance of Müller glia homeostasis and retinal architecture: The role of Brevican
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Purpose: microRNAs (miRNAs) are negative regulators of gene expression and known to play a role in retinal development and regeneration. However, there is not much known about the role of miRNAs in Müller glia function and whether or not they are required for glial state *per se*.
Methods: We created a conditional knock out (CKO) of Dicer1 (Dicer-CKO_{MG}) specific for MG (Rlbp-CreER: $Dicer^{fl/fl}$: $Stop^{fl/fl}$ tdTomato; wild type: Rlbp-CreER: $Stop^{fl/fl}$ tdTomato). To activate the CreER in the Dicer-CKO_{MG} mice, we injected tamoxifen intraperitoneally at postnatal days (P) 11-14. Retinal cross sections of eyes were used to analyze the Dicer-CKO_{MG} phenotype one or six months after Dicer deletion by means of immunofluorescent labeling and confocal microscopy. For RNA analysis, Müller glia from wild type and Dicer-CKO_{MG} mice were purified by FACS and analyzed using the NanoStrings nCounter® System and RNA-Seq. In addition, we used *in vitro* (Müller glia cell culture) and *ex vivo* (retinal explants) cultures to confirm observations made *in vivo* and to identity a possible underlying mechanism.
Results: In the Dicer-CKO_{MG}, we found a reduction in miRNA levels in Müller glia by an average of 70% and a temporary increase in the number of Müller glia due to increased proliferation. However, after six months, we observed a significant decline in Müller glia number. Moreover, we saw Müller glial migration from their normal position in the inner nuclear layer, ultimately disrupting normal retinal architecture within six months. RNA-Seq analysis and *in vitro* experiments suggest that an increase in the expression of Brevican in the Dicer-CKO_{MG} partly explains the observed changes in their migratory behavior and that miR-9 is a potential regulator of Brevican. Brevican is a chondroitin sulfate proteoglycan, usually absent in MG, and over-expression of Brevican causes Müller glia to migrate and form aggregations *in vitro* and *ex vivo*. These aggregations can be prevented/resolved in the glia from Dicer-CKO_{MG} mice by miR-9 mimics or Brevican inhibitory antibodies.
Conclusions: Our results show that miRNAs and downstream targets are important for the maintenance of the stable Müller glia state and these results could have implications for the retinal remodeling that occurs in long-term degenerative diseases.
Commercial Relationships: Stefanie G. Wohl, None; **Thomas A. Reh**, None
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Program Number: 806
Presentation Time: 4:15 PM–4:30 PM
Microglia homeostasis in the adult mouse retina: restoration of microglial distribution, morphology, and function following acute depletion
Yikui Zhang, Lian Zhao, Xu Wang, Wenxin Ma, Adam Lazere, Haohua Qian, Robert N. Fariss, Jun Zhang, Mones S. Abu-Asab, Wai T. Wong. National Eye Institute, Bethesda, MD.
Purpose: Microglia are long-lived resident innate immune cells that are found as ramified cells distributed across the retina in a regularly tiled pattern. The factors that maintain the organization of retinal microglia are not understood. We investigate how microglia undergo repopulation in the retina following acute depletion and examine the origin and function of "new" repopulating microglia.
Methods: Two models for microglial depletion were used: (1) a genetic model involving tamoxifen-induced microglial-specific expression of diphtheria toxin (DTA) subunit alpha, and (2) a pharmacological model involving PLX5622, a small molecule antagonist of CSF1R whose signaling is required for microglial viability. Microglia repopulation following depletion was monitored with *in vivo* retinal imaging, fate mapping technology, and Iba1 immunohistochemistry. Characterization of repopulating microglia and their effects in the retina were performed using *ex vivo* live cell imaging, electroretinography (ERG), electron microscopy (EM) and immunohistochemistry.
Results: In both depletion models, Iba1+ microglia repopulated the retina following short-term depletion of endogenous retinal microglia. Initial repopulating cells emerged primarily in the peripapillary region in the inner plexiform layer (IPL) of the retina, subsequently spreading over the entire retina. Early repopulating microglia are small and simple, but increase progressively in ramification to fully recapitulate original microglial morphology. Time-lapse *in vivo* fundus imaging demonstrated prominent cell migration and division in repopulating microglia. Fate mapping of endogenous microglia indicated that repopulating microglia originated mostly from the few residual microglia remaining after depletion. Repleted microglia demonstrated similar functions as endogenous microglia in terms of: (1) surveillance process motility, (2) response to light injury, and (3) maintenance of retinal synapse structure and function as evaluated by ERG and EM analysis.
Conclusions: Homeostasis exists in the retina to facilitate a robust repopulation of microglia following depletion that fully and precisely restores the original numbers, distribution, morphology and functions of endogenous microglia via cellular division, migration of residual microglia.
Commercial Relationships: Yikui Zhang, None; **Lian Zhao**, None; **Xu Wang**, None; **Wenxin Ma**, None; **Adam Lazere**, None;

Haohua Qian, None; **Robert N. Fariss**, None; **Jun Zhang**, None; **Mones S. Abu-Asab**, None; **Wai T. Wong**, None

Program Number: 807
Presentation Time: 4:30 PM–4:45 PM
Effect of Surfactant Protein A on Retinal Cytokines and Microglia in Systemic Inflammation
Faizah Bhatti[1, 2], Netsanet Kassa[1], Johannes Kung[1], Phillip S. Coburn[2]. [1]Pediatrics, OUHSC, Oklahoma City, OK; [2]Ophthalmology, OUHSC, Oklahoma City, OK.
Purpose: Activation of retinal microglia is associated with retinal neovascularization (NV) in the mouse model of oxygen induced retinopathy (OIR) and in the presence of systemic inflammation (SI). SP-A expression is also known to be up regulated in the retinas of wild type (WT) mice after intravitreal injections of toll like receptor (TLR) 2 and 4 ligands. SP-A expression is also increased in NV in WT mice. We hypothesize that retinal SP-A is upregulated in SI and that it also modulates retinal cytokine and microglial expression.
Methods: SI was induced in WT mice by intraperitoneal (IP) injection of lipopolysaccharide (LPS) at Day 4. SP-A protein was measured in the retina after 48 and 72 hrs. mRNA of IL-1β, TLR-4, TNFα and a microglial specific marker Iba1 were quantified by real time PCR on P6 and P10 in SP-A$^{-/-}$ mice compared to WT. Total number of microglia were compared between WT and SPA$^{-/-}$ mice on flat mounts (FM) stained with antibody for Iba1.
Results: SP-A protein was significantly increased in WT mice 48 hours after LPS injection (899 ± 93.53 ρg /mg retinal protein) vs controls (374 ± 62.93 ρg /mg retinal protein) but there was no difference at 72 hrs. At P6, VEGF, cytokines and Iba1 were increased in WT vs SP-A$^{-/-}$. However, at P10, there was an increase in TLR-4, TNFα and Iba1 in SP-A$^{-/-}$ vs WT. VEGF remained high in WT mice at P10 but there was no difference in IL-1β. At P6, the number of total microglia in central and peripheral retinal zones were significantly higher in WT vs SPA$^{-/-}$ mice. At P10, the number of microglia in the central zone of retina were increased in SP-A$^{-/-}$ vs WT mice, but there was no difference in the periphery.
Conclusions: SP-A is up regulated in the neonatal mouse retina in SI. Absence of SP-A is associated with an initial decrease in retinal cytokines 2 days after induction of SI but the cytokines were higher at 6 days. These results suggest that SP-A may play differing roles during the acute and late phase of SI. Furthermore, absence of SP-A was associated with a decrease in microglia on retinal flatmounts 48 hour in SI, suggesting that SP-A may play a role in chemotaxis of microglia. The decreased retinal VEGF expression in SP-A deficient mice also suggests that SP-A may be important in the progression of inflammation leading to vaso-obliteration and NV.
Commercial Relationships: Faizah Bhatti, None; **Netsanet Kassa**, None; **Johannes Kung**, None; **Phillip S. Coburn**, None

Program Number: 808
Presentation Time: 4:45 PM–5:00 PM
Hypoxia-induced phosphorylation of astrocytic Cx43 is associated with misdirected angiogenesis in oxygen induced retinopathy
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Purpose: Hypoxia-induced degeneration of astrocytes contributes to the misdirected angiogenesis in oxygen-induced retinopathy (OIR). We studied the role of Cx43, the connexin isoform that forms gap junctions and hemichannels in astrocytes, in this degeneration and the associated pathology in OIR. To gain mechanistic insight, we examined changes in Cx43 phosphorylation, a key regulatory event known to affect Cx43 channel function, that occur prior to astrocyte loss. Finally, we explored whether preventing hypoxia-induced Cx43 phosphorylation increases astrocyte density and promotes reparative angiogenesis in OIR.
Methods: Mouse litters were exposed to 75% oxygen from postnatal day (p) 7 to p12 and then returned to room air. Cx43 was conditionally deleted from astrocytes using Cre-LoxP recombination (KO mice). Cx43 phosphorylation during relative hypoxia was examined with Western blot using phospho-specific antibodies. Kinase inhibitors as well as site-specific Cx43 phosphorylation-deficient mice (KI mice) were used to inhibit phosphorylation of Cx43. The degrees of vasobliteration (VO) and neovascularization (NV), and the astrocyte density were quantified using immunohistochemistry on retinal wholemounts from wild type (WT), KO and KI mice.
Results: Deletion of Cx43 markedly improved astrocyte density in OIR by p14 (WT, 257.8±14.5 cells/mm^2 vs KO, 471±37.7 cells/mm^2; n=5, p=0.005), accelerated retinal revascularization (62.5±1.6% decrease in VO in KO mice compared to WT; n=8 mice, p<0.001) and reduced neovascularization (63±3.2% decrease in NV in KO mice compared to WT; n=8 mice, p<0.001). A 3-fold increase in Cx43 phosphorylation at CK1δ sites was detected within 6 hours of exposure to hypoxia (n=6, p=0.001). Retinae from mice injected with CK1δ inhibitors or KI mice that express Cx43 with CK1δ-site serine to alanine mutations exhibited enhanced directed angiogenesis (decreases of 41±2% in VO and 40±5% in NV in KI compared to WT; n=8, p<0.001) and elevated astrocyte density.
Conclusions: Phosphorylation of Cx43 by CK1δ occurs early in the hypoxia phase of OIR and facilitates astrocyte degeneration leading to pathologic angiogenesis. Targeting Cx43 channels or their phosphorylation are viable options for the treatment of ischemic retinopathies.
Commercial Relationships: NEFELI SLAVI; **Paul D. Lampe**, None; **Miduturu Srinivas**, None