513 Oxidative damage, ER stress and autophagy
Thursday, May 11, 2017 8:30 AM–10:15 AM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 5352–5374/B0509–B0531
Organizing Section: Retinal Cell Biology

Program Number: 5352 **Poster Board Number:** B0509
Presentation Time: 8:30 AM–10:15 AM
Neuroprotectin D1 (NPD1) upregulates Iduna expression and provides protection against uncompensated oxidative stress (UOS) in Human Retinal Pigment Epithelial Cells
Pranab K. Mukherjee, Veronica Bender, Jorgelina M. Calandria, Nicolas G. Bazan. Neuroscience Cntr/Ophthalmology, LSU Health Sciences Center, New Orleans, LA.
Purpose: Ring finger protein 146 (Iduna) facilitates DNA repair and protects against cell death induced by NMDA receptor-mediated glutamate excitotoxicity or by cerebral ischemia. NPD1 promotes cell survival under UOS. The purpose of this study is to define the underlying molecular mechanism of NPD1-mediated Iduna neuroprotection against UOS-induced cell death.
Methods: ARPE-19 cells were grown and maintained in a T-75mM flask. Transient transfection of ARPE-19 cells by Iduna-siRNAs constructs were done by transfecting with 5 μg of constructs using Fugene-6 (Roach, NJ, USA). In addition to the Iduna-siRNA constructs, we also used shRNA-Iduna or YRAA-Iduna, an Iduna mutant lacking PAR binding sequences. A GFP-Iduna construct was used as transfection control. Transfected cells were incubated 24h at 37°C, then serum starved for 8h at 37°C, exposed to UOS (600 μM H_2O_2) and treated with NPD1 (50-100 nM) for 6h followed by Western blot analysis and 16h for apoptotic cell death assessment. Iduna protein abundance was detected by Western blot analysis using either anti-Iduna antibody (RNF146) or clone N201/35 containing anti-Iduna antibody (UC Davis/NIH Neiro Mab Facility, CA).
Results: Our data demonstrates that NPD1 potently upregulates Iduna expression and provides remarkable protection against UOS. NPD1 induction of Iduna expression requires interaction binding at the poly (ADP-ribose) (PAR) sites. Use of Caspase and PARP inhibitors indicates that NPD1 induced Iduna-mediated cytoprotection involved in apoptotic and parthanatos signaling cascades. Iduna-specific and non-specific siRNAs established as controls for Iduna off-target effects. Western blot analysis of the Iduna protein in whole cell, cytoplasmic and nuclear preparations indicated that NPD1 triggered Iduna translocation into the nucleus.
Conclusions: NPD1 enhances Iduna expression under disruptive neuro-homeostasis. These findings provide a conceptual advancement for survival of neural cells undergoing challenges to homeostasis because a lipid mediator (NPD1), made "on demand," modulates abundance of a critically important protein (Iduna) for cell survival. Further unraveling of the molecular details of DHA-NPD1-Iduna expression signaling may contribute to therapeutic interventions for retinal degenerations.
Commercial Relationships: Pranab K. Mukherjee, None; **Veronica Bender**, None; **Jorgelina M. Calandria**, None; **Nicolas G. Bazan**, None
Support: Supported by NEI grant EY005121, NIGMS grant GM103340, the Eye, Ear, Nose and Throat Foundation (NGB) and Research to Prevent Blindness.

Program Number: 5353 **Poster Board Number:** B0510
Presentation Time: 8:30 AM–10:15 AM
Combination of oral progesterone and lipoic acid treatment and its effect on oxidative stress in an animal model of retinal degeneration
Maria Miranda[1], Ramírez-Lamelas T. Dolores[1], Rosa López-Pedrajas[1], Roberto Gimeno-Hernández[1], Á Fernández-Carbonell[1], Inmaculada Almansa[1], Javier Araiz[2, 3].
[1]Physiology, Univ CEU-Cardenal Herrera, Moncada, Valencia, Spain; [2]ICQO, Bilbao, Spain; [3]Universidad del País Vasco, Leioa, Spain.
Purpose: We have previously shown the beneficial effects of progesterone (P4) on the evolution of cell death in two animal models of retinitis pigmentosa (RP), the rd1 and the rd10 mice. RP is a complex disease that affects photoreceptors and that have been related to oxidative stress. Indeed, the possible P4 action mechanism may be related to its ability to regulate thiol metabolism and to decrease lipid peroxidation. In an attempt to improve the positive effects of P4, the aim of this study was to combine P4 treatment with a well known and potent antioxidant (lipoic acid (LA)) and to establish the changes induced by this combined treatment in the retina of rd1 mice.
Methods: LA (100mg/kg), P4 (100mg/Kg) and a combination of both (LA+P4), were administered orally to rd1 mice at different postnatal (PN) days. On PN11, eyes were enucleated and sectioned (N=6). Histological evaluation was performed using in situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) assay. Glial fibrillary acidic protein (GFAP) and glutamate cisteyne ligase (GCL) retinal immunohistochemistry were also performed. Additionally, we measured the total antioxidant capacity of LA and P4 and three of their related metabolites (dihydrolipic acid (DHLA), allopregnenolone and estradiol). SPSS software package version 16.0 was used, and the level of significance was set at $p<0.05$, one-way ANOVA with the corresponding post hoc test was performed.
Results: The combination of P4 and LA administration improved cell survival and showed better photoreceptor protection effect than when administered alone, as shown by the decrease in the number of TUNEL positive cells. In addition, AL, P4 and their combination oral treatment are able to prevent the characteristic gliosis observed in the retina of the rd1 mouse model (74,74% of reduction at the peripheral retina) and a slight decrease of GCLC expression (37,20% of reduction at the peripheral retina) was observed in the retina of rd1 mice treated with the combination of LA and P4 with respect to mice treated only with LA or P4. LA and P4 metabolites have shown higher total antioxidant capacity.
Conclusions: LA and P4 may have neuroprotective actions in the retina and probably their action mechanism is due to their metabolization in the eye.
Commercial Relationships: Maria Miranda, None; **Ramírez-Lamelas T. Dolores**, None; **Rosa López-Pedrajas**, None; **Roberto Gimeno-Hernández**, None; **Ángel Fernández-Carbonell**, None; **Inmaculada Almansa**, None; **Javier Araiz**, None
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Program Number: 5354 **Poster Board Number:** B0511
Presentation Time: 8:30 AM–10:15 AM
Protective effect of N-Acylsphingosine Amidohydrolase 1 (acid Ceramidase) in RPE cells against oxidative stress
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Purpose: RPE cells have multiple functions to protect the neural retina from oxidative stresses. It has been reported that the inhibition of de novo ceramide synthesis protects rat retina from light-induced degeneration. Ceramide is a key metabolic cellular factor that acts as 2nd messenger for cell death. To determine the role of ceramide metabolism in RPE cell death, we induced oxidative stress in RPE cells and studied the role of ceramide by modulating its level with N-Acylsphingosine Amidohydrolase (ASAH1).
Methods: ARPE-19 cells were plated and after 24hrs of the cultivation, medium was replaced with serum free medium. To induce the oxidative stress, 4-hydroxy-2-nonenal (4-HNE), H2O2 or C2-ceramide was added. The expression of ASAH1 in ARPE-19 cells was investigated by RT-PCR. Two isoforms of ASAH1s were cloned from ARPE-19 cells and inserted into the mammalian expression vector with the fusion to Venus gene. Vectors were linearized and electroporated into ARPE cells. Following the isolation of venus positive cells by a cell sorter, 2 types of stable cells that expressed ASAH1 or its transcript variant were established. The localization of the ASAH1s were examined with Cell Navigator Lysosome Staining. The protective effect of ASAH1s against oxidative stresses were studied by cell viability assay.
Results: The expression of ASAH1 was not detected in non-oxidative condition, however, up-regulated by 4-HNE and H2O2. Two isoforms of ASAH1s were detected in ARPE cells. One was expressed in the cytoplasm (cy-ASAH1, NM_004315) and the other was in lysosome (ly-ASAH1, BC_016481). The Cy-ASAH1 was the transcript variant, however, the function has not been elucidated. Cy-ASAH1 and Ly-ASAH1 was extremely increased by 4-HNE and H2O2, respectively. Both of stably ASAH1- expressing cells (ARPE-cy-ASAH1 and ARPE-Ly-ASAH1) survived more than that of native ARPE cells under oxidative stresses.
Conclusions: ASAH1 is the key enzyme that hydrolyzes cytotoxic ceramide and generates sphingosine and cytoprotective sphingosine 1-phosphate. We conclude, the stably ASAH1 expressing ARPE-19 cells protected these cells from oxidative stress by reducing cellular ceramides. We found two types of ASAH1s were up-regulated in ARPE cells during the oxidative stress that might be dependent on the type of oxidative agents and may indicate different ceramide generation pathways in different form of stresses.
Commercial Relationships: Eriko Sugano, None; **Mandal A. Nawajes**, None; **Kitako Tabata**, None; **Makoto Tamai**, None; **Hiroshi Tomita**, None
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Program Number: 5355 **Poster Board Number:** B0512
Presentation Time: 8:30 AM–10:15 AM
Characterization of pathological mechanisms during aging of the *Prpf31*-mutant mouse model of retinitis pigmentosa
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Purpose: Mutations in the ubiquitously-expressed *Pre-mRNA Processing Factors 3*, *8*, and *31* (*PRPF3*, *8* and *31*) constitute the second most prominent cause of non-syndromic autosomic dominant retinitis pigmentosa (adRP) in humans. We previously determined that young *Prpf*-mutant animals display abnormal RPE functions and develop a phenotype with age. Despite the difference in pathological timing between patients and corresponding mouse models, human ARPE-19 cells down-regulated for *PRPF31* and *Prpf31*-mutant primary RPE cells display a similar defect in retinal phagocytosis, suggesting this mouse model can be used as a paradigm to identify related pathological processes. Thus, we set out to characterize RPE-related stress pathways occuring during the aging of *Prpf31*-mutant mice.
Methods: Studies were conducted on 3 to 24-month-old animals in order to dissect the full series of molecular events. Gene and protein expression levels for the mitochondrial respiratory chain and detoxification pathways were assessed by qPCR and immunoblots. Accumulation of lipids was evaluated on histological sections by Oil-Red-O and Luxol Blue stainings.
Results: *ND4* (complex I) expression is decreased from 6 months of age in the RPE/Choroid fraction, while *CoxIV* (complex IV) expression increases. *ATP synthase* (complex V) expression diminishes in young animals before augmenting after 12 months of age. Expression of the detoxifying enzyme *SOD1* (CuZn-SOD, cytosol) increases with age in mutant mice in contrast to wildtype controls. *SOD2* (Mn-SOD, mitochondrial matrix) shows less variation of its expression than *SOD1*. Lipids coloration were validated on control beta5 knockout integrin mouse sections that accumulate lipofuscin and lipids with age. Stainings on *Prpf31*-mutant sections confirm our hypothesis that *Prpf31*-mutant RPE cells accumulate more lipid droplets than wildtype littermates during aging.
Conclusions: Our results suggest that mitochondrial defects could contribute to the RPE phenotype. In addition, oxidative processes appear to take place in *Prpf31*-mutant RPE cells. Taken together, we strongly believe our data will help us decipher the etiology of tissue-specific adRP cases linked to ubiquitously-expressed splicing factors and could contribute to define a new potential common therapeutic approach for all *PRPF* genes.
Commercial Relationships: Emeline F. Nandrot, None; **Nawel Hadjout**, None; **Déborah Lew**, None; **Abdallah Hamieh**, None
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Program Number: 5356 **Poster Board Number:** B0513
Presentation Time: 8:30 AM–10:15 AM
Effect of Bax Inhibitor 1 overexpression on Tunicamycin-induced ER Stress in Human RPE Cells
Muhammad A. Abu-Rmaileh, Victoria Treboschi, Ping Yang, Glenn J. Jaffe. Opthalmology, Duke University, Durham, NC.
Purpose: Progression of age-related macular degeneration (AMD) is linked to an increase in cellular stress, especially in the endoplasmic reticulum (ER). ER proteins that maintain cellular homeostasis, such as 78 kDa glucose-regulated protein (GRP78) and protein kinase-like endoplasmic reticulum kinase (PERK), and that mediate apoptosis, such as C/EBP homologous protein (CHOP) determine the cell's longevity through the unfolded protein response (UPR). Bax Inhibitor 1 (BI-1) is an ER-associated protein that protects against BAX-mediated cell-death. In the current study, we investigated the

effect of BI-1 overexpression on ER stress proteins in RPE cells subjected to tunicamycin, an RPE cell death-inducing drug.
Methods: Cultured human RPE cells were infected with adenovirus encoding either LacZ or BI-1 at a multiplicity of infection (MOI) of 8 for 24 hours. Media were replaced with 1% FBS-MEM, the infected cells were cultured for additional 4 days in 1% FBS-MEM, and then treated with tunicamycin at various concentrations for 24 hours. Cell morphology was assessed by light microscopy. Cell death was determined by lactose dehydrogenase (LDH) assay. GRP78, PERK, and CHOP protein levels were evaluated by Western blot.
Results: On morphological analysis, and by LDH assay, BI-1 infected cells when compared to LacZ infected cells were more resistant to tunicamycin-induced death, regardless of concentration; LacZ infected cells released nearly twice as much LDH into media when compared to BI-1 infected cells. Tunicamycin induced increased levels of CHOP and GRP78 when compared to untreated cells, and produced higher CHOP and PERK levels in BI-1-treated cells than in LacZ-treated cells. In contrast, similar levels of GRP78 were observed in LacZ and BI-1 infected cells treated with tunicamycin.
Conclusions: When exposed to tunicamycin, BI-1 induces increased levels of specific ER stress proteins, while at the same time protecting cells from tunicamycin induced cell death. Understanding how BI-1 relates to the adaptive unfolded protein response characteristic of ER stress proteins may guide methods to limit RPE cell death in eyes with AMD.
Commercial Relationships: Muhammad A. Abu-Rmaileh, None; **Victoria Treboschi**, None; **Ping Yang**, None; **Glenn J. Jaffe**, None
Support: NIH 5P30EY005722 (Core grant), Research to Prevent Blindness, Inc. (RPB)

Program Number: 5357 **Poster Board Number:** B0514
Presentation Time: 8:30 AM–10:15 AM
Effects of two SERPINS on ARPE-19 Cells after Acute Induced-$NaIO_3$ Damage
Francisco M. Nadal-Nicolas, Sofia P Becerra. National Eye Institute (NEI) - LRCMB, National Institute of Health (NIH), Bethesda, MD.
Purpose: Oxidative stress is one of the risk factors associated in neurodegenerative diseases such as age-related macular degeneration (AMD). One hallmark of AMD is degeneration of the retinal pigment epithelium (RPE) that eventually leads to blindness. Sodium iodate ($NaIO_3$) induces a selective retinal degeneration targeting the RPE by triggering oxidative injury. Two serpins, pigment epithelium-derived factor (PEDF) and protease nexin-1 (PN-1), have neurotrophic properties and are present extracellularly in the retina. While PEDF lacks protease inhibitory activity, PN-1 is a serine protease inhibitor. The aim of the study is to investigate the effects of PEDF and PN-1 on RPE treated with a selective oxidative agent
Methods: Human ARPE-19 cells were seeded in 96-well plates. After 16h of serum starvation, the cells were pretreated with full-length recombinant human PEDF, PN-1 and PN-1-[R346A], an altered version at its serpin reactive center, as well as synthetic peptides derived from PEDF termed 34mer, 44mer and 17mer, and from PN-1 termed 17mer and incubated at 37C for 2 h. Freshly prepared $NaIO_3$ solutions were added to the cultures and incubated at 37C for 2 h. After changing the media and incubating for 16 h, cytotoxicity was determined by measuring the product of the cytoplasmic Lactate DeHydrogenase (LDH) enzyme in the media. Media with serum was replaced and incubated for 48 h before cell viability was determined by measuring the intracellular ATP levels
Results: PEDF and PN-1 decreased the activity of LDH in the cultures to 30 and 50% respectively, relative to 100% of untreated cultures. They increased cell viability to 142% and 132%, respectively, relative to untreated cultures. The PEDF peptides 17mer, 44mer and PN-1 peptide 17mer decreased the LDH levels to 60% - 74%, whereas they increased cell viability, respect to untreated cultures, by 1.5–fold. The inactive serpin PN-1-[R346A] decreased cytotoxicity and increased cell viability similar to its wild type counterpart PN-1. However, the PEDF 34mer had no significant effect on cytotoxicity or viability of ARPE-19 damaged with NaIO3
Conclusions: The two serpins PEDF and PN-1 share protective properties against $NaIO_3$-mediated injury. A small fragment from both proteins with 17 residues contains a biological active region. The inhibition of serine proteases is dispensable for the protection by these two serpins
Commercial Relationships: Francisco M. Nadal-Nicolas, None; **Sofia P Becerra**

Program Number: 5358 **Poster Board Number:** B0515
Presentation Time: 8:30 AM–10:15 AM
MicroRNA-302d promotes dedifferentiation of the retinal pigment epithelium by targeting the CDKN1A
chao jiang, Xue Chen, Chen Zhao. Jiangsu Province People's Hospital, NANJING, China.
Purpose: MicroRNA plays a critical role in dedifferentiation of retinal pigment epithelium (RPE) cells. In our previous studies, we found the hsa-miR-302d-3p was down-regulated in RPE differentiation process. However, the microRNA mediated dedifferentiation in human RPE cells has not been thoroughly reported. In our study, we aim to analyze the role of miR-302d in RPE dedifferentiation
Methods: We respectively transfected hsa-miR-302d mimic and inhibitor into hiPSC-RPE at 30 day to see its role on cell differentiation. The related rpe genes and proteins expression in the rpe differentiation process were observed by Q-PCR and western blotting. We also observe the expression of ZO-1 in ips-rpe cells according to immunofluorescence. We next determined whether miR-302d would promote proliferation and migration in ARPE-19 cells. Furthermore, cell cycle were observed by flow cytometry after transfected hsa-miR-302d mimic and inhibitor. We confirm that CDKN1A as a direct target of miR-302d in ARPE-19 cells. Also, we established an AMD model for the treatment of hydrogen peroxide with H2O2. We observed the phagocytosis and apoptosis of RPE cells after transfected hsa-miR-302d mimic and inhibitor
Results: We found relative mRNA expressions were significantly decreased of RPE65, RLBP1, MERTK, BEST1, CTNNB1 and TJP1 in hiPS-RPE at 30 days after transfected with miR-302d mimic compared to NC mimic. Also the relative mRNA expressions were significantly increased of RPE65, RLBP1, MERTK, BEST1, and TJP1 in hiPSC-RPE at 30 days transfected with miR-302d inhibitor compared to NC inhibitor. Both proliferation and migration were promoted by miR-302d overexpression after transfection with ARPE19 cells. And the luciferase reporter gene assay showed that CDKN1A is the target gene of microRNA302d. After transfected with miR-302d minics and inhibitor, we then treated the cells with 200uM H2O2. We found that with the oxidative stress model, the cell apoptosis rates significantly increased after transfected with miR-302d inhibitor compared to NC inhibitor
Conclusions: Mir-302d is associated with RPE cells proliferation and migration as well as apoptosis under oxidative stress, indicating that Mir-302d negatively regulates RPE cells differentiation.
Commercial Relationships: chao jiang; **Xue Chen**, None; **Chen Zhao**, None

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Program Number: 5359 **Poster Board Number:** B0516
Presentation Time: 8:30 AM–10:15 AM

The retinal pigment epithelium of the canine area centralis is uniquely susceptible to mitochondrial toxicant damage

Melanie L. Foster[1], Regan L. Lane[1], Annie Oh[1], Steven Nagar[2], Mary C. McGahan[2], Priyatham S. Mettu[3], Scott W. Cousins[3], Freya M. Mowat[1]. [1]Department of Clinical Sciences, North Carolina State University, Raleigh, NC; [2]Molecular Biomedical Sciences, North Carolina State University, Raleigh, NC; [3]Duke Eye Center/ Ophthalmology, Duke University School of Medicine, Durham, NC.

Purpose: Mitochondrial dysfunction has been implicated in the ageing process, and may contribute to pathology of the retinal pigment epithelium (RPE) in age-related macular degeneration. The canine retina, unlike that of rodents, has a well-defined cone photoreceptor enriched region known as the area centralis, comparable to the human macula. We tested the hypothesis that the canine area centralis RPE has features that make it more sensitive to toxicity from hydroquinone, a mitochondrial toxicant component of cigarette smoke.

Methods: We harvested and cultured RPE cells from 4 different regions of the eye (area centralis, nasal visual streak, superior and inferior periphery) from young, healthy adult mixed-breed dogs following humane euthanasia. We quantified the individual cellular mitochondrial content in RPE cells immediately after harvest and in confluent culture from different regions using Mitotracker Red staining and ImageJ image analysis tools. Mitochondrial basal activity and response to stressors were measured using a Seahorse XF Mito Stress assay. Cultures were treated with 6h exposures of 0, 25, or 50 μM hydroquinone, every 3 days for a total of 5 times. Cell viability was quantified following exposure. An ANOVA was used to compare values from the area centralis with other regions of the eye.

Results: The basal and maximal mitochondrial respiratory capacity of the area centralis was lower than the superior peripheral retina. There was decreased viability of area centralis cells exposed to hydroquinone compared to other regions. There was a 24.5% increase in cell death compared to control in area centralis cells treated with 50 μM hydroquinone, a concentration which produced no increase in cell death in other regions.

Conclusions: The retinal pigment epithelium of the area centralis has reduced mitochondrial respiratory capacity compared with the superior periphery. When exposed to the mitochondrial toxicant hydroquinone, RPE of the area centralis undergoes a greater amount of cell death. The canine area centralis therefore has the potential to be used to study mechanisms of RPE injury in diseases such as age-related macular degeneration.

Commercial Relationships: Melanie L. Foster, None; **Regan L. Lane**, None; **Annie Oh**, None; **Steven Nagar**, None; **Mary C. McGahan**, None; **Priyatham S. Mettu**, None; **Scott W. Cousins**, None; **Freya M. Mowat**, None

Support: Comparative Medicine Institute, North Carolina State University

Program Number: 5360 **Poster Board Number:** B0517
Presentation Time: 8:30 AM–10:15 AM

Effects of Ginkgo biloba Extract on Endoplasmic Reticulum Stress in A2E-containing Retinal Pigment Epithelial Cells Exposed to Blue Light

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Purpose: Cumulative exposure to blue light may play an etiological role in age-related macular degeneration (AMD). Endoplasmic reticulum (ER) stress is involved in the pathogenesis of AMD. In this study, we investigated the effects of Ginkgo biloba Extract (GBE) on ER stress in retinal pigment epithelial (RPE) cells exposed to blue light.

Methods: RPE cells (ARPE-19) were incubated with N-retinylidene-N-retinylethanolamine (A2E) for 2 h, and exposed to blue light for 30 min in media with and without 100 μg/ml GBE. Cell viability was measured by MTT assay. Expressions of binding protein/glucose-regulated protein 78 (BiP/GRP78) and C/ EBP homologous protein-10 (CHOP) mRNA were measured using quantitative real-time polymerase chain reaction (qRT-PCR). BiP/ GRP78 and CHOP protein levels were assessed with Western blot.

Results: Compared with the group not exposed to blue light, cell viability decreased, and mRNA and protein levels of two ER stress indicators, BiP/GRP78 and CHOP, increased in the group exposed to blue light (BL group). In the group treated with GBE (BL+GBE group), cell viability improved and the expressions of both mRNA and the protein of BiP/GRP78 and CHOP reduced compared with the BL group.

Conclusions: This *in vitro* study demonstrates that GBE protects RPE cells from blue light-induced damage by mitigating ER stress.

Commercial Relationships: Jong-Hyun Oh, None; **Ariunaa Togloom**, None; **Moosang Kim**, None

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Program Number: 5361 **Poster Board Number:** B0518
Presentation Time: 8:30 AM–10:15 AM

The mitochondrial-derived peptide humanin improves mitochondrial bioenergetics after endoplasmic reticulum stress in human RPE cells

Hiroto Terasaki[1, 2], Parameswaran G. Sreekumar[1], Hemal H. Mehta[3], Pinchas Cohen[3], David R. Hinton[4, 5], Ram Kannan[1]. [1]Doheny Eye Institute, Los Angeles, CA; [2]Ophthalmology, Kagoshima University, Kagoshima, Japan; [3]USC Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA; [4]Ophthalmology, University of Southern California, Los Angeles, CA; [5]Pathology, University of Southern California, Los Angeles, CA.

Purpose: We have recently reported that the mitochondrial-derived peptide humanin (HN) protected human retinal pigment epithelial cells (hRPE) from endoplasmic reticulum (ER)-induced stress by a mechanism that involved ER-mitochondrial crosstalk and selective increase in mitochondrial glutathione (Matsunaga et al. PLoS ONE 11(10): e0165150, 2016). The aim of the present work was to study the effect of ER stress on mitochondrial bioenergetics in hRPE cells and to determine the effect of HN on ER-induced changes on key mitochondrial parameters.

Methods: Early passage primary cultured hRPE cells were treated with tunicamycin (TM, 3 μg/ml or 10 μg/ml) for 12 h with or without the co-treatment of HN (10 μg/ml or 20 μg/ml). Mitochondrial respiration parameters (oxygen consumption rate (OCR) and ATP

production, expressed as pmol/min/total DNA) were analyzed by a Seahorse XF96 Analyzer. In separate experiments, TM-induced changes as well as the effect of HN co-treatment on ER stress biomarker GRP78 (Western blot) and apoptotic cell death (TUNEL) were measured.
Results: TM caused a significant two fold increase in GRP78 expression over the untreated hRPE controls. HN co-treatment resulted in a decrease in GRP78 as compared to TM-treated cells; however, GRP78 expression remained higher than in untreated controls. TM caused 12.7 % cell death from apoptosis with 10 μg/ml TM treatment (TUNEL) which was significantly inhibited to 5.1% and 3.9% by co-treatment with HN (10 μg/ml and 20 μg/ml respectively). Both OCR and ATP production by hRPE cells were significantly decreased with 3 μg/ml TM treatment (52.1% and 63.7% respectively; $p<0.01$ vs untreated controls). HN co-treatment with 10 μg/ml or 20 μg/ml elevated OCR over TM group by 42.0% and 43.2% respectively. ATP production rates were also increased over the corresponding rates with TM treatment. However, the restoration of OCR and ATP production was partial as compared to untreated controls or hRPE cells treated with HN alone.
Conclusions: Our results show for the first time that ER stress significantly inhibits mitochondrial respiration in RPE cells. HN partially protects RPE from ER stress-induced attenuation of mitochondrial function and represents a novel therapeutic approach for ER-related retinal diseases.
Commercial Relationships: Hiroto Terasaki, None; **Parameswaran G. Sreekumar**, None; **Hemal H. Mehta**, None; **Pinchas Cohen**, CohBar,Inc. (C), CohBar,Inc. (I); **David R. Hinton**, None; **Ram Kannan**, None
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Program Number: 5362 **Poster Board Number:** B0519
Presentation Time: 8:30 AM–10:15 AM
The fundus camera delivered-light induced retinal degeneration model is mediated by oxidative stress
Cynthia X. Wang[1], Bogale Aredo[1], Yi Ding[1], Xin Zhong[2], Kaiyan Zhang[3], Rafael Ufret-Vincenty[1]. [1]Ophthalmology, UTSW Medical Center, Dallas, TX; [2]Ophthalmology, The First Affiliated Hospital of Guangxi Medical University, Nanning, China; [3]Ophthalmology, Hainan Provincial People's Hospital, Haikou, China.
Purpose: Retinal light injury models can be useful in understanding aspects of retinal degeneration and retinal oxidative stress. We recently developed and reported on a new model of fundus camera-delivered light-induced retinal degeneration (FCD-LIRD). In the current study our aim was to test whether oxidative stress response enzymes have an impact on the level of retinal injury induced by FCD-LIRD.
Methods: Age-matched C57BL/6J and SOD1/DJ1/Parkin TKO mice (triple-KO or "TKO" mice are deficient in oxidative stress response enzymes SOD1, DJ1 and Parkin) were used at 6-12 wk of age and exposed to FCD-LIRD. Both male and female mice were used. Eyes were monitored with fundus photography and OCT. On separate experiments, eyes were collected 4h or 24h after FCD-LIRD to test expression changes in cell signaling-related genes using an RT^2 Profiler PCR Array (Qiagen), and results were confirmed by qPCR. The effect on RPE cells was evaluated with ZO-1 staining of flat mounts.
Results: There was a significant increase in retinal damage after FCD-LIRD in TKO mice compared to C57BL/6J, indicating that oxidative stress is an important aspect of the mechanism of injury in FCD-LIRD. Increased damage was confirmed by fundus photography and OCT volume measurements. Gene expression changes in genes related to oxidative stress response and signaling were noted at 4 and 24h after FCD-LIRD. Prominent changes in RPE cells were seen in TKO mice compared to B6J mice.
Conclusions: FCD-LIRD leads to oxidative stress-mediated retinal damage, which is exacerbated in the absence of oxidative stress response enzymes. Further studies will be aimed at elucidating the specific pathways involved in the retinal response to oxidative stress.
Commercial Relationships: Cynthia X. Wang, None; **Bogale Aredo**, None; **Yi Ding**, None; **Xin Zhong**, None; **Kaiyan Zhang**, None; **Rafael Ufret-Vincenty**, None
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Program Number: 5363 **Poster Board Number:** B0520
Presentation Time: 8:30 AM–10:15 AM
The Nrf2 and PGC-1α deficient murine retina reveals retinal pigment epithelium damage that coincides with autophagy decline and damaged mitochondria
Jussi J. Paterno[1, 2], Niko Kivinen[1, 2], Johanna Viiri[1], Juha Hyttinen[1], Ali Koskela[1], Mateusz Winiarczyk[3], Deborah A. Ferrington[4], Szabolcs Felszeghy[5], Anu Kauppinen[6], Kai Kaarniranta[1, 2].
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Purpose: Increased oxidative stress has been linked to the degeneration of retinal pigment epithelial cells (RPE) during aging process. Nuclear factor-erythroid 2-related factor-2 (Nrf2) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) are transcription factors that regulates reactive oxygen species (ROS) balance. We generated Nrf2–/– PGC-1α–/– double knock-out (DKO) mouse model to study morphological alterations and autophagy markers in RPE.
Methods: Retinal tissue sections of DKO mice and age-matched wild-type controls on a C57BL/6J background, sacrificed at one years of age, were processed for transmission electron microscopy (TEM) and immunohistolochemical microscopy analysis. Immunohistochemical stainings were done for oxidative stress marker 4-HNE and autophagy regulators p62/SQSTM1, Beclin-1, LC3 and ubiquitin protein conjugates. Eye fundus imaging was performed to assess retina *in vivo*.
Results: In the RPE of DKO mice, number of autophagosomes were increased ($p<0.01$), together with upregulation of p62/SQSTM1, Beclin-1, LC3 and ubiquitin protein conjugates. DKO mice model showed clearly increased number of damaged mitochondria ($p<0.001$), while number of melanosomes were decreased ($p<0.05$). No signs of retinal or choroidal neovascularization were seen *in vivo*.
Conclusions: Our findings support that dissecting the ROS regulating pathways in the RPE of the Nrf2 and PGC-1α deficient mice is associated with autophagy decline and mitochondrial dysfunction. As these are important features in the ageing eye and degeneration of RPE cells, this animal model appears to have applications for experimental research of dry age-related macular degeneration and its treatment.
Commercial Relationships: Jussi J. Paterno, None; **Niko Kivinen**, None; **Johanna Viiri**, None; **Juha Hyttinen**, None; **Ali Koskela**,

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Presentation Time: 8:30 AM–10:15 AM
Protective action of a novel HDL mimetic peptide, HM-10/10 in RPE in vitro and in a murine model of sodium iodate induced retinal degeneration
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Purpose: Age-related macular degeneration (AMD) is a leading cause of blindness in the developed world. The retinal pigment epithelium (RPE) is a critical site of pathology in AMD. Oxidative stress plays a key role in the development of AMD. We developed a chimeric high density lipoprotein (HDL) - mimetic peptide (HM-10/10) that not only reduces oxidative stress but also prevents oxidative stress-induced cell death in cell culture models. The purpose of this study was to investigate the effect of HM-10/10 in the regulation of human RPE cell death from oxidative and sodium iodate ($NaIO_3$) and to determine the effect of HM-10/10 in a model of $NaIO_3$-induced retinal degeneration.
Methods: Early passage (2-4) primary cultured human RPE cells were treated with 200 uM tBH or $NaIO_3$ (200 μg/ml or 500 μg/ml) for 24 h with or without the co-treatment of HM-10/10 (10 μg/ml). Apoptotic cell death was measured using TUNEL assay and caspase-3 by western blot. Twelve C57Bl6/J male mice were divided into three groups of four mice per group and treated as follows: control mice (PBS-treated), $NaIO_3$-treated (20 mg/kg) mice, and $NaIO_3$-treated (20 mg/kg) mice with HM-10/10 (100 mg/kg) added to their chow. At the end of three weeks, eyes were enucleated, and frozen sections of retinal tissue were used for histology. Retinal sections were scanned and retinal thickness was quantified (Aperial Scan Scope, Leica Biosystems).
Results: HM-10/10 (10 μg/ml) significantly inhibited RPE cell death from 24h tBH treatment ($p<0.01$ vs tBH-treated). Incubation of RPE with either 200 μg/ml or 500 μg/ml of $NaIO_3$ resulted in increased TUNEL-positive cells and co-treatment with HM-10/10 (10 μg/ml) at either $NaIO_3$ concentration significantly reduced apoptotic cell death. Caspase-3 activation was significantly elevated in RPE cells treated with 200 μM H_2O_2 or NaIO3 alone, whereas co-treatment with HM-10/10 inhibited activation of caspase-3. In mice, intravenous $NaIO_3$ caused a decrease in retinal thickness vs PBS controls and HM-10/10 in the diet significantly ($p<0.05$ vs $NaIO_3$-treated) restored retinal thickness to control values.
Conclusions: Our results demonstrate that HM-10/10 protects RPE and retina from oxidant injury and can serve as a potential therapeutic agent for the treatment of retinal degeneration.
Commercial Relationships: Ram Kannan, None; **Feng Su**, None; **Robin Farias-Eisner**, Salvaregen (I); **Christine Spee**, None; **Eric Barron**, None; **David R. Hinton**, None; **Srinivasa T. Reddy**, Salvaregen (I)
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Program Number: 5365 **Poster Board Number:** B0522
Presentation Time: 8:30 AM–10:15 AM
Monomethylfumarate induces NAD+ and SIRT1 in Human Retinal Pigment Epithelial Cells: Relevance to Development of New Therapies for Dry AMD
Ravirajsinh Jadeja[1], Folami Powell[1], Malita Jones[1], Manuela Bartoli[2], Pamela M. Martin[1]. [1]Biochemistry and Molecular Biology, Augusta University, Augusta, GA; [2]Ophthalmology, Augusta University, Augusta, GA.
Purpose: Of the millions affected by Age-related macular degeneration (AMD), the vast majority have the dry "atrophic" form of the disease, a type of AMD for which options to prevent and treat are most limited. Age and race are non-modifiable risk factors; however, oxidative stress, inflammation, altered cholesterol and dysregulated energy metabolism, factors that can potentially be modified, also play major causative roles. Nicotinamide adenine dinucleotide (NAD^+) plays an essential role in many processes (i.e., cellular metabolism, ATP production and DNA repair) and is a required factor for the anti-aging protein deacetylase, SIRT1. Decreased NAD^+ and SIRT1 are common in aging and are linked strongly to the development of age-related pathologies such as Alzheimer's, various cancers, and potentially AMD. Our prior work has shown monomethylfumarate (MMF) to impact oxidative stress, inflammation and cholesterol metabolism in retinal pigment epithelial (RPE) cells, a cell type whose dysfunction is integral to AMD development. In the present study, we evaluated the effect of the compound on cellular energy metabolism and its potential therapeutic utility in the context of pathology relevant to AMD.
Methods: Human retinal pigment epithelial (ARPE-19) cells were cultured in the presence of FK866 (0.01-10μM), a highly specific non-competitive inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme in NAD^+ biogenesis, in the presence or MMF (100-500μM). The impact of the treatments on markers of cell viability, senescence, oxidative stress and inflammation as well as SIRT1 expression and activity was evaluated using RT-qPCR, Western blotting and commercial activity assay kits.
Results: FK866 treatment led to the time and dose-dependent depletion of NAD^+, a factor associated with reduced SIRT1 and the increased expression of markers of cellular senescence, inflammation and oxidative stress. Co-culture of cells with MMF reversed these factors in a time- and dose-dependent manner.
Conclusions: Therapies to boost NAD^+/SIRT1 and limit oxidative stress, inflammation and cholesterol retention in retina may be of value in AMD. Our work shows MMF to be a strong potential candidate.
Commercial Relationships: Ravirajsinh Jadeja; **Folami Powell**, None; **Malita Jones**, None; **Manuela Bartoli**, None; **Pamela M. Martin**, None
Support: NH Grant EY022704

Program Number: 5366 **Poster Board Number:** B0523
Presentation Time: 8:30 AM–10:15 AM
The effects of zinc supplementation on autophagy in primary human fetal retinal pigment epithelial cells – new molecular target pathway
Eszter Emri[1, 2], Xinyi Yang[2], Janos Kriston-Vizi[3], Robin Ketteler[3], Imre Lengyel[1, 2]. [1]Centre for Experimental Medicine, Queens University, Belfast, United Kingdom; [2]Ocular biology and Therapeutics, University College London, London, United Kingdom; [3]Bioinformatics Image Core, MRC Laboratory for Molecular Cell Biology, London, United Kingdom.
Purpose: Autophagy is thought to be a response to increased oxidative stress in age related macular degeneration (AMD) and it

has been suggested that modulation of autophagy flux could become a treatment strategy for the disease. The ARED study showed that zinc supplementation can reduce the progression of AMD, raising the possibility, that zinc might be directly involved in regulating cellular processes during disease progression. In this study we tested the hypothesis that zinc supplementation can directly affect autophagy.
Methods: Primary human fetal RPE cells (ScienceCell) were cultured on polyester transwells (Corning), coated with Geltrex for 4 and 31 days in the presence or absence of 125 μM externally added $ZnSO_4$. Autophagosome number was induced by Chloroquine or Bafilomycin, visualized using CytoID kit (Enzo) and the Opera high content screening system (PerkinElmer) and quantified by ImageJ. Distribution of autophagosomes were examined by confocal (Zeiss LSM 700) and transmission electron microscopy (Jeol 1010).
Results: There was a small, but significant decrease in autophagosome numbers with increasing differentiation in non-treated cells (1.77±0.31 vs. 0.65±0.15; $p<0.05$). Treatment with Chloroquine or Bafilomycin increased autophagosome numbers to 7.92±0.45 and 11.25±2.34 respectively, at day 4. The treatment with Bafilomycin did not affect the cells at day 31, while Chloroquine had a small, but significant effect (0.65±0.15 vs 1.17±0.21; $p<0.05$). Treatment with zinc significantly attenuated the effects of both Chloroquine and Bafilomycin ($p<0.05$). Examination of the autophagosomes with confocal and transmission electron microscopy showed that autophagosomes were not only present in the cells but also in the sub-RPE space.
Conclusions: Our results support the hypothesis, that zinc supplementation can directly affect autophagy. The observation that autophagosomes are present in the sub-RPE space suggest that they may contribute to deposit formation in AMD. Therefore, the effects observed in the ARED study, at least in part, might be due to a direct influence on autophagy and this could indeed be a target for future treatment strategies for AMD.
Aknowledgement: This work was supported by COST TD 1304 'The Network for the Biology of Zinc (Zinc-Net)'and EYE-RISK European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 634479.
Commercial Relationships: Eszter Emri, None; **Xinyi Yang**, None; **Janos Kriston-Vizi**, None; **Robin Ketteler**, None; **Imre Lengyel**, None

Program Number: 5367 **Poster Board Number:** B0524
Presentation Time: 8:30 AM–10:15 AM
Nrf2 Protects Mitochondrial Function from Cigarette Smoke (CS) Independent of its Mitochondrial Antioxidant Capability
Marisol D. Cano, Sayantan Datta, Lei Wang, Tongyun Liu, Sonny Dike, James T. Handa. Wilmer Eye Institute/Ophthalmology, Johns Hopkins University, Westminster, MD.
Purpose: Oxidative stress is hypothesized to contribute to RPE apoptosis in Age-related macular degeneration (AMD). CS is the strongest environmental risk factor for AMD, yet how smoking affects the RPE is poorly understood. Nrf2 is a cytoprotective transcriptional factor that controls a cascade of antioxidant genes in the RPE. Nrf2 decreases with age and chronic oxidative stress. The purpose herein is to determine how CS and Nrf2 deficiency affects RPE mitochondrial function.
Methods: Confluent ARPE-19 cells were treated with 100-500 μg/ml CS extract for 24h, after treating cells with Nrf2 or scrambled siRNA. Viability was determined by MTS assay. Protein was extracted and used for western blot. ATP was determined by Cell titer glow, cellular and mitochondrial superoxide by DHE and Mitosox, respectively, NAD/NADH and Lactate using commercial kits.
Results: Cells exposed to 100-500 μg/ml CS for 24h remained viable. CS induced an increase in cellular and mitochondrial superoxide ($p\leq0.01$). Nrf2 knockdown (KD) further increased cellular, but not mitochondrial derived superoxide. CS greatly reduced ATP levels ($p\leq0.01$) without cytochrome C release as an indicator of apoptosis. Nrf2KD maximized this reduction in ATP to render the cell susceptible to apoptosis with cytochrome c release and caspase 3 cleavage. The mitochondrial impairment, as suggested by the decreased ATP levels, prompted us to evaluate mechanisms other than oxidative stress that can affect ATP production. Nrf2KD causes impairment of oxidative phosphorylation due to a lack of NAD substrate. Indeed, NAD was reduced after Nrf2KD compared to controls ($p\leq0.02$). To compensate, the cells increased glycolysis, with increased lactate levels after Nrf2KD compared to controls ($p\leq0.0001$). CS exposure (4 hrs) decreased glycolysis in both control cells ($p\leq0.01$) and after Nrf2KD ($p\leq0.0004$). However, glycolysis in Nrf2KD cells remained above Nrf2 competent cells for the same CS concentration ($p\leq0.01$). After 24 hrs of CS exposure, glycolysis was impaired, with decreased lactate levels after Nrf2KD compared to controls ($p\leq0.009$). The lack of glycolysis as a source of ATP in cells already with mitochondrial dysfunction, rendered cells susceptible to apoptosis.
Conclusions: Nrf2 deficiency does not exacerbate mitochondrial derived oxidative stress, but does impair mitochondrial derived metabolic balance that is exacerbated by CS.
Commercial Relationships: Marisol D. Cano; **Sayantan Datta**, None; **Lei Wang**, None; **Tongyun Liu**, None; **Sonny Dike**, None; **James T. Handa**, None
Support: EY 14005, EY019904, Unrestricted grant from RPB (Wilmer Eye Institute), Dr. Handa is the Robert Bond Welch Professor.

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Presentation Time: 8:30 AM–10:15 AM
Neuroprotectin D1 (NPD1) downregulates amyloid beta (Aβ42) oligomer-induced senescence in human retinal pigment epithelial (RPE) cells
Khanh Do, Nicolas G. Bazan. LSU-Health Science Center, New Orleans, LA.
Purpose: Aβ42, one of the end products of the amyloidogenic pathway, is a component of drusen in age-related macular degeneration (AMD) and of senile plaques of Alzheimer's disease (AD) (Bazan NG.Future Neurol. 2016; 11:99). In our previous studies NPD1 was shifts the amyloid precursor protein (APP) processing from toxic amyloidogenic to non-amyloidogenic (Zhao Y, et al. PLoS One. 2011). Molecular mechanisms of senescence, and how to regulate them, in the RPE are key for the discovery of disease modifying therapies in AMD (Liu, et al. Int. J. Mol. Med. 2015). Here we aim to determine if Aβ42-oligomers (O-Aβ42) on hRPE cells or NPD1 are able to prevent Aβ42-induced senescence in RPE cells. Thus we explored mainly the p16INK4a, that is induced by cellular stress, acts as a late response to telomeric DNA damage and expression increases with age.
Methods: The primary hRPE cells were incubated with 1 μM or 10 μM of O-Aβ42. After 4 and 7 days, cells were fixed and subjected to apoptosis assay (TUNEL and Caspase-3 staining) and senescence assay (β-Galactosidase and p16INK4a staining). The TNF-α and H_2O_2 induced-oxidative stress (OS) was used as a positive control of apoptosis. Then we examined the ability of NPD1 (200 nM) to rescue senescent RPE. The abundance of p16INK4a, and p21Waf1/Cip1 were evaluated by Western blot (WB) and the transcriptional level

of senescence-associated inflammation genes (IL-1, IL-6, IL-8) was analyzed by RT-PCR.
Results: NPD1 shifts the APP processing away from amyloidogenic pathway towards sAPP-α. For chronic uncompensated OS (ARPE-19, H_2O_2 300μM, for hRPE cells, H_2O_2 500μM, treated continuously for 5 days), NPD1 rescues the RPE cells. The formation of Aβ42 oligomers was confirmed by WB. In hRPE cells, O-Aβ42 induced senescence as evidenced by a higher abundance of β-Galactosidase and p16INK4a, while apoptosis was unaffected as reflected by low expression of TUNEL and Caspase-3-positive cells. The WB and RT-PCR results showed that NPD1 decreased the level of both senescent markers and senescence-associated inflammation genes expression.
Conclusions: NPD1 protects hRPE cells from uncompensated oxidative stress (acute or chronic) and shifts the APP processing away from the amyloidogenic pathway. NPD1 also potently protects hRPE cells from O-Aβ42 induced senescence by down regulating senescent markers and senescence-associated inflammation genes.
Commercial Relationships: Khanh Do; **Nicolas G. Bazan**, None
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Program Number: 5369 **Poster Board Number:** B0526
Presentation Time: 8:30 AM–10:15 AM
Sigma 1 Receptor (Sig1R) regulates retinal ER & Oxidative (OX) Stress
Jing Wang[1, 2], Sylvia B. Smith[1, 2]. [1]Cellular Biology and Anatomy, Medical College of Georgia at Augusta University, Augusta, GA; [2]James & Jean Culver Vision Discovery Institute, Augusta University, Augusta, GA.
Purpose: Sig1R is a unique transmembrane protein whose biological role is uncertain. We reported recently that activation of Sig1R affords profound rescue of cone photoreceptor (PRC) function in *Pde6b*^{rd10} *(rd10)* mice (Wang et al, *PNAS*, 2016). *rd10* mice show peak rod loss at P25 and cone loss at P35. Interestingly, although *Sig1R*$^{-/-}$ mice have mild, late-onset ganglion cell loss and no PRC loss (Ha et al, 2011), *rd10* mice lacking Sig1R (*rd10/Sig1R*$^{-/-}$) have markedly accelerated cone dysfunction at P25 with only ~3 rows of PRC nuclei *v.* 6-7 in *rd10* mice (Smith et al, ARVO, 2016). We hypothesized that elevated ER and OX stress contribute significantly to the accelerated retinal dysfunction in *rd10/Sig1R*$^{-/-}$ mice.
Methods: Neural retinas isolated from P21 *rd10/Sig1R*$^{-/-}$, *rd10*, WT mice were used for qRT-PCR (*n*=4/group) and western blotting (*n*=11-16/group) to assess ER stress *(Ire1α, Xbp1, Atf4, Chop, Bip, Perk, Atf6)* and OX stress *(Nrf2, Keap1, Sod1, Cat, Nqo1, Hmox1)* genes/proteins. Since microRNAs can regulate multiple components of ER and OX stress pathways in response to changes in cellular environment, we quantified retinal miRNA levels using miScript miRNA PCR array (*n*=3/group). Data were analyzed by ANOVA.
Results: ER stress: at P21 there were few differences between *rd10* and WT mice (except for CHOP). However, a significant increase in ER stress was detected in *rd10/Sig1R*$^{-/-}$ mice, notably in gene/protein levels of CHOP (6 fold), BIP (6 fold) & XBP1 (12 fold), and 1-2 fold increases in PERK & ATF4. OX stress: there were ~1 fold increases in NRF2, NQO1, HMOX1 in *rd10* v. WT mice, but more significant increases in these proteins & the encoding genes in *rd10/Sig1R*$^{-/-}$ retinas including NRF2 (3.5±0.5 fold), NQO1 (4.4±0.3 fold), HMOX1 (8.47±1.3 fold). Consistent with these findings, were significant increases in 12 miRNAs in *rd10/Sig1R*$^{-/-}$ *v. rd10,* most notably miR-199a-5p (3.5 fold), miR-214-3p (5.1 fold), and miR-155-5P (2.6 fold).
Conclusions: Absence of Sig1R in the *rd10* model of retinal degeneration is accompanied by increased ER and OX stress, which may account for accelerated PRC loss and cone dysfunction. Sig1R represents a novel, promising therapeutic target for retinopathy and, while its biological role is not certain, our data provide strong evidence for Sig1R in modulating retinal homeostasis.
Commercial Relationships: Jing Wang, None; **Sylvia B. Smith**, None
Support: NIH Grant EY014560

Program Number: 5370 **Poster Board Number:** B0527
Presentation Time: 8:30 AM–10:15 AM
N-Acetyl Cysteine Amide (NACA) Provides Greater Protection from Oxidative Damage in the Retina Than N-Acetyl Cysteine (NAC)
Lili Lu, Yogita Kanan, Peter A. Campochiaro. Ophthalmology, Johns Hopkins Univ, Baltimore, MD.
Purpose: To compare the effect of NACA and NAC on oxidative damage-induced retinal degeneration
Methods: C57BL/6 mice were untreated or treated with 7 mg/ml of NAC or NACA in drinking water and given daily intraperitoneal (IP) injections of 2.57 mg/kg of paraquat (PQ) daily for 7 days, or a single intravitreous injection of 514 μg of PQ. ERGs were performed 14 days after starting IP PQ or 7 days after single intravitreous injection of PQ. Then reduced to oxidized glutathione ratio (GSH/GSSG), protein carbonyl content, and malondialdehyde (MDA) was measured.
Results: After 14 days of IP PQ, mean scotopic b-wave amplitude (μV) (1.5 cdxs/m^2) in PQ control, NAC-treated, and NACA-treated mice (n=6) was, 216±27.7, 397±77.6, and 498±54.6, and 553±40.4 in normal mice ($p<0.05$ by ANOVA for difference between NACA and NAC) and mean photopic b-wave amplitude (1.4 cdxs/m^2) was 91±19.6, 191±50.4, and 255±50.1, and 289±40.6 in normal mice ($p<0.05$ by ANOVA for difference between NACA and NAC). Retinal levels of MDA (μg) in PQ control, NAC, and NACA groups were 9.03±0.32, 7.75±0.16, and 6.45±0.33, and 6.3±0.56 in retinas of mice not treated with PQ). Carbonyl content (nmol/mg protein) was 2.58±0.16, 1.97±0.19, 1.47±0.14, and 1.19±0.11 in normal retina. GSH/GSSG ratio was 1.37±0.15, 1.86±0.16, and 2.98±0.18, and 3.13±0.14 in normal retina ($p<0.05$ by ANOVA with Bonferroni for NACA vs NAC for each of these 3 variables). Seven days after intravitreous injection of PQ, mean scotopic b-wave amplitude (μV) in PQ control, NAC-treated, and NACA-treated mice (n=6) was 156±20.4, 267±31.4, and 503±33.6, and 583±26.2 in normal mice ($p<0.05$ by ANOVA for difference between NACA and NAC) and mean photopic b-wave amplitude (1.4 cdxs/m^2) was 97±18.4, 219±9.8, and 273±14.2, and 295±14.6 ($p<0.05$ NACA vs NAC). Retinal levels of MDA (μg) in PQ control, NAC, and NACA groups were 1339±0.39, 10.82±0.21and 8.16±0.21, and 6.9±0.14 in retinas of mice not treated with PQ. Carbonyl content (nmol/mg protein) was 3.39±0.29, 2.27±0.16, 1.69±0.18, and 1.28±0.18 in normal retina. GSH/GSSG ratio was 2.54±0.12; 3.52±0.16, and 4.15±0.1 compared with 4.41±0.15 in normal retina ($p<0.05$ for NACA vs NAC by ANOVA with Bonferroni for each of the 3 variables).
Conclusions: Oral NACA is superior to oral NAC for prevention of PQ-induced oxidative damage and loss of function in the retina.
Commercial Relationships: Lili Lu; **Yogita Kanan**, None; **Peter A. Campochiaro**, None

Program Number: 5371 **Poster Board Number:** B0528
Presentation Time: 8:30 AM–10:15 AM
Exogenous thioredoxin attenuates oxidative stress in retinal degeneration but does not modify retinal expression of other growth factors
Javier Araiz[1, 2], Roberto Gimeno-Hernández[3], Á Fernández-Carbonell[3], Francisco Bosch-Morell[3], Teresa Olivar[3], Inmaculada Almansa[3], Maria Miranda[3]. [1]ICQO, Bilbai, Spain; [2]Universidad del Pais Vaco, Leioa, Spain; [3]Universidad CEU-Cardenal Herrera, Valencia, Spain.
Purpose: Thioredoxin (TRX) is a well-known and potent antioxidant and antiinflamatory molecule. It is particularly interesting the recent finding of two proteins encoded by the same gene (Rod-derived Cone Viability Factor (RdCVF) and RdCVFL), the first one is involved in the survival of the cones in retinitis pigmentosa (RP) and does not have any thioredoxin effect, but the second one is an active thioredoxin. The purpose of this study was to examine the effect of thioredoxin administration at different time points of the characteristic retinal degeneration observed in the rd1 mice.
Methods: Animals were treated in accordance to the ARVO statement for the use of animals in ophthalmic and vision research. TRX-1 (Sigma-Adrich) was administered intraperitoneally (3 MG/KG) to rd1 mice at postnatal day 8 and 14, animals were sacrificed at postnatal days 11 and 17 respectively. In another set of experiments TRX was administered at postnatal days 11, 14, 17, 21 and 25 and sacrificed at postnatal day 28. Retinas were dissected and retinal glutathione and related metabolites were determined by high pressure liquid chromatography (HPLC). Western blot analysis was performed to assess expression of several growth factors (brain-derived neurotrophic factor (BDNF) and vascular endothelial factor (VEGF)), beclin, lysosomal associated membrane protein-2 (LAMP2A) and Atg7.
Results: TRX administration was able to attenuate retinal oxidative stress and regulate thiol metabolism, this change was more important at postnatal day 28 (after 5 doses of TRX). No changes were observed in retinal BDNF and VEGF in both control and rd1 mice after TRX treatment. Beclin, LAMP2A and Atg7 (autophagy markers) expression change in control and rd1 mice with age, but their expression was not affected by TRX treatment in any of the experimental procedures performed.
Conclusions: TRX administration may be an important tool to attenuate oxidative stress in retinal alteration, though further studies are needed to determine the effective dose and pattern of administration.
Commercial Relationships: Javier Araiz, None; **Roberto Gimeno-Hernández**, None; **Ángel Fernández-Carbonell**, None; **Francisco Bosch-Morell**, None; **Teresa Olivar**, None; **Inmaculada Almansa**, None; **Maria Miranda**, None
Support: This research has been supported by Proyecto Precompetitivo Banco Santander UCH-CEU

Program Number: 5372 **Poster Board Number:** B0529
Presentation Time: 8:30 AM–10:15 AM
Photoreceptor expression of functional Crx is required to maintain retinal pigment epithelium and retinal vasculature integrity
Jerome E. Roger, Elodie-Kim Grellier, Nareh Sahakian, Muriel Perron. NEUROPSI, CERTO / CNRS, Orsay, France.
Purpose: We previously identified a spontaneous mouse mutant with a dominant frameshift mutation in the photoreceptor-expressed gene *Crx* (*Crx*^{Rip}) causing congenital blindness due to arrested photoreceptor differentiation. Photoreceptors degenerate in *Crx*$^{Rip/Rip}$ mice while they are preserved for an extended period of time in *Crx*$^{Rip/+}$. Since they represent an excellent pre-clinical model, we initiated an in-depth characterization of both autonomous and non-autonomous effects during aging of *Crx*^{Rip} mice to better assess future therapeutic outcomes.
Methods: Histology and TUNEL staining were used to assess photoreceptor loss in *Crx*$^{Rip/Rip}$ compared to *Crx*$^{Rip/+}$ mice. Immunohistochemical analyses were performed on retinal sections and flat mount retinal pigment epithelium (RPE). The retina and the vasculature were observed by fundus imaging and angiography, respectively. Gene expression analysis was performed at P21 by RNA-Seq.
Results: In *Crx*$^{Rip/+}$ mice, limited photoreceptor apoptosis was detected up to P30. Müller cell activation was observed from P30 onward. The integrity of the retinal vasculature was preserved (up to 7 months). Fundus imaging showed a slight pigmentation of the back of the eye from P60 but a good preservation of the RPE. In contrast, a large number of photoreceptors died in *Crx*$^{Rip/Rip}$ mouse retina starting at P21 with a peak at P30 and a complete loss at 7 months. Concomitantly, Müller cell interkinetic migration occurs into the photoreceptor layer. Massive blood leakage occurred as early as P30, when the thickness of the outer nuclear layer is not significantly decreased compared to control and *Crx*$^{Rip/+}$ retina. Unexpectedly, massive RPE atrophy was observed starting at P30 from the center to the periphery. In comparison, such large defects were observed in *rd10* mice but only weeks after complete loss of rods. Using RNA-Seq data from P21, we are currently seeking for deregulated genes causing the non-cell autonomous effects observed in *Crx*$^{Rip/Rip}$ retina compared to *Crx*$^{Rip/+}$.
Conclusions: Our work revealed that lack of functional CRX in *Crx*$^{Rip/Rip}$ photoreceptors not only impairs their survival but also leads to massive RPE atrophy and retinal vasculature defects. Such non-cell autonomous defects strongly suggest that CRX regulates photoreceptor-expressed factors required for RPE and retinal vasculature maintenance and yet to be identified.
Commercial Relationships: Jerome E. Roger, None; **Elodie-Kim Grellier**, None; **Nareh Sahakian**, None; **Muriel Perron**, None
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Program Number: 5373 **Poster Board Number:** B0530
Presentation Time: 8:30 AM–10:15 AM
The role of pro-apoptotic proteins Bak and Bax in *rd1* rodent model of retinitis pigmentosa
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Purpose: In retinal degeneration, such as retinitis pigmentosa (RP), apoptosis is the common pathway involved in photoreceptor death. The mitochondrial-mediated pathway requires the activation of the Bcl-2 family members Bak and Bax, without which this form of apoptosis cannot proceed. The purpose of this study is to determine whether *Bax* and/or *Bak* have a role in the degeneration observed in the *rd1* mouse model of RP.
Methods: *Bak*$^{-/-}$and *Bax*$^{-/-}$ on a *C57Bl6/J* background were bred with *C57Bl6/J*$^{rd1/rd1}$ mice to generate mice with *C57Bl6*$^{rd1/rd1}$*/Bak*$^{-/-}$*/Bax*$^{+/+}$, *C57Bl6*$^{rd1/rd1}$*/Bak*$^{-/-}$*/Bax*$^{+/-}$ and *C57Bl6*$^{rd1/rd1}$*/Bak*$^{-/-}$*/Bax*$^{-/-}$ genotypes. Eyes were collected at post-natal day (P) 14, 18 and 21 and the structure of the retina was assessed morphometrically using 5 µm haematoxylin and eosin stained paraffin sections. *Rd1* retinas with *Bax* and *Bak* mutations were compared to *C57Bl6*$^{rd1/rd1}$and *C57bl6/J* controls (n>6).

Results: Genetic ablation of *Bak* with or without a *Bax* null allele (*C57Bl6*$^{rd1/rd1}$/*Bak*$^{-/-}$/*Bax*+/- and *C57Bl6*$^{rd1/rd1}$/*Bak*$^{-/-}$/*Bax*$^{+/+}$) resulted in a slightly thicker outer nuclear layer (ONL) compared to the age-matched *C57Bl6*$^{rd1/rd1}$ controls at P14 (27.5 ± 1 μm and 25.6 ± 0.4 μm compared to 20.9 ± 0.4 μm, $p<0.0001$), but no significant diffrences in total retinal thickness. The thickness of ONL and total retina remained significantly less than *C57BL6* mice (142.1 ± 2.4 μm and 138.7 ± 2.7 μm compared to 185.36 ± 3.1 μm- $p<0.0001$). This increase in outer retinal thickness was transient, with no significant differences found at P18 and P21 among *Bax/Bak/rd1* mutant and *rd1* mice. While deletion of both *Bak* and *Bax* is associated with neonatal lethality, a single animal survived until P13. The ONL and total retina (65.1 μm and 291.3 μm) were considerably thicker than *Bak*$^{-/-}$/*Bax*$^{+/+}$/*rd1* and *rd1*.
Conclusions: Genetic elimination of *Bak*, with or without heterozygosity for *Bax*, in the *rd1* mouse caused a transient rescue of photoreceptor cells at early stages of retinal degeneration (P14). However, deletion of both *Bak* and *Bax* resulted in rescue of cell death at P13. These data suggest both *Bak* and *Bax* are involved in photoreceptor degeneration in the *rd1* mouse, and that they may act redundantly.
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Characterization of autophagy in normal and degenerating rod photoreceptors of *Xenopus laevis* using dually fluorescent LC3 markers
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Purpose: Previous results suggest that enhanced autophagy is a feature of retinal degeneration (RD) caused by the P23H rhodopsin mutation in *Xenopus laevis* models of retinitis pigmentosa (RP). We therefore established transgenic *Xenopus laevis* lines expressing fluorescent reporters of autophagy and used them to further characterize autophagy in wild-type and degenerating rods.
Methods: We established transgenic *Xenopus laevis* lines expressing dually fluorescent mRFP-eGFP-LC3 under control of either the *Xenopus* Rod Opsin promoter (XOP) for rod-specific expression, or the hsp70 promoter (hsp70) for inducible expression. F1 animals were sacrificed at various time-points under various lighting conditions. We also generated LC3 reporter animals co-expressing a bovine P23H rhodopsin transgene. Animals were also exposed to potential inducers of autophagy, including HDAC inhibitors. Cryosectioned eyes were examined by confocal microscopy, and fluorescent puncta were quantified.
Results: Autophagic structures were observed in the inner segments of wildtype rods, suggesting autophagy is a normal feature of rod photoreceptors.
Experiments using heat-shock induction of fluorescent LC3 demonstrated that in rods, autophagosomes persist for 6 to 8 hours before fusing with lysosomes and acidifying. Acidified autolysosomes persist for 28 hours at least.
We observed diurnal non-circadian variation in the number of autophagic structures, with more autophagosomes generated under light illumination; the number of autophagic structures increased 1.9 fold after 10 hours of 1700 lux light illumination.
In rods expressing bP23H rhodopsin, the number of autophagic structures increased by 52% compared to that of wildtype rods.
The histone deacetylase (HDAC) inhibitors VPA and CI994, which can rescue retinal degeneration associated with P23H rhodopsin, promoted autophagy in rods by 23% and 37% respectively.
Conclusions: Autophagy was enhanced in rods expressing P23H rhodopsin, suggesting it plays a role in retinal degeneration; autophagy induced by light may be a related phenomenon associated with decreased stability of WT rhodopsin.
HDAC inhibitor treatment, especially CI994, promoted autophagy in rods. Upregulation of autophagy is a candidate protective mechanism by which HDAC inhibitor treatment may prevent RD associated with P23H rhodopsin in *X. laevis* models of RP.
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