Program Number: 3378
Presentation Time: 3:45 PM–4:00 PM
Expression of complement and other inflammatory pathway genes is co-ordinated in the human RPE cell transcriptome

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Purpose: To investigate the expression of complement genes in primary cultures of human RPE cells.

Methods: RPE cells isolated from adult human donor eye tissue were cultured for 7-10 days prior to RNA extraction. Quantitative polymerase chain reaction (qPCR) was used to determine the expression of a range of complement genes including complement factor H (CFH) and complement component C3 (C3). Illumina Hi-Seq RNA expression analysis was performed on RNA from 12 x RPE cultures; 6 x expressing high levels of CFH and C3 and 6 x expressing low levels.

Results: qPCR analysis of 108 primary human RPE cell cultures from different donors demonstrated that the expression levels of CFH and C3 correlate significantly (R2 = 0.521, p < 0.0001). This observation was investigated further by RNA-seq transcriptome analysis. RNA-seq detected, in total, RNA from 14,187 genes (13,141 of which were protein-coding) expressed in RPE cell cultures. Ingenuity Pathways Analysis identified 1289 genes which were differentially expressed (fold change > 50%, p < 0.05) in RPE cells which had high CFH and C3 expression, versus those with low expression. The complement pathway was shown to be the most upregulated canonical pathway, with 76% of complement genes detected being significantly increased in expression in high expressers of CFH and C3. Several canonical pathways relating to inflammation and immunology were upregulated in donors with elevated CFH/C3 expression, with the highest fold gene changes in cytokines, transcription factors, lipid metabolism and adhesion molecules. Top upstream regulators of altered canonical pathways in the high CFH/C3 group included TNF, IL1B, IFNG and TGFβ. Spearman’s rank analysis against CFH and C3 expression similarly identified members of the TNF superfamily, vasoregulatory and retinoic acid metabolism genes as significantly correlating with altered expression (p < 0.001).

Conclusions: Expression of the majority of complement genes detected in primary cultures of RPE cells is co-ordinated. This co-ordination of expression extends to genes involved in other pathways including cytokines, transcription factors, lipid metabolism and adhesion molecules.

Commercial Relationships: Paul N. Bishop, None; Selina McHarg, None; Nicole Brace, None; Alexander W. Langford-Smith, None; Richard Unwin, None; Rahat Perveen, None; Graeme C. Black, None; Anthony Day, None; Simon J. Clark, None.

Support: Supported by Fight for Sight (1517/1518) and The Macular Society (12928)
Human fetal RPE and ARPE-19 cells were maintained in β-None; None; None; None.

Method: In 3 out of 4 AMD donor eyes, LC3 immunostaining in the macular RPE was qualitatively increased relative to age-matched controls (Fig. 1).

Results: Increased levels of LC3 in the RPE of AMD eyes could result from a compensatory increase in phagocytosis in response to protein buildup or dysfunction in another component of the RPE’s proteolytic machinery. Alternatively, increased levels of LC3 could reflect a buildup of autophagosomes which are not being degraded by lysosomes. Future directions will include staining for lysosome markers in the macular RPE of AMD patients to determine if lysosome levels increase in concordance with autophagosome levels in AMD patients.

Hence, these findings indicate that PGC-1β may promote RPE oxidative damage and choroidal neovascularization.

Conclusion: All together, these findings indicate that PGC-1β exerts paradoxical functions in RPE and that pathologic induction of PGC-1β may promote RPE oxidative damage and choroidal neovascularization.

Program Number: 3382
Presentation Time: 4:45 PM–5:00 PM
High-resolution and multispectral imaging of autofluorescent retinal pigment epithelium (RPE) granules

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Purpose: To image and analyze individual RPE melanosomes (M), lipofuscin (LF), and melanolipofuscin (MLF) granules using high-resolution structured illumination microscopy (hrSIM) and confocal multispectral laser scanning microscopy (cmLSM).

Methods: Human donor RPE-flatmounts (n=35; normal macular status: 9-51yrs, 9-80yrs; age-related macular degeneration (AMD): 17) were scanned apical to basal through RPE cells at the fovea, perifovea, and near periphery using hrSIM (Zeiss Elyra.S1; ex488 nm; em488-695 nm; 390 nm step size) and cmLSM (Zeiss LSM780; ex488 nm; em 490-695 nm; 390 nm step size; 8.9 nm spectral channel width). The hrSIM and lower-resolution cmLSM images were processed using a customized algorithm to localize fluorescent objects.

Support: The Grimshaw Foundation (MSG), a Research to Prevent Blindness Unrestricted Grant (MSG), and donations to the Macular Degeneration Research, a program of the BrightFocus Foundation (MSG).
were co-registered by linear 3D registration and choice of mutual
information as the image match criterion (PMID16545965). This
results in a 1:1 mapping between the single channel hrSIM and
multichannel cmLSM data. Individual granules were segmented from
the hrSIM data by expert-guided 3D level-set segmentation. Via the
hrSIM-cmLSM mapping, the spectra of individual granules can be
extracted for quantitative analysis. M, LF, MLF granules/cell were
also counted using a custom FIJI plugin.

**Results:** HrSIM imaging and segmentation enables clear delineation
and identification of M, LF, and MLF granules (Fig. A,B). Individual
granules can be tracked in the z-direction, and size, shape,
dimensions, and intracellular position can be monitored. Each cell
contains several hundred granules. A cushion of M localizes apically,
while LF/MLF prefer basolateral accumulation. Software-assisted
mapping of corresponding z-sections (hrSIM/cmLSM) for spectral
characterization (Fig. C,D) demonstrates spectral variability among
granules.

**Conclusions:** With the combination of hrSIM and cmLSM imaging,
individual autofluorescent RPE granules can be identified, localized
in three-dimensions, and spectrally analyzed. The examination of
spectral characteristics and changes related to intracellular and tissue
localization, age, and disease status, on an individual granule basis,
might reveal LF/MLF metabolism, and help elucidate LF’s role in
human RPE physiology. Ongoing studies are examining RPE granule
properties in aging and AMD.

A. Cropped (from an overview, yellow box) and mapped hrSIM and
cmLSM images. B. Further zoom in with tagged LF (green) and MLF
(red) granules. C. The spectral information from the cmLSM data set
delivers spectra (D) for both granules.

**Commercial Relationships:** Thomas Ach, Novartis
(R); Sungmin Hong, None; Rainer Heintzmann, None;
Jost Hillenkamp, None; Kenneth R. Sloan, Spouse - Novartis
(C), Spouse - Roche (C), Spouse - Janssen (C); Neel S. Dey, None;
Guido Gerg, None; Theodore Smith, None; Christine Curcio,
Novartis (C), Janssen (C), Roche (C); Katharina Bermond, None

**Support:** Dr. Werner Jackstädt Foundation (TA); IZKF Würzburg
(KB, TA); R01 EY015520 and R01 EY021470 (RTS); EY06109
(CAC).

**Program Number:** 3383
**Presentation Time:** 5:00 PM–5:15 PM
**Mechanistic Insight into the Effects of the αCT1 Peptide on RPE
Cell Integrity in Models of AMD**

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**Purpose:** A critical target tissue in age-related macular
degeneration (AMD) is the retinal pigment epithelium (RPE), which forms
the outer blood-retina barrier (BRB). RPE-barrier dysfunction might
result from attenuation/disruption of intercellular tight junctions
(TJs). Zonula occludens-1 (ZO-1) is a major structural protein
of TJs. A connexin43 (Cx43)-based peptide mimetic, αCT1 was
developed to competitively block interactions at the PDZ2 domain
of ZO-1, thereby inhibiting ligands that selectivity bind to this
domain. We hypothesized that targeting ZO-1 signaling using αCT1
would maintain BRB integrity and reduce RPE pathophysiology by
stabilizing gap- and/or tight-junctions and reducing cytokine activity.

**Methods:** Transepithelial resistance (TER) experiments on ARPE-
19 (express Cx43) and MDCK (express negligible levels of Cx43)
monolayers were employed to study the integrity of TJs. In mice,
choroidal neovascularization (CNV) was induced using laser-
photocoagulation; this wet AMD-like model is known to lead to
VEGF-dependent loss of cell junctions as well as increased cytokine
production. Animals were injected with anti-VEGF antibody in
addition to αCT1 eye drops to measure a potential synergistic effect
on CNV development. A cytokine array assay determined the relative
quantity of cytokines and chemokines in αCT1-treated animals
compared to vehicles three and six days after CNV induction.

**Results:** Barrier dysfunction was induced by VEGF or calcium
chelation (EGTA) in ARPE-19 and MDCK cells, respectively, αCT1
ameliorated reduction in TER in both experimental paradigms. In
mice, the VEGF and αCT1 co-treatment study revealed no synergistic
effects on reducing CNV, but were equally effective on their own.
Furthermore, decreased cytokine activity was measured in αCT1-
treated animals after induction of laser-photocoagulation compared to
non-treated animals.

**Conclusions:** *In vitro* experiments using RPE and MDCK
monolayers indicated that αCT1 stabilizes TJs, independent of
its effects on Cx43. *In vivo* experiments in mouse also suggest
that αCT1 stabilizes epithelial monolayers by inhibiting the
VEGF-dependent breakdown of TJs, but in addition, reduces an
inflammatory immune response. Targeting TJ stability may serve as
a promising new treatment paradigm in retinal diseases in which the
RPE barrier is affected, either as monotherapy, or in combination
with existing therapeutics.

**Commercial Relationships:** Elisabeth Obert, None;
Gautam Ghatnaker, FirstString Research Inc. (E), FirstString
Research Inc. (P); Robert Gourdie, FirstString Research Inc. (C),
FirstString Research Inc. (P); Baerbel Rohrer, FirstString Research
Inc. (C), FirstString Research Inc. (P)

**Support:** Feldberg Endowment and NIH R01 EY019320
Program Number: 3384  
Presentation Time: 5:15 PM–5:30 PM

βA3/A1-Crystallin/SLC36A4/V-ATPase complex in the RPE is a novel therapeutic target for AMD

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Purpose: We have previously demonstrated that βA3/A1-crystallin in the RPE is localized to the lysosomal lumen, where it regulates endo-lysosomal acidification by binding to ATP6V_{A1/V_{0}}-ATPase. It is now clear that amino acids and V-ATPase are essential for mechanistic target of rapamycin, complex 1 (mTORC1) signaling. This study investigated whether βA3/A1-crystallin interacts with the amino acid transporter SLC36A4 (solute-linked carrier 36 family, member 4) and thereby regulates the amino acid pool in the lysosomes of RPE.

Methods: Co-immunoprecipitation was performed to investigate the binding between βA3/A1-crystallin and SLC36A4. An L-amino acid quantification kit was used to measure intracellular L-amino-acid concentration in RPE cells from both Cryba1^{fl/fl} (control) and Cryba1^{ KO} mice. Autophagy was induced by 24h fasting of animals or by withholding serum and L-glutamine from cell cultures to investigate the mTORC1 pathway.

Results: We showed that βA3/A1-crystallin binds to SLC36A4 in RPE cells from Cryba1^{fl/fl} mice, but not in cells from Cryba1^{ KO} mice. SLC36A4 expression both at the mRNA and protein level was down-regulated in Cryba1^{fl/fl} mice after 24h fasting. However, in Cryba1^{ KO} mice, SLC36A4 expression was up-regulated after 24h fasting. Cryba1^{ KO} mice appear to have a lower basal level of SLC36A4 expression compared to control mice. Our data also indicate that after 24h fasting the concentration of L-amino acids in RPE cells of Cryba1^{ KO} mice was elevated, whereas this increase was not seen in RPE cells of Cryba1^{fl/fl} mice. After autophagy induction, the level of phosphorylated mTORC1(Ser2448) and phosphorylated p70s6k (T421/S424) decreased in RPE cells of Cryba1^{fl/fl} mice, but stayed at consistently higher levels in RPE cells of Cryba1^{ KO} mice, suggesting mTORC1 activation and autophagy inhibition in Cryba1^{ KO} RPE cells. Moreover, the protein levels of Ragulator and Rag GTPases were generally higher in Cryba1^{ KO} mice, especially after autophagy induction.

Conclusions: We observed that βA3/A1-crystallin binds to SLC36A4, providing a direct link between amino acid availability and mTORC1 signaling. Therefore, targeting the βA3/A1-crystallin/SLC36A4/V-ATPase complex in the RPE may be an effective means of preventing or delaying the progression of age-related macular degeneration.

Support: This work was supported by funding from National Eye Institute, National Institutes of Health grant EY019037-S (DS), BrightFocus Foundation (DS) and Research to Prevent Blindness (unrestricted grant to Wilmer Eye Institute).