5879 - 1:00PM
Regulation of RPE Phagocytosis by Integrin Receptor-Tetraspanin Surface Membrane Domains
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Purpose: Retinal pigment epithelial (RPE) cells depend on αvβ5 integrin receptors and their downstream signaling pathways to maximize their phagocytic activity following circadian photoreceptor outer segment (POS) shedding in the retina. We hypothesize that the activity of αvβ5 integrin itself may be regulated in the RPE to synchronize phagocytosis. Integrin receptors may associate with proteins of the tetraspanin family in specialized membrane microdomains to control integrin signaling. Here, we test whether tetraspanins functionally interact with αvβ5 integrin in RPE cells.

Methods: RT-PCR and immunoblotting established tetraspanin expression in RPE cells in vitro and in vivo. We used confocal microscopy following live cell labeling with receptor antibodies and cholera toxin B to co-localize αvβ5 integrin and tetraspanins to plasma membrane sub-domains at the apical surface of the RPE. We used optispred gradient fractionation of RPE cell lysates and co-immunoprecipitation assays to further test subcellular co-fractionation of tetraspanins and αvβ5 integrin. We used isolated POS fragments in quantitative uptake assays to determine effects of surface cholesterol depletion, tetraspanin antibody blocking and tetraspanin overexpression on the phagocytic function of RPE cells in culture.

Results: We found that tetraspanins CD9, CD63 and CD81 localize to the apical, phagocytic surface of RPE cells in culture. CD81 partially co-localized with αvβ5 integrin. Furthermore, we identified CD31 in a complex with αvβ5 integrin in RPE cells in culture and in intact retina. CD81 co-fractionated with αvβ5 in low-density membrane domains of RPE cell lysates. Cholesterol depletion, which altered CD81 and αvβ5 surface distribution, as well as specific inhibition of CD81 reduced POS phagocytosis by human and rat RPE cell cultures. In contrast, CD81 overexpression increased POS phagocytosis.

Conclusions: Our results demonstrate that the apical plasma membrane tetraspanin CD81 regulates the phagocytic activity of the RPE. Functional interaction with tetraspanins including CD81 and microdomain recruitment may contribute to the temporal control of αvβ5 integrin receptor signaling in the RPE that is necessary for rhythmic phagocytosis of shed POS. Ongoing experiments study CD81 and integrin αvβ5 complex formation and distribution during active RPE phagocytosis in vitro and in vivo.

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5880 - 1:15PM
The Integrin Ligand MFG-E8 Promotes RPE Phagocytosis of Photoreceptor Outer Segments in vitro and in vivo
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Purpose: Retinal pigment epithelial (RPE) cells use αvβ5 integrin and Mer tyrosine kinase (MerTK) receptors to recognize and engulf shed photoreceptor outer segments (POS). Lack of αvβ5 integrin receptors in mice abolishes rhythmic POS phagocytosis causing loss of vision and lipofuscin accumulation with age. Despite their potential importance in synchronizing POS uptake in the retina, ligands activating αvβ5 integrin at the RPE apical surface are thus far unknown. Here we identify an important role for the soluble integrin ligand MFG-E8 in POS phagocytosis by RPE cells in vitro and in vivo.

Methods: We studied MFG-E8 mRNA and protein expression in rat RPE-Js, whole retina and RPE tissues as well as its secretion by RPE in culture. We compared effects of wild-type and mutant recombinant MFG-E8 on uptake of isolated POS fragments by RPE cells in culture. We performed similar phagocytosis assays using unpassaged primary RPE cells isolated from MFG-E8 null and heterozygote mice. We compared MerTK phosphorylation in MFG-E8 null and heterozygote retina in vivo by quantitative immunoblotting.

Results: We detected MFG-E8 transcript and protein in rat RPE-J cells, mouse retina and RPE. Moreover, RPE cells in culture secreted MFG-E8 constitutively and during in vitro phagocytosis. Addition of recombinant wild-type MFG-E8 to RPE cells in culture increased surface POS binding and engulfment. In contrast, recombinant mutant MFG-E8 lacking the RGD integrin recognition motif inhibited POS binding. Primary RPE cells isolated from MFG-E8 null mice showed reduced phagocytic activity compared to MFG-E8 heterozygote RPE cells despite normal epithelial morphology and apical surface localization of αvβ5 integrin receptors. Strikingly, MFG-E8 null retina in vivo failed to stimulate peak MerTK phosphorylation that follows light onset in wild-type retina.

Conclusions: Increase of POS phagocytosis by recombinant MFG-E8 in vitro suggests that this integrin ligand promotes POS binding to αvβ5 integrin receptors and subsequent activation of signaling pathways essential for particle engulfment. Furthermore, in vivo results suggest that MFG-E8 may be solely responsible for the rhythmic daily signaling of αvβ5 integrin to MerTK that synchronizes retinal phagocytosis. Studies are underway to fully elucidate the role of MFG-E8 in daily RPE phagocytosis and its regulation in the retina.

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5881 - 1:30PM
Recruitment of Annexin 2 to the Nascent Phagosome is an Essential Step in the Phagocytosis of Outer Segments by RPE Cells
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Purpose: To examine the role of annexin 2 in the formation and internalisation of phagosomes in retinal pigment epithelial (RPE) cells.

Methods: Human ARPE19 cells were cultured on the glass inserts of Matek dishes and transfected with a constitutively active annexin 2 expression construct. Cells were exposed to siRNA for annexin 2 for three days prior to experimentation. Outer segments were prepared from porcine retina and washed thoroughly with PBS, trypsinized, re-suspended in PBS and examined by imaging experiments.

Results: We detected annexin 2 transcript and protein in rat RPE-J cells, mouse retina and RPE. Moreover, RPE cells in culture secreted annexin 2 constitutively and during in vitro phagocytosis. Addition of recombinant wild-type MFG-E8 to RPE cells in culture increased surface POS binding and engulfment. In contrast, recombinant mutant MFG-E8 lacking the RGD integrin recognition motif inhibited POS binding. Primary RPE cells isolated from MFG-E8 null mice showed reduced phagocytic activity compared to MFG-E8 heterozygote RPE cells despite normal epithelial morphology and apical surface localization of αvβ5 integrin receptors. Strikingly, MFG-E8 null retina in vivo failed to stimulate peak MerTK phosphorylation that follows light onset in wild-type retina.

Conclusions: Increase of POS phagocytosis by recombinant MFG-E8 in vitro suggests that this integrin ligand promotes POS binding to αvβ5 integrin receptors and subsequent activation of signaling pathways essential for particle engulfment. Furthermore, in vivo results suggest that MFG-E8 may be solely responsible for the rhythmic daily signaling of αvβ5 integrin to MerTK that synchronizes retinal phagocytosis. Studies are underway to fully elucidate the role of MFG-E8 in daily RPE phagocytosis and its regulation in the retina.

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5882 - 1:45PM
The Effect of Oxidative Stress Induced by Photossensitized Oxidation Reaction on Phagocytic Activity of ARPE-19 Cells
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Purpose: To determine how photodynamic damage to ARPE-19 cells affects their phagocytic activity.

Methods: ARPE-19 cells were subjected to sub-lethal doses of photodynamic treatment by irradiating cells with green light in PBS for 30 min. in the presence of various concentrations of meroxyamine 540 or Rose Bengal. The survival of cells was determined by standard cellular techniques such as MTT reduction assay, trypan blue exclusion method and the release of LDH. In all experiments, the cell survival was in the range of 90-100%. After exposure to FITC-labeled latex beads or bovine ROS, cells were thoroughly washed with PBS, re-suspended in PBS and examined by flow cytometry. To determine whether the photodynamic treatment resulted in a measurable oxidation of the cell lipids, chloroform-methanol extracts of the control and treated cells were examined by HFLC-EC(H)g set for detecting characteristic cholesterol hydroperoxides.

Results: The photodynamic treatment of ARPE-19 cells induced a dose-dependent inhibition of both their specific and non-specific phagocytic activity. The extent of the inhibition of the specific phagocytic activity was significantly higher than that of the non-specific phagocytic activity. At the cell survival close to 100%, the photodynamic treatment resulted in 40-60% inhibition of the phagocytosis. Our preliminary results indicate that the photodynamic treatment of ARPE-19 cells was accompanied by a measurable formation of singlet oxygen- and free radical-dependent cholesterol hydroperoxides.

Conclusions: Photodynamic damage to ARPE-19 cells is accompanied by a distinct inhibition of the cell phagocytic activity. Our data suggest that the degree of phagocytosis inhibition can be correlated with the extent of the cell lipid peroxidation. Therefore, it can not be ruled out that age-related increase in the membrane lipid peroxidation contributes to the reduction of the cell phagocytic activity.

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Altered Cholesterol Homeostasis in the Retinal Pigment Epithelium in the Presence of the Lipofuscin Fluorophore A2E


Purpose: Accumulating evidence suggests that cholesterol and lipid metabolism in the RPE is important for photoreceptor health and function. Further, histopathological studies of retinas from donors affected with age-related macular degeneration (AMD) indicate that alterations in RPE cholesterol homeostasis may contribute to disease progression. Here, we investigated the regulation of cholesterol trafficking in the RPE in the presence or absence of the lipofuscin component A2E.

Methods: Determinants of cholesterol ester (CE) metabolism and cholesterol efflux were studied in human ARPE-19 cells and in primary cultures of bovine RPE. Bodipy-labeled cholesterol ester was used as a probe to monitor the kinetics of CE degradation. Filipin staining and thin-layer chromatography were used to assess intracellular and total cholesterol pools, respectively.

Results: The presence of A2E in RPE endolysosomes inhibited CE metabolism. As exogenous cholesterol esters are hydrolyzed by acid lipase in the low pH of late endosomes/lysosomes, we first asked whether A2E affected either of these two determinants. Amazingly, both late endosomal/lysosomal pH and acid lipase activity were normal in A2E-loaded cells. We then studied cholesterol trafficking in the RPE because cholesterol efflux from late endosomes is crucial for driving further CE hydrolysis in these organelles. Three independent lines of evidence showed that cholesterol transport out of late endosomes is essential for CE metabolism in RPE cells: (i) pharmacological inhibition of cholesterol efflux from late endosomes inhibited CE hydrolysis; (ii) activation of peroxisome proliferator activator gamma (which increases cholesterol efflux via increased expression of the ABC cholesterol transporters) increased CE degradation in A2E-loaded cells; and (iii) acid lipase activity was inversely proportional to the amount of free cholesterol (product) in the RPE. Our findings suggest that alterations in RPE cholesterol homeostasis may contribute to disease progression.

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