Program Number: 1682
Presentation Time: 11:15 AM–11:30 AM
Quantitative Proteomic Analysis of Lipid-Raft Domains from Lens Fiber Cells
Kevin L. Schey, Zhen Wang. Biochemistry, Vanderbilt University, Nashville, TN.

Purpose: Lipid rafts are domains within cell membranes that are rich in cholesterol and sphingolipids and serve to concentrate specific protein activities. Lens fiber cell membranes contain high concentrations of sphingomyelin and cholesterol. Lipid rafts could play critical roles in regulating protein function and in maintaining lens transparency. The purpose of this study is to characterize lens proteins that are localized in raft domains and in non-raft membranes using quantitative proteomic methods.

Methods: Bovine lenses were decapsulated and dissected into cortex and nucleus regions. The water-insoluble fraction from each region was divided into two samples and one was treated with methyl-β-cyclodextrin to deplete cholesterol and disrupt rafts. Samples were incubated with detergent (1% Brij 98, 35 mM octyl-glucoside, 600 mM NaCl) at 4 °C for 30 min. and subjected to sucrose density gradient centrifugation. Proteins from low density to high density were isolated and precipitated using chloroform/methanol, digested by trypsin, and analyzed by LC-MS/MS. Raft fractions were identified based on the enrichment of lipid raft markers. Using the iTRAQ quantitative proteomics method, raft proteins were distinguished from non-raft membrane proteins based on abundance changes upon cholesterol-depletion.

Results: Lipid raft fractions were identified as fractions recovered from the interface of the 5% and 35% sucrose layers since raft markers such as flotillin, erlin and prohibitin, were enriched in these fractions and moved to higher density fractions after cholesterol depletion. Additional proteins identified as highly enriched in the raft fractions included caveolins, AQP5, Lim2, and Voltage-dependent calcium channel subunit alpha-2/delta-1. Some proteins were detected in both raft and non-raft fractions including AQP0, neural cell adhesion molecule, Voltage-dependent anion-selective channel protein 1, paralemmin, ras-related C3 botulinum toxin substrate 1, and protein kinase C.

Conclusions: Quantitative proteomic analysis revealed components of the lens fiber cell lipid raft-like, detergent resistant membranes. These data provide clues to protein sorting into distinct membrane microdomains and possibly how protein function is altered depending on the lipid environment.

Commercial Relationships: Kevin L. Schey, None; Zhen Wang, None

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Program Number: 1683
Presentation Time: 11:30 AM–11:45 AM
Ankyrin-B Haploinsufficient Lenses Uncover the Importance of Membrane Subdomain Organization for Fiber Cell Hexagonal Packing and Mechanical Properties
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Purpose: Fiber cell hexagonal symmetry, membrane organization and tensile properties are considered to be critical for lens architecture, transparency and deformability. The molecular determinants of membrane organization that govern these lens characteristics, however, are not well defined. In this study, we...
Results: Interlocking protrusions in young differentiating fiber cells developed normally but showed minor abnormalities at approximately 50 μm deep from the surface in the absence of AQP0 in all ages studied. Strikingly, interlocking protrusions in maturing fiber cells specifically underwent uncontrolled elongation, deformation and fragmentation while the cells still possessed fairly normal configurations in the early process. These changes eventually resulted in fiber-cell separation, breakdown and cataract formation in the lens core. Immunolabeling at the light and electron microscopic levels demonstrated that AQP0 was particularly enriched in interlocking protrusions in wild-type lenses.

Conclusions: This study suggests that AQP0 exerts its primary adhesion or suppression role specifically to maintain the normal structure of interlocking protrusions that is critical to the integrity and transparency of the lens.

Commercial Relationships: Woo-Kuen Lo, None; Sandip K. Biswas, None; Lawrence Brako, None; Alan Shiel, None; Sumin Gu, None; Jean X. Jiang, None
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Program Number: 1685
Presentation Time: 12:00 PM–12:15 PM
Extracellular loop positive charges of lens aquaporin 0 play a significant role in cell-to-cell adhesion
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Purpose: Investigate the role of extracellular loops of Aquaporin 0 (AQP0) on cell-to-cell adhesion (CTCA) function.

Methods: Extracellular loops A, and C of mouse AQP0 were substituted with those of AQP1 through polymerase chain reaction using specific oligonucleotide primers. We have also replaced the positively charged residues in loop A of AQP0 with structurally compatible neutral residue to create R33Q and H40Q mutants. Water permeability (Pw) and CTCA properties of the chimeric and mutant AQP0 were studied by expressing them in Xenopus oocytes through cRNA injection and by transecting into adhesion-deficient mouse fibroblast L-cells, as appropriate. Pw was studied using the shrinking and swelling assay. CTCA was tested using a method devised by our laboratory. Intact (wild type) AQP0 and E-cadherin served as positive controls while AQP1 served as a negative control for CTCA studies.

Results: AQP0-AQP1-loop A chimera trafficked to the plasma membrane like the intact AQP0 while AQP0-AQP1-loop C chimera did not. Pw of AQP0-AQP1-loop A was 45.3 ± 5.8 μm/s while that of intact AQP0 was 46.4 ± 3.9 μm/s; Pw of control oocytes injected with distilled water was 10.9 ± 2.5 μm/s. CTCA assays showed that the adhesion property of AQP0-AQP1-loop A chimera was significantly reduced (24%; P< 0.001) compared to that of intact AQP0. Mutants AQP0-R33Q and AQP0-H40Q trafficked and localized at the plasma membrane like the intact AQP0. Functional studies conducted in Xenopus oocytes showed no significant difference (P>0.05) in Pw of AQP0-R33Q (45.7 ± 6.9 μm/s) and AQP0-H40Q (44.7 ± 7.2 μm/s) compared to that of intact AQP0. However, the CTCA property of AQP0-R33Q (~22%) and AQP0-H40Q (19%) was significantly reduced (P< 0.001) in comparison to that of intact AQP0.

Conclusions: The data suggest that extracellular loop A substitution or point mutation of AQP0 might not have caused significant alterations in protein folding since there was no obstruction in protein trafficking or Pw but extracellular loop C substitution inhibited protein trafficking suggesting alteration/s in protein folding. Reduction in the CTCA observed for AQP0-AQP1-loop A chimera as well as AQP0-R33Q and AQP0-H40Q mutants suggest that the
conserved positive charges in the Loop A may be critical for the CTA function of AQP0.

**Commercial Relationships:** Kulandaiappan Varadaraj, None; Sindhu Kumari, None

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**Program Number:** 1686
**Presentation Time:** 12:15 PM–12:30 PM

**A Role for Microtubules in Lens Fiber Cell Elongation and Lens Morphogenesis**

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**Purpose:** Tissue development and regeneration involve high-ordered morphogenetic processes that are governed by elements of the cytoskeleton in conjunction with cell adhesion molecules. Such processes are particularly important in the lens whose structure dictates its function. Microtubules have many roles in the cell, among them as determinants of directional migration and as the highways for vesicle transport. Here we investigated the possible role of microtubules and their interactions with N-cadherin in providing directionality to fiber cell elongation.

**Methods:** Co-immunoprecipitation analysis was performed on chick embryo lenses microdissected into four distinct zones of differentiation to analyze association of tubulin/acetylated tubulin with N-cadherin. The role of tubulin in fiber cell elongation was examined by treating E10 lenses in organ culture with the microtubule depolymerizing drug nocodazole. Treated lenses were microdissected as above and immunoblotted for tubulin expression/acetylation. Lens cryosections were labeled for α-tubulin, acetylated tubulin, N-cadherin, and/or F-actin.

**Results:** N-Cadherin interacts with tubulin primarily in the cortical fiber zone, where lens fiber cells elongate. No association was detected between N-cadherin and the acetylated form of tubulin. Disassembly of microtubules with nocodazole affected fiber cell elongation and directionality. High doses of nocodazole also affected interactions between fiber cells and epithelial cells along the epithelial-fiber interface (EFI). These effects were accompanied by changes in levels of F-actin and increased localization of N-cadherin along the EFI. These results provide the first demonstration of a role for microtubules in lens fiber cell elongation, in lens morphogenesis and in the maintenance of lens tissue integrity.

**Conclusions:** Microtubules have an important role in the determination of proper lens morphogenesis.

**Commercial Relationships:** Caitlin Logan, None; Liping Zhang, None; A S. Menko, None

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**Presentation Time:** 12:30 PM–12:45 PM

**Lens fiber cell denucleation and cataractogenesis**

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**Purpose:** To investigate the mechanisms that control nuclei distribution, chromatin behavior or denucleation process during lens fiber cell differentiation and maturation in normal and cataractous mice caused by various mutations, such as alpha-crystallin gene mutations, intermediate filament protein CP49 gene deletion and connexin gene knockouts. Moreover, to evaluate how aging affects fiber cell differentiation and maturation.

**Methods:** Transgenic expression of histone 2B (H2B)-GFP and DNA-staining dyes were used to monitor the distribution and disassembly of fiber cell nuclei and chromatin behavior in live lenses by 3-dimensional confocal laser microscopic imaging. Western blotting and immunostaining were performed to elucidate the molecular and cellular changes.

**Results:** Distinct nuclei distribution was observed along the anterior and posterior equatorial panel during fiber cell differentiation and elongation. Chromatins in fiber cell nuclei displayed sequential changes during fiber cell maturation. All inner fiber cells lost their nuclei at about 150 μm distance from the lens surface. However, denucleation of fiber cells often occurred long before reaching the nuclei-free zone. During denucleation, H2B-GFP proteins were diffused along both anterior and posterior directions and then were evenly distributed with cell boundaries of inner fibers. Altered distribution of fiber cell nuclei and aberrant distribution and/or aggregation of the H2B-GFP proteins were detected in different cataractous lenses. In addition, aberrant aggregation of H2B-GFP proteins was also detected in newly formed cortical mature fiber cells in aged wild-type lenses.

**Conclusions:** This work reveals that inner fiber cell denucleation occurs at around 100-150 μm distance from the lens surface rather than an old assumption that fiber cell denucleation proceeds only in several cell layers before the nuclei-free zone. Aging impairs fiber cells denucleation by blocking the disassembly of nuclear proteins and induces the aggregation of nuclear proteins such as H2B. Different types of cataracts caused by various gene mutations are associated with uniquely altered distribution and/or disassembly of fiber cell nuclei. Coordinated functions of intercellular gap junction communication, intermediate filaments and alpha-crystallin chaperons precisely regulate the distribution and disassembly of fiber cell nuclei during cell maturation.

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