432 Cell biology of gap junctions in the eye: Connexin signaling in health and disease - Minisymposium

Wednesday, May 04, 2016 11:00 AM–12:45 PM
615/617 Minisymposium
Program #/Board # Range: 4751–4756
Organizing Section: Lens
Contributing Section(s): Cornea, Visual Neuroscience

Program Number: 4751
Presentation Time: 11:03 AM–11:20 AM
Wound repair is regulated by the gap junction protein connexin43
Paul Lampe1, 2. 1 Translational Research Program, Fred Hutchinson Cancer Research Center, Seattle, WA; 2 University of Washington, Seattle, WA.

Presentation Description: Gap junctions composed of connexin43 (Cx43) are present in most of the epithelial tissues of the eye including the lens, cornea, ciliary body and retina. During epithelial wound repair, gap junctions composed of Cx43 are highly regulated. They are necessary to initiate early steps in wound healing but later are downregulated to allow proper proliferation and migration. Thus, a variety of strategies have been devised to speed wound healing via changing gap junctional communication and varying the levels/function of Cx43. This presentation will show how Cx43 is regulated during wounding. Specifically it will show how sequential phosphorylation at specific serines and tyrosines in the C-terminal tail via Akt, PKC, MAPK and Src occurs during response to epithelial wounding, and the different effects of the activation of these kinases on Cx43 and gap junctional communication and turnover.

Commercial Relationships: Paul Lampe, None

Program Number: 4752
Presentation Time: 11:20 AM–11:37 AM
Connexin 43 in corneal and retinal injury and disease

Presentation Description: The two hemichannels (connexons) that make up a gap junction channel need to have low opening probability prior to docking with a neighboring cell. Open hemichannels form a large, relatively non-selective membrane channel directly exposing the cell cytoplasm to the extracellular milieu. Under injury and disease conditions gap junction coupling may be reduced, but both Connexin 43 expression and hemichannel opening are increased. The hemichannel has therefore been referred to as a pathological pore and it is a key component in the inflammasome pathway during both its initiation and propagation. In this seminar the effect of pathological opening of connexin hemichannels in ocular injury and the benefits of intervention are described. In a rat cornea photorefractive keratectomy model, down regulation of Connexin 43 expression prevents edema and myofibroblast activation. In a rabbit trabeculectomy model, Connexin 43 down regulation reduces inflammation and scarring which can result in cannula block. In the bright light retinal injury model intravitreal injection of connexin hemichannel blocking peptides reduces inflammation and significantly improves functional outcomes (electroretinograms). In a rat retinal-ischemia reperfusion model systemic delivery or intravitreal injection of hemichannel blocking peptides prevents loss of vascular integrity, reduces glial cell activation and cuts downstream retinal ganglion cell (neuron) loss by over two thirds. These data, correlated with ex vivo human donor tissue analysis, further indicates that loss of vascular integrity may be common component in ocular disease, including retinal pigment epithelium loss, age related macular degeneration and diabetic retinopathy.

Commercial Relationships: Colin Green, CoDa Therapeutics, Inc.

Program Number: 4753
Presentation Time: 11:37 AM–11:54 AM
Neuronal Gap Junctions form Novel Therapeutic Targets for Neuroprotection in Glaucoma
Stewart A. Bloomfield. Biological and Vision Sciences, State University of New York College of Optometry, New York, NY.

Presentation Description: The death of retinal ganglion cells is a hallmark of glaucoma, which leads to diminished visual function. In addition to the well-characterized intracellular cascades responsible for primary cell death, intercellular movement of toxic molecules via gap junctions between dying neurons and their neighbors appears to play a crucial role in the progression of cell death across the retina. This finding suggests that targeting neuronal gap junctions forms a novel therapeutic strategy for neuroprotection in glaucomatous retinas to thereby preserve visual function.

Commercial Relationships: Stewart A. Bloomfield, None

Program Number: 4754
Presentation Time: 11:54 AM–12:11 PM
Regulation of Gap Junctional Coupling between Photoreceptors
Christophe P. Ribelayga. University of Texas Medical School at Houston, Houston, TX.

Presentation Description: Among retinal photoreceptors, gap junction coupling occurs between photoreceptors of the same type (homologous coupling; cone-cone, rod-rod) and of different types (heterologous coupling; rod-cone). Distinct functional roles have been elucidated for photoreceptor homologous and heterologous coupling, and the gap junction expressed in cones has been identified as connexin35 (Cx35) in cold-blooded vertebrates and its ortholog Cx36 in mammals. However, despite decades of research on the molecular basis and function of photoreceptor coupling, some of its fundamental aspects remain unknown, in particular, if Cx36 is the rod connexin in mammals and the plasticity of coupling suggested by indirect measures but not yet demonstrated by direct measurements of the junctional conductance. Through a recent technical advance, we have developed the capability to record from pairs of adjacent photoreceptors in the mouse retina using a perforated-patch clamp technique. This permits direct measurement of the gap junction conductance between two coupled cells. We have also developed several mouse models that are deficient for Cx36, specifically in rods or cones. In this presentation, I will review recent research in the field and present our findings in the conditional knock out models. As a whole, current research in this area supports the view that 1) Cx36 is the rod connexin; and 2) photoreceptor coupling is not static but tuned with precision according to the time of day and/or environmental lighting conditions.

Commercial Relationships: Christophe P. Ribelayga, None

Program Number: 4755
Presentation Time: 12:11 PM–12:28 PM
Differences in second messenger permeability of lens connexins
Thomas W. White. Stony Brook University, Stony Brook, NY.

Presentation Description: Intercellular channels assembled from different connexin proteins have unique molecular permeability. Gap junctions have been historically described as relatively non-selective, permeable to a wide variety of molecules with a size
smaller than ~1200 Daltons. However, experiments carefully examining the movement of ions and dyes between cells expressing different connexins have revealed that there are connexin-dependent differences in permeation. We have extended this type of analysis to the signaling molecules cAMP, IP$_3$, and Ca$^{2+}$. Most cell types express more than one connexin, and the permeability data suggest that loss of a single connexin species within a given eye tissue (i.e. by genetic mutation or gene knockout) not only changes the macroscopic levels of communication, but also significantly alters the range of molecules being exchanged between the coupled cells.

**Commercial Relationships:** Thomas W. White, None

**Program Number:** 4756

**Presentation Time:** 12:28 PM–12:45 PM

**Gap junctions provide metabolic coupling and adhesive niches for the morphogenesis of lens fibers**

Xiaohua Gong. School of Optometry and Vision Science Program, University of California at Berkeley, Berkeley, CA.

**Presentation Description:** Purpose. To test a hypothesis that gap junctions and the cytoskeleton synergistically control lens metabolism and the surface interlocking structure of fiber cells to establish lens transparency and stiffness. To identify and characterize genetic variances in common mouse strains that influence the severity of cataracts in Gja3 knockout (-/-) mice.

Methods. Morphogenesis of lens fiber cells was characterized by confocal imaging and electron microscopic analysis. Lens phenotypes were evaluated by slit-lamp *in vivo* and by light scattering measure *in vitro*. Linkage markers were used for mapping genetic variances. Lens proteins were characterized with various biochemical and cellular techniques.

Results. Disrupted morphogenesis of mature fibers includes a loss of interlocking structures such as tongue-and-groove in Gja3-/- lenses. Both periaxin (prx) gene variances and CP49 deletion between C57BL/6J (B6) and 129SvJae (129) mouse strains manifested the severity of nuclear cataracts and lens stiffness. 129-Prx proteins were extensively associated with the membrane/F-actin network of peripheral and interior fibers while B6-Prx was restricted to peripheral differentiating fibers. The 129 Prx/F-actin complexes impaired the organization of surface interlocking structure from the vertices of hexagonally shaped fibers to disrupt organization of maturing fibers.

Conclusions. Lens gap junctions serve dual functions: one is to provide a pathway for metabolism and the other is to act as adhesion niches for the arrangement of surface interlocking structures required for lens transparency and stiffness. 129-Prx gene variance and CP49 deletion disrupted F-actin network or beaded intermediate filament to alter surface morphogenesis of lens fibers. Dysfunctional gap junctions and aberrant cytoskeletons synergistically impair lens homeostasis and the interlocking structures of maturing fibers, which trigger fiber cell degeneration mediated by calcium-dependent proteases, ultimately leading to dense nuclear cataracts.

**Commercial Relationships:** Xiaohua Gong, None

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