Hot Topics in Glaucoma 254

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Y. Du a, M.M. Mann b, D.S. Roh b, M.L. Funderburgh a, J.S. Schuman a, J.L. Funderburgh a, a Ophthalmology, University of Pittsburgh, Pittsburgh, PA;

Purpose: Glaucoma is a leading cause of blindness. Reduced cellularity within the trabecular meshwork (TM) is observed with age and correlates with increased outflow resistance and elevated IOP, important risk factors for glaucoma. Cell-based therapy of TM for restoration of aqueous outflow has not been fully explored. Such therapy would require cells capable of assuming a TM phenotype. This study aimed to identify and culture stem cells from TM to and to explore the potential of such cells to differentiate into functional TM cells.

Methods: Human corneal-scleral tissues were immunostained as whole-mounts using stem cell markers: ABCG2, PAX6, Ankyrinh, Nestin; or TM cell markers: Aquaporin1, MGP, NCAM, CHI3L1, NCAM, and TIMP3. Fresh TM was dissected for explant culture and propagated at low cell density to promote stem cell expansion. Immunofluorescent staining, qPCR, and immuno blotting were used to assess cell phenotypes. Side-population cells were isolated by fluorescence-activated cell sorting using DyeC. Viability of TM side-population cells induced to differentiate to TM using aqueous humor or fetal bovine serum. Differentiation was confirmed by expression of TM-specific proteins and phagocytic function.

Results: Cells immunostaining for stem cell proteins were not limited to the insert region but found throughout the TM. Passaged TM cells contained a side-population sensitive to fumitremorgin C, which grew clonally, exhibiting a stem cell phenotype. Expanded stem cells maintained stem cell phenotype but lost TM-specific gene expression. Stem cells cultured in aqueous humor or fetal bovine serum lost stem cell marker expression and markedly upregulated TM tissue-specific gene expression. Phagocytic function also increased as the cell differentiated to TM.

Conclusions: Stem cells are found distributed throughout human TM cells. These cells are distinct from differentiated TM cells, can be isolated as a side population using cell sorting, and can be expanded in culture maintaining their stem cell phenotype. These stem TM cells can be induced to express differentiated TM marker genes and exhibit phagocytic activity typical of TM cells.

CR: Y. Du, None; M.M. Mann, None; D.S. Roh, None; M.L. Funderburgh, None; J.S. Schuman, None; J.L. Funderburgh, None.

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255. Characteristics of Trabecular Meshwork Stem Cells

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Optic Nerve Head (ONH) Lamina Cribrosa Insertion Migration and Pialization in Early Non-Human Primate (NHP) Experimental Glaucoma

H. Yang, G. Williams, J. Downs, I. Sigal, M. Roberts, J. Grimm, H. Thompson, C. Burgoyne. Devers Eye Institute, Portland, OR; School of Public Health, LSU/HSC, New Orleans, LA.

Purpose: To geometrically characterize the lamina cribrosa insertion into the scleral canal wall in normal and early experimental glaucoma (EENG) NHP ONH to test whether regional posterior (outward) migration of the lamina cribrosa insertion occurs in NHP EEG leading to pialization (laminar beam insertion into the pia instead of the sclera).

Methods: All NHP ONHs from both eyes of 12 normal (N) and 9 unilateral EENG NHPs were perfusion fixed, serial sectioned, 3D reconstructed and delineated. Regional and overall values for the following distances were calculated for each ONH: anterior scleral canal opening to anterior laminar insertion distance (ASCO-ALI); anterior to posterior laminar insertion distance (ALI-PLI); and posterior scleral canal opening to posterior laminar insertion distance (PSCO-PLI). The outward ALI and PLI migrations were defined to be statistically significant changes in ASCO-ALI and PSCO-PLI distances in the outward direction. Data were pooled into 4 groups based on perfusion IOP and eye status: N/10/10 (n=6); N/30/30/45 (n=6); EGG/10/10 (n=3) and EGG/30/45/45 (n=6). Treatment and region effects were accessed within each NHP and overall by ANOVA.

Results: ALI was outwardly migrated in the EGG 30/45 group (P<0.0001). PLI was outwardly migrated in EGG 10/10 (22 mm) and EGG 30/45 (44 mm) (P<0.0001) groups. Laminar insertion thickening (ALI-PLI expansion) was significant (32 mm in both EEG groups). These changes of EEG eyes held true in a majority of EGG monkeys. Regionally, 3 of the 9 EEG eyes demonstrated outward PLI migration to the degree of posterior lamina pialization (Fig 1) in at least 2 adjacent regions.

Conclusions: Outward migration of the ALI may be the result of physical trauma and/or remodeling of the anterior laminar beams. Outward migration of the PLI may be the result of laminar remodeling that includes retrolaminar septal recruitment. (Roberts et al, IOVS, 2009). The implications of these findings on the pathogenesis of ONH splinter hemorrhages, cupping, blood flow alterations and axon loss will be discussed.

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