POSTER 39

GENE TRANSFER TECHNOLOGY: A TOOL FOR STUDYING GENE FUNCTION AND ROLE IN CORNEAL PATHOGENESIS

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Purpose: Transforming growth factor β (TGFb) is associated with many corneal pathologies, diseases and dystrophies. The function of TGFb in adult corneas cannot be studied using conventional transgenic approach because TGFb1 and TGFb2 deficient transgenic animals suffer multiple inflammatory diseases, severe developmental defects, and death by 3-4 weeks of age. This study tested the hypothesis that selective tissue-targeted gene transfer approaches will permit examination of TGFb gene function in the adult cornea without altering TGFb expression in vital organs.

Methods: Female black C57 mice were used. Animals were anesthetized with intramuscular injection of ketamine (130mg/kg) and xylazine (8.8mg/kg). Topical solution of 1% proparacaine hydrochloride was instilled to each eye for local anesthesia. Two microliters of AAV5 naked vector or expressing TGFb1 gene (titer 10^9 genomic copies/µl) was administered into the cornea. Eyes were collected at various time-points post-AAV application. Visual eye exam, stereomicroscopy, and slit-lamp biomicroscopy were used to monitor corneal health. Immunocytochemistry, western blotting and real-time PCR techniques were used to study corneal tissues.

Results: Tissue-selective targeted delivery of TGFβ1 gene via AAV5 induced haze and opacity in the mouse cornea in a time-dependent manner as evident from slit-lamp biomicroscopy and preliminary immunocytochemistry experiments. Experiments are underway to study expression of collagens, extracellular matrix proteins and signaling pathways linked to TGFβ-mediated pathologies.

Conclusions: Tissue-specific controlled gene transfer approaches are a powerful tool to study gene function and identify therapeutic targets for mechanism-based innovative therapies to treat and prevent corneal abnormalities.