POSTER 100

SMAD-SIGNALING INHIBITION: POTENTIAL FOR DEVELOPING NEWER TREATMENTS FOR CORNEAL FIBROSIS

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Purpose: Transforming growth factor β (TGFβ) is known to cause fibrosis in the cornea following injury and/or infection. Effective reduction in corneal fibrosis has been reported by inhibiting TGFβ activity. However, associated molecular mechanism is still unknown. The aim of study was to test the hypothesis that the alteration in SMAD signaling is a novel approach for treating corneal fibrosis using an established *in vitro* model.

Methods: Primary corneal fibroblast (HSF) cultures generated from donor human corneas were exposed to TGFβ1 (1ng/ml). To test the hypothesis gene transfer approach was used. Decorin (a natural inhibitor of TGFβ) cDNA was introduced into HSF with non-viral (lipids) or viral (AAV5) vector. Real-time PCR, immunoblotting and/or immunocytochemistry measured the markers of fibrosis (αSMA, F-actin and fibronectin). Immunoblotting and/or immunocytochemistry examined the non-phosphorylated and phosphorylated forms of SMAD2 and SMAD7 proteins.

Results: TGFβ1 treatment significantly induced myofibroblast formation and fibrosis in the HSF as shown by mRNA and protein levels of αSMA (myofibroblasts marker). Decorin-transfected HSF showed significant decrease in TGFβ1-induced fibrosis in the human cornea *in vitro*. Detection of significant increase in Smad7 and decrease in Smad2 levels in decorin-overexpressing clones was detected compared to naked vector-transfected clones. The effects were more pronounced in AAV-transduced clones than the plasmid-transfected clones, most likely due to the higher transgene delivery with AAV than the plasmid vector.

Conclusions: Inhibition of SMAD signaling pathway can be used for developing mechanism-based newer anti-fibrotic therapies for the cornea.