

## POSTER 100

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

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**Purpose:** Transforming growth factor  $\beta$  (TGF $\beta$ ) is known to cause fibrosis in the cornea following injury and/or infection. Effective reduction in corneal fibrosis has been reported by inhibiting TGF $\beta$  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 TGF $\beta$ 1 (1ng/ml). To test the hypothesis gene transfer approach was used. Decorin (a natural inhibitor of TGF $\beta$ ) 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 ( $\alpha$ SMA, F-actin and fibronectin). Immunoblotting and/or immunocytochemistry examined the non-phosphorylated and phosphorylated forms of SMAD2 and SMAD7 proteins.

**Results:** TGF $\beta$ 1 treatment significantly induced myofibroblast formation and fibrosis in the HSF as shown by mRNA and protein levels of  $\alpha$ SMA (myofibroblasts marker). Decorin-transfected HSF showed significant decrease in TGF $\beta$ 1-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.