Role of Inhibitor of Differentiation (Id) Proteins in Fibrosis of the Human Cornea

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Background
- Ocular trauma and infection result in corneal fibrosis and vision loss in human and veterinary patients.
- Transforming Growth Factor β1 (TGFβ1) plays a major role in fibrosis and in the differentiation of fibroblasts to myofibroblasts.
- Inhibitor of differentiation (Id) genes are known to regulate cellular differentiation. Expression of Id protein is modulated in human corneal fibroblasts (HCF) by TGFβ1.
- However, the role of Id genes in HCF differentiation and corneal healing is still unknown.

Hypothesis
The purpose of the present study is to understand how Id proteins regulate corneal fibrosis in the presence of TGFβ1. We hypothesize that Id2 and Id3 over-expression in HCF functions as a molecular switch and drives cellular fate for corneal fibroblast transdifferentiation.

Experimental Design
- Human corneal fibroblasts were transfected with either Lipofectamine 3000 or jetPEI Nanoparticle systems to overexpress Id2 or Id3 with the vector as an internal control.
- Transfected cells were selected for using the antibiotic G418.
- Nontransfected HCF and Lipofectamine transfected cells were split and grown in serum-free medium with or without TGFβ1 for 72 hours.
- RNA was isolated for the production of cDNA for all cells. Quantification of expression of Id 2 and Id3 genes as well as fibrotic markers αSMA, XYL, Collagen I, III, and β-Acin was done via qRT-PCR.

Preliminary Data

Id Gene Expression

Figure 1: Images A, B, and C show the expression of Id2 via immunofluorescence staining in a normal human corneal cross section.

Id Gene Expression with TGFβ

Figure 2: Expression of all mammalian Id genes were analyzed via qRT-PCR in human corneal fibroblasts (HCF) as is shown in the representative image with β-actin as the internal control (A). The average expression of each Id gene was also plotted (B).

Results
Fibrotic Marker Analysis of TGFβ Treatment

Figure 3: Id genes were differentially expressed when treated with TGFβ in a time-dependent manner. This is shown for Id2 (A) and Id3 (B) expression which was analyzed via qRT-PCR.

Conclusions & Future Studies
- Comparing the two transfection systems, the Lipofectamine 3000 system, in our study, had a higher transfection rate than the jetPEI Nanoparticle system.
- For the TGFβ1-treated cells, the control showed an increase in fibrotic markers as expected. The Id2 overexpressing cells showed a decrease in fibrotic markers where as Id3 overexpressing cells showed little effect.
- Future studies include: quantifying the effects of Id2 and Id3 overexpression when cells are treated with TGFβ1 at 24, 48 and 72 hours of treatment via qRT-PCR, immunocytochemistry, and cell migration studies at all time points.

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- Zoets Animal Health

Reference