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 cell groups. Quantification of expression of Id 2 and Id3 genes as well as fibrotic markers αSMA, XYLT, Collagen I, III, and β-Actin was done via qRT-PCR.

Preliminary Data

Normal Human Cornea

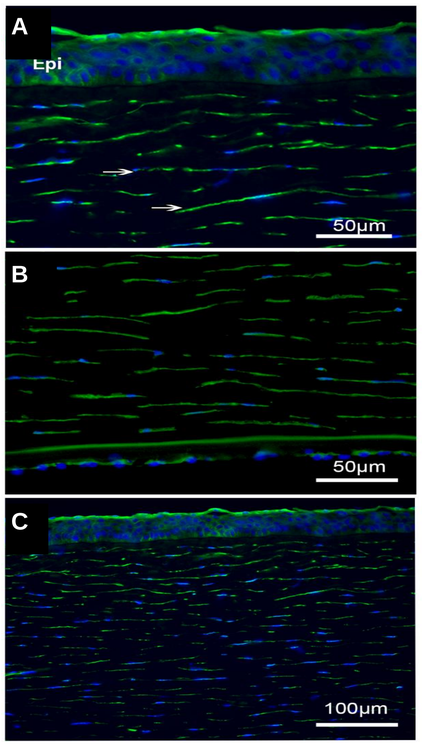

Figure 1: Images A, B, and C show the expression of Id2 via immunofluorescence staining in a normal human corneal cross section.

Id Gene Expression

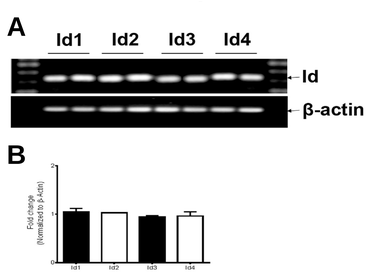

Figure 2: Expression of all mammalian Id genes were analyzed via qRT-PCR in human corneal fibroblasts (HCF) as is shown in the representative image with B-actin as the internal control (A). The average expression of each Id gene was also plotted (B).

Id Gene Expression with TGFβ

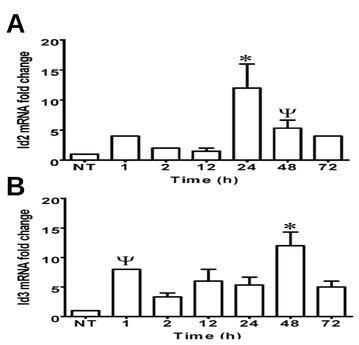

Figure 3: Id genes were differentially expressed when treated with TGFβ in a time-dependent manner. This is shown for Id2 (A) and Id3 (B) expression which was analyzed via qRT-PCR.

Differentiation of Fibroblasts

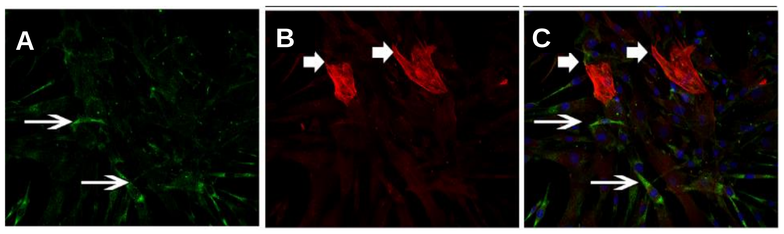

Figure 4: Human corneal fibroblasts were prepared in complete medium and serum-free, TGFβ-containing medium to obtain fibroblasts (thin arrow) and myofibroblasts (thick arrow), respectively. Cells were stained with Id2 (A), α-SMA (B), and Id2 + αSMA (C) showing distinct staining for each other with no co-localization.

Results

Fibroblast Transfection

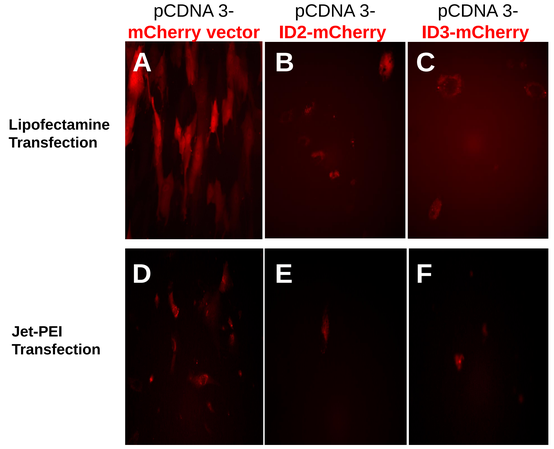

Figure 1: Human corneal fibroblasts were transfected with a plasmid via either Lipofectamine 3000 (A, B, C) or jetPEI Nanoparticle (D, E, F) systems. The plasmids used contained the fluorescent marker m-Cherry for visualization. The vector plasmid (A, D) showed a higher rate of transfection over plasmids containing Id2 (B, E) and Id3 (C, F).

Fibrotic Marker Analysis of TGFβ Treatment

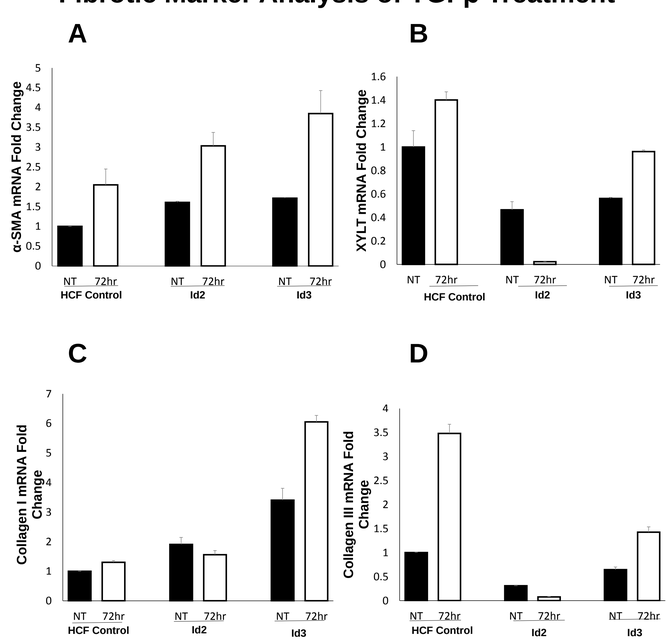

Figure 2: These graphs show mRNA expression of several fibrotic markers within each control and treatment group. The fibrotic marker expression was analyzed using qRT-PCR and include α-Smooth Muscle Actin (α-SMA) (A), xylosyltransferase 1 (XYLT1) (B), Collagen I (C), and Collagen III (D). Nontransfected human corneal fibroblasts (HCF) were used as a control group for comparison against the Id2 and Id3 overexpression groups. All cell groups were cultured in serum-free medium and treatment groups were treated with TGFβ1 for 72 hours.

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.

Acknowledgements

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- Zoetis Animal Health

Reference

- Rajiv R. Mohan, et al. (2016) Characterization of Inhibitor of differentiation (Id) proteins in human cornea. Experimental Eye Research 146: 145-153