Altered gene and protein expression in corneal epithelium from keratoconus patients

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ABSTRACT

This PhD study was performed at the Department of Ophthalmology and Molecular Diagnostic Laboratory, Aarhus University Hospital, Denmark.

The purpose was to identify keratoconus-associated genes by the use of microarray analyses on the human corneal epithelium. Keratoconus-associated proteins were identified from 2D-gel electrophoresis and mass spectrometry.

Nineteen epithelial samples were analyzed on the Hu6800FL GeneChip (Affymetrix). From stringent data mining using the Microarray Suite 5.0 software, 56 differentially expressed genes were identified. The microarray data of ten genes were validated with quantitative RT-PCR and similar expression profiles were obtained. The altered genes comprised a variety of functions; half of them related to the cytoskeleton, cell signaling, and the extracellular matrix.

Five genes (CLC, EMP3, DSG3, S100A2, and SLPI) were selected for more intense expression studies. The gene expression profiles were validated on additional and larger sample set, and were all found to be significantly expressed. Commercial antibodies against DSG3, S100A2, and SLPI were available. The protein expression was quantified with Western blotting and all proteins showed a significant differential expression, especially detected for DSG3. This protein resembles a good potential as a marker for keratoconus.

A trial to characterize the genes CLC, EMP3, and S100A2 was attempted in the human epithelial cell line, HCE-T. Unfortunately, an over-expression negatively influenced the cell growth and appeared to be toxic. S100A2-transfected cells died, and CLC and EMP3 transfected cells were largely retarded in growth. RNA was isolated from the surviving cells and quantitative RT-PCR showed a massive expression of the CLC and EMP3 gene.

Proteins from 12 epithelial samples were separated on 2D-polyacrylamide gels. Protein spots were identified on the silver stained gels and quantified. The most differentially expressed proteins were identified by mass spectrometry. Enolase 1, gelsolin, and S100A4 were observed as altered in keratoconus.

The role of the discussed genes and proteins in the pathogenesis of the keratoconus disease still needs to be investigated.

In this study, a broad range of molecular biological techniques have been optimized and are now easy-applicable to other corneal diseases.