



THE EFFECT OF CYCLICAL MECHANICAL STRETCH ON DIFFERENTIAL GENE EXPRESSION IN LAMINA CRIBROSA CELLS

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Objective: To create an in-vitro model for raised intraocular pressure by exposing lamina cribrosa cells to cyclical mechanical stretch and examine the effect of this stimulus on gene expression patterns of components and modulators of the extracellular matrix (ECM) of the lamina cribrosa.

Methods: Confluent normal primary human lamina cribrosa were transferred onto flexible and rigid six well culture plates. Cells were stretched in the Flexercell strain unit by 20% at a rate of 60 cycles per minute. Total RNA was extracted from the lamina cribrosa cells using TRIzol. Complimentary DNA was synthesised by RT-PCR using a T7 RNA polymerase. Biotinylated cRNA was synthesised in an in-vitro transcription reaction using the cDNA from the 24 hour time point. This was then fragmented and hybridised to the human Affymetrix gene chip.

Results: At 24 hours, there was up-regulation and down regulation of approximately 300 genes. Differentially expressed genes (stretch versus control) included:

1. Components of the ECM: collagens I, V, VI, elastin, biglycan, profilin and dermatan sulphate proteoglycan.
2. Modulators of the ECM: TIMP-3, TIMP-4.
3. Signal transduction pathway proteins: MAP-kinase-8, serine/threonine protein kinase-19, phosphodiesterase-3B, regulator of G-protein signalling-3 and SMAD 6.
4. Ion channel proteins.
5. Cytoskeletal proteins: plectin, α -actin, myosin heavy chain-4, a gelsolin homologue and an endothelial actin binding protein homologue.
6. Apoptosis related proteins:

Conclusion: This experimental in-vitro study has identified changes in genes coding for proteins known to play a role in connective tissue remodelling of the lamina cribrosa in glaucoma in-vivo. The stretch model should help to provide insights into the pathogenesis and therapy of glaucomatous optic neuropathy.