00296 Cortactin in the Pathogenesis of Pterygium

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Aims: Cortical actin binding protein (cortactin; CTTN), involved in actin polymerization and rearrangement, has been implicated in upregulation of matrix metalloproteinases and extracellular matrix degradation. Its role in pterygium, a common ocular surface disease, is unknown. This project aims to investigate the role of cortactin phosphorylation and relevance of cortactin in pterygium pathology.

Methodology: Human pterygium and conjunctiva tissue explants excised from the same eyes were used to generate primary cultured fibroblasts. These fibroblasts were immortalised by stable transfection with retroviral vectors expressing human telomerase reverse transcriptase gene and p53/p16 shRNAs. Phosphorylation of fibroblasts was activated by pervanadate. Using fibroblast lysates, phosphoproteins were pulled down and mass spectrometry and peptide sequencing were utilised to identify differentially phosphorylated peptides. Western blot and immunofluorescence were performed to quantify and localize cortactin phosphorylation and expression in the immortalised tissue-derived pterygium and conjunctival cells.

Result: In pull down assays, pterygium cells showed greater phosphorylation and expression of cortactin compared to conjunctiva. Pervanadate stimulation of the cultured fibroblasts in both serum free and serum-containing media increased expression in pterygium-derived fibroblasts to a greater extent than conjunctiva fibroblasts. Cortactin is shown to be localised to the invadopodia-like filapodia in pterygium cells. There was no localization of cortactin to the advancing edge of cell sheets in scratch assays.

Conclusion: Pterygium fibroblasts exhibit increased expression and phosphorylation of cortactin. The activation of cortactin may increase fibroblast migration or extracellular matrix production to cause pterygium progression. This pathway may present therapeutic targets to treat pterygium disease.