Characterization of a Standard Strain of Human Scleral Fibroblasts

Abstract: : **Purpose:** Abnormal wound healing and ocular scarring play a role in either the pathogenesis or treatment failure of most blinding diseases, including post-operative failure of glaucoma filtration surgery. Although use of human cells derived from primary tissue is optimal for studying these processes, such cells exhibit a reduced division capacity leading to senescence in vitro, which limits opportunities for research. In human dermal fibroblasts it has been shown that this "replicative senescence" is triggered by progressive telomeric attrition and can be prevented by ectopic expression of the catalytic subunit of human telomere reverse transcriptase (telomerase or hTERT). Telomerase expression results in immortal cell lines with a phenotype similar to that of normal primary cells. The goal of this project is the derivation of an immortalized ocular fibroblast cell line, which would facilitate the study of therapeutic targets in ocular disease. **Methods:** Human scleral fibroblasts derived from donor tissue were infected with pBABE-hTERT, an amphoteric retrovirus expressing hTERT. Infected cells were expanded and subcloned under puromycin selection. Fibroblasts were compared with uninfected parental cells and cells infected with the control vector (pBABE-puro). The level of telomerase activity was determined and the replicative life-span of the fibroblasts was subsequently assessed by serial passage. Immunocytochemical analysis demonstrated the lineage of origin of the cell strains. An in vitro model of wound healing, the free-floating fibroblast populated collagen lattice (FPCL) contraction assay, was used to assess changes in fibroblast function in response to hTERT immortalization with lattice diameters recorded at specific time points. The role of MMP-1, an important matrix remodelling enzyme, was also examined. Cells were transiently transfected with the MMP-1 promoter ligated to a luciferase reporter. A luciferase assay was used to determine the activation of the MMP-1 promoter in each cell strain, normalised against ß-galactosidase expression. **Results:** The hTERT cell strain has a life-span of greater than 120 population doublings. Data suggests that hTERT immortalization does not significantly affect the contractile properties of the cell strain or ability to activate the MMP-1 promoter. However hTERT cells undergo significantly greater activation of the MMP-1 promoter than the parental cell strain ($p<0.01$). **Conclusions:** It is anticipated that the hTERT cell strain will prove a useful tool in the in vitro study of stromal wound healing.