

Articular Chondroprogenitors as tools for cartilage tissue engineering

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INTRODUCTION: The use of stem/progenitor cells whose progeny have the capacity to form good quality cartilage matrix has been targeted as a therapeutic goal for the treatment of large defects of articular cartilage that result from serious trauma or widespread osteoarthritic lesions. Previously we reported the isolation and characterisation of a progenitor cell population that resides in the surface layer of neonatal bovine articular cartilage [1]. Here, we extend our observations by assessing progenitor cell chondrogenic potential as a function of their ability to elaborate a cartilage matrix in pellet cultures, maintenance of *sox9* expression, analysis of telomere length and telomerase activity following long-term clonal expansion.

METHODS Isolation and cultivation of chondroprogenitor clones. Chondroprogenitor cells were isolated on the basis of differential adhesion to fibronectin as previously described by Dowthwaite *et al* (2004) [1]. Isolated chondrocytes were subjected to differential adhesion on fibronectin-coated 60 mm dishes for 20 minutes in 4 mls 1:1 DMEM/F12 plus 10% foetal calf serum (Invitrogen, UK) at a concentration of 700 cells ml⁻¹. Colonies of >32 cells (chondroprogenitor clones) were isolated using cloning rings. Full-depth and superficial zone cells were enzymatically isolated using surgical dissection from the MCP joints. Telomere lengths of samples were detected using the TeloTAGGG telomeric length assay kit (Roche Diagnostics, Sussex, UK). Comparative quantitative analysis of telomerase activity in samples was performed using a previously validated RTQ-TRAP methodology [2]. The ABI Prism TaqMan quantitative polymerase chain reaction (qPCR) system (Applied Biosystems, CA, USA) was used to study the relative expression levels of collagen type II, aggrecan, *sox9* and Notch-1 between different cell populations.

RESULTS: Cloned chondroprogenitors exhibited exponential growth for the first 20 population doublings (PD), then slower linear growth with evidence of replicative senescence at later passages. Mean telomere lengths of exponentially growing chondroprogenitors were

significantly longer than dedifferentiated chondrocytes that had undergone a similar number of PD ($P < 0.05$). Chondroprogenitors also had 2.6-fold greater telomerase activity and maintained *sox9* and Notch-1 mRNA expression whereas in dedifferentiated chondrocytes there was little or no detectable expression of these genes. Chondroprogenitors were induced to differentiate into cartilage in 3D pellet cultures, immunological investigation of *sox9*, Notch-1, aggrecan and PCNA expression showed evidence of co-ordinated growth and differentiation within the cartilage pellet.

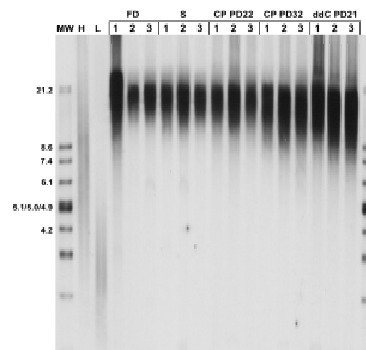


Fig. 1: Telomere length analysis of chondrocytes and chondroprogenitor clones. A. Southern blot analysis of telomere lengths of restriction enzyme digested DNA of full depth articular cartilage chondrocytes (FD), surface zone chondrocytes (S), chondroprogenitor clones (CP) that had undergone ~22PD or ~32PD, and dedifferentiated chondrocytes (ddC, ~21PD).

DISCUSSION & CONCLUSIONS: Maintenance of telomerase activity, Notch-1 and *sox9* gene expression distinguish clonal chondroprogenitor cells from dedifferentiated chondrocytes. When placed in chondrogenic medium chondroprogenitors appear to engage in co-ordinated growth and differentiation in stark contrast to dedifferentiated chondrocytes that exhibit dysregulated growth.

REFERENCES: ¹Dowthwaite *et al*, *J Cell Sci.* 2004 Feb 29;117(Pt 6):889-97 ²Hou *et al*. *Clinical chemistry* 2001, 47:519-524.