

ARTICULAR CARTILAGE-DERIVED CHONDROPROGENITORS MAINTAIN TELOMERE LENGTH THROUGH TRANSIENT TELOMERASE ACTIVATION.

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INTRODUCTION: The therapeutic goal for the treatment of large defects of articular cartilage that result from serious trauma or widespread osteoarthritic lesions is to use stem/progenitor cells whose progeny have the capacity to form good quality cartilage matrix has been targeted as a. 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 report on the long-term clonal expansion of the progenitor cells in monolayer and assess their chondrogenic potential.

METHODS Isolation and cultivation of chondroprogenitor clones. Chondroprogenitor (CPs) cells were isolated on the basis of differential adhesion to fibronectin as previously described by Douthwaite *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 (FD) and superficial zone (SZ) 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: There was no difference in mean telomere lengths between unpassaged FD or SZ chondrocytes and low passage CPs. All three

latter cell populations had significantly longer mean telomere lengths than mid-passage CPs and dedifferentiated chondrocytes. Further, we analysed telomerase activities of individual cell populations using a real-time TRAP assay, Low-passage CPs exhibited greater telomerase activity than either FD or SZ chondrocytes (mean threshold cycle (CT); FD, SZ and chondroprogenitor chondrocytes. When converted into HL60 cell equivalent units CPs had 2.6 fold more telomerase activity than FD or SZ chondrocytes,

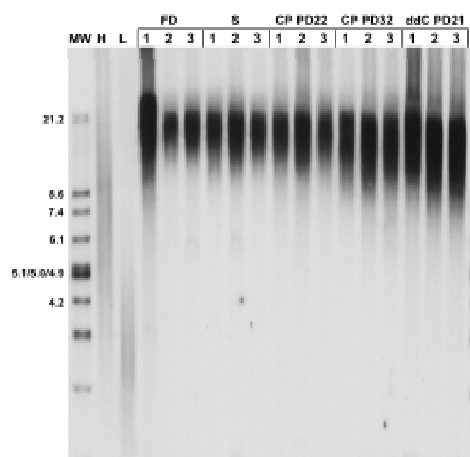


Fig. 1: Telomere length analysis of chondrocytes and chondroprogenitor clones. A. Southern blot analysis of telomere lengths of restriction enzyme digested DNA of full depth articular cartilage chondrocytes (FD), surface zone chondrocytes (S), chondroprogenitor clones (CP) that had undergone ~22PD or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

DISCUSSION & CONCLUSIONS: We discovered that CPs possess 2.6-fold more telomerase activity than unpassaged chondrocytes whereas as dedifferentiated chondrocytes have no detectable activity. Therefore we believe the transient maintenance of telomere ends in CPs during the early growth phase is promoted in part by the activity of telomerase. Therefore, maintenance of telomere length during extended culture expansion may potentially contribute to enhanced repair due to protection from replicative stress.

REFERENCES: ¹Douthwaite *et al*, *J Cell Sci*. 2004 ²Hou *et al*. *Clinical chemistry* 2001,