PLASTICITY OF DE-DIFFERENTIATED HUMAN ARTICULAR CHONDROCYTES

Introduction. Mesenchymal progenitor cells with the capacity to differentiate along different connective tissue lineages have been identified from an increasing number of tissues, including bone marrow stroma (1), periosteum (2), synovial membrane (3), and blood vessels (4) (5). Chondrocytes from articular cartilage are known to de-differentiate during in vitro culture in monolayer and to display a pre-chondrogenic mesenchymal-like phenotype (6) (7). In this work, we studied if human adult articular chondrocytes (HAAC) behave similarly to mesenchymal progenitor cells; in particular we addressed the following specific questions: (i) Can de-differentiated HAAC re-differentiate into different connective tissue lineages, similarly to mesenchymal progenitor cells? (ii) Can media enhancing de-differentiation modulate the chondrocyte capacity to re-differentiate? (iii) Can single colony-derived strains of HAAC differentiate into multiple lineages after monolayer expansion?

Methods. Human articular cartilage biopsies were collected from the knee joints of 3 individuals (age range 25-43). Isolated cells were expanded in medium containing 10% foetal bovine serum (CTR) or further supplemented with 1 ng/ml TGFβ1, 5 ng/ml FGF-2 and 10 ng/ml PDGFβb (TFP) (these factors have been shown to enhance HAAC proliferation and capacity to re-differentiate (8)). After two passages in culture, cells were cultivated (i) as 3D pellets in a serum-free medium containing 10 ng/ml TGFβ1 and 100 nM dexamethasone (chondrogenic medium); (ii) in monolayers in medium containing 10% FBS, 10 nM dexamethasone and 10 nM b-glycerophosphate (osteogenic medium), with or without 100 ng/ml BMP-2; (iii) in monolayers in medium containing 10% FBS, 1µM insulin, 1µM dexamethasone, 1µM indomethacin and 0.5 µM methyl-isobutylxanthine (adipogenic medium).

Cell differentiation of multi- and single-colony derived strains of HAAC was assessed biochemically (sulfated glycosaminoglycans and calcium quantifications), histologically/histochemically (Safranin-O, Oil red-O and Alkaline Phosphatase stains) and by the mRNA expression of collagen type II (CII), collagen type X (CX), bone sialoprotein (BSP), osteocalcin (OC) and adipsin (Adip) at the mRNA level using real-time quantitative RT-PCR. Values of expression for each gene, normalized to the rRNA 18S, were plotted as fold difference from those measured prior to exposure to differentiation stimuli. Statistical differences were calculated on the logarithmic values using a double-sided T-test, assuming independent variances; values of p<0.05 were considered to indicate statistically significant differences.

Essential results. After two weeks in chondrogenic medium, de-differentiated HAAC were able to re-express collagen type II and generate cartilaginous pellets. As compared to HAAC expanded in CTR medium, TFP-expanded cells upregulated collagen type II expression at a higher level, formed tissues more intensely stained for Safranin-O (Fig. 1A,E) and accumulated 2.5 fold higher sulfated glycosaminoglycans. Culture of expanded chondrocytes for 3 weeks in osteogenic medium without BMP-2 induced them to become strongly positive for alkaline phosphatase positive, to deposit large amounts of calcium and to upregulate the expression of BSP and OC (significantly only for the TFP expanded cells), but not of collagen types II and X (Fig 1B,F), indicating that cells reached an osteoblast-like phenotype. Further supplementation of the osteogenic medium with BMP-2 induced additional upregulation of collagen types II and X (significantly only in TFP expanded chondrocytes) (Fig 1C,G), indicating that cells reached a stage of hypertrophic differentiation. De-differentiated chondrocytes cultured for 3 weeks in adipogenic medium accumulated lipid droplets stained with Oil red-O, although none of the investigated genes was significantly upregulated (Fig. 1D,H). The density of adipocytic cells was 6.5 fold higher for CTR- than for TFP-expanded cells. A total of 20 clones were isolated from one primary culture and expanded with TFP. Clones proliferated at different rates and had different morphologies, indicating an overall heterogeneity of chondrocyte subpopulations. Of the total number of TFP expanded clones, 30% had no differentiation capacity in any lineage, 45% could differentiate into only one lineage (mostly chondrocytic), 15% into two lineages (chondrocytic and osteogenic or adipogenic) and only 10% into all lineages.

Discussion. De-differentiated HAAC could be re-differentiated into chondrocytes (hypertrophic or non-hypertrophic), osteoblasts and adipocytes, indicating – at least in vitro – extensive plasticity of the chondrocyte phenotype. TFP expanded cells had more chondrogenic, osteogenic and less adipogenic ability than CTR expanded chondrocytes. Although most clones of HAAC were only chondrogenic or nullipotent, some clones of HAAC displayed features of mesenchymal progenitor cells. Identification of markers for specific clonal HAAC subpopulations could allow sorting of isolated chondrocytes for enhanced performance of cell-based cartilage repair procedures.

Fig. 1. Histological appearance (A-D) and gene expression levels (E-H) of HAAC cultured under chondrogenic (A,E), osteogenic (B,F), hypertrophic (C,G) and adipogenic (D,H) conditions.


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