DISTRIBUTION AND CHANGES IN PROGENITOR CELL MARKERS IN NORMAL AND OSTEOARTHRITIC HUMAN ARTICULAR CARTILAGE

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Purpose: Recent findings suggest the presence of mesenchymal progenitor cells in articular cartilage. We analyzed their spatial organization and involvement in tissue homeostasis and in the development of osteoarthritis (OA).

Methods: Progenitor cells throughout normal and OA adult human articular cartilage were identified using antibodies directed towards Notch-1, Strom-1 and VCAM-1 via immunohistochemistry and flow cytometry. We also monitored Sox9, a transcriptional regulator of chondrocyte-specific genes. DNA microarrays were used to detect differences in progenitor markers in the cartilage zones and between normal and OA tissues.

Results: In normal cartilage, over 68% of cells within the superficial zone (SZ) were positive for the stem cell markers, which decreased to approximately 32% in the middle zone (MZ) and deep zone (DZ) (Fig. 1). Non-fibrillated OA cartilage SZ showed reduced and MZ increased progenitor marker staining frequency, which was decreased in the DZ of fibrillated OA samples. Most cells in OA clusters were positive for the stem cell markers. Sox9 staining was nuclear only in normal cartilage, while cluster cells showed both nuclear and cytoplasmic staining. Flow cytometry and DNA arrays support zone specific and OA-related differences in stem cell marker expression.

Conclusions: These results show a surprisingly high number of cells that express progenitor cell markers throughout cartilage, with a greater proportion localized in the SZ and upper MZ. The increased presence of progenitor markers in OA cell clusters implicates the involvement of these cells in the abnormal cell activation and differentiation process characteristic of OA.

THE SIDE POPULATION IN HUMAN ARTICULAR CARTILAGE RETAINS CHONDROGENIC CAPACITY

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Purpose: The side population population (SP) as identified by exclusion of the Hoechst 33342 dye is considered to represent a stem cell population and recent studies indicate the presence of a SP in bovine cartilage. In this study, we isolated SP and non-SP (NSP) cells from adult human articular cartilage (AHAC) and tested their chondrogenic capacity.

Methods: Chondrocyte isolation and culture. AHAC (approved by the TSRI human subjects committee) was digested in collagenase. Isolated cells were cultured in DMEM supplemented with 10% CS (Invitrogen) and cultured until confluence and split once. Confluent passage 1 cells were harvested for sorting. Hoechst 33342 staining. Cells were incubated in Hoechst 33342 (4 μg/ml) at 37°C for 90 minutes, washed in ice cold HBSS and maintained on ice. Propidium iodide (PI) (2 μg/ml) was added just prior to sorting to exclude dead cells. FACS sorting. The FACSVantage SE flow cytometer (BD Biosciences) was used to isolate SP and non-SP (NSP) chondrocytes. Sorted cells were placed in culture and expanded. Chondrogenic pellet cultures. Cells from each population were placed into pellet cultures (0.5-10³/pellet) in ITS+ serum free medium (Sigma) supplemented with TGFβ1 (10 ng/ml) for 2 weeks. Pellets were processed for histology and RT-PCR analyses. Histology. Pellets were sectioned and stained for Safranin O. RNA extraction and RT-PCR. Total RNA was extracted using RNA easy kit (Qiagen) and cDNA was generated using a reverse transcription kit. Expression of Sox9, aggrecan and Col10a1 were determined using RT-PCR. Statistical analysis. Statistical analysis was performed using the Student's t-test.

Results: SP cells expressed a 3-fold higher level of transmembrane ABCG2 protein compared to NSP (Fig. 1A). Although Col2a1 IIA and Col2a1 IIB were not detected in monolayer-cultured cells, both Sox9 and aggrecan were highly expressed in the SP compared to NSP (8-fold and 20-fold, respectively). Safranin O staining (Fig. 2B) was more extensive in SP stained pellets, which possessed higher gene expression levels of Sox9 (8-fold) and aggrecan (60-fold) compared to the NSP. Col1a1 IIA and Col10a1 IIB mRNA expression were detected in SP, but were undetectable in NSP pellets. Conversely, NSP expressed higher levels of Col1a1 and Col10a1 compared to SP (Fig. 1C).

Conclusions: The enhanced ABCG2 expression in SP cells signifies successful isolation of SP cells from AHAC. The superior chondrogenic capacity and virtually undetectable Col10a1 expression levels in SP cells compared to NSP indicate that the SP may be more suitable for cell based cartilage repair. Extensive characterization including proliferation, flow cytometry and multipotential capacity of these populations are currently underway.

THE EFFECT OF CYTOKINE ON HYALURONAN METABOLISM OF CHONDROCYTE

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Purpose: Hyaluronan is not only a major non-protein glycosaminoglycan component of the extracellular matrix but also a signaling molecule. High molecular weight hyaluronan exhibits anti-inflammatory activities. On the other hand, low molecular weight hyaluronan has pro-inflammatory effects. The enzymes associated with hyaluronan metabolism such as hyaluronan synthesis and digestion have been called hyaluronan synthase and hyaluronidase. In this study, we evaluated the effects of cytokines on hyaluronan metabolism by chondrocyte.

Methods: Human articular chondrocytes from knee joint (NHAc-Kn, purchased from LONZA) were stimulated with IL-1β, TNF-α, TGF-β1, and IL-1β, TNF-α, TGF-β1. Total RNAs were prepared from cells and reverse transcribed to cDNA. The expression levels of hyaluronan synthases (HAS1, HAS2 and HAS3) and hyaluronidases (Hyal1 and Hyal2) were assessed using real-time quantitative PCR method and normalized to β-actin expression. Results: IL-1β induced HAS2 and especially HAS3 mRNA expression. There were no significant changes in the expression levels of Hyal1 and Hyal2 with or without IL-1β, TGF-β1 induced HAS1 mRNA expression and strongly reduced HAS3 mRNA expression. The expression levels of Hyal1 and Hyal2 were reduced by TGF-β1. TNF-α had little effect on the expression of these enzymes.

Conclusions: IL-1β markedly upregulated HAS3, which is known to synthesize the lower size of HA. It may explain that increase of low molecular weight HA in synovial fluid of patients with rheumatoid arthritis. TGF-β1 promoted the production of high molecular weight hyaluronan and inhibited the production of low molecular weight hyaluronan. Thus, in view of the hyaluronan function as a signaling molecule, TGF-β1 may also have an anti-inflammatory effect through the hyaluronan size regulation.