Discriminating Chondrogenic Progenitor Cells (CPCs) as a Distinct Cell Type, Apart from Normal Chondrocytes

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Disclosures:

Introduction: Articular cartilage is an avascular and aneural tissue with a structure consisting of a superficial, a middle and a deep zone [1]. Cartilage cells (chondrocytes) residing in the superficial zone had been thought to be a subpopulation of chondrocytes [2]. However, a second cell population, later proved to be chondrogenic progenitor cells (CPCs), was distinguishable from chondrocytes based on their clonogenicity, multipotency, migration, higher proliferate rate and substantial morphological differences [3]. Our continuing studies have shown that CPCs are less chondrogenic than normal chondrocytes (NCs) and their function is to protect the cartilage surface rather than to regenerate cartilage matrix as previously supposed. In addition, we found evidences to suggest that CPCs act as pro-inflammatory cells in the context of cartilage injury. In this study, we undertook a more comprehensive comparison of the phenotypic differences between CPCs and NCs and between CPCs and joint cells (synoviocytes and cells present in synovial fluid) which have been shown to play roles in joint inflammation.

Methods: Cell procurement and isolation: matrix tears were created to stimulate CPCs migrate to cartilage surface; CPCs were trypsinized to be isolated 7-10 days post tear injury. Remaining cartilage was serially digested by protease (0.4% for 1.5 hrs) and collagenase (0.02% for 16 hrs) to isolate NCs, synoviocytes and synovial fluid cells (SFCs) were obtained from same bovine knee joint.

Microarray analysis: RNA from each cell types was reversed transcribed to cDNA, biotinylated cDNA was then hybridized to Bovine Genome Arrays (Affymetrix), which were scanned with Affymetrix model 3000. Heat maps based on 5-fold difference between CPCs and NCs, as well as the hierarchical cluster, were created through internal data base.

Characterized comparison: Gene expression study was taken for matrix forming markers, inflammatory related markers. sGAG assay was used to assess the proteoglycan (PG) deposition. Chondrogenic, osteogenic differentiation was applied in pellet culture or monolayer culture, respectively.

Statistical analysis: Statistical assay was performed using a one-way analysis of variance (ANOVA) through SPSS. A P value less than 0.05 via Tukey’s post hoc test was considered to indicate statistical differences among groups (*: p < 0.05, **: p < 0.01, ***: p < 0.001).

Results: The 5-fold based heat map and annotated heat maps clearly showed the CPCs are remarkably different compared to NCs, sharing substantial similarities with SFCs and synoviocytes. The hierarchical clustering analysis straightforwardly divided all cell types into chondrocyte and non-chondrocyte phenotypes (Figure 1). qPCR exhibited dramatic over-expression in NCs than CPCs, SFCs and synoviocytes in the context of matrix forming genes, while opposite gene expression pattern can be applied to the comparison in term of inflammatory related genes (Figure 2), all of which validated the microarray results. sGAG assay legibly demonstrated the highest proteoglycan (PG) contents in NCs, significantly overtopping CPCs, which stay in the same level with SFCs and synoviocyte. However, no significant differences of PG deposition among CPCs, NCs SFCs and synoviocytes observed both in chondrogenesis and osteogenesis differentiation assays.
Figure 1. 5-fold based heat map and annotated groups. Left: The 5-fold (>±5-fold or <−5-fold) change between NCs and CPCs based heat map revealed the substantial difference between NCs and CPCs, as well as showed the similarities among CPCs, synoviocytes and SFCs. The hierarchical cluster analysis exhibited the similarities among four cell types and directly divided all four cell types into two major categories (NCs and the other combined cell types). Right: Five annotated heat maps (A. metalloendopeptidase related, B. collagen related, C. inflammatory related, D. extracellular related, E. cytokine related) were generated based on specific gene functions, exhibiting the differences among four cell types, which are essentially in accord with the 5-fold based heat map.
Figure 2. Gene expression analysis. Left: qPCR showed dramatically higher expression of all matrix forming genes (Collagen II, Aggrecan, Link protein and COMP) in NCs than in the other three cell types. Right: qPCR showed lower expression of CXCL12 (one of inflammatory related genes) in NCs than in the other three cell types.

**Discussion:** Cartilage is thought to possess poor healing capacity post injury due to its native properties (avascular, aneural, alymphatic). However the chondrogenic progenitor cells (CPCs), which can be activated and migrate onto the cartilage surface by the injury, may provide us a new insight to self-repair the cartilage. Through this series of experiments, we validated that these CPCs with less cartilage matrix gene expression, as well as higher inflammatory gene expression, are essentially distinct from chondrocytes, contradicting the universal opinion that all cells residing in cartilage superficial zone are chondrocytes. Since many intrinsic properties of CPCs are shared with SFCs and synoviocytes, we could thus build the concept that the function of these cells is to prevent the cartilage from further injury through the formation of a protective layer on the very top of the cartilage.

**Significance:** This study established a novel comparison among CPCs, NCs, SFCs and synoviocytes using microarray technology, discriminating CPCs from NCs based on multiple evidences. The higher expression in inflammatory genes provides us clues that CPCs may be actively involved in inflammation pathology.

**Acknowledgments:**

**References:**

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