

A COMPARISON OF CHONDROCYTE RESPONSE IN CARTILAGE AND 2D CULTURES

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Introduction

Cartilage cells, known as chondrocytes, are the only cells in cartilage and a key player in joint diseases such as Rheumatoid Arthritis (RA) and Osteoarthritis (OA). Together with synovial cells, they orchestrate a complex interplay of cytokines and chemokines that can detrimentally influence cartilage integrity [de Lange-Brokaar et al, 2012]. During the above pathologic conditions, the altered environment of chondrocytes affects their function and phenotype [Recklies et al, 1992]. The vast majority of studies have used monolayer (2D) cell culture experiments to capture the mechanisms underlying these diseases omitting the effect of the extracellular matrix (ECM). Herein, we try to address this matter and compare how chondrocytes are responding in their natural environment and how this response is altered in 2D cultures.

Methods

OA cartilage tissue was obtained from two donors, male (donor1) and female (donor2) of age 65 and 67 respectively, undergoing total knee arthroplasty. Cartilage was removed from the underlying bone using a scalpel and then the one half was digested in pronase/collagenase medium to isolate chondrocytes, while the other half was cut to cartilage disks of 3mm in diameter with a biopsy punch. Both cartilage disks and chondrocytes were stimulated for 24 hours with 11 inflammatory mediators at high concentrations. Controls were stimulated with culture media (DMEM). For each condition, the release of 23 cytokines was measured using custom Xmap assay on a Luminex FlexMap instrument. Meanwhile, one specimen of each donor was formalin fixed, decalcified, embedded in paraffin and stained with toluidine blue.

Results

The release of 23 cytokines from cartilage tissue and chondrocytes derived from two donors was measured under the stimulation with 11 inflammatory ligands. Overall, there was 5.8% similarity between cells and tissue releases (figure 1). Moreover, histological sections revealed high OA grade in both donors.

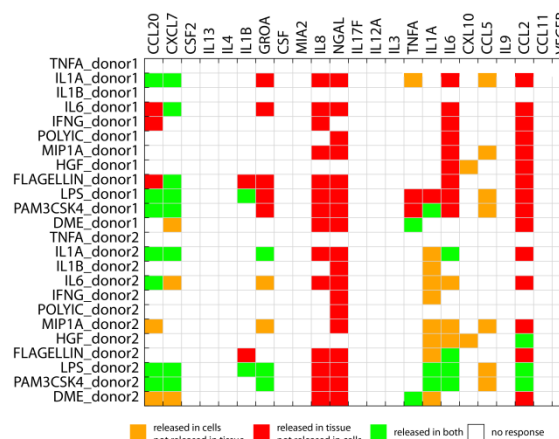


Figure 1: The relationship between cell and tissue cytokine releases (columns) under every stimulus (rows).

Discussion

These results indicate that the extracellular matrix of cartilage has a profound effect on chondrocyte function. Cartilage disks seem to be more responsive under the stimuli used, thus giving a more detailed knowledge of the mechanisms underlying joint diseases. On the other hand, isolated cells demonstrate significant basal levels of inflammatory cytokine secretion such as Il6, Il8, NGAL and CCL2 even in non-stimulated situation, indicating that ECM significantly affects chondrocyte phenotype. Uncovering the exact role of the ECM will provide valuable information regarding the design and development of in-vitro models of cartilage degeneration.

References

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