7 ELR+ CXC CHEMOKINE SIGNALLING IN ARTICULAR CHONDROCYTE PHENOTYPIC STABILITY

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10.1136/annrheumdis-2011-201237.7

Background and objectives The production of ELR+ CXC chemokines is widely studied in arthritis and is postulated to contribute to the inflammatory phenomena that lead to cartilage breakdown. Healthy articular chondrocytes however, also express their own chemokine receptors and ligands. The function of CXC chemokine receptors in these cells is puzzling because chondrocytes are encased in a dense extracellular matrix and are not known to migrate in vitro. This study aims to identify the function of this signaling mechanism in articular cartilage.

Materials and methods Adult human articular chondrocytes (AHAC) were expanded in monolayer culture under standard conditions. Receptor expression was confirmed using semi-quantitative RT-PCR, western blot and immunohistochemistry. CXCR1/2 combined and individual functionality was tested using an in vitro calcium mobilisation assay. CXCR1/2 signalling was blocked at specific receptor level using validated blocking antibodies and siRNA, or at the downstream level using Pertussis toxin, PI3K inhibitors and intracellular calcium chelators. Chondrocyte phenotypic gene expression was assessed using real time RT-PCR. The content of highly sulphated proteoglycans in chondrocyte micromasses was analysed using Alcian blue staining followed by guanidine HCl extraction and spectrophotometric quantification. CXCL6 and CXCL8 were detected in heparitinase digested, chondroitinase ABC digested and un-digested paraffin sections from

healthy and osteoarthitis full thickness human articular cartilage using immunohistochemistry.

Results Receptors were expressed in normal human articular cartilage. Disruption of CXCR1/2 signalling at receptor level or by downstream blockade in chondrocytes resulted in reduced extracellular matrix sulphated glycosaminoglycan content, which was found to be PI3K-dependant, and reduced expression of the chondrocyte differentiation markers COL2A1, Aggrecan, and SOX9. CXCL6 and CXCL8 were found in cartilage extracellular matrix in healthy tissue in distinct localisation patterns, which were disrupted in osteoarthritic tissue. CXCL8 was lost from territorial matrix following heparitinase digestion, but not following digestion in chondroitinase ABC.

Conclusions Our findings indicate that CXCR1/2 signalling is required for the maintenance of phenotypic stability in articular chondrocytes. Interactions with heparan sulphate proteoglycans and distribution patterns of ligands within the ECM, together with their disruption during pathology, indicate the presence of a homeostatic mechanism whereby CXCL8 is retained within the articular cartilage matrix via its interaction with heparan sulphate proteoglycans, contributing to chondrocyte phenotypic stability.