A46 CHONDROCYTES FROM ARTHRITIC CARTILAGE IN A SUBSET OF OSTEOARTHRITIS PATIENTS EXPRESS LOWER LEVELS OF NFκB-P65: A SURVIVAL FACTOR AGAINST TUMOUR NECROSIS FACTOR α-INDUCED CELL DEATH

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Chondrocytes play a central role in cartilage pathology, as seen in patients with rheumatoid arthritis (RA) and osteoarthritis (OA), by a deranged synthesis of extracellular matrix (ECM) components and the enhanced release of ECM destructive metalloproteinases (MMPs). Nuclear factor κB (NFκB) is an important transcription factor in the regulation of MMPs, but it is also regarded as a survival factor in cells. We measured the level of NFκB-P65 in chondrocytes of arthritic cartilage obtained from joint replacement surgery. In freshly isolated chondrocytes of patients with OA and RA, the levels of NFκB-P65 protein were measured by western blotting. It was apparent that patients with OA could be divided into two groups, one with high and the other with low amounts of NFκB, whereas chondrocytes from all patients with RA showed high levels of NFκB. In a spontaneous OA mouse model (STR/ORT) the levels of NFκB decreased when joints became affected, as determined by immunohistochemistry and quantitative real-time PCR. In contrast, the level of chondrocyte NFκB-P65 increased twofold directly after induction of either streptococcal cell wall- or antigen-induced arthritis and returned to baseline when the disease went into remission. Next we studied the functional consequences of a decreased level of NFκB-P65 in chondrocytes. The murine H4 chondrocyte cell line was transduced with a lentivirus expressing a short-hairpin RNA against NFκB-P65 to reduce the NFκB-P65 protein levels by an RNA interference approach. We selected several cell lines that expressed different amounts of NFκB-P65 protein. A negative correlation was found between TNFα-induced apoptosis, as detected by 7-amino-actinomycin D staining, and the levels of NFκB in these chondrocyte cell lines. To study the biological consequences, conditioned medium of OA synovium was added to the murine chondrocyte cell line with the lowest NFκB level. This resulted in apoptosis of these cells which could be prevented by preincubation of the conditioned media with Enbrel. The presence of TNFα protein was confirmed in these conditioned OA media using a Luminex assay. This study clearly demonstrated that NFκB is downregulated in chondrocytes in murine OA and in a subset of patients with OA, and that these chondrocytes are more vulnerable to TNFα-induced cell death.