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Cartilage degradation in knee joint

## Regulation of Mitogen-Activated Protein Kinases and Matrix Metalloproteinases by Reactive Oxygen Species and Secondary Messengers

Osteoarthritis (OA) is a chronic, disabling disease involving articular joints and characterized by cartilage destruction. Pathological changes associated with osteoarthritis have been attributed to the activation of MAP kinase (MAPK) pathways by reactive oxygen species (ROS) in response to catabolic stimuli. Additionally, cartilage cells, also known as chondrocytes, produce nitric oxide (NO), a secondary messenger, which has been shown to readily interact with superoxide to form peroxynitrite, which is in turn thought to mediate cartilage damage. Additionally, NO is thought to promote signals that increase matrix metalloproteinase (MMP) production, which is implicated in the breakdown of the extracellular matrix of cartilage. The purpose of this study was to observe how ROS and NO regulate the cellular mechanisms of inflammation and cartilage degradation in osteoarthritis. Chondrocytes isolated from articular cartilage were treated with endotoxin-free recombinant human fibronectin fragment (Fn-f) (42 kD), recombinant human interleukin-1 beta (IL-1 beta). Both treatments elicited increased levels of nitric oxide in the media. However, pre-treatment with the antioxidant MnTBAP or inhibition of NO synthase by L-NMMA did not block the effects of Fn-f or IL-1B on the stimulation of MMP-13. These results suggest that NO and/or peroxynitrite may play a role in contributing to the production of degradative factors such as MMP-13. Chondrocytes were also treated with hydrogen peroxide on a dose curve [0.5 to 50  $\mu$ M]. The cells were then oxidatively labeled and probed for total protein using total antibodies or probed for phosphorylated MAPKs (JNK, p38) using phospho-antibodies. Low levels of ROS were required for JNK activation and both low and high levels activated p38, suggesting that differential activation of MAPKs by ROS levels, represents an additional layer of regulation that distinguishes stress and non-stress signaling.