The interplay between JNK pathway activation, proteoglycan turnover and endocytosis in chondrocytes

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Introduction:
The JNK pathway (c-jun N-terminal kinase) is activated by inflammatory and physically stressful stimuli. Recently, we identified a new important role for the JNK pathway in the turnover of the cartilage proteoglycan aggrecan. Loss of JNK2 isoform in human chondrocytes and murine cartilage abolished IL1- induced aggrecan degradation and aggrecanase activity. Furthermore, JNK2-null mice were protected from surgically-induced osteoarthritis. Here we show that the JNK2-dependent effect on aggrecan degradation is linked to an LRP1-dependent endocytic mechanism.

Materials & Methods:
Human articular chondrocytes were overlaid with bovine aggrecan in combination with siRNAs. Aggrecanase activity and TIMP3 levels were measured using ELISA. Murine epiphyseal cartilage was cultured ±IL1. Aggrecan fragments were analysed by western blotting. Endocytic rate of LRP1 was analyzed by flow cytometry using Alexa647-labelled alpha-2 macroglobulin ligand.

Results and Discussion:
In human articular chondrocytes, ADAMTS-5 is a major aggrecanase and its activity in the extracellular matrix was highly dependent on JNK2 activation. Loss of JNK2 reduced aggrecanase activity and caused accumulation of TIMP3 in culture medium of human chondrocytes and murine cartilage. Knowing that TIMP3 and ADAMTS5 are internalized through the endocytic receptor LRP1 and the lack of effect of JNK2 knockdown on their gene expression, we then investigated if JNK2 would control aggrecan turnover through endocytosis. IL1 changed the balance between the aggrecanase and TIMP-3 in the extracellular matrix by increasing the uptake of TIMP-3 favouring aggrecanase activity, while knockdown of JNK2 decreased IL1-induced LRP1 shedding. Knockdown of LRP-1 caused accumulation of TIMP-3 in culture medium and inhibition of aggrecan degradation. Using Alexa 647-labelled alpha-2-macroglobulin ligand, we studied the involvement of JNK2 in LRP1-dependent endocytosis in response to IL1. Interestingly, IL-1 had a marked effect on endocytic rate of LRP1 and increased Alexa-647-α2M uptake that was abolished when JNK2 is knocked down.

These results reveal a major role of the JNK2 signalling pathway in proteoglycan turnover involving the control of LRP1-dependent endocytosis. It appears that increased function of LRP1 (either through increased expression or endocytic rate) favours the balance between TIMP3 and ADAMTS5 in favour of the aggrecanase.