

JNK-DEPENDENT MODULATION OF THE PROTEASE SECRETOME PROFILE OF OSTEOARTHRITIC CARTILAGE

Authors H. M. Ismail, L. Troeberg, T. L. Vincent, J. Saklatvala; Univ. of Oxford, Oxford, United Kingdom

Abstract:

Purpose: Recently, we identified a significant role for the intracellular signalling pathway, the c-jun N-terminal kinase (JNK) in the regulation of aggrecanase activity in vitro and in vivo. Specifically, we showed that JNK2 knockout mice exhibited slower progression of experimental osteoarthritis. Targeting the JNK pathway by knockdown or inhibition by small molecules significantly reduced aggrecanase activity and caused accumulation of the TIMP3 protease inhibitor in human and mouse cartilage. One putative mechanism for this is a JNK-dependent shift in the balance between ADAMTS5 and TIMP3 through the endocytic receptor LRP1. Here we aim to understand whether the JNK pathway modulates the broader secretome profile of proteases of osteoarthritic cartilage.

Methods: Human normal and osteoarthritic cartilage samples were collected after surgery following ethical approvals. Freshly dissected cartilage was cultured in the presence or absence of the JNK specific inhibitor JNK-IN8 for 24 hrs. The secretion patterns of 34 proteases were compared in the conditioned medium of osteoarthritic and normal cartilage tissues using R&D Proteome Profiler Human Protease Array Kit. Western blotting of TCA-precipitated proteins was used for validation of identified proteases.

Results: Osteoarthritic cartilage secreted a number of proteases that are significantly induced compared to normal cartilage including Cathepsin B, Cathepsin D, Cathepsin X/Z/P, Kallikrein 10 (KLK10), MMP1 and MMP13. Culturing OA cartilage in the presence of the JNK inhibitor significantly reduced the levels of secreted KLK10 and MMP13 proteases. The effect of the JNK signalling pathway on secreted levels of KLK10 was further validated in multiple OA samples by western blotting and a consistent reduction in KLK10 levels was observed in the presence of the JNK inhibitor.

Conclusions: Profiling of articular cartilage identified 8 secreted proteases that were regulated in OA cartilage compared to normal tissue. Inhibition of JNK pathway significantly lowered the secreted levels MMP13 and KLK10. KLK10 is one of the fifteen kallikrein subfamily members of serine proteases. Kallikreins can process kininogens, growth factors, and extracellular matrix (ECM) molecules to regulate inflammation, pain and a number of other diverse functions. These data highlight KLK10 as a potential JNK-dependent protease in OA. Functional analysis of KLK10 is required to test its importance in the development of osteoarthritis in vivo.