The Role Of Ank Interactions With Mybbp1a And Sphk1 In Catabolic Events Of Articular Chondrocytes

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Disclosures:


Introduction: The progressive ankylosis protein (ANK) is a transmembrane protein that transports intracellular pyrophosphate (PPI) to the extracellular milieu [1]. Extracellular PPI (ePPI) has been shown to inhibit basic calcium phosphate (BCP) crystal formation [2]. Consequently, lack of functional ANK in ank/ank mice or ank knockout mice leads to pathological mineralization of articular cartilage, ligaments, menisci and tendons resulting in arthritic changes [1,3]. Surprisingly, however, several studies including those from our laboratory have shown that ANK is expressed at low levels in healthy articular cartilage, and ANK expression levels drastically increase during osteoarthritis (OA) initiation and progression [4-6]. These findings raise the possibility that ANK plays more complex and unknown functions in articular cartilage and OA pathology than the prevention of BCP crystal formation. The aim of this study was to determine the role of ANK in cartilage destruction during OA. Specifically, we identified novel interactions between ANK, Myb-binding protein 1a (MYBBP1a) and sphingosine kinase 1 (SphK1) and determined the role of these interactions in catabolic events of articular chondrocytes.

Methods: ANK/MYBBP1a and SphK1 interactions were identified using yeast two hybrid screening and co-immunoprecipitation. To determine the role of these interactions in catabolic events of articular chondrocytes, ank/ank and wild type mouse articular chondrocytes or femoral heads were treated with interleukin-1beta (IL-1β) in the absence or presence of a SphK inhibitor, or a sphingosine 1-phosphate receptor (S1PR) inhibitor. Catabolic marker mRNA levels were analyzed by real time PCR; proteoglycan loss using safranin O staining and MMP-13 immunostaining were determined in femoral head explants; NF-κB activity was determined by transfecting chondrocytes with a NF-κB-specific luciferase reporter and by analyzing nuclear translocation of p65 by immunoblotting; MYBBP1a nuclear or cytoplasmic amounts were determined by immunohistochemistry and immunoblotting.

Results: The N-terminal region of ANK interacted with SphK1, whereas the C-terminal region interacted with MYBBP1a. Lack of ANK in ank/ank chondrocytes resulted in increased nuclear amounts of MYBBP1a, decreased SphK1 activity, decreased NF-κB activity, decreased mRNA levels of catabolic genes, decreased proteoglycan loss and decreased MMP-13 immunostaining in IL-1β-treated ank/ank articular chondrocytes or femoral heads compared to WT cells or femoral head explants. Transfection of ank/ank chondrocytes with ank expression vector fully rescued nuclear MYBBP1a and SphK1 activity to levels similar to WT cells, whereas PSL or P5T chondrocalcinosis ANK mutations only decreased nuclear MYBBP1a and the C331R craniometaphyseal dysplasia (CMD) ANK mutations only increased SphK1 activity. Consequently, the PSL, P5T and C331R ANK mutations only partially increased NF-κB activity in ank/ank chondrocytes, whereas transfection with ank rescued NF-κB activity in ank/ank chondrocytes to levels similar to WT cells.

Discussion: This study shows that ANK interacts via its N-terminal and C-terminal cytoplasmic regions with SphK1 and MYBBP1a, respectively. The interaction with MYBBP1a results in the inhibition of the translocation of MYBBP1a to the nucleus, where it acts as a co-repressor of NF-κB transcriptional activity [7]. The interaction with SphK1 is a major regulator of SphK1 activity in IL-1β-treated articular chondrocytes. Both interactions stimulate NF-κB activity and ultimately catabolic events in IL-1β-treated articular chondrocytes. Since IL-1β and NF-κB play major roles in OA pathogenesis [8,9], our findings suggest that increased ANK expression in OA cartilage may be a major contributor to cartilage destruction. Future experiments are needed to fully understand the mechanisms of these interactions between ANK, MYBBP1a and SphK1 and to develop strategies to interfere with these interactions as a novel therapy for the treatment of OA.

Significance: This study identified novel mechanisms that play major roles in the stimulation of catabolic events of articular chondrocytes.

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References:

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