SirT1 represses MMP13 expression in human articular chondrocytes.

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Introduction

Osteoarthritis (OA) is a degenerative joint disease of the articular cartilage resulting in the depletion of collagens and proteoglycans due in part to accelerated turnover and inadequate repair. Maintenance of healthy articular cartilage in human adults relies on the optimal expression of a number of unique extracellular matrix genes, such as aggrecan and collagen type II, by the chondrocytes in this tissue. MMP13 is a proteolytic enzyme that degrades the extracellular matrix, including the cartilage-specific component, type II collagen. It plays a critical role in bone remodeling and arthritis. SirT1 is an NAD-dependent histone deacetylase that regulates gene expression, differentiation, development and organism life span. The role this enzyme plays in cartilage specific gene expression in human adult chondrocytes has not been explored. Here we found that MMP13 expression and activity is downregulated by SirT1 in human chondrocytes.

Methods

Human chondrocytes (hChs) were isolated from the knees of osteoarthritic patients undergoing total knee arthroplasty, supplied by the National Disease Research Interchange (NDRI), Philadelphia, PA. Cells were cultured in monolayer. Chondrocyte transfections were carried out using the Amaxa Nucleofector technology. Cells were processed for protein or RNA extraction followed by immunoblotting or RT-PCR according to standard procedures, using the indicated antibodies and primer pairs.

Results

MMP13 levels were assessed in OA human chondrocytes stably overexpressing SirT1. Immunoblot assays confirmed SirT1 overexpression (Figure 1A) and the SirT1-expressing cells possessed reduced MMP13 protein expression (Figure 1A) and RNA levels (Figure 1B) compared to control cell lines. Figure 1C indicates an increase in SirT1 activity in cells expressing wild type SirT1 indicating that the ectopic SirT1 is active within these chondrocytes. As shown in Figure 1D, the activity of MMP13 is decreased in SirT1-overexpressing cells. Taken together, these results show that MMP13 expression is suppressed by SirT1.

We then tested the effect of nicotinamide (NAM, 10mM), a SirT1 inhibitor, on MMP13 expression. NAM treatment resulted in a significant increase of MMP13 mRNA levels in in SirT1-overexpressing cells (Figure 2A) and MMP13 protein levels (Figure 2C). SirT1 levels were also knocked down by SirT1 siRNA leading to increased MMP13 expression (Figure 2B). Additionally, treatment of cells with IL1beta led to induction of MMP13, yet SirT1 was partially able to block this IL1beta-mediated induction (data not shown).

Discussion

MMP13 is a critical player in joint destruction in OA. Since SirT1 is believed to play a critical supporting role in enhancement of longevity it was thought that this chromatin modifying enzyme may regulate MMP13 levels. As shown here we find this to be the case; SirT1 in represses MMP13 gene expression. This appears to occur even in the presence of the potent MMP13 inducer IL1beta. The underlying mechanism by which SirT1 represses MMP13 is not known however Lef1 is a known regulator of MMP13 transcription. That Lef1 is suppressed by SirT1 is consistent with the suppression of MMP13 expression in these cells. It should be noted that we have examined a number of other MMP and ADAMTS proteins in these chondrocytes and find that while some are affected (i.e. repressed) by SirT1 others are not. Thus it would appear that SirT1 may play an important role in cartilage pathology.

References

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