Leptin as a potential molecular target for osteoarthritis therapeutic intervention

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Introduction: Osteoarthritis (OA) is a complex disease with genetic, mechanical and environmental components leading to the destruction of the articular cartilage. As articular chondrocytes seem to be involved in the initiation and progression of osteoarthritis, the investigation of the molecular changes that occur in chondrocytes during the development of osteoarthritis is of outmost importance. Recently, we have shown that one of the genes up-regulated in osteoarthritic chondrocytes is leptin. Leptin acts as a regulator of bone growth inducing osteoblast proliferation, collagen synthesis and bone mineralization and also stimulating endochondral ossification. The potential role of leptin in osteoarthritis has been supported by the relationship between obesity and increased risk of OA development. MMP-9 and MMP-13 are among the major enzymes that mediate the destructive process in OA and their immunocytochemical localization and increased expression has been confirmed in OA cartilage. We investigated the effect of leptin on matrix metalloproteinases expression in normal and osteoarthritic chondrocytes. Furthermore using small interference RNA technology against leptin, we investigated its role on MMP-13 regulation, testing thus its potential use as a molecular target for therapeutic intervention in osteoarthritis.

Materials and Methods: We collected osteoarthritic articular cartilage tissue samples from two distinct locations of the knee (n=15); from the main defective area of maximal load (advanced OA) and from adjacent macroscopically intact regions (minimal OA) and proceeded with chondrocyte cultures. Normal and osteoarthritic chondrocytes were seeded and treated with different leptin concentrations (0–100 ng/mL). mRNA and protein levels were tested by Real-Time PCR and Western Blot analyses respectively. Additionally, osteoarthritic chondrocytes were transfected using siRNA against leptin and monitored leptin’s protein expression by western blot analysis. The siRNA against leptin was transferred with liposomes.

Results: We observed that 5 and 7 days after leptin treatment of OA and normal chondrocytes, there was a 3.7-fold and 5.6-fold as well as a 8.6-fold and 14.5-fold increase in MMP-9 and MMP-13 levels, respectively. In addition we detected a leptin dose dependent increase in MMP-13 and MMP-9 mRNA and protein levels in OA and normal chondrocytes. Very low leptin concentrations of 0.1 and 1 ng/mL in OA and normal chondrocytes were not able to induce MMP-9 and MMP-13 levels, however 10ng/mL were able to activate MMP-9 (8.6-fold) and MMP-13 (8.2-fold) mRNA levels, respectively. After 7 days of treatment with leptin (100 ng/mL) in OA chondrocytes we detected an increase in protein expression of MMP-9 (7.8-fold) and MMP-13 (5.4-fold) [Figure 1]. Furthermore, we blocked leptin’s expression completely 48h after liposomal siRNA transfection [Figure 2A]. Leptin’s down-regulation actually affected MMP13 expression levels. Specifically 48h after siRNA treatment against leptin, MMP13 expression was significantly reduced and continued to reduce even 96h after treatment (p<0.001) [Figure 2B]. Except MMP13 we did not find any modulation of MMP3 or MMP9 levels after leptin down-regulation. The specificity of this interaction between leptin and MMP13 is essential for osteoarthritis therapy.

Discussion: It has been shown in our previous study[1] that in patients with osteoarthritis there is a unique microenvironment in the cartilage characterized by enhanced locally produced leptin levels, which induce IL-1β production. Along with the observed leptin dose dependent increase in MMP-13 and MMP-9 mRNA and protein expression in OA and normal chondrocytes, it can be suggested that leptin acts as a pro-inflammatory cytokine with a catabolic role on cartilage metabolism.

Small interference RNA against leptin deactivated directly MMP-13, raising the issue of leptin’s therapeutic potential for osteoarthritis[2]. Taken all together we propose for the first time that targeted gene therapy using small interference RNA transferred with liposomes in chondrocytes chondrocytes could possibly have therapeutic potential for osteoarthritis treatment especially in early stages. However further studies are required to fully understand the molecular profile of osteoarthritis.