demonstrated that siRNA for Ank reduced 4-fold more than siRNA for PC-1 the inducing effect of TGF-β1 on ePPi levels. We showed that neither SB203580 (a p38 MAPK inhibitor), nor RcAMP (a PKA inhibitor) were able to modulate Ank induction, but that PD98059 (a MEK 1 inhibitor) was strongly active, ensuring a 50% inhibition of TGF-β-1-induced Ank expression. On the other hand, the effect of PKC inhibitors depended on their selectivity: PKCδ inhibitor (rottlerin) was weakly effective compared to Ca2+-dependent PKC inhibitor (Gö6976), that diminished Ank expression by 60%, suggesting the involvement of calcium-dependent PKC in the regulation of Ank gene by TGF-β1. Finally, we demonstrated with plasmids transfection the involvement of Ras, Raf-1 and confirmed the contribution of ERK in Ank mRNA regulation. However, overexpression of Smad 7 did not affect Ank expression, showing that Smad-dependent signaling events downstream to TGF-β1 were not implicated.
Conclusions: These results indicate that TGF-β1 mediates an increase in ePPi concentration, mainly by induction of Ank expression, which depends on activation of Ras, Raf-1, ERK and Ca2+-dependent PKC pathways in chondrocyte. Our results underline the interest to modulate Ras/Raf-1/ERK or PKC signaling pathways in chondrocytes, as it could open insights for the treatment of sporadic chondrocalcinosis.

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MOLECULAR INTERACTION BETWEEN BCL-XL AND BNIP3 DETERMINES THE CELL FATE OF HYPERTROPHIC CHONDROCYTES

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Purpose: It has been reported that the early stage of osteoarthritis (OA) is characterized by hypertrophic differentiation and apoptosis of chondrocytes, which mimics the developmental process of growth plate chondrocytes. The level of inorganic phosphate (Pi) elevates at the site of cartilage mineralization, and previous studies have proposed that Pi entry into hypertrophic chondrocytes may act as an apoptogen. The Bcl-2 family proteins, which consist of pro- and anti-apoptotic members, are major regulators of mitochondria-initiated apoptosis. In this study, we investigated how they determine the cell fate of chondrocytes.
Methods: We developed an *in vitro* system to analyze the mechanism of hypertrophic differentiation and apoptosis of chondrocytes using chondrogenic ATDC5 cells. Using this culture system, the expression levels of pro- and anti-apoptotic Bcl-2 members were analyzed by real time RT-PCR or western blotting, and their roles in chondrocyte apoptosis by gene overexpression and gene silencing. Association between anti-apoptotic member Bcl-xL and pro-apoptotic member Bnip3 was examined by immunoprecipitation, and their expression patterns in growth plate chondrocytes were investigated by immunohistological staining. Finally, we generated chondrocyte-specific knockout mice of anti-apoptotic Bcl-2 family member protein Bcl-xL using the Cre-loxP recombination system, and examined its roles *in vivo*.
Results: When differentiated ATDC5 cells were treated with Pi, they mineralized the surrounding matrix and underwent rapid apoptosis as evidenced by nuclear condensation, Caspase-3 & 7 activation, and Lamin proteolysis. In this culture system, among 15 pro-apoptotic Bcl-2 family members, 7 molecules increased their expression levels during the course of hypertrophic differentiation, and two in response to Pi stimulation. Of these, gene silencing of a proapoptotic BH3-only molecule *bnip3* by RNA interference significantly suppressed Pi-induced apoptosis. Conversely, among anti-apoptotic members examined, overexpression of *bcl-xL* suppressed, and its knockdown promoted apoptosis. The susceptibility to apoptosis by *bcl-xL* knockdown was partially restored by simultaneous silencing of *bnip3*. Pi treatment markedly upregulated the protein levels of Bnip3 without affecting Bcl-xL levels. Bnip3 was associated with Bcl-xL and attenuated its anti-apoptotic effect in chondrocytes. Immunohistological examination of murine growth plates revealed that Bcl-xL expressed uniformly in the growth plate chondrocytes, whereas Bnip3 expression was exclusively localized in the hypertrophic chondrocytes. Finally, we generated chondrocyte-specific *bcl-xL* knockout mice using the Cre-loxP recombination system, and provided evidence that the hypertrophic chondrocyte layer was markedly shortened in those mice owing to massive chondrocyte apoptosis and that the mice exhibited dwarfism as a result. Furthermore, Bnip3 expression was decreased in hypertrophic chondrocytes in mice fed with a low phosphate diet, and the abnormalities in the growth plate were almost completely rescued.
Conclusions: Increase in Pi levels in hypertrophic zone causes upregulation of Bnip3 in chondrocytes, which binds to Bcl-xL and consequently impairs its anti-apoptotic effect, and finally causes apoptosis of the cells. Our observations will open a new therapeutic approach for OA by modulating the function of Bcl-xL/Bnip3 axis and consequently regulating chondrocyte apoptosis.

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INTERLEUKIN 6 (IL-6) SIGNALING MODULATES ANABOLIC AND CATABOLIC PATHWAYS IN HUMAN ARTICULAR CHONDROCTYES

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Purpose: Interleukin 6 (IL-6) production is elevated in affected joints of osteoarthritis (OA) patients. The central role played by IL-6 in joint inflammation and destruction has suggested therapies targeting IL-6 for treatments of arthritic diseases. However, increased severity of spontaneous cartilage damage in IL6-/- mice suggests a protective role by IL-6 in joint homeostasis. The aim of this study was to examine the effects of blocking IL-6 signaling on both catabolic and anabolic gene regulation in human primary chondrocytes (HACs) by small interfering RNA (siRNA) technology and antibody treatment.
Methods: Small interfering RNA-mediated gene-specific knockdown of IL-6 signaling components (IL-6 or IL-6R) was applied to achieve blockade of IL-6 signaling in human primary chondrocytes (HACs) treated with catabolic (IL-1β) or anabolic (TGF-β or IGF-1+OP-1) stimuli. To confirm the siRNA effects in chondrocytes, a neutralizing anti-IL-6 antibody was also studied for effects in response to IL-1β treatment. Expression of the mRNA for chondrocytic genes was determined by quantitative real-time PCR (Taqman). The protein level of IL-6 and MMP-13 were measured using immunochemical based assays.
Results: Transfection of IL-6 and IL-6R siRNA into chondrocytes resulted in significant gene specific knockdown of IL-6 and IL-6R mRNA, respectively. In addition, knockdown of IL-6 led to a drastic reduction in IL-1β-induced expression of IL-6 mRNA and protein. Decreased IL-6 expression also led to a marked reduction in IL-1β-induced expression of pro-inflammatory cytokine TNF-α, and cartilage degrading enzymes such as MMP-13 and ADAMTS-4. The inhibitory effects by IL-6 siRNA were specific to IL-6 signaling, as similar effects were exhibited by both IL-6 neutralizing antibody and IL-6R siRNA. Surprisingly, IL-6 appears to modulate gene expression in chondroctyes under anabolic stimulation. We found that the expression of several essential ECM and cartilage components, in particular collagen II (Col2a), was not induced by TGF-β or IGF-1/OP-1 in cells transfected with IL-6 siRNA.