**TGF-BETA REGULATES PTHRP EXPRESSION IN CHONDROCYTES**

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Introduction: Within the growth plate, chondrocytes undergo a series of physical and biochemical changes resulting in matrix calcification and primary bone formation. This process of endochondral ossification is regulated at the local and systemic levels, and PTHrP has been shown to be a critical autocrine/paracrine factor which stimulates chondrocyte proliferation and prevents premature maturation. TGF-β is also a factor which is produced by chondrocytes and has phenotypic effects on proliferation and maturation in the growth plate, in part resembling those of PTHrP, with mitogenic and proteoglycan synthesis stimulating properties. PTHrP production in the epiphysis is stimulated by Indian hedgehog (IHH) through a well characterized paracrine mechanism involving the perichondrium. PTHrP reciprocally suppresses IHH expression in the growth plate, creating a feedback loop which controls the rate of chondrocyte maturation. PTHrP is produced at low levels within the growth plate itself, and regulatory interactions with IHH and other signals may be operant in an autocrine fashion as well. The present study investigated the role of TGF-β in regulation of PTHrP expression in cartilage.

Methods: Chondrocytes were isolated from 4- to 6-week old chicks by removal of the growth plates or epiphyses from the distal femur and proximal tibia, followed by sequential enzymatic digestion with trypsin, hyaluronidase, and collagenase. The cells were plated in monolayer cultures in DMEM supplemented with 5% fetal bovine serum. PTHrP mRNA levels were measured by RNAse protection assay and competitive polymerase chain reaction (PCR) assay. Generally RNAse protection was used for epiphyseal chondrocytes, which express higher levels of PTHrP, and PCR for the growth plate cells. For some experiments, growth plate chondrocytes were separated by size using countercurrent centrifugal elutriation into maturationally distinct populations. RNA was isolated from chondrocytes using Rnasey (Quiagen, Germany). Northern analysis for type X collagen was performed using denaturing formaldehyde/agarose gels and a synthetic end-labeled oligonucleotide cDNA probe.

Results: TGF-β, like PTHrP, suppresses the expression of type X collagen in a dose-dependent manner. The expression of PTHrP was examined in freshly isolated epiphyseal chondrocytes, growth plate chondrocytes, and growth plate chondrocytes separated by countercurrent centrifugal elutriation into populations with distinct maturational characteristics. The expression of PTHrP mRNA was much higher in epiphyseal chondrocytes compared to the other cells populations. In contrast, the expression of PTHrP varied according to the maturational state of cells in the growth plate. The highest levels of PTHrP expression occurred in the small cell fraction, representing chondrocytes from the resting/proliferating zones of the growth plate. PTHrP expression gradually decreased during chondrocyte differentiation, with only low levels present in hypertrophic cells. Consistent with the paracrine theory of PTHrP regulation, the epiphyseal cells had 10-fold higher levels of PTHrP, and may provide the major source of this peptide in the growth plate. In order to study regulation of PTHrP, epiphyseal chondrocytes were plated in monolayer culture and treated with several growth factors thought to be important in endochondral ossification. Both TGF-β1 and trans retinoic acid caused a marked increase in PTHrP mRNA expression in epiphyseal chondrocytes (Fig 1). In contrast, PTHrP suppressed expression of its own message. TGF-β1 also stimulated mRNA levels in growth plate chondrocytes, as measured by competitive PCR assay. Dose-response experiments showed maximal responses at between 3-5 ng/ml of TGF-β (shown for epiphyseal chondrocytes in Fig 2). Time course experiments also showed that PTHrP is induced within 6 hours of treatment with TGF-β, and increasing expression occurs for 48 hours. TGF-β2 and TGF-β3 also stimulated PTHrP expression, with TGF-β2 being slightly less effective than the other isoforms.

We and others have shown that growth plate chondrocytes synthesize TGF-β, and it is possible that expression of PTHrP by epiphyseal chondrocytes may be regulated by the expression of TGF-β or other factors produced by growth plate chondrocytes. In turn, PTHrP from the epiphysis may regulate the rate of terminal differentiation in the growth plate. In order to determine whether a paracrine interaction is possible between the growth plate and the epiphysis, these two chondrocyte types were placed in coculture on opposing plastic culture disks and the mRNA from each population was harvested separately after 24 hr. Epiphyseal cells had increased expression of PTHrP following co-culture, while growth plate chondrocytes had decreased expression of type X collagen and IHH, demonstrating potentially important paracrine interactions between these cell populations.

Discussion: The current study demonstrates high levels of PTHrP expression in isolated epiphyseal chondrocytes. While much lower levels of PTHrP are expressed by growth plate chondrocytes, the highest levels are in the smallest, least mature chondrocytes adjacent to the epiphysis. The least mature cells in the growth plate. Despite the importance of PTHrP in endochondral ossification, little is known about the factors that regulate its expression. While IHH plays a role in PTHrP regulation during embryogenesis, its role in regulation of the growth plates postnatally is unclear. Our study indicates that TGF-β stimulates PTHrP expression in chondrocytes, and all isoforms are effective. The effects of TGF-β1 on growth plate chondrocytes are similar to those of PTHrP in some respects (stimulation of cell proliferation and proteoglycan synthesis, and inhibition of type X collagen expression), suggesting that these effects could be in part mediated by stimulation of endogenous expression of PTHrP. Although IHH in the growth plate signals through the receptor patched and the transcription factor gli in the perichondrium, the downstream signals leading to increased epiphyseal production of PTHrP are unknown. TGF-β1 may function as a regulator of PTHrP in parallel with IHH or downstream of this factor. Clearly the interrelationships of the regulatory factors which control the rates of chondrocyte proliferation and maturation are more complex than initially believed, and TGF-β may play an important role.