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REVIEW ARTICLE

The Genetics of Childhood Disease and Development: A Series of Review Articles

The following article is the third in this series. It describes the genetic and molecular factors controlling the development of long bones. It focuses on Indian Hedgehog-parathyroid hormone related protein regulation of normal chondrocyte development and how aberrations in this pathway lead to tumor formation.

Alvin Zipursky
Editor-in-Chief

Developmental Pathways in Musculoskeletal Neoplasia: Involvement of the Indian Hedgehog–Parathyroid Hormone-Related Protein Pathway

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ABSTRACT

There are many crucial genes and signaling pathways in the proper development of an organism. Pathologies may arise from a deregulation of these pathways. The Indian Hedgehog–PTH-related protein (Ihh-PTHrP) pathway is vital in the proper development of endochondral bones, such as the long bones. The Ihh-PTHrP pathway regulates the rate at which chondrocytes within the growth plate proliferate and differentiate. Thus, this pathway allows for the longitudinal growth of bones. However, a disruption in this pathway may lead to enchondromas and osteochondromas, which are both childhood cartilaginous neoplasms. Recently, our lab identified a mutant receptor for PTHrP in enchondroma samples. Mice expressing this mutant receptor and mice with increased Ihh activity develop conditions similar to human enchondromatosis. Linkage analysis shows an association between EXT genes and osteochondromas in hereditary multiple exostoses syndrome. Studies in *Drosophila* and mice suggest EXT gene products play a role in the diffusion of hedgehog proteins. A mutation in EXT genes may result in an abnormal Ihh diffusion pattern leading to an osteochondroma. There are agents that inhibit Hedgehog signaling. These agents may be useful in the treatment of enchondromas and osteochondromas. This review will discuss the discovery of the Ihh-PTHrP pathway and its involvement in neoplasia, and will suggest possible novel therapeutic agents in the treatment of these cartilaginous neoplasms. **(*Pediatr Res* 53: 539–543, 2003)**

Abbreviations

Ihh, Indian Hedgehog
PTHrP, PTH-related protein
PTHR1, PTH-related protein receptor
HME, hereditary multiple exostoses
Hh, Hedgehog
Ttv, Tout-velu
HS, heparin sulfate

The coordinated control of genes and signaling pathways allows for the growth, movement, and death of cells, resulting in the proper development of an organism. Later in life, cells in pathologic processes may use these same pathways, disrupting homeostasis (1). Understanding the deregulation of these pathways in pathologic processes will not only advance our understanding of pathophysiology, but may also suggest novel therapeutic approaches (1). Recent studies have elucidated pathways important in regulating endochondral bone growth and how deregulation of these pathways leads to the development of cartilaginous neoplasia. In this review, we will dem-

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onstrate how developmental signaling pathways are active in cartilaginous neoplastic processes, and suggest how the development of novel therapeutic approaches may arise from this knowledge.

SKELETAL FORMATION

Bone formation is a well-organized and highly regulated process. During embryogenesis, mesenchymal cells migrate to areas destined to become bone. The mesenchymal cells differentiate into skeletal elements by directly forming bone (intramembranous ossification) or by forming a cartilaginous model, which then induces bone formation (endochondral ossification) (2). Chondrogenesis, a precursor to ossification, occurs in many different bones, including the vertebral column and long bones (3).

Long bones begin as cartilage miniatures of their adult counterparts. Through endochondral ossification, there is a conversion of cartilage into bone. The primary ossification site occurs at the center of the bone shaft (diaphysis). The ossification event radiates from the center out toward the ends of the shaft (epiphysis). A secondary site of ossification arises at either end of the bone. Ossification of the cartilage proceeds away from these secondary sites, resulting in cartilage being sandwiched between the secondary and primary ossification sites (Fig. 1). This region of cartilage, the growth plate, is responsible for longitudinal bone growth (2).

The growth plate is a tightly regulated area of chondrocyte differentiation and maturation. Within the growth plate, chondrocytes differentiate, progressing through the resting, proliferation, prehypertrophic, and hypertrophic stages, eventually undergoing programmed cell death. During the resting phase, the chondrocytes arrange themselves in small clusters. These clusters of cells enter the proliferation stage by undergoing successive mitotic divisions to form the characteristic columns of chondrocytes associated with the growth plate. Chondrocytes that cease dividing begin to hypertrophy and may undergo apoptosis (3). Upon chondrocyte degeneration, osteoblasts and capillaries from the diaphysis begin to invade the cartilage matrix, producing the new bone (3). This ultimately leads to growth of endochondral bones (Fig. 2).

Vortkamp and her colleagues (4) demonstrated the involvement of Ihh and PTHrP in regulating the differentiation of growth plate chondrocytes. In particular, they showed that cells in the prehypertrophic stage produce Ihh, a secreted signaling molecule, and that increasing levels of Ihh slow the rate of differentiation of chondrocytes. An examination of the expression of Gli and Patched (downstream targets of Ihh), suggests that Ihh affects the perichondrium, the connective tissue surrounding the growth plate (4), where expression of PTHrP occurs (5). Furthermore, there is acceleration in the transition from proliferative to hypertrophic phase in mutant mice lacking PTHrP (6, 7). Addition of extra hedgehog (Hh) increases PTHrP levels in the perichondrium, however, addition of Hh does not reduce the increased rate of differentiation in mice lacking PTHrP (4). Overall, these results suggest that PTHrP is the mediator of Ihh signaling.

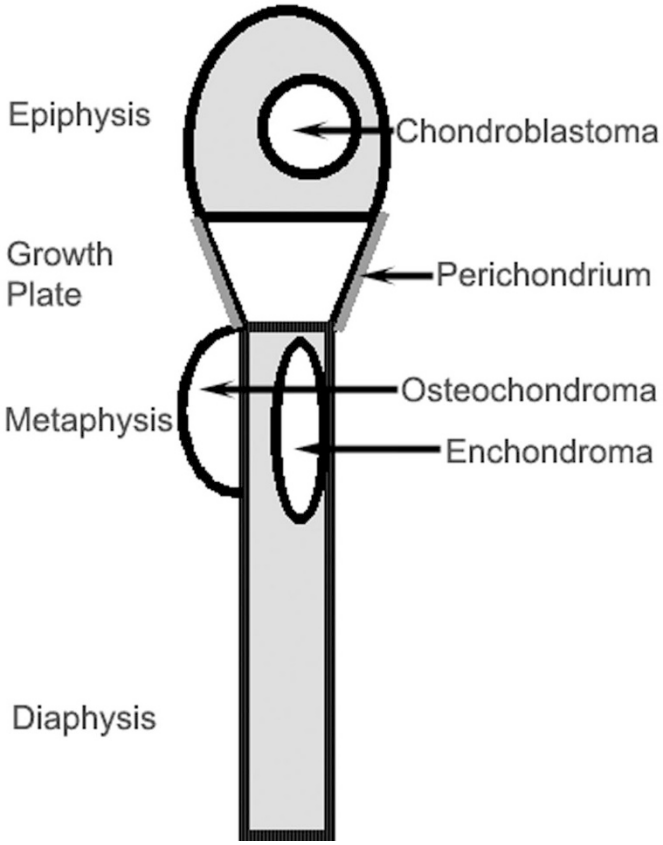

Figure 1. Cartilage neoplasms. The divisions of the bone are the epiphysis, physis (growth plate), metaphysis, and diaphysis. The perichondrium, a layer of connective tissue, envelops the growth plate. Each cartilage neoplasm forms in a specific area of the bone. Chondroblastomas show a preference for the epiphysis. Endochondromas are on the metaphyseal side. Osteochondromas, typically manifest as outgrowths from the metaphysis.

This and other studies have led to the development of a model where Ihh and PTHrP are involved in a feedback loop controlling chondrocyte differentiation and proliferation in the growth plate (Fig. 2). Chondrocytes at the prehypertrophic and hypertrophic boundary express Ihh (4, 8). Ihh may stimulate growth plate chondrocytes to proliferate (9) and induce perichondrial cells enveloping the growth plate to increase the expression of PTHrP (8, 10). The expression of PTHrP, in turn, signals to the PTHrP-receptor expressing cells (mainly proliferating and prehypertrophic cells), slowing the differentiation into hypertrophic cells (11). Chondrocytes that eventually enter hypertrophic differentiation will express Ihh (8). However, because increased PTHrP production results in an overall decrease of differentiation into hypertrophy, there is a general decrease in Ihh. There is also evidence that PTHrP directly down-regulates Ihh (12). Thus, the balance of Ihh expression *versus* PTHrP expression controls the rate of chondrocyte differentiation, laying the foundation for normal longitudinal bone development.

These studies show that the Ihh-PTHrP pathway plays an important role in regulating the rate of maturation of the normal growth plate. Furthermore, the critical importance of this pathway becomes increasingly clear when one examines the consequences that result from a deregulation of the Ihh-

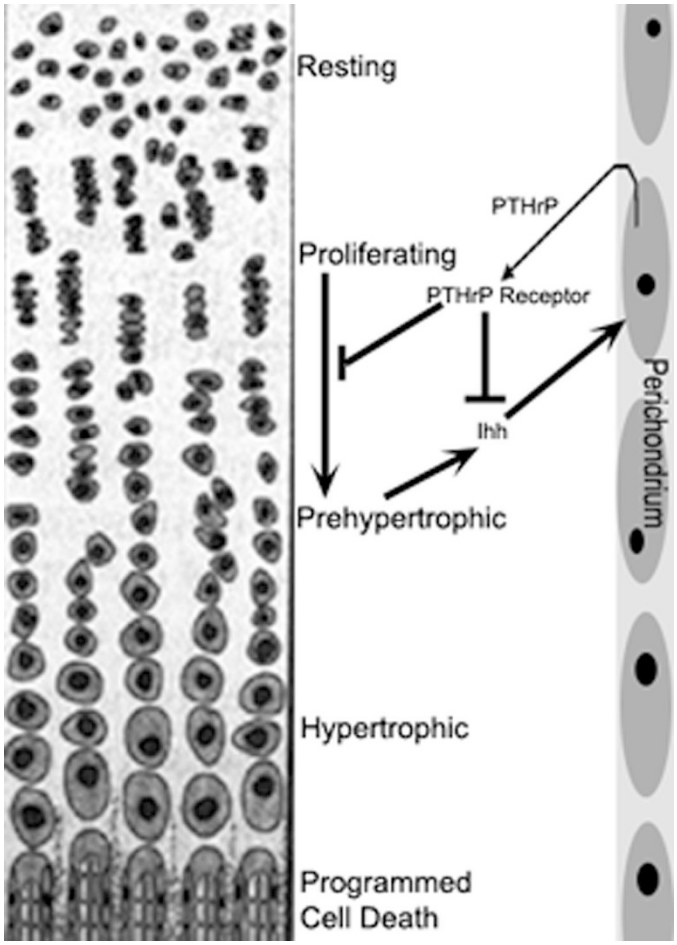

Figure 2. A model for the regulation of chondrocyte proliferation and differentiation. As chondrocytes begin to hypertrophy, they express Ihh. Ihh stimulates chondrocyte proliferation. In addition, Ihh also stimulates perichondrial cells to express PTHrP. PTHrP inhibits Ihh expression and prevents proliferative cells from differentiating. Thus, the Ihh-PTHrP pathway works to regulate the differentiation and proliferation of chondrocytes within the growth plate.

PTHrP pathway. For example, a disruption in this pathway can lead to enchondromas and osteochondromas, benign cartilaginous tumors of bone.

ENCHONDROMAS

Enchondromas are common benign cartilaginous neoplasms in children. These tumors arise on the metaphyseal side of the growth plate in bones that undergo endochondral ossification (Fig. 1). Enchondromas can occur as solitary lesions, or as multiple lesions in enchondromatosis (13). Although there is a broad spectrum of clinical manifestations of enchondromatosis, they are often categorized into two syndromes: Ollier and Maffucci. Multiple enchondromas, resulting in a short, angulated limb characterize Ollier disease (14). Maffucci syndrome also consists of multiple enchondromas, but is associated with vascular malformations. An enchondroma may be completely asymptomatic and the discovery may be an incidental finding on a radiograph. Enchondromas may cause pain, skeletal deformity, bony weakness leading to pathologic fracture, and malignant change to chondrosarcoma (13, 15). Clinical data show a high risk of malignant transformation to chondrosarcoma in multiple enchondroma syndromes. The actual incidence is unknown. However, some studies report close to 100% malignant transformation in Maffucci syndrome (16). Enchondromas may disappear with time after skeletal maturity, possibly because of gradual completion of endochondral ossification.

For the majority of these tumors, observation is usually a sufficient therapeutic modality. However, further treatments are exclusively surgical, consisting of curettage of the tumors with bone grafts for reconstruction (14), fixation of pathologic fractures (16), and osteotomy for limb realignment and lengthening (17) may also be necessary.

Enchondromas are composed of cells cytologically similar to growth plate chondrocytes, possibly representing foci of incomplete endochondral ossification (18). The cytological similarity between enchondromas and growth plate chondrocytes prompted our lab to investigate the Ihh-PTHrP pathway in these tumors. We found that enchondromas express key components of this pathway and that the down-regulation of Ihh by PTHrP was lost in these tumor samples (19). An investigation of enchondroma samples resulted in the finding of a mutant PTHrP receptor (PTHR1) in two unrelated males. It appears that this mutant receptor localizes to the membrane at a lesser extent than the wild-type receptor. However, this mutation seems to constitutively activate the PTHrP pathway. Increase in PTHrP signaling leads to a decrease in chondrocyte differentiation, thus leading to the formation of enchondromas (19).

Transgenic mice expressing the mutant PTHR1 develop tumors similar to human enchondromatosis. The bones of these mice have cartilage islands in the metaphyses. These islands contain proliferating cells and occasional binucleate lacunae arranged in lobular patterns in a hyaline cartilage matrix. In addition, columns of cartilage commonly connect the cartilage islands to the adjoining growth plate (19).

Because we found that regulation of Ihh by PTHrP is lost in enchondromas, we generated transgenic mice overexpressing the Hedgehog (Hh) transcriptional regulator, Gli2. The Gli family of transcription factors is responsible for transducing the Hh signal to the cell nucleus, and Gli2 is the key activating transcription factor. These mice develop ectopic cartilage islands similar to those of the mutant PTHR1 mice (19). Thus, the Ihh signaling pathway as a whole seems to play a crucial role in the formation of enchondromatosis (19).

OSTEOCHONDROMAS

Osteochondromas manifest as outgrowths of bone and cartilage from the metaphyseal region of long bones (Fig. 1). The lesions have a cartilage cap that develops during the individual's growing years (20). In a sense, osteochondromas behave like displaced epiphyseal cartilage that grows at an angle from the normal bone (15, 21). Osteochondromas can occur as solitary lesions or as multiple lesions, as seen in hereditary multiple exostoses (HME) syndrome (21, 22). These lesions are usually not painful. However, vascular complications and functional hindrance of the surrounding soft tissue and nerves

may occur. There may be growth disturbances of the bones in individuals with multiple lesions (14). There is also the possibility of a malignant transformation. Although the absolute incidence is unknown, it is thought to be lower than Ollier disease or Maffucci syndrome (14, 16).

Because there is an obvious inheritance pattern for HME, the search for possible candidate loci gained interest (20). Buhler and Malik (23) noted that a subset of individuals with Langer-Giedion syndrome also have multiple exostoses. A large germline deletion in a region of chromosome 8 causes this syndrome. Thus, the gene responsible for HME is located in this deleted region. Cook *et al.* (24) investigated 11 families with HME. Using genomic markers, this group of researchers was able to link HME to a gene in chromosome 8 in the q24.1 region, denoted EXT1. However, because only 70% of HME links to this region, there may be additional loci. Linkage analysis on affected and unaffected individuals within large pedigrees isolated a second EXT gene on chromosome 11, EXT2 (25). Currently, there are investigations into other EXT and EXT-like genes (26–28).

Evidence is accumulating on the functional role of the EXT genes. Tout-velu (Ttv), the *Drosophila* homologue of EXT (26, 29), is involved in heparin sulfate (HS) biosynthesis (30, 31). Analysis of *Drosophila* that lack Ttv function indicates that this protein plays a role in the short-range diffusion of Hh (29). Therefore, Ttv is important in turning on downstream targets of Hh.

Studies of the EXT1 and EXT2 proteins support a similar role in mammals. In the growth plate, the enhancement of Ihh diffusion by EXT gene products may allow Ihh to exhibit its effects on the proliferating and perichondral chondrocytes. *In situ* hybridization studies in wild-type mice show EXT1 and EXT2 expression in proliferative and prehypertrophic chondrocytes, but not in hypertrophic chondrocytes (32). In addition, Ihh is unable to associate with the surface of target cells in mice embryos lacking the EXT1, suggesting that HS expression is essential for Ihh binding (31).

The role for EXT in normal bone formation may help explain the etiology of HME. Mutations in the EXT genes could lead to a defect in HS biosynthesis. This may result in a localized disruption of the negative feedback loop that regulates chondrocyte proliferation and differentiation by causing a change in the diffusion pattern of Ihh. A change in the Ihh diffusion could potentially result in premature differentiation of nearby growth plate chondrocytes. This may alter the direction of growth of the chondrocytes and ultimately result in the development of an exostosis or osteochondroma (33). However, further investigations are required to clarify the mechanism behind the formation of an exostosis.

CLINICAL SIGNIFICANCE

Developmental pathways may be ideal targets for novel pharmacological agents, such as cyclopamine, a drug derived from the plant *Veratrum californicum*. This plant, when ingested during pregnancy by Ewes, results in the birth of lambs with holoprosencephaly (34). Later studies identified that the teratogenic effect of cyclopamine results from the drug's ability to block hedgehog signaling (35). Because this agent affects the developing fetus and not the mature sheep ingesting the plant, the drug has potential to inhibit hedgehog signaling in mature animals with minimal side effects. As a result, cyclopamine and other hedgehog-inhibiting agents may have a role in treating cartilage neoplasia. Thus, understanding the role of developmental signaling pathways may assist in elucidating novel treatments for pathologic conditions.

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