Gene Expression and Tibial Dyschondroplasia

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ABSTRACT Tibial dyschondroplasia (TD) is a skeletal deformity associated with rapid growth in a number of avian species. The disease is the result of a disruption in the cascade of events that occur in the epiphyseal growth plate. Whereas the incidence of TD is susceptible to genetic selection, no specific genetic defect has been identified. Although there are extensive data describing the morphological and biochemical characteristics of the lesion, the mechanism of lesion formation is unknown. However, naturally occurring or induced genetic mutations in other species can provide important clues to possible mechanisms responsible for lesion development. Disruption of normal chondrocyte differentiation by constitutive activation of the parathyroid hormone/parathyroid hormone-related peptide (PTH/PTHrP) receptor, inactivation of the fibroblast growth factor receptor-3 (FGFR-3) receptor, and blocking vascular endothelial growth factor (VEGF) signaling all result in lesions that resemble TD. Impairment of vascular penetration due to the ablation of matrix metalloproteinase-9 (MMP-9) or tartrate-resistant acid phosphatase (TRAP) activity also results in similar cartilage abnormalities. We have integrated these observations with our current knowledge of TD to describe a hypothesis for the sequence of events responsible for the development of tibial dyschondroplastic lesions.

(*Key words*: tibial dyschondroplasia, gene expression, epiphyseal growth plate, angiogenesis)

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INTRODUCTION

Tibial dyschondroplasia (TD) is a disease found in the proximal tibiotarsus and tarsometatarsus of rapidly growing chickens and resembles osteochondrosis in mammals. This disorder is characterized by the formation of a lesion composed of noncalcified, nonvascularized cartilage that can extend from the epiphyseal growth plate into the metaphysis. Since its initial description by Leach and Nesheim (1965), this cartilage abnormality has been studied extensively by many laboratories. These investigations have identified many factors that influence the occurrence of TD (reviewed by Leach and Lilburn, 1992). Growth rate is a major contributor to the spontaneous occurrence of this disorder, because restricted feeding, at ages 10 to 14 d, virtually eliminates the condition in broiler chicks. Nutritional factors that influence incidence include electrolyte balance, calcium to phosphorus ratio, 1,25-dihydroxy vitamin D3, and ascorbic acid. The drug thiuram and the mycotoxin fusarochromanone can also induce high incidence. In order to facilitate study of this disease, Leach and Nesheim (1965) established a line of broiler chickens with a high incidence of TD. The observation that the incidence of TD is susceptible to genetic selection has subsequently been confirmed by a number of investigators (Riddell, 1976; Sheridan et al., 1978; Thorp et al., 1993; Wong-Valle et al., 1993). However, the pattern of inheritance has not been established.

CHARACTERISTICS OF THE TD LESION

The lesion is characterized by the accumulation of chondrocytes that differ in morphological and histochemical properties when compared to normal hypertrophic chondrocytes (Leach and Neshiem, 1965; Hargest et al., 1985). It appears that the cells comprising TD lesions are prehypertrophic (transitional) chondrocytes that have not matured into hypertrophic chondrocytes. Haynes and Walser (1982) used a chorioallantoic membrane assay to determine that TD lesions are resistant to vascular invasion. Apparently, the chondrocytes and surrounding matrix of the TD lesion fail to provide the proper angiogenic signals to stimulate normal growth plate vascularization. These observations have stimulated extensive study of

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Abbreviation Key: FGFR-3 = fibroblast growth factor receptor-3; MMP-9 = matrix metalloproteinase-9; PTH = parathyroid hormone; PTHrP = parathyroid hormone-related peptide; SLS = spider lamb syndrome; TD = tibial dyschondroplasia.

the composition of TD tissue. Biochemical analysis has shown that large TD lesions contain less protein, DNA, and RNA than chondrocytes of the normal growth plate (Freedman et al., 1985; Praul and Leach, unpublished data). Chondrocytes comprising large lesions have reduced metabolic activity, as measured by ^{3}H-thymidine and $^{35}SO_4$ uptake, in comparison to normal chondrocytes (Rosselot et al., 1994). Researchers have shown, using a variety of techniques, that the presence of a number of proteins, including aggrecan, alkaline phosphatase, basic fibroblast growth factor (bFGF), biglycan, bone sialoprotein, carbonic anhydrase, matrix metalloproteinase (MMP), osteonectin, osteopontin, and transforming growth factor-beta (TGF-β), is greatly diminished in TD lesions compared with the normal growth plate (Gay et al., 1985; Thorp and Jakowlew, 1994; Knopov et al., 1995; Tselepis et al., 1996; Twal et al., 1996; Wu et al., 1996; Rath et al., 1997; Pines et al., 1998). In situ hybridization has also revealed that expression of collagen II, collagen X, and osteopontin is substantially reduced in TD lesions (Chen et al., 1993; Knopov et al., 1995). Interestingly, Chen et al. (1993) observed that although collagen X expression is absent in the center of large lesions, it is present in the proximal and distal edges of the lesion. Recent studies by Praul et al. (1997) and Rath et al. (1998) have determined that large TD lesions contain many apoptotic chondrocytes, whereas smaller lesions contain fewer or no apoptotic chondrocytes. Paradoxically, Ohyama et al. (1997) have reported a complete absence of apoptosis in TD lesions. Extensive apoptosis in large TD lesions would explain 1) the reduced metabolic activity, 2) reduced overall levels of protein, DNA, and RNA, and 3) the reduced expression of the many factors listed previously.

GENETIC MUTATIONS RESEMBLING TIBIAL DYSCHONDROPLASIA

Mutation of genes that control normal endochondral bone growth and are expressed in the epiphyseal growth plate could underlie the formation of TD lesions. By examination of diseases and genetic manipulations that alter endochondral bone growth in other animal species, we may obtain valuable clues as to the type of metabolic defect that is responsible for TD. For example, Jansen's metaphyseal dysplasia is a human disease with a cartilage lesion similar to TD. A mutation in the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor gene results in constitutive activation of the receptor (Schipani et al., 1995). Extensive research using transgenic animals has demonstrated the significance of PTHrP and Indian Hedgehog (Ihh) as factors that form a regulatory loop essential for normal cartilage development (Lanske et al., 1996; Vortkamp et al., 1996). Overexpression of PTHrP results in a delay of chondrocyte maturation and subsequent vascularization (Henderson et al., 1996; Lee et al., 1996; and Weir et al., 1996). Ablation of the PTHrP signal produces the opposite effect, causing accelerated maturation of growth plate chondrocytes (Amizuka et al., 1994; Karaplis et al., 1994).

It is well established that diets imbalanced for calcium and phosphate (marginal calcium, excess phosphate) can induce hyperparathyroidism. Edwards and Veltmann (1983) found that these diets induced a higher incidence of TD. Thus, in addition to acting on kidney and bone tissue, systemic PTH could be influencing chondrocyte activity through the stimulation of the PTH/PTHrP receptors in cartilage tissue. These observations, combined with the results of transgenic studies, led us to hypothesize that TD is the result of perturbation of PTH/PTHrP signaling. Thus, either excessive systemic PTH or overproduction of PTHrP in the epiphyseal growth plate could initiate TD.

We have tested this hypothesis by feeding a low-calcium, TD-inducing diet to three strains of broiler chicks. At 21 d of age, blood samples were taken, and chicks were autopsied for bone lesions. Circulating PTH levels were determined with a cell-based in vitro assay (Pines et al., 1994). The data presented in Table 1 show that chicks exhibiting rachitic cartilage lesions had significantly elevated levels of circulating PTH, whereas those with TD lesions did not. In addition, immunohistochemistry has revealed no difference in the intensity of immunostaining for PTHrP in normal chondrocytes versus chondrocytes within small (mostly nonapoptotic) TD lesions (Medill, 2000).

In situ hybridization has been used to study PTH/PTHrP receptor gene expression in normal, rachitic, and TD growth plate tissue. This receptor is expressed in the prehypertrophic and early hypertrophic zone of normal and TD tissue. However, receptor expression is absent in rachitic cartilage (Ben-Bassat et al., 1999). Therefore, it does not appear that a direct perturbation of PTH/PTHrP signaling is responsible for the development of TD. However, it is possible that signaling in the pathway downstream from the PTH/PTHrP receptor is disrupted.

The fibroblast growth factor family and their receptors also play an important role in regulating normal cartilage development. Thus, disruption of this signal transduction system could be involved in the formation of TD lesions. Sheep afflicted with a deformity called spider lamb syndrome (SLS) have an area of nonvascularized cartilage that extends from the epiphyseal growth plate into the metaphysis. This disorder has recently been mapped to fibroblast growth factor receptor-3 (FGFR-3) (Cockett et al., 1999). Transgenic mice with an inactive FGFR-3 have an extended hypertrophic zone (Deng et al., 1996). However in contrast to TD, SLS sheep and FGFR-3-disrupted mice have an enlarged proliferative zone and enhanced endochondral bone growth, neither of which is associated with TD. Many other FGFR-3 mutations have been identified that constitutively activate this receptor (reviewed by Tavormina et al., 1999). These mutations, and the overexpression of fibroblast growth factor, do not resemble TD because they result in a diminished hypertrophic zone and in reduced endochondral bone growth.

TABLE 1. The effect of diet on body weight, incidence of tibial dyschondroplasia (TD), and plasma parathyroid hormone (PTH) bioactivity in chickens[1]

Diet	Body weight	Incidence of TD	Incidence of Rickets	Plasma PTH bioactivity[2]		
				Growth plate lesions		
				Normal	TD	Rickets
	(g)	(%)				
Marginal calcium	518 (45)	58 (45)	24 (45)	1,757[b] (4)	2,297[b] (10)	2,957[a] (3)
Normal calcium	553 (50)	18 (50)	0 (50)	1,758 (12)	1,689 (5)	

[a,b]Parathyroid hormone.

[1]Numbers in parenthesis are the number of chickens.

[2]As measured by cAMP response (p*M*/mL).

The importance of normal vascularization in endochondral bone growth is highlighted by the recent study by Gerber et al. (1999). These researchers demonstrated that injection of a soluble vascular endothelial growth factor (VEGF) receptor into mice resulted in an impairment of normal growth plate vascularization, resulting in an expanded hypertrophic zone and an inhibition of metaphyseal bone formation. Morphologically, the growth plates of these mice resemble growth plates afflicted with TD.

In addition to regulatory pathways intrinsic to the epiphyseal growth plate, factors outside the growth plate also play an important role in normal endochondral bone growth. Physical disruption of the metaphyseal vasculature results in the formation of lesions very similar to TD (Trueta and Amato, 1960; Kleinman et al., 1991). However, TD is not associated with traumatic interruption of the vasculature. Impairment of osteoclast function has also been observed to disrupt growth plate vascularization and cause formation of TD-like lesions. For example, transgenic mice lacking tartrate-resistant acid phosphatase, which is highly expressed by osteoclasts, have an expanded, nonvascularized, hypertrophic zone that extends into the metaphysis (Hayman et al., 1996). Matrix metalloproteinase-9 (MMP-9) knockout mice also have an extended zone of nonvascularized hypertrophic chondrocytes (Vu et al., 1998). Areas of apoptotic cells appear within the center of this extended hypertrophic zone, as observed in TD. The importance of osteoclasts in the perturbation of the growth plate is highlighted by the fact that osteoclasts express MMP-9 in normal mice, and a bone marrow transplant from a normal mouse rescues the null phenotype.

HYPOTHESIS OF THE DEVELOPMENT OF THE TD LESION

The fact that TD is confined to the proximal ends of the tibiotarsus and tarsometatarsus and that lesions develop focally on the medial side of the joints suggests that mechanical perturbation and pressure may be involved in initiation of the lesion (Trueta and Trias, 1961; Thorp, 1992). We propose that mechanical stimulation in combination with genetic background and a variety of dietary factors disrupts the exquisite chain of events involved in chondrocyte differentiation. Initiation of the lesion occurs when the transition of chondrocytes from prehypertrophy to hypertrophy is inhibited (Figure 1A). This inhibition produces a layer of chondrocytes, which synthesize a matrix that does not calcify and is resistant to vascularization. Because chondrocytes continue to proliferate normally, there is an increase in the number of prehypertrophic chondrocytes, bounded by the zone resistant to vascularization (Figure 1B). As the lesion grows in size, the chondrocytes in the center of the lesion are deprived of nutrients and oxygen, which results in widespread apoptosis (Figure 1C). Eventually the environmental trigger inhibiting normal chondrocyte hypertrophy disappears, the lesion is gradually resorbed, and normal endochondral bone growth continues until sexual maturity.

Although a great deal of data describing the morphological and biochemical attributes of this disease have been collected, there is little information that directly demonstrates the mechanism responsible for TD lesion formation. The apparent lack of widespread effect on endochondral bone growth, the focal nature of the lesion, the variety of nutritional factors that can influence lesion formation, and the inability to map the genetic component of the disorder make TD research a significant challenge. At present, the best hope of understanding TD seems to be in examining other diseases of endochondral bone growth with known etiology and by studying transgenic models that produce similar lesions.

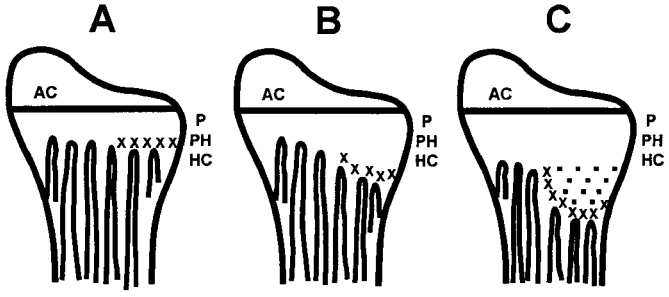

FIGURE 1. Development of tibial dyschondroplasia. A) Early stage of dysplastic lesion, B) developing dysplastic lesion, C) severe dysplastic lesion. AC = articular cartilage, P = proliferative zone, PH = Prehypertrophic zone, HC = hypertrophic chondrocytes, xx = chondrocytes with altered gene expression that provide a barrier to vascularization, and ■■ = apoptotic lesion chondrocytes.

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