Journal of Pediatric Rehabilitation Medicine: An Interdisciplinary Approach 3 (2010) 129–138
DOI 10.3233/PRM-2010-0117
IOS Press

Basic Science Review

Chondroitin sulfate and growth factor signaling in the skeleton: Possible links to MPS VI

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Accepted 25 March 2010

Abstract. Mucopolysaccharidosis type VI (MPS VI), also called Maroteaux-Lamy syndrome, is an autosomal recessive lysosomal storage disorder caused by deficiency of a specific enzyme required for glycosaminoglycan catabolism. Deficiency in the N-acetylgalactosamine-4-sulfatase (4S) enzyme, also called arylsulfatase B (ARSB), may have profound skeletal consequences. In MPS VI, partially degraded glycosaminoglycans (GAGs) such as dermatan sulfate and chondroitin sulfate accumulate within lysosomes. Through mechanisms that remain unclear, the abnormal GAG metabolism impacts several aspects of cellular function, particularly in the growth plate. This article explores the hypothesis that accrued partially degraded GAGs may contribute to deregulation of signaling pathways that normally orchestrate skeletal development, with a focus on members of the transforming growth factor-β (TGF-β) family. Understanding the molecular mechanisms disrupted by MPS VI may yield insight to improve the efficacy of MPS VI therapies, including bone marrow transplantation and enzyme replacement therapies.

Keywords: MPS VI, TGF-β, chondroitin sulfate, growth plate, osteoblast and chondrocyte differentiation

1. Growth plate sensitivity to GAG accumulation in MPS VI

MPS VI is associated with a spectrum of skeletal manifestations, as detailed by Beck in this issue. While the skeleton is relatively unaffected in some individuals, MPS VI is often accompanied by short stature, degenerative joint disease, dysostosis multiplex, and craniofacial dysmorphia [48]. Osteopenia is common due to reduced trabecular bone volume. All of these result from abnormalities in cartilage and bone development. The most severely affected bones – even in the skull – are those of endochondral origin [58], implicating the growth plate as a tissue that is particularly sensitive to the accumulation of partially degraded GAGs.

The growth plate coordinates the development and longitudinal growth of bones formed through endochondral ossification [34]. Immature chondrocytes in the resting zone near the periarticular surface proliferate and form columnar stacks. As these cells differentiate, they produce a collagen II and proteoglycan-rich matrix that is subsequently calcified. Terminally differentiated chondrocytes hypertrophy and undergo apoptosis, making way for the resorption of the calcified cartilage matrix and deposition of new bone matrix. Cells at each stage of differentiation possess a characteristic histologic appearance and molecular signature. These features have facilitated identification of numerous biochemical, chemical and physical cues that are required for skeletogenesis. Precise calibration of the timing, intensity, and localization of these cues establishes and maintains the characteristic order of this tissue to direct skeletal growth.

Investigation of MPS VI animal models has revealed that 4S (arylsulfatase B, N-acetylgalactosamine 4-sulfatase) insufficiency causes severe disorganization of the growth plate [23]. In MPS VI cats, resting zone chondrocytes are larger, more vacuolated, and

may be more proliferative, with a corresponding reduction in the amount of cartilage matrix in this region [51]. Although chondrocytes in the proliferative zone do not exhibit increased proliferation, the characteristic columns of chondrocytes are poorly organized or lost [1,14]. The hypertrophic zone chondrocytes are more vacuolated [1]. A recent study characterized the growth plates of MPS VII mice that are deficient in β-glucoronidase [45]. As in MPS VI, the enzyme deficiency in MPS VII also causes lysosomal accumulation of chondroitin sulfate and dermatan sulfate and skeletal malformations. MPS VII mice also show disorganized growth plates with a marked reduction in chondrocyte proliferation [45]. No apparent differences in chondrocyte apoptosis were observed in MPS VII growth plates. Clearly, the careful control of chondrocyte progression through the growth plate is disturbed by accumulation of partially degraded chondroitin sulfate and/or dermatan sulfate. Additional studies are needed to better understand the mechanisms responsible for the progressive growth plate disorganization in MPS VI and MPS VII.

Deficiency in 4S also alters the normal control of articular chondrocytes which may contribute to the degenerative joint disease in MPS VI. Chondrocyte apoptosis is increased in articular cartilage and in cultured articular chondrocytes from MPS VI cats and rats [62]. The increased chondrocyte apoptosis is thought to be responsible for the diminished levels of proteoglycan and collagen in articular cartilages. On the other hand, articular chondrocytes from MPS VI rats and cats exhibit a two-fold or five-fold increased proliferation rate in vitro, respectively, relative to cells from normal animals [60]. Although the increased apoptosis may be 'offset' by the increased proliferation to maintain somewhat normal cell numbers, the consequences on articular cartilage are significant.

2. MPS VI effects on bone

Abnormal regulation of growth plate cartilage impacts bone mass and quality. Bone mass is reduced in MPS VI due to smaller bones and reduced trabecular bone volume. The precise reason for shortened long bones in MPS VI has not been described but is likely affected by the altered dynamics of chondrocyte hypertrophy and growth plate closure. Following chondrocyte hypertrophy, calcified cartilage is normally resorbed to facilitate deposition of bone matrix. Insufficient resorption of calcified cartilage in the feline MPS VI growth plate can leave persistent islands of cartilage embedded in cortical bone [1]. The heterogeneity of the bone matrix may contribute to its inferior mechanical behavior in felines [49]. Abnormalities in endochondral ossification can also lead to defects in trabecular bone architecture, another key aspect of bone quality [26]. Therefore, some abnormalities in trabecular bone in MPS VI felines may originate from the effects of GAG accumulation on the growth plate during development.

Developmental defects in trabecular microarchitecture can be exacerbated by an imbalance in bone metabolism. Normally, the bone-depositing activity of osteoblasts is coupled to the bone-resorbing activity of osteoclasts [63]. The low bone mass in osteopenia or osteoporosis can result from insufficient bone formation or from excessive bone resorption. The bone formation rate, a dynamic measure of osteoblast function, is reduced in MPS VI felines [6,51]. The low bone mass in MPS VI is likely a combination of abnormal growth plate development and impaired osteoblast function.

Additional studies are needed to clarify the extent to which osteoclasts contribute to the low bone mineral density in MPS VI. There is a lack of consensus in the current literature, with some studies suggesting that osteoclast numbers are reduced or unchanged in MPS VI [1,50], while others suggest they are increased [61]. Differences may be due to animal models, stages of skeletal development or the skeletal sites examined. Recent data suggest that osteoclasts may be inappropriately recruited to joints in MPS VI [61]. Sphingosine-1-phosphate was recently shown to enhance osteoclast recruitment to sites of bone remodeling [29]. Interestingly, sphingosine-1-phosphate is increased in MPS VI chondrocytes [61]. MPS VI chondrocytes and synoviocytes also express elevated levels of receptor activator of nuclear factor-kappa B ligand (RANKL), a cytokine that is required for osteoclast differentiation and tissue resorption [61]. Therefore, deregulation of osteoclastogenic factors in MPS VI may cause inappropriate tissue resorption.

Understanding the regulation of osteoclast recruitment, differentiation, and activity in MPS VI will yield particularly valuable clinical insights. Since RANKL activates the tumor necrosis factor (TNF) pathway, clinically available TNF-inhibitors may reduce osteoclast-mediated catabolism in bone or in the joint for the treatment of MPS VI. Furthermore, sphingosine-1-phosphate agonists inhibited ovariectomy-induced osteoporosis by interfering with osteoclast recruitment in mice [29]. These agents may also have therapeutic implications for the treatment of osteopenia or degenerative joint disease in MPS VI.

3. Molecular clues from glycobiology

Though not responsible for all of the skeletal manifestations of MPS VI, the growth plate plays an critical role in this disorder. Many of the major developmental signaling pathways, including Wnts, fibroblast growth factors (FGFs), TGF-βs, bone morphogenetic proteins (BMPs), and hedgehogs, act on specific growth plate cell populations at distinct stages of development to ensure proper coordination of chondrocyte and osteoblast differentiation, vascularization, and patterning of bone size, shape, and position [34]. Through direct and indirect interactions with these ligands and receptors, GAG-containing proteoglycans are well known to influence the activity of these signaling pathways [40]. Consequently, a defect in GAG metabolism or compartmentalization has the potential to deregulate numerous essential regulators of skeletogenesis.

The mechanisms by which lysosomal GAG accumulation in MPS disorders cause skeletal malformations are unclear. One hypothesis is that release of partially degraded GAGs into the extracellular space may interfere with the function of GAG-containing proteoglycans in the growth plate. Interestingly, a recent study shows that the normal compartmentalization of chondroitin sulfate is disrupted in MPS VII, resulting in skeletal manifestations that are very similar to those in MPS VI [45]. Examining the interactions between the GAGs affected by 4S deficiency and critical growth plate signaling molecules may elucidate the unique vulnerability of the MPS VI growth plate.

The MPS disorders collectively reveal the tissue-specificity of GAG metabolism and function. Synthesis and catabolism of the diversity of GAGs requires a large number of enzymes, many of which are expressed in tissue-specific patterns [38]. Several of these enzymes are physiologically essential and non-redundant. A gross clinical classification of the MPS disorders correlates loosely with the family of GAG accumulated [8]. MPS with lysosomal storage of GAGs in the dermatan sulfate/chondroitin sulfate or keratan sulfate families are associated with skeletal abnormalities (MPS VI, MPS IVA, MPS VII). In contrast, storage of heparan sulfate is associated with central nervous system pathology (MPS III). Deficiency in enzymes common to multiple GAG pathways causes skeletal and CNS manifestations (MPS I, MPS II). Even within MPS VI, the accumulation of GAGs is tissue-specific, such that 80% of accumulated GAGs in hepatocyte lysosomes are iduronate-modified dermatan sulfates, whereas up to 90% of GAGs in bone and kidney cell lysosomes are glucuronate-modified chondroitin sulfates [8]. This result suggests that abnormal metabolism of GAGs of the chondroitin sulfate family may play a particularly important role in the skeletal manifestations of MPS VI.

Chondroitin sulfate is a major component of cartilage proteoglycans such as aggrecan. These proteoglycans, decorated with GAG chains, are well known for their mechanical and biological functions in articular cartilage. The negatively charged GAGs form ionic bonds with cations and water, thereby establishing hydrostatic and osmotic pressure within the tissue. The hydrostatic pressure allows cartilage to resist compressive loads equivalent to ten times the body weight [44]. With each loading cycle, the osmotic gradient facilitates nutrient exchange between the chondrocytes embedded within an avascular extracellular matrix and the synovial fluid. Loss of proteoglycan content in pathologies such as osteoarthritis diminishes the mechanical integrity of the tissue and nutrient and gas exchange. The work of Metcalf, et al. suggests that GAG compartmentalization is disrupted in MPS VII [45]. Additional studies are needed to determine if the release of partially degraded GAGs into the extracellular space in MPS disorders compromises the normal mechanical function of GAG-containing proteoglycans, and consequently, the viability of articular cartilage.

However, the role of GAGs in cartilage and bone is much more extensive. These highly charged molecules and the protein cores on which they are assembled bind several growth factors that direct the development of the skeleton and other organ systems [36,40]. Because of their essential roles in development, genes encoding GAG metabolic enzymes and growth factors in the TGF-β, Wnt, FGF, and hedgehog families are highly conserved. Taking advantage of these evolutionary relationships, genetic studies in Drosophila have identified several mechanisms by which proteoglycans influence growth factors signaling [40]. These fundamental mechanisms, elucidated primarily using heparan sulfate-containing proteoglycans, are conserved in mammalian systems and likely extend to chondroitin sulfate and dermatan sulfate-containing proteoglycans [67]. Studies by Radek, et al. show that dermatan sulfate enhances FGF-10 activity, and also illustrate the specificity of functional interactions between distinct ligands and GAG-containing proteoglycans [54].

Heparan sulfate containing proteoglycans have been shown to bind growth factors to concentrate, sequester, or pattern morphogenic signals [36]. GAG binding

of growth factors can stabilize ligands or modify their affinity for transmembrane receptors to augment or alter their bioactivity. Specificity is provided by the regulated, tissue specific expression of growth factors, core proteins, and enzymes required for GAG metabolism, as well as by differential affinity between growth factors and each GAG-modified proteoglycan. Proteoglycans, therefore, have an array of tools to modulate the specificity, localization, and activity of growth factor signaling. Conversely, deficiency of any enzyme that alters GAG availability, presumably including deficiency of 4S in MPS VI, can impact not only GAGs, but also the localization and activity of the growth factors to which it binds.

4. Critical role for chondroitin sulfate synthetic and catabolic enzymes in the growth plate

A mouse model deficient in one of the enzymes required for chondroitin sulfate synthesis clearly illustrates the growth factor regulatory role of this GAG in the growth plate. Since some aspects of the chondroitin 4-sulfotransferase (C4ST) deficient growth plate are reminiscent of features present in MPS VI [37], it is valuable to explore the role of C4ST and the consequences of its deletion in some detail. C4ST encodes a Golgi enzyme that catalyzes transfer of a sulfate group to the 4-O position of chondroitin sulfate and dermatan sulfate. Early in development, C4ST is expressed in the branchial arches which give rise to musculoskeletal and neurovascular structures in the head and neck and in the apical epidermal ridge of the limb bud, a key signaling center that dictates limb morphogenesis [37]. Later in development, expression localizes within skeletal primordia. Though mice deficient in C4ST survive through late embryonic development, they die within six hours of birth due to severe dwarfism and respiratory distress. At embryonic day 19.5, long bones and pelvic bones of C4ST-deficient mice are shorter and wider than those of wild-type littermates. Facial bones and vertebrae are also affected [37].

As in MPS VI, bones affected by C4ST deficiency were primarily formed by endochondral, but not intramembranous, ossification [58]. Growth plate organization was disrupted in C4ST-deficient mice, showing a loss of columnar organization and shortened proliferative and hypertrophic zones [37]. Molecular markers failed to identify a severe defect in the ability of chondrocytes to differentiate. However, C4ST-deficient growth plates showed a two-fold increase in cell division in the proliferative and columnar zones. The increased proliferation was overcome by a large increase in chondrocyte apoptosis throughout the growth plate. These defects were attributed to an overall reduction in chondroitin sulfate and a shift from predominantly chondroitin-4-sulfate in wild-type growth plates to predominantly chondroitin-6-sulfate in C4ST-deficient mice.

The C4ST-deficient mice are not identical to MPS VI. However, several similarities are striking. Specifically, the shortened and widened endochondral bones, the loss of columnar chondrocyte organization in the growth plate, and the loss of balanced chondrocyte proliferation and apoptosis are common features. Therefore, defects in either chondroitin sulfate synthesis (C4ST deficiency) or degradation (4S deficiency) cause cellular deregulation that impairs growth plate coordination of normal skeletal development. In addition, mutations in the chondroitin 6-O-sulfotransferase-1 gene are associated with human chondrodysplasia [66]. Together with observations that 90% of the GAG stored in MPS VI bone cell lysosomes is chondroitin sulfate [8], and that chondroitin sulfate compartmentalization is disrupted by deficiency in a lysosomal GAG-catabolic enzyme [45], these studies focus attention on the role of chondroitin sulfate in the MPS VI growth plate.

Using the C4ST-deficient mice, Wrana and colleagues evaluated the effect of altered chondroitin sulfate metabolism on hedgehog, TGF-β and BMP signaling activity. Although hedgehog signaling remained mostly intact, C4ST deficiency shifted the balance of TGF-β and BMP activity in the growth plate [37]. BMP and TGF-β activity was monitored by immunofluorescent detection of their phosphorylated intracellular effectors, Smad1 and Smad2, respectively. Normally, BMP-activated phospho-Smad1 is prominent and nuclear in hypertrophic chondrocytes, but very little nuclear phospho-Smad1 was detected in C4ST-deficient mice. Conversely, TGF-β-activated phospho-Smad2 was increased with more prominent nuclear localization in C4ST-deficient mice relative to wild-type controls. These studies collectively illustrate that C4ST deficiency hyperactivates TGF-β signaling, perhaps at the expense of BMP function. Chondroitin sulfate balances the activity and localization of TGF-β and BMP in the growth plate.

Which functions of chondroitin sulfate are essential for its role in the growth plate and could contribute to the skeletal manifestations of MPS VI? Relatively little is known about the mechanisms by which abnormal CS

metabolism – synthesis or catabolism – leads to skeletal malformations. Recent studies provide important clues and suggest a number of possibilities. First, deficiency in lysosomal enzymes that catabolize GAGs may cause abnormal chondroitin sulfate compartmentalization [45]. Conceivably, this could interfere with the normal interactions between extracellular proteins and chondroitin-sulfate-containing proteoglycans and glycoproteins that are essential for skeletal development and function. Second, Wilson, et al. showed that accumulation of GAG in the growth plate of MPS I mice inhibits osteoclast mediated-resorption of subepiphyseal cartilage, which may contribute to the skeletal abnormalities associated with MPS I [69]. Third, defects in chondroitin sulfate synthesis shift the balance of TGF-β and BMP signaling with significant consequences on growth plate organization [37].

To assist in the consideration of the many possible mechanisms, the remainder of this article explores the hypothesis that abnormal chondroitin sulfate metabolism, due to deficiency in 4S, disrupts the balance of TGF-β signaling in the growth plate and contributes to the skeletal manifestations of MPS VI. First, the numerous and functionally significant interactions between GAG-containing proteoglycans and the TGF-β family are reviewed. Second, the role of TGF-β and BMP in skeletal development, skeletal homeostasis, and skeletal disease are described. Finally, hypothetical and established connections between TGF-β, BMP and MPS VI are discussed. Although many lines of evidence suggest the possible involvement of the TGF-β family pathways in the skeletal manifestations of MPS VI, it is equally likely that other growth factor pathways could be affected by loss of normal GAG-metabolism. Indeed, Metcalf, et al. recently suggested that LIF/Stat-mediated signaling is impaired in MPS VII. Many of the mechanisms described here for TGF-β have also been implicated in other signaling pathways. By detailing the interactions of the TGF-β signaling pathway with GAGs and GAG-containing proteoglycans, this review aims to illustrate general mechanisms that are applicable to other growth factor signaling pathways.

5. Interaction of proteoglycans with TGF-β family signaling

The TGF-β family consists of 33 growth factors, including 3 isoforms of TGF-β itself, the BMPs, GDFs (growth differentiation factors), inhibins and others [12]. These secreted polypeptide ligands regulate a diversity of cellular functions throughout the body and have been implicated in the biology of healthy tissue and disease. In spite of their wide-ranging functions, this family of growth factors signals through conserved mechanisms that were initially described for TGF-β. These mechanisms have since been extended to other family members and elaborated through extensive research.

Several extracellular matrix proteins influence the localization, level, and activity of ligands in the TGF-β family [4,11]. For example, fibrillin binds the latent TGF-β complex to prevent its processing to an active form [47]. Fibrillin mutations that cause promiscuous TGF-β activation are linked to Marfan syndrome. Marfan syndrome, along with Loeys-Dietz syndrome and Camurati-Engelmann disease, exhibit skeletal malformations that result from aberrant control of TGF-β activity [41,55]. To counteract the effects of hyperactivated TGF-β signaling, pharmacologic inhibitors of TGF-β function have been proposed as a therapy for some members of this group of developmental disorders [22].

TGF-β is also bound by chondroitin sulfate-containing proteoglycans such as biglycan and decorin. Decorin and biglycan can modulate TGF-β-dependent cellular responses, apparently in a tissue-specific manner [24,64,71]. Enzymatic removal of the chondroitin and dermatan sulfate chains increases TGF-β affinity for decorin and biglycan and may impact interactions between the ligand and its receptor [27]. Biglycan and decorin-deficient mice both have osteopenic phenotypes [10,70]. Much additional research is needed to better understand the mechanisms by which matrix proteins and proteoglycans control the latency and signaling of TGF-β and other growth factors, particularly in the skeleton.

The activated TGF-β family ligand binds to a tetrameric complex of transmembrane receptors, each of which possesses serine-threonine kinase activity. Ligand-induced aggregation of the two type I receptors and two type II receptors induces receptor cross-phosphorylation and phosphorylation of an array of effector molecules, the most famous of which are the receptor-activated Smad proteins [15,59,65]. Betaglycan, also a chondroitin sulfate-containing proteoglycan, functions as a TGF-β type III coreceptor with distinct affinities for different TGF-β family member ligands. In this way, betaglycan can shift the balance of signaling among TGF-β, inhibin, and BMP [35].

Inside the cell, Smads confer TGF-β and BMP-activated signals to the nucleus to regulate gene expression [15,59,65]. BMP signaling activates Smads

1, 5, and 8, whereas TGF-β activates Smads 2 and 3. Following phosphorylation by the type I receptor, two receptor-activated Smads form a trimeric complex with the co-Smad, Smad4. This complex then translocates into the nucleus where it interacts with other transcription factors that bind DNA with greater affinity and specificity than the Smads themselves. The interaction of Smads with these sequence-specific transcription factors directs the association of transcriptional coregulators that activate or repress transcription. For example, TGF-β-activated Smad3 binds the transcription factor AP-1 at the MMP-1 promoter, facilitating recruitment of CBP, a histone acetyltransferase [16,30, 73]. The increased histone acetylation promotes the recruitment of RNA polymerase II to transcribe AP-1 target genes. Conversely, Smads can also recruit corepressors with histone deacetylase activity to repress gene transcription [33]. The differential regulation of histone acetylation, therefore, is one mechanism by which TGF-β family members influence the level of gene expression. TGF-β controls expression of many chondroitin sulfate-containing proteoglycans, including biglycan and decorin [32].

The basic components outlined here represent the most distilled version of the TGF-β family signaling pathway. Importantly, the TGF-β family pathways interact with numerous other signaling pathways, effectors, and regulatory proteins [74]. Each of these interactions has the capacity to modify signaling location, duration, function, intensity or specificity.

6. TGF-β and BMP in the skeleton

The importance of the TGF-β family in the skeleton is most apparent when considering the many human skeletal disorders associated with mutations in this pathway. Human mutations in GDF5 and the BMP receptor Ib (BMPRIb) have been linked to chondrodysplasia and brachydactyly, while mutations in the BMP antagonist noggin cause symphalangism and synostoses [2]. Mutations in TGF-β family signaling components have also been linked to human osteoporosis, osteoarthritis, ectopic bone formation and sclerosing bone disorders. Using genetic mouse models, many additional participants in TGF-β family pathways have been implicated in skeletal biology and disease.

Another indication of the physiologic necessity of TGF-β family signaling in the skeleton is the widespread and tightly regulated expression of TGF-β family ligands, receptors and effectors throughout skeletal development [2]. The expression of each TGF-β and BMP ligand is temporally and spatially regulated in a unique but overlapping manner that confers both specificity and redundancy. Each cell population in the skeleton has the capacity to respond to TGF-β and BMP signaling including osteochondroprogenitors, chondrocytes, osteoblasts, and osteoclasts. In this way, TGF-β and BMP exert their critical and multifaceted roles in bone and cartilage.

The mechanisms of TGF-β and BMP activity in the skeleton have been studied extensively in vitro and in vivo and are beyond the scope of this article but are detailed in several sources [2,9,11,13,18,31]. The effects of both TGF-β and BMP require interaction with several other regulatory pathways. In general, BMPs act in growth plate cartilage by promoting chondrocyte proliferation and differentiation, but also by promoting chondrocyte hypertrophy and apoptosis [9]. In bone, BMP drives commitment of osteochondroprogenitors to an osteogenic lineage and then induces osteoblast proliferation, differentiation, and matrix mineralization. Hyperactive BMP signaling can cause premature osteocyte apoptosis and lead to osteopenia [25].

TGF-β, initially isolated as a cartilage-inducing factor, promotes mesenchymal cell recruitment to sites of condensation and selection of the chondrogenic lineage. In general, TGF-β promotes early stages of chondrocyte differentiation and matrix production but inhibits terminal differentiation, hypertrophy and apoptosis [13,31]. Unlike BMP, TGF-β does not promote osteoblast commitment. It can, however, promote recruitment and proliferation of osteoprogenitors at sites of new bone formation. TGF-β antagonizes terminal osteoblast differentiation. Although an oversimplification, TGF-β drives both chondrocytes and osteoblasts towards matrix production and prevents their exit from this synthetic state [11,13,31].

One mechanism by which TGF-β inhibits terminal osteoblast differentiation is the repression of Runx2, a critical osteoblast transcription factor, through a Smad3 and histone deacetylase-dependent mechanism [3,33]. TGF-β repression of Runx2 function inhibits transcription of Runx2-target genes, including Runx2 itself and osteocalcin. Accordingly, osteocytes in both Smad3-deficient mice and in mice that overexpress Runx2 in bone exhibit an osteopenic phenotype with premature osteocyte apoptosis, the most terminal event in osteoblast differentiation [7,20].

TGF-β and BMP also act on osteoclasts. Both growth factors can bind and activate specific receptors on osteoclasts and their monocyte/macrophage progen-

itors to regulate osteoclast differentiation and function [18,52]. In addition, TGF-β can induce and repress the expression of several osteoclast regulatory factors in osteoblasts, including osteoclast-inducing RANKL, osteoclast-inhibitory osteoprotegerin (OPG), ephrin B2 and EphB4 [18,46]. This osteoclast-osteoblast crosstalk couples the activity of the bone resorbing and bone-forming cells to maintain stable bone mass. TGF-β participates in the osteoclast-mediated bone resorption that accompanies and promotes metastatic invasion of osteolytic tumors into bone [21]. Consequently, the molecular mechanisms of TGF-β action are more well-established than for BMPs. Although effects of TGF-β on osteoclast differentiation and function are dose, time and differentiation-stage specific [18], we recently found that inhibition of TGF-β signaling in mice using small molecule inhibitors of the TGF-β type I receptor reduced osteoclast numbers, which together with increased osteoblast function, greatly increased bone mass [46].

TGF-β family members influence bone quality at multiple levels of hierarchical organization. Alteration of TGF-β signaling affects bone mass, which in turn impacts the ability of bone to resist fracture [5,17,19, 46]. In addition, we have implicated TGF-β as a regulator of the material quality of bone matrix. Bone matrix from mice with increased TGF-β signaling has lower mineral concentration and a reduced elastic modulus, whereas a reduction in TGF-β signaling increases mineral concentration and elastic modulus [5,46]. TGF-β can regulate bone matrix material properties independently of its effects on bone mass, though the mechanisms remain unknown. In macromechanical testing, bones from MPS VI felines have decreased stiffness and strength, likely due to the osteopenia and imbalanced bone metabolism [49]. Clinically, however, bone fracture is not a common problem in MPS VI patients.

7. Balanced TGF-β and BMP signaling is essential for skeletogenesis and may be disrupted in MPS VI

TGF-β and BMP can act cooperatively or antagonistically in the coordination of endochondral ossification. Signaling activity depends on many factors, including the activity of other pathways, the stage of cell differentiation, and other cues present in the microenvironment, including the availability of specific chondroitin sulfate isoforms. As shown in C4ST-deficient mice, loss of balanced TGF-β and BMP function disrupts growth plate organization, cell proliferation, and apoptosis [37]. The hypothesis proposed here is that impaired chondroitin sulfate metabolism in MPS VI disrupts the normal balance of TGF-β and BMP signaling. Furthermore, such deregulation of TGF-β and BMP signaling may be responsible for specific skeletal manifestations of MPS VI. Testing these hypotheses may provide insight into the mechanisms of MPS VI and reveal novel therapeutic targets for its treatment.

These hypotheses are supported by a number of mouse models in which disruption of the balance of TGF-β and BMP signaling impairs growth plate organization. While chondroitin sulfate calibrates TGF-β and BMP bioavailability in the extracellular space, effectors such as the Smads modulate their relative activities inside the cell. Targeted mutation or deletion of Smads in the mouse growth plate, including the common Smad4, BMP receptor-activated Smad1 and Smad5, or inhibitory Smad6, disrupt growth plate function and organization [28,57,72]. Interestingly, these studies have revealed additional complexity in TGF-β and BMP signaling in the growth plate, highlighting the importance of 'non-canonical' pathways. In vitro studies have clarified mechanisms of TGF-β and BMP crosstalk in chondrocyte and osteoblast differentiation. BMP-induced phosphorylation of Smads 1, 5, and 8 is enhanced in Smad3-deficient chondrocytes [39]. Treatment of C2C12 myoblasts with TGF-β type I receptor inhibitors increases BMP function by repressing Smad6 expression, leading to their osteogenic differentiation [43]. The potential mechanisms by which TGF-β ligands and chondroitin sulfate may contribute to the skeletal manifestations of MPS VI are numerous. Of note, a recent immunohistochemical evaluation of BMP and TGF-β-activated Smad phosphorylation showed no differences between wild-type and MPS VII growth plates [45]. While this does not discount the involvement of TGF-β signaling pathways in either MPS VI or MPS VII, it highlights the need for additional research to test the integrity of canonical and non-canonical TGF-β signaling mechanisms in the context of MPS VI.

8. Conclusions

Clearly TGF-β family members and chondroitin sulfate play essential roles in the control of skeletogenesis. Several pieces of evidence point to a role for the TGF-β family and chondroitin sulfate in MPS VI. First, chondroitin sulfate is the most abundant lysosomal GAG

in MPS VI bone [8], consistent with the deficiency in MPS VI of the 4S enzyme responsible for chondroitin sulfate catabolism. Second, in the related disorder, MPS VII, chondroitin sulfate compartmentalization is disrupted, resulting in high levels of partially degraded chondroitin sulfate in the extracellular space [45]. Third, mice deficient in the chondroitin sulfate synthetic enzyme, C4ST, exhibit growth plate disorganization and shortened bones, much like bones in MPS VI. In addition to these phenotypic similarities with MPS VI, C4ST-deficient mice reveal that chondroitin sulfate maintains the balanced activity of two critical growth plate morphogens, TGF-β and BMP [37]. The expression of TGF-β and several TGF-β-regulated genes is disrupted in MPS VI. TGF-β levels are elevated in MPS VI chondrocytes [60]. The expression of RANKL, MMP2, MMP9, and MMP13, each of which is a reported TGF-β target gene [3,18,42,53,56], is abnormal in MPS VI [60,61]. Several of these are also regulated by chondroitin sulfate [68]. Whether or not the effects of chondroitin sulfate on expression of these genes are TGF-β-dependent remains unclear.

In conclusion, abundant clinical, cellular, biochemical and genetic evidence has provided compelling rationale to investigate the deregulation of chondroitin sulfate homeostasis and its effects on TGF-β family and other growth factor signaling in the MPS VI skeleton. This area is not only scientifically ripe with opportunity to understand the mechanisms controlling skeletogenesis, but also has significant potential to improve the clinical management of MPS VI.

Acknowledgements

This work was supported by NIH RO3 DE016868, NIH RO1 DE019284, and the Sandler Program in Basic Sciences.

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