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Fibrillin microfibrils in bone physiology

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Abstract

The severe skeletal abnormalities associated with Marfan syndrome (MFS) and congenital contractural arachnodactyly (CCA) underscore the notion that fibrillin assemblies (microfibrils and elastic fibers) play a critical role in bone formation and function in spite of representing a low abundance component of skeletal matrices. Studies of MFS and CCA mice have correlated the skeletal phenotypes of these mutant animals with distinct pathophysiological mechanisms that reflect the contextual contribution of fibrillin-1 and -2 scaffolds to TGF β and BMP signaling during bone patterning, growth and metabolism. Illustrative examples include the unique role of fibrillin-2 in regulating BMP-dependent limb patterning and the distinct impact of the two fibrillin proteins on the commitment and differentiation of marrow mesenchymal stem cells. Collectively, these findings have important implication for our understanding of the pathophysiological mechanisms that drive age- and injury-related processes of bone degeneration.

Keywords

autopod patterning; bone marrow niche; fibrillin; Marfan syndrome; mesenchyme stem cells; osteopenia; TGF β and BMP signaling

Introduction

Reciprocal interactions between extracellular matrix (ECM) proteins, resident cells and soluble biochemical signals play a critical role in tissue differentiation, growth and homeostasis [1]. Fibrillin assemblies (microfibrils and elastic fibers) are ubiquitous elements of the architectural matrix that impart specific physical properties to tissues, transmit mechanical forces across them, communicate with cells through integrin receptors, and modulate the concentration, presentation and activation (bioavailability) of locally produced TGF β and BMP complexes [2–4]. Consonant with this multiplicity of functions, severe systemic manifestations are associated with mutations in fibrillin-1 and fibrillin-2 in Marfan syndrome (MFS) and congenital contractural arachnodactyly (CCA), respectively [5]. Here

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we review the findings relevant to skeletal abnormalities in MFS and CCA mice that have revealed unsuspected new roles of fibrillin assemblies in bone physiology.

Fibrillins; a brief history

In 1951, a report by Hall et al. [6] described for the first time an elastase-resistant mucoprotein-containing overcoat tightly bound to elastic fibers later, which was subsequently found to consist of 10-nm-diameter microfibrils lacking the characteristic collagen banding [7]. In 1986, Sakai et al. [8] identified a 350Kda, cysteine-rich glycoprotein (later re-named fibrillin-1) as the major structural component of the 10-nm-diameter microfibrils. Soon after that report appeared, Hollister et al. [9] showed a significant paucity of fibrillin-1 microfibrils exclusively in tissues and cultured cells isolated from patients afflicted with MFS. In 1991, three back-to-back articles were published that established the primary structure of fibrillin-1; demonstrated genetic linkage between MFS and fibrillin-1; identified a spontaneous *FBN1* mutation in two unrelated MFS patients; and cloned a second fibrillin gene (*FBN2*) co-segregating with the CCA locus on chromosome 5 [10–12]. The generation and characterization of mouse models of MFS and CCA subsequently led to the surprising discovery that the fibrillin proteins are also involved in regulating the bioavailability of local TGF β and BMP signals [13, 14].

Structure and function of fibrillin proteins

Fibrillin-1 and fibrillin-2 share a superimposable modular structure that almost entirely consists of calcium-binding EGF (cb-EGF) and 8-cysteine (TB/8-Cys) motifs, which are also the principle elements of the primary structure of latent TGF β -binding proteins (LTBPs) [3]. The two fibrillins differ from each other for the number of putative glycosylation and integrin-binding sites, and in the composition of a short internal domain devoid of cb-EGF and TB/8-Cys sequences. Fibrillins can bind in vitro to the pro-peptide of some BMPs and to several ECM proteins, including LTBPs and elastin in the elastic fibers [2]. Fibrillins give rise to 10-nm-diameter microfibrils through an as yet to be characterized process whereby individual molecules are organized into head-to-tail polymers that associate laterally with one another and incorporate other ECM proteins [3]. Fibrillins co-distribute in most tissues with fibrillin-2 representing the least abundant of the two proteins and the one principally expressed during tissue morphogenesis and remodeling [3, 15]. While it is unclear if fibrillin-1 and fibrillin-2 form homotypic and/or heterotypic assemblies in vivo [15], studies of mice lacking either or both fibrillins have revealed both unique and partially overlapping non-structural functions during embryonic and postnatal tissue differentiation.

TGF β and BMP molecules specify a plethora of cellular activities, including ECM formation and remodeling [2]. TGF β and BMP signaling are regulated at multiple levels, including extracellularly through ligand's storage in and release from the ECM. By binding to both small latent TGF β complexes and fibrillins, LTBPs tether TGF β molecules to the ECM from where they are released and activated through non-proteolytic and proteolytic mechanisms [16]. Direct binding of bioactive pro-BMPs to fibrillins similarly results in growth factor latency [17]. Fibrillin-mediated sequestration of TGF β and BMP complexes in the ECM is thought to promote the spatial distribution and proper concentration of bioactive ligands for

either immediate presentation to cells (positive regulation) or for subsequent release during tissue remodeling/repair (negative regulation) [2]. Owing to the clinical severity of MFS, most functional studies have focused on the pathogenesis of cardiovascular abnormalities in *Fbn1* mutant mice. As a result, instructive functions of fibrillin-1 assemblies that have been associated with cardiovascular physiology include transducing mechanical signals from the periphery to cardiomyocytes and modulating TGF β -dependent homeostasis of the aortic wall [4, 18, 19]. Studies described later in this review have unraveled additional non-structural roles of fibrillin assemblies in the developing and adult skeleton of mice.

Fibrillin assemblies in skeletal tissues

Fibrillins constitute only a small fraction (1–3%) of the ECM proteins deposited in the soft and hard tissues of the skeleton; like in other organ systems, fibrillin-2 represents the less abundant component of these fibrillin assemblies [5]. Onset of fibrillin expression in the developing skeleton precedes mesenchyme differentiation, continues throughout post-natal growth and is reactivated during bone remodeling or fracture repair [5, 13, 20, 21]. Fibrillin expression eventually leads to the formation of the uninterrupted elastic fibers running along the entire length of the perichondrium, the circumferential microfibril bundles wrapped around the Ranvier's groove and growth plate's chondrocytes, and the compact microfibril meshworks deposited at the endochondral surface, within trabecular and cortical matrices, and around mesenchymal stem cells (MSCs) in the adult bone marrow [3, 22]. Fibrillin assemblies in tendon/ligament tissue include the elastic fibers of the fibrocartilaginous, avascular/tensional and bone insertion regions, and the microfibrils located around putative stem/progenitor cells and differentiated tenocytes [23, 24].

Despite their relatively low abundance, mutations in fibrillin-1 and fibrillin-2 lead to severe skeletal abnormalities. The most striking and immediately evident manifestation of MFS patients is a disproportionate increase in longitudinal bone growth that results in serious malformations of the limbs, spine and anterior chest wall [3]. Additional skeletal abnormalities include joint laxity and dural ectasia. Low bone mass (osteopenia) has long been a controversial finding due to the lack of robust normative data for children and standardized protocols for bone mineral density (BMD) measurements in MFS vs. unaffected individuals [25]. However, more recent clinical studies performed with improved imaging modalities and larger cohorts of MFS patients have concluded that reduced BMD during childhood may lead to a low peak bone mass, thus increasing the risk of fractures during adulthood [26–28]. Multiple joint contractures and generalized osteopenia are clinical hallmarks of CCA [3]. In rare instances, mutations in fibrillin-1 can cause Weill-Marchesani syndrome (WMS), geleophysic dysplasia (GD) and acromicric dysplasia (AD), connective tissue diseases that manifest joint and bone growth abnormalities different from those of MFS [29]. The unique impact of these rare, domain-specific *FBNI* mutations on microfibril biogenesis may explain the distinct skeletal phenotypes of this group of connective tissue diseases [30]. The additional finding that WMS, GD and AD are also associated with mutations in other ECM components has led to the demonstration that these structurally unrelated proteins interact with fibrillin-1 during microfibril biogenesis [29,31].

Fibrillin-2 and limb patterning

Fibrillin-2 deficiency in mice causes multiple joint contractures that, like those of CCA patients, disappear concomitantly with the switch from fetal (*Fbn2*) to postnatal (*Fbn1*) gene expression, thus reiterating the distinct contributions of fibrillin proteins to tissue formation and growth [13]. Similarly, characterization of a unique limb-patterning defect in *Fbn2*^{-/-} mice, syndactyly, has provided the first demonstration that tissue-, stage- and fibrillin-specific mechanisms underlie microfibril regulation of TGFβ and BMP bioavailability [13]. Digit formation is a complex morphogenetic process that involves several signaling molecules, including BMPs, which drive the naïve mesenchyme of the forming autopod toward either chondrogenic outgrowth or interdigital cell death [32]. Fibrillin-2 deficiency has been associated with disrupted microfibril organization in the autopod and failed activation of BMP-induced apoptosis of resident cells of the presumptive interdigital space (Fig. 1) [13]. The additional finding of syndactyly in otherwise normal *Fbn2*^{+/-};*Bmp7*^{+/-} mice has validated the notion of functional interaction between these two proteins exclusively at a specific developmental stage and anatomical site of the limbs. Fibrillin-1 inability to compensate for the loss of fibrillin-2, in spite of robust co-expression and comparable BMP binding, has further underscored the diverse contributions of fibrillins to modulating growth factor bioavailability. In contrast to the positive role of fibrillin-2 in BMP signaling during autopod formation, characterization of myopathy in *Fbn2*^{-/-} mice has revealed that this ECM protein actually restricts BMP signaling during early postnatal development of skeletal muscles [33]. Together, these findings reiterate the notion that hierarchical assembly of fibrillin macro-aggregates specifies the contextual activity of TGFβ family members in individual tissues and at defined stages of tissue differentiation.

Fibrillins and bone remodeling

Regulators of bone remodeling include systemic (extrinsic) signals, such as circulating hormones, and local (intrinsic) factors, such as ECM-bound growth factors and ECM components that directly or indirectly influence bone cell differentiation and/or matrix mineralization [34]. In this view, the spatiotemporal balance between locally generated TGFβ and BMP signals is one of the key intrinsic factors that enable bone remodeling to properly progress [35]. Studies of osteopenia in MFS and CCA mice have correlated the gradual progression of bone disease with distinct pathophysiological mechanisms that are principally driven by unique, stage-specific perturbations of local TGFβ and BMP signaling (Fig. 2) [20, 36]. More generally, these findings have implied that fibrillin assemblies are integral components of the dynamic networks of intrinsic signals that control bone anabolism and bone catabolism.

Reduced BMD and trabecular bone content were originally described in *Fbn1* hypomorphic mice (*Fbn1*^{mgR/mgR} mice), which recapitulate the lethal cardiovascular complications of early onset, progressively severe MFS [37]. These in vivo findings were correlated in vitro with accelerated maturation of calvarial osteoblasts and increased osteoblast-dependent osteoclast activity. Parallel analyses of *Fbn2*^{-/-} mice have revealed severely impaired maturation of calvarial osteoblast cultures and a modest increase of osteoblast-supported osteoclastogenesis [20]. While TGFβ-dependent stimulation of RANKL expression by

osteoblasts was demonstrated to account for augmented bone resorption in both strains of mutant mice [36, 37], deficiency of either fibrillin-1 or fibrillin-2 was found to perturb TGF β - and BMP-regulated programs of osteoblast maturation and matrix mineralization in a distinct manner [20]. Whereas impaired differentiation of *Fbn2*^{-/-} osteoblasts was associated with TGF β hyperactivity, accelerated differentiation of *Fbn1*^{mgR/mgR} osteoblasts was associated with both TGF β and BMP hyperactivity. As result of unbalanced TGF β and BMP signaling in *Fbn2*^{-/-} but not in *Fbn1*^{mgR/mgR} mice, only osteoblast cultures from the former mice displayed a dramatic reduction in Osx-driven collagen synthesis and consequentially, in matrix mineralization (Fig. 2). While these findings were consistent with the opposing roles of the growth factors in osteoblast maturation [35], they did not explain how structurally related components of the same ECM assemblies could differentially modulate growth factor bioavailability signals of bone formation. Along the same lines, additional experiments using *Fbn1*- or *Fbn2*-silenced Kusa-A1 osteoprogenitor cell lines have reiterated the distinct contributions of the two matrix proteins to the activity of intrinsic regulators of bone cell proliferation and differentiation [21]. Furthermore, biophysical analyses have indicated that fibrillin-2 plays a more prominent role than fibrillin-1 in specifying the mechanical properties of cortical bone [38].

Fibrillin-1 and the bone marrow niche

MSC maintenance, commitment and differentiation during bone growth and remodeling largely depends on the partition of adult bone marrow tissue into functionally distinct microenvironments (niches) [39]. A few non-structural ECM proteins have been implicated in regulating MSC activity within bone marrow niches. For example, characterization of mice deficient for both biglycan and decorin has suggested that these small leucine-rich proteoglycans (SLRPs) that interact with both collagen fibrils and TGF β molecules modulate the proliferation and survival of marrow stem/progenitor cells [40]. In line with these findings, it has been argued that perturbed interactions between collagen I fibrils and SLRPs may account for TGF β -dependent bone loss in mouse models of osteogenesis imperfecta [41]. Recent analyses of mice with fibrillin-1 deficient bones have demonstrated that this component of the architectural matrix defines the functional microenvironment of adult bone marrow niches by regulating TGF β -stimulated activities of resident MSCs (Fig. 2).

Osteopenia in mice with restricted *Fbn1* inactivation in either the limb mesenchyme cells or early pre-osteoblasts (*Fbn1*^{Prx-/-} and *Fbn1*^{Osx-/-} mice, respectively) was the first evidence that accelerated osteoprogenitor cell differentiation may be a primary determinant of bone loss in MFS [42]. Indeed, subsequent longitudinal analyses of *Fbn1*^{Prx-/-} mice have revealed that premature depletion of MSCs and osteoprogenitor cells combined with constitutively enhanced bone resorption account for age-related, progressive loss of cancellous bone in these mutant animals [22]. Additional findings have included a surprising paucity of marrow fat due to impaired MSC commitment to adipogenesis, and over-activation of latent TGF β complexes in marrow cell cultures derived from *Fbn1*^{Prx-/-} bones. Consistent with the latter observation, systemic treatment of *Fbn1*^{Prx-/-} mice with a TGF β neutralizing antibody normalized the number of MSCs, osteoprogenitor cells, osteoblasts, osteoclasts and adipocytes with the result of improving bone mass and trabecular microarchitecture, and restoring marrow adipogenesis [22].

While the above studies have established that fibrillin-1 assemblies regulate MSC fate by modulating TGF β bioavailability within marrow niches, they have also raised several new questions. First and foremost is the question of how fibrillin-1 deficiency may influence osteoclastogenesis, aside from stimulating RANKL production by osteoblasts. One possibility is that the ECM defect may indirectly influence osteoclast progenitor cell differentiation as result of the functional crosstalk between MSC and hematopoietic stem cell (HSC) niches. Recent experimental evidence of abnormal hematopoiesis in *Fbn1*^{Prx-/-} mice strongly supports this possibility [Smaldone et al, manuscript submitted]. Another probable determinant of increased bone catabolism in MFS is the negative role that fibrillin-1 plays in osteoclastogenesis by sequestering RANKL in the ECM and by preventing translocation of transcription factor NFATc1 in the nuclei of differentiating osteoclasts [43]. Additional unanswered questions include the identity of the MSC population and stage of adipogenesis affected by fibrillin-1 deficiency, and the potential relationship, if any, between reduced bone mass and increased linear length of MFS bones.

In contrast to the findings with fibrillin-1-deficient mice, studies of mice with a unique *Fbn1* mutation causing tight skin (*Tsk*/+ mice) have correlated bone loss with decreased osteogenesis and increased adipogenesis due to defective MSC lineage determination [44]. One probable explanation for this apparent discrepancy is that osteopenia in MFS and *Tsk*/+ mice represents an interesting example of mutations in the same gene that trigger distinct pathophysiological mechanisms leading to the same phenotype. On the one hand, MFS mutations decrease the threshold of functional microfibrils with the result of influencing MSC fate by perturbing the structural microenvironment that controls TGF β signaling within the adult marrow niche [22]. On the other hand, the *Tsk* mutation exerts a dominant-negative effect on integrin-mediated microfibril assembly that apparently modifies MSC commitment through improper activation of ILK4/mTOR signaling [44, 45]. However, it has been noted that the reported improvement of MSC differentiation in *Tsk*/+ mice by genetic or pharmacological inhibition of mTOR signaling is contrary to the well-established notion that this pathway is absolutely required to support the continuous, high level protein synthesis required for osteogenesis to progress [46]. Future analyses of bone mass in a related mouse model of *Fbn1*-caused systemic sclerosis - namely mice that replicate human stiff skin syndrome [47] - may shed some light on this intriguing conundrum.

Conclusions and medical perspectives

Studies of the skeletal phenotypes of mice with distinct dysfunctions of fibrillin assemblies have significantly advanced our understanding of the structural and instructive factors that underlie the dynamic cell/matrix interactions promoting and supporting tissue differentiation and homeostasis. By documenting the distinct spatiotemporal contributions of fibrillins to TGF β - and BMP-driven tissue differentiation, these investigations have revealed that the compositional diversification of microfibrils specifies the contextual outcome of TGF β and BMP signaling. More generally, these findings imply that the architectural ECM directly controls intrinsic determinants of stem cell fate during bone patterning and remodeling. As such, a new view is emerging that suggests functional coupling between tissue-specific assembly of an architectural matrix and extracellular localization of signaling molecules.

Studies described in this review have translational implications for the clinical management of MFS patients, particularly severely affected pediatric patients. The discovery that TGF β neutralization can maintain normal bone mass and microarchitecture in MFS mice represents an attractive new treatment option against increased risk of fractures in MFS patients [22]. However, implementation of such therapeutic strategy should be weighed against the reported detrimental impact of TGF β neutralization on aortic growth in severely affected MFS mice [19]. It is therefore safe to predict that more research effort will be devoted in the future toward identifying new drug treatments that can normalize pathogenic TGF β hyperactivity in the cardiovascular and musculoskeletal systems, while sparing physiological TGF β signaling in the growing aorta.

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Highlights

- Fibrillin assemblies are low abundance structural components of skeletal tissues that regulate the bioavailability of locally produced TGF β and BMP complexes.
- Mutations in fibrillin-1 and fibrillin-2 cause severe skeletal abnormalities in patients afflicted with Marfan syndrome (MFS) and congenital contractural arachnodactyly (CCA), respectively.
- Progressive bone loss in mouse models of MFS and CCA is associated with distinct defects in mesenchymal stem/progenitor cell differentiation that reflect the discrete contributions of fibrillin proteins to bone formation and metabolism.
- Fibrillin assemblies therefore represent a unique experimental model to interrogate the dynamics of cells/matrix interactions that orchestrate the differentiation, homeostasis and repair of skeletal tissues.

Figure 1. Fibrillin-2 is a positive regulator of BMP signaling in the developing autopod

Unlike the presumptive digital (D) region of the forming autopod, fibrillin-1 and fibrillin-2 molecules (orange and purple bar, respectively) are abundantly deposited in the interdigital (ID) matrix where BMP complexes (yellow arrowheads) are concentrated via interaction with fibrillin-2 assemblies so to promote apoptosis of naïve mesenchymal cells (pink ovals) after been released from the matrix (red arrowhead) [top three panels]. Loss of fibrillin-2 perturbs ID matrix organization, blunts BMP signaling and prevents mesenchymal cell death with the tissue-level outcome of fused digits [bottom two panels].

Figure 2. Fibrillins are negative regulators of TGFβ-and BMP-driven bone cell differentiation
 A schematic overview of microfibril modulation of local TGFβ and BMP signaling during bone remodeling that is based on the characterization of osteopenia in mice deficient for either fibrillin-1 or fibrillin-2.