Review

Fibrillin microfibrils in bone physiology

Silvia Smaldone and Francesco Ramirez

Department of Pharmacology and Systems Therapeutics, *Institute for Systems Biomedicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States*

Correspondence to Francesco Ramirez: *Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10021, United States. Francesco.ramirez@mssm.edu*
http://dx.doi.org/10.1016/j.matbio.2015.09.004
Edited by Marian Young

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 TGFβ 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.

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 TGFβ 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 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 350 kDa, 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 TGFβ 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 principal elements of the primary structure of latent TGFβ-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 a 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.

TGFβ and BMP molecules specify a plethora of cellular activities, including ECM formation and remodeling [2]. TGFβ 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 TGFβ complexes and fibrillins, LTBPs tether TGFβ 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 TGFβ 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 TGFβ-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 *Fbn1* 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