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Review

Extracellular microfibrils in development and disease

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Abstract. Fibrillins are the structural components of extracellular microfibrils that impart physical properties to tissues, alone or together with elastin as elastic fibers. Genetic studies in mice have revealed that fibrillin-rich microfibrils are also involved in regulating developmental programs and homeostatic processes through the modulation of TGF-β/BMP signaling events. A new paradigm has thus emerged whereby the spatiotemporal organization of microfibrils dictates both the cellular activities and physical properties of connective tissues. These observations have paved the way to novel therapeutic approaches aimed at counteracting the life-threatening complications in human conditions caused by dysfunctions of fibrillin-rich microfibrils.

Keywords. Aortic aneurysm, development and morphogenesis, emphysema, fibrillin, extracellular matrix, Marfan syndrome, myopathy, TGF-β/BMP signaling molecules.

Introduction

The fundamental mechanisms that determine cell proliferation, migration, differentiation and survival depend on a variety of dynamic interactions with the surrounding matrices. The extracellular matrix (ECM) is a highly heterogeneous amalgam of macromolecules capable of self assembling into tissue-specific multimolecular structures that impart mechanical and physiological properties to connective tissues, define histological boundaries within different organs, provide structural information to resident cells, and regulate the biological activity of intercellular signaling molecules. Interactions amongst structural components, soluble signaling factors, resident cells, and adjacent matrices are key determinants of the development, growth and function of every organ system. Conversely, mutations in ECM components negatively impact the orderly processes that shape higher order tissue structures, resulting in deleterious consequences for the fitness and survival of the whole organism. A case in point is the spectrum of human phenotypes and underlying pathogenetic mechanisms that are associated with mutations of microfibril and elastic fiber proteins.

Microfibrils are thin, filamentous assemblies of fibrillin polymers that are widely distributed in both elastic and non-elastic connective tissues [1–6]. Fibrillin-rich microfibrils can associate with a large variety of ECM constituents, including the structurally related fibulins and latent TGF-β-binding proteins (LTBPs), as well as

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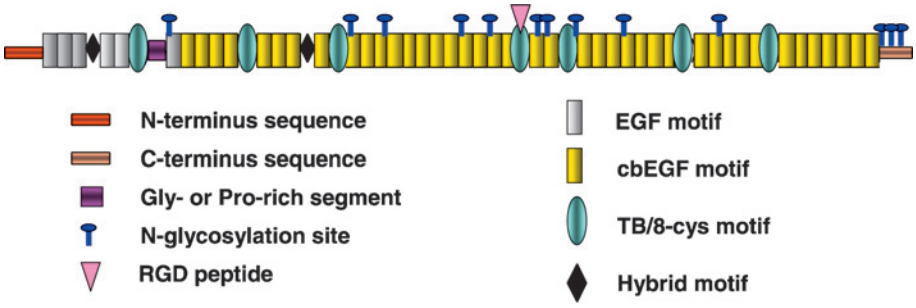

Figure 1. Protypical structure of fibrillin. The scheme illustrates the main structural features of a fibrillin molecule and includes only the putative *N*-glycosylation sites of fibrillin-1.

with cross-linked elastin molecules to form the elastic fibers. Microfibrils and elastic fibers display a variety of tissue-specific architectures that range from uninterrupted elastic fibers in the periosteum of long bones, to parallel bundles of microfibrils in the suspensory ligament of the lens, to concentric rings of elastic fibers with microfibril-mediated attachments in the tunica media of the aorta. The spectrum of human disorders caused by mutations in microfibril and elastic fiber components include Marfan syndrome (MFS; fibrillin-1), congenital contractural arachnodactyly (CCA; fibrillin-2), supravalvular aortic stenosis (SVAS; elastin) and cutis laxa (CL; elastin, fibulin-4 and fibulin-5) [6–8]. Characterization of the underlying pathogenesis of these conditions in mouse models has revealed unexpected new functions that microfibrils and elastic fibers play in regulating morphogenetic and tissue homeostatic programs through direct cell-matrix interactions or indirectly by modulating the activity of TGF-β/bone morphogenetic protein (BMP) signals [4–6, 9]. Importantly, the seminal discovery by Neptune at al. [10] that perturbed TGF-β activation is a critical determinant of clinical severity in MFS has not only revolutionized the whole field of matrix biology, but has also opened exciting new opportunities for targeted therapy in heritable disorders of the connective tissue. This review focuses on the scientific and clinical ramifications of that discovery, in addition to providing background information about the structural features, molecular interactions and biogenesis of fibrillin-rich microfibrils.

Structural features of fibrillins

Biochemical and immunolocalization studies originally identified fibrillin-1 as the principal component of 10-nm microfibril assemblies [11, 12]. Additional investigations using tissues from MFS patients revealed a significant reduction in fibrillin-1 immunodectection compared with samples from unaffected individuals [13]. This last observation was subsequently corroborated by the finding of fibrillin-1 mutations in MFS patients [14]. At the same time, cloning experiments discovered a second fibrillin protein (fibrillin-2) that linkage studies and mutational analyses causally associated with CCA [15, 16]. A third fibrillin (fibrillin-3) was more recently discovered with the completion of the human genome project [17]. Fibrillin-3 is mostly expressed in fetal tissues where it is found associated with microfibrils close to amorphous elastin. However, neither the physiological function of fibrillin-3 nor its role in microfibril biogenesis is currently known.

Fibrillins are large cysteine-rich glycoproteins (~350 kDa) that consist primarily of domains homologous to the calcium-binding epidermal growth factor-like (cb-EGF) module and domains containing eight cysteines (TB/8-cys) found uniquely in the fibrillins and LTBPs (Fig. 1) [1, 2, 5]. The structure of cb-EGF domains in fibrillin is characterized by an invariably arranged six-cysteine sequence that gives rise to a disulfide-bond-stabilized domain dominated by a central anti-parallel β-sheet, and in which the juxtaposition of four conserved amino acid residues promotes calcium binding. Calcium binding stabilizes contiguous cb-EGFs into a rigid linear structure, perhaps favoring the proper assembly of microfibrils, and protects fibrillin molecules from proteolysis [18, 19]. The TB/8-cys motif results in a globular fold composed of six anti-parallel β strands and two α helices. Discrete fibrillin fragments have been shown to interact *in vitro* with molecules as diverse as proteoglycans, LTBP-1, and BMP-7 [20–26]. An RGD sequence in the TB4/8-cys module of fibrillin-1 and fibrillin-2 has also been shown to reside within a favorable structural context to interact with integrins $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_V\beta_3$ and $\alpha_V\beta_6$ [27–30]. Additional structural features of the fibrillin proteins include several *N*-glycosylation sites dispersed throughout the molecule, hybrid domains made of both TB/8-cys and cb-EGF sequences, a glycine- or proline-rich segment, and N and C termini, homologous, respectively, to the N termini of LTBPs and the C-terminal domains of fibulins (Fig. 1) [1, 2, 5].

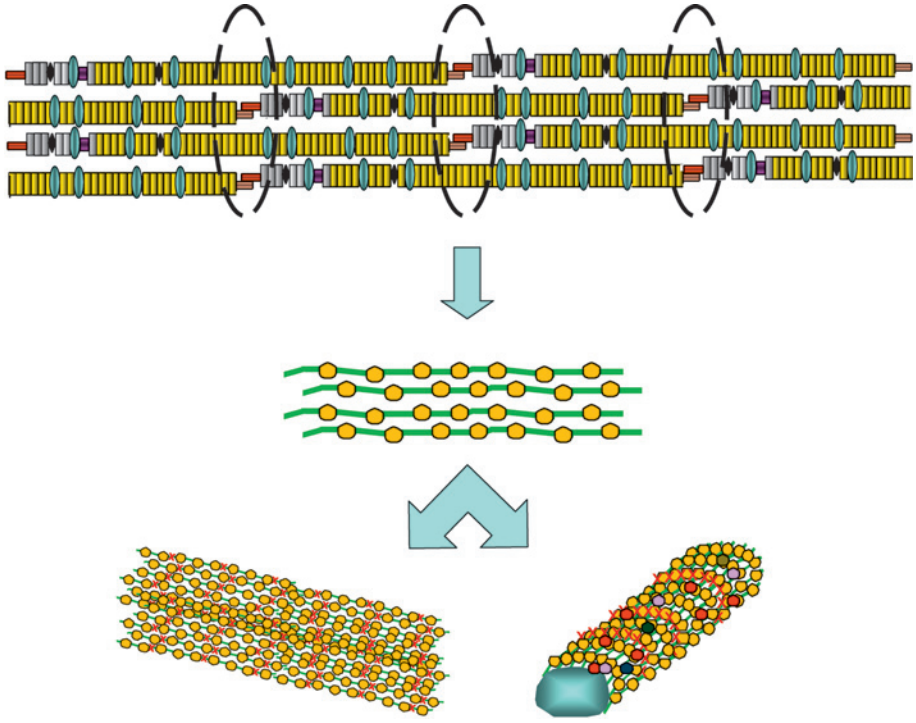

Figure 2. Microfibril and elastic fiber biogenesis. From top to bottom are shown the hypothetical assembly of fibrillin monomer into the staggered conformation with the dotted oval indicating the bead position, and the lateral association of bead-on-a-string polymers that leads to the formation (below) of microfibril (left) and elastic fiber (right) assemblies.

Fibrillin monomers polymerize extracellularly into microfibrils in which individual molecules are organized in a head-to-tail arrangement and can also interact laterally (Fig. 2) [1, 2, 5]. Fibrillin microfibrils incorporate or are decorated by additional proteins, including the structurally related fibulins and LTBPs as well as amorphous elastin in the elastic fibers (Fig. 2). During development, fibrillin-2 is generally synthesized before and in lower amounts than fibrillin-1, and both molecules can be part of the same microfibrils [31–33]. Fibrillin polymers are visualized by rotary shadowing electron microscopy as multiple strings with regularly spaced beads, the periodicity of which can extend from ~50 nm to ~200 nm depending on tissue source and conditions used for microfibril extraction. While initial epitope-mapping studies demonstrated that the beads correspond to where the N and C termini of contiguous parallel monomers reside, they could not resolve the issue of whether fibrillin molecules are staggered or un-staggered [1]. Consequently, molecular models of fibrillin microfibrils remain controversial and revolve around the extent to which fibrillin microfibrils can stretch and recoil. The so-called "jack-knife" model envisions that un-staggered fibrillin monomers progressively fold into the ~50-nm microfibril period [34]. An alternative model proposes that each molecule is 1/3 staggered to fit evidence for trans-glutaminase-mediated cross-link between fibrillins [35]. A third model postulates that fibrillins are 1/2 staggered with the N-terminal halves forming an outer surface of the microfibril to fit the multiple protein-protein interactions mediated by the N-terminal half of the molecule [36].

Extracellular control of TGF-β/BMP activity

Members of the TGF-β/BMP superfamily of signaling molecules are multifunctional cytokines that regulate a host of cellular activities and developmental programs in a context-dependent manner [37]. TGF-β/BMP molecules signal through trans-membrane serine/threonine kinase receptors that activate intracellular Smad proteins, which regulate gene activity by binding to specific DNA elements and by interacting with nuclear co-factors. There is increasing evidence that TGF-β/BMP can also signal through molecules other than Smads, and that the canonical Smad pathway can intersect with several other transducing molecules [38]. These interactions amongst different pathways represent an important mechanism that the cells use to modulate the nature, intensity and duration of their responses to environmental stimuli. Similarly, it has become clear that differences in ECM architecture and composition during physiological and pathological processes provide a spatiotemporal context to modulate TGF-β/BMP signaling activities [39]. A currently evolving case in point is the func-

tional interaction between TGF-β/BMP and fibrillin-rich microfibrils.

TGF-β signaling is controlled at several different levels, including extracellularly through the diffusion, storage, presentation, release and activation (collectively referred to as bioavailability) of the cytokine [40, 41]. TGF-β is secreted as a 100-kDa latent complex (small latent complex; SLC) consisting of the cytokine homodimer in non-covalent association with its propeptide (latency-associated protein; LAP), or as a larger 290-kDa complex (large latent complex; LLC) composed of the SLC covalently bound to LTBPs1, 3 or 4 (Fig. 3). Association of the SLC with LTBPs promotes folding and secretion of TGF-β as well as targeting of the LLC to the ECM through interactions between LTBPs and fibronectin, and LTBPs and fibrillin assemblies. LTBPs also undergo trans-glutaminase-mediated cross-linking to the ECM (Fig. 3). There is also evidence that the small leucine-rich proteoglycans biglycan, decorin and fibromodulin, as well as the microfibril-associated protein emilin bind and neutralize TGF-β activity [42–46]. Latent TGF-β activators include low pH, reactive oxygen species, proteases, thrombospondin-1 and integrins, factors that are generally associated with physiological modifications or pathological perturbations of the ECM [40, 41].

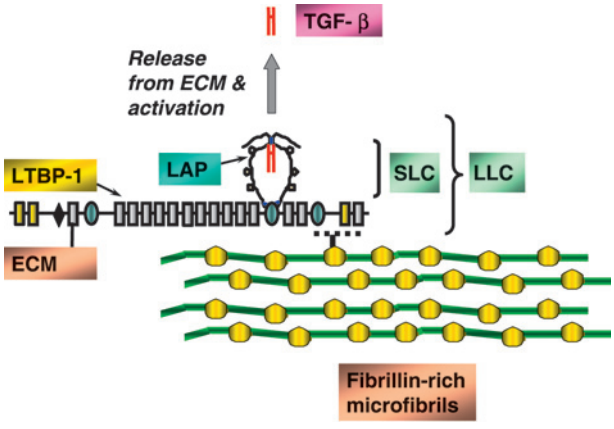

Figure 3. Matrix-bound large latent TGF-β complex. The diagram shows the components of the small and large latent TGF-β complex, its sites of interaction with the ECM and microfibrils, and the active form of the cytokine.

The ECM-bound latent TGF-β complex has aptly been compared to a molecular sensor that translates changes in the extracellular milieu into threshold responses of TGF-β signals [40]. Function of the LLC sensor depends on its appropriate assembly (LTBP-driven secretion and matrix targeting), competence to be turned-on (intracellular cleavage of proLLC) and detector activation (extracellular removal of LAP, which inhibits ligand/receptor interactions). Indeed, mice harboring null or hypomorphic mutations of the *Ltbp-3, Ltbp-4, and Ltbp-1L* genes display phenotypic abnormalities consistent with decreased TGF-β signaling [47–49]. Examples include the association of developmental emphysema in *Ltbp-3* null or *Ltbp-4* hypomorphic mice with decreased nuclear localization of phosphorylated Smad2/3 or decreased release of TGF-β into the ECM, respectively [48, 49]. Likewise, skeletal defects in *Ltbp-3*$^{-/-}$ mice mimic some of the manifestations seen in mice with altered TGF-β signaling in bone [50, 51]. Furthermore, unpublished studies of *Ltbp-1L*-deficient mice have revealed impaired morphogenesis of the cardiac outflow tract and pharyngeal arch arteries, developmental processes that normally involve TGF-β regulation of cardiac neural crest cells (Todorovic et al., manuscript in preparation; [52, 53]). Thus, there is good reason to conclude that dysfunctions of LTBPs may lead to diminished TGF-β signaling due to decreased LLC secretion, inappropriate localization, and/or altered activation. Interestingly, none of the *Ltbp* mutant mice phenocopies in full the *Tgfb* null mice, perhaps because of functional redundancy between LTBPs or because the SLC can be activated in certain contexts. In addition to controlling TGF-β secretion and targeting it to the matrix, LTBPs may also function as structural components of the ECM independent of their binding to SLC. This contention is based on the structural similarity and tissue co-localization of LTBPs and fibrillins, and on the ability of some LTBPs to modulate cell adhesion *in vitro* [40, 41].

Two lines of evidence indicate that fibrillin-rich microfibrils participate in modulating BMP signaling as well. Early genetic work in mice linked fibrillin-2 and BMP-7 to a developmentally regulated program of bone patterning, namely digit formation [54]. Shaping of the digits in the developing autopod is the combined result of chondrogenic outgrowth and interdigital cell death. BMPs have been implicated in specifying the chondrogenic and apoptotic fate of mesenchymal cells, and in regulating digit identity as well [55]. Like CCA patients, *Fbn2* null mice have joint contractures that disappear with aging. These mutant mice also display bilateral syndactyly in forelimbs and hindlimbs, a phenotype that is not observed in *Fbn1* null mice [56]. This last finding strongly suggests that the differential expression of fibrillin genes is another important diversifier of microfibril function during and after development. Syndactyly in *Fbn2* null mice is associated with a disorganized matrix and the inability of interdigital cells to undergo apoptosis. Importantly, combined *Fbn2* and *Bmp7* haploinsufficiency results in digit abnormalities in the absence of additional manifes-

tations in other organ systems [54]. More recently, biochemical studies have documented the ability of the BMP-7 prodomain and the BMP-7 complex, but not the separated BMP-7 dimer, to interact directly with N-terminal regions of fibrillin-1 [26]. This interaction may target the BMP-7 complex to fibrillin-rich microfibrils in the ECM. Indeed, the same study co-localized BMP-7 to extracellular microfibrils in several but not all the tissues known to be active sites for BMP-7 signaling. This last finding has been interpreted as suggesting that fibrillins posses a tissue-specific function in positioning and concentrating cytokines in the ECM [26]. Collectively, these results demonstrate that the organization of extracellular architectural elements, notably fibrillin-rich microfibrils, participate in controlling the activity of signaling molecules, and that the instructive role of microfibrils is developmental stage, tissue and fibrillin isoform specific. These studies also raise the possibility that prodomains of other members of the TFG-β/BMP superfamily may interact with fibrillins and/or LTBPs and that these cytokines are also targeted to and modulated by the ECM.

Roles of fibrillins in organ development and function

Several different strains of mice that harbor distinct mutations in the *Fbn1* gene have yielded interesting new insights into the diverse roles that microfibrils play in organ formation and function [4, 6, 9]. Importantly, the mouse studies have also been instrumental in elucidating the pathophysiological mechanisms responsible for the life-threatening manifestations of MFS. This autosomal dominant connective tissue disorder has an estimated frequency of 1 in 5000 individuals and is chiefly characterized by abnormalities in the cardiovascular, skeletal, and ocular systems; additionally, the skin, lung, muscle and dura may also be affected to varying degrees [8]. Whereas skeletal and ocular ailments as well as cardiac valve defects contribute significantly to MFS morbidity, dissecting aortic aneurysm is the leading cause of death in affected individuals. By analogy to the dominant-negative model previously established for the collagenopathies, it was originally argued that fibrillin-1 mutations in MFS may adversely affect the deposition, assembly and/or function of the normal pool of fibrillin-1 molecules [57, 58]. However, at least three indirect lines of evidence did not support a model of MFS pathogenesis, which is based primarily on the loss of tissue integrity. First, inability to predict clinical severity in MFS from the identity of fibrillin-1 (cb-EGFs 28–33) mutations suggested the additional involvement of non-structural mechanisms in tissue failure. Second, MFS manifestations like bone overgrowth, craniofacial abnormalities, myxomatous valve changes and muscle hypoplasia were thought to be more plausibly accounted for by altered cellular performance. Third, wide distribution of LTBPs in connective tissues affected by fibrillin-1 mutations indicated potential for a dysfunction in the extracellular regulation of TGF-β bioavailability.

Phenotypical characterization of *Fbn1* mutant mice has mostly centered on the ascending aorta, owing to the importance of this organ in the survival of MFS patients. Aortic media formation in the mouse begins at about mid-gestation with the deposition by vascular smooth muscle cells (VSMC) of ECM proteins that are gradually organized into elastic fibers and interconnecting microfibrils [59]. This process extends to early neonatal life with the progressive maturation of elastic fibers and microfibrils into the structural network of mature elastic lamellae that separate layers of quiescent VSMC. The resulting organization of the tunica media into a multilayered structure of alternating VSMC and elastic lamellae (a.k.a.: the lamellar unit) is the main determinant of arterial function. The so-called mgR mouse produces structurally normal fibrillin-1 protein at ~15% of the normal level and in homozygosity replicates the adult lethal form of MFS [58]. Ruptured aortic aneurysm in mgR/mgR mice is preceded by a series of secondary events that include medial calcification, ECM accumulation, intimal hyperplasia and adventitial inflammation [60, 61]. Overall, these secondary events suggested the activation of an unproductive tissue repair response, which results in intense elastolysis and consequent exacerbation of the structural collapse of the aortic wall. Subsequent studies of other *Fbn1* mutant mice indirectly implicated enhanced TGF-β activity in stimulating the tissue repair response in this mouse model of MFS. Addition of synthetic fibrillin-1 peptides or aortic extracts from mgR/mgR mice to cell culture systems has suggested more recently that proteolysis of fibrillin-rich microfibrils may also contribute to aneurysm progression by stimulating the expression of metalloproteinases and macrophage chemotaxis [62, 63]. Along the same lines, another study has indicated that a specific C-terminal peptide of fibrillin-1 (cb-EGFs 26–31) can modulate TGF-β bioavailability by competing LTBP-mediated binding of the LLC to the N terminus of fibrillin-1 [64].

MFS-like vascular disease was also observed in mice heterozygous for a missense mutation (C1039G) that eliminates one of the obligatory cysteines of cb-EGF11 [8, 65]. However, unlike mgR/mgR mice, C1039G/+ mice do not routinely progress to aortic dissection despite showing all of the hallmark histo-

logical features of MFS and progressive aortic root dilation. Owing to their survival, C1039G/+ mice have been employed to document that TGF-β antagonism can effectively prevent histological signs of aneurysm progression in this MFS model [66]. Collectively, these observations demonstrated that fibrillin-1 microfibrils serve an essential role in aortic matrix homeostasis during postnatal life, and that structural deficits in microfibrils promote abnormal activation of TGF-β with deleterious consequences on VSMC performance and tissue remodeling. Mice that completely lack fibrillin-1 (mgN/mgN mice) have documented the critical role of this protein in the fetal to neonatal maturation of the aortic wall [56]. *Fbn1*$^{-/-}$ mice in fact die soon after birth due to ruptured aortic aneurysm and show severe disorganization of the medial layer and impaired maturation of the vessel wall. Aortic wall collapse in these mice is preceded by the up-regulation of genes normally involved in TGF-β-driven vascular remodeling, such as PAI-1 and CYR61/CCN1. The contribution of fibrillin-1 molecules to aortic wall maturation is strictly dependent on earlier fibrilllin-2 deposition, in that all *Fbn1*$^{-/-}$*;Fbn2*$^{-/-}$ and half of *Fbn1*$^{-/+}$*;Fbn2*$^{-/-}$ mice die at embryonic day 14.5 showing impaired/delayed assembly of the aortic matrix [56].

Additional studies of *Fbn1* mutant mice have underscored the central role that fibrillin-1 and TGF-β signaling play in organ development and function, and in the genesis of the pleiotropic manifestations in MFS. First and foremost is the groundbreaking discovery by Neptune et al. [10] of a causal correlation amongst severe fibrillin-1 deficiency, promiscuous activation of latent TGF-β signaling and developmental emphysema in mice homozygous for a hypomorphic in-frame deletion (mgΔ) in *Fbn1* that replicate the neonatal lethal form of MFS [67]. Importantly, this study was also the first to document phenotypic rescue by TGF-β antagonism *in vivo*. As such, the work of Neptune et al. [10] provided genetic validation to subsequent biochemical evidence of molecular interaction between fibrillins and LTBP-1 [24]. Other genetic observations include the association of architectural alterations in the mitral valves of C1039G/+ mice with increased cell proliferation, decreased apoptosis and abnormally high TGF-β activity, as well as the ability of TGF-β neutralizing antibodies to prevent these mitral valve manifestations [68]. Furthermore, excessive latent TGF-β activation and signaling has also been reported in the dura of mgR/mgR mice, and in the aortic wall and skeletal muscles of C1039G/+ mice [66, 69, 70]. Once again, *in vivo* administrations of TGF-β neutralizing antibodies have been shown to effectively rescue aortic aneurysm and myopathy in C1039G/+ mice [66, 70].

It is worthwhile comparing the pathogenetic mechanism responsible for developmental emphysema in mgΔ/mgΔ mice with the decreased TGF-β signaling that causes a similar lung phenotype in *Ltbp-4* hypomorphic mice [10, 48, 49]. One potential explanation to reconcile the paradox that opposite effects on TGF-β activity lead to identical phenotypes may be the pleiotropic nature of the cytokine, its bimodal action, and the unique manner in which TGF-β is presented in the extracellular milieu. Within the developing lung, TGF-β isotypes perform multiple activities, including modulation of ECM synthesis and regulation of ECM catabolism. Decreased levels of TGF-β isotypes may result in abnormal expression of matrix molecules, such as elastin, a molecule crucial for septation [71]. In addition, TGF-β has both growth inhibitory and growth promoting activities, depending upon its concentration, the cell target, and the overall milieu of other expressed cytokines [37–41]. Thus, it is entirely possible that decreased TGF-β in *Ltbp-4* mutant mice may prevent the differentiation of septal progenitors because of a failure to stimulate cell growth, whereas excess TGF-β in *Fbn-1*-deficient animals may inhibit the proliferation or induce apoptosis of these same or different cells within the developing lung.

Clinical implications

An important corollary to the discovery that perturbed TGF-β signaling contributes to MFS pathogenesis is that conditions with MFS-like manifestations may be caused by mutations in different components of the TGF-β signaling network (regulators or transducers). Loeys-Dietz syndrome (LDS) is an illustrative example of this prediction [72]. LDS is an autosomal dominant disorder with widespread systemic involvement that includes both unique and MFS-like manifestations, such as aortic root aneurysm, aneurysms and dissections throughout the arterial tree and generalized arterial tortuosity. LDS is caused by heterozygous mutations (largelyin the kinase domains) of the types I or II TGF-β receptor (TGFBR1 or TGFBR2), which in principle should blunt TGF-β signaling. Contrary to this prediction, the vessel wall of LDS patients has been found to exhibit increased TGF-β signaling and cells from these patients have been shown to maintain TGF-β responsiveness [72]. This paradoxical finding suggests that heterozygous TGFBR mutations either trigger unproductive compensatory events *via* non-canonical pathways, or themselves have gain-of-function properties. These possibilities could be causally related during development and in a context-dependent

manner. For example, lower than threshold levels of TGF-β signaling during a temporally constrained developmental event might activate alternative or redundant signaling pathways, and this compensatory loop might remain constitutively active in the absence of a normal complement of signal transducers or regulators. It is therefore plausible that complex connective tissue phenotypes, such as MFS and LDS, may integrate both an excess and deficiency of signaling by multiple cytokines.

A subset of patients with substantial overlap with the vascular form of Ehlers-Danlos syndrome (vascular EDS), a condition typically caused by mutations in type III collagen, harbor heterozygous mutations in TGFBR1 or TGFBR2 [73]. Such patients (designated LDS type II) do not have the typical craniofacial features previously associated with LDS (now termed type I), but show arterial tortuosity and similarly aggressive vascular disease. Arterial tortuosity syndrome (ATS) is another disorder characterized by enhanced TGF-β activity and by vascular and skeletal manifestations that overlap with LDS [74]. The underlying defect in ATS is loss of function of the glucose transporter GLUT10, which impairs glucose-dependent expression of decorin, a potent extracellular inhibitor of TGF-β [75]. Finally, there are other disorders that are occasionally caused by fibrillin-1 mutations, such as Shprintzen-Goldberg syndrome and Weill-Marchesani syndrome [74, 76, 77]. It is yet to be determined whether or not the majority of these patients harbor mutations in discrete components of the TGF-β signaling network.

Therapeutic applications

The demonstration that TGF-β antagonism can rescue aortic aneurysm in C1039G/+ mice prompted the idea to test the efficacy of losartan, a widely used angiotensin II type 1 receptor (AT1) antagonist, because of its ability to counteract TGF-β action in animal models of chronic renal disease and cardiomyopathy [78, 79]. In a recent pre-clinical trial, losartan was administered to 2-month-old C1039G/+ mice and 14-day-old C1039G/+ embryos for 6 and 10 months, respectively [66]. Losartan treatment blocked the development of histological signs of aortic aneurysm in both cases, in addition to ameliorating impaired alveolar septation and muscle hypoplasia. Although the precise mechanism of losartan action on systemic TGF-β blockade is yet to be elucidated, signaling through AT1 has been shown to stimulate the expression of TGF-β ligand and receptor as well as thrombospondin-1, a potent activator of TGF-β. These experiments have provided proof-of-principle that TGF-β antagonism is a general strategy against aneurysm progression in MFS and other disorders caused by dysfunctions in the TGF-β signaling network.

Losartan may also prove to be an effective therapy in the clinical management of congenital and acquired myopathies. As already mentioned, muscle hypoplasia characterizes both *Fbn1* mutant mice and MFS patients. Experimental evidence from the C1039G/+ model has associated the muscle phenotype with impaired tissue regeneration in response to injury or physiological signals for hypertrophy [70]. This phenotype is also correlated with an increase in TGF-β signaling, a known inhibitor of myoblast differentiation [70, 80]. Consistent with these observations, TGF-β antagonism using either neutralizing antibodies or losartan was shown to effectively rescue muscle hypoplasia in C1039G/+ mice [70]. Importantly, the same TGF-β-dependent failure of muscle regeneration were also seen in a dystrophin-deficient mouse model of Duchenne muscular dystrophy (*mdx* mouse, [81]) with significant improvement of muscle degeneration, architecture and function upon treatment with losartan.

Conclusions and perspectives

The studies highlighted in this review underscore the significant progress made during the past decade in our understanding of the multiple roles that extracellular microfibrils play in development and disease. First and foremost is the novel concept that these architectural elements of the connective tissue also modulate critical signaling events during morphogenesis and in physiological and pathological tissue remodeling. Second is the renewed appreciation that the compositional diversification of microfibrils in different tissues and developmental stages provides a spatiotemporal context for the structural, signaling and cellular interactions that orchestrate the formation, growth and function of every organ system. Third is the unexpected realization that fibrillin-rich microfibrils are also part of a larger and complex network of extracellular, cell surface, and intracellular molecules that are causally associated with disorders characterized by impaired morphogenesis and homeostasis of multiple organ systems. While exciting and novel, these insights have also raised additional questions that affect our ability to immediately capitalize on these observations. As a result, we are still confronted with the unmet challenge of understanding how the orderly process of ECM assembly ultimately leads to the shaping of higher order tissue structure and the acquisition of organ function and thus, how best to

ameliorate the phenotypic consequences of genetic perturbations of this complex biological process. Whereas new technologies are needed to follow molecular interactions of ECM components *in vivo* and in real time, it is safe to predict that biochemical and cellular approaches in combination with genetic observations both in human patients and mouse models will continue to inform our investigative efforts.

In spite of the intrinsic limitations in studying ECM biology, the new paradigm that matrix sequestration critically regulates the local activation of latent TGF-β has already had several practical benefits. They include the use of pharmacological therapies to counteract TGF-β action in aneurysm progression and myopathic states. Indeed, evidence gathered from the losartan studies in *Fbn1* mutant mice has justified the launch of a multi-center, NIH-sponsored clinical trial to examine the effectiveness of this treatment in pediatric MFS patients. The same translational outcome may happen for muscular dystrophies, based on the beneficial effects of losartan in the *mdx* mouse model. Additionally, TGF-β involvement in MFS pathogenesis has helped conceptualize the origin of clinical variability by providing a number of candidate modifiers that are part of the TGF-β signaling network. Lastly, the expanding concept of the ECM as both a structural and instructive entity will likely to be relevant in the therapy of more common disorders, such as fibrotic lung, liver and skin diseases. In conclusion, the study of microfibril pathophysiology has been a highly successful example of how the combination of basic science findings and clinical observations can synergize to shed new light on fundamental principles of cellular and developmental biology, and on disease pathogenesis and therapeutic applications.

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