J. Cell Commun. Signal. (2015) 9:309–325
DOI 10.1007/s12079-015-0307-5

REVIEW

Fibrillin-containing microfibrils are key signal relay stations for cell function

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Received: 11 September 2015 / Accepted: 29 September 2015 / Published online: 8 October 2015
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Abstract Fibrillins constitute the backbone of microfibrils in the extracellular matrix of elastic and non-elastic tissues. Mutations in fibrillins are associated with a wide range of connective tissue disorders, the most common is Marfan syndrome. Microfibrils are on one hand important for structural stability in some tissues. On the other hand, microfibrils are increasingly recognized as critical mediators and drivers of cellular signaling. This review focuses on the signaling mechanisms initiated by fibrillins and microfibrils, which are often dysregulated in fibrillin-associated disorders. Fibrillins regulate the storage and bioavailability of growth factors of the TGF-β superfamily. Cells sense microfibrils through integrins and other receptors. Fibrillins potently regulate pathways of the immune response, inflammation and tissue homeostasis. Emerging evidence show the involvement of microRNAs in disorders caused by fibrillin deficiency. A thorough understanding of fibrillin-mediated cell signaling pathways will provide important new leads for therapeutic approaches of the underlying disorders.

Keywords Fibrillin · Microfibrils · Cell signaling · Connective tissue disorders · Integrins · Matrix metalloproteinases · MicroRNA

Abbreviations

AT1/2	Angiotensin II type 1/2 receptor
BMP	Bone morphogenetic protein
(cb)EGF domain	(calcium binding) epidermal growth factor-like domain
EBP	Elastin-binding protein
ECM	Extracellular matrix
ERK1/2	Extracellular signal-regulated kinase 1/2
GDF	Growth and differentiation factor
GTPase	Guanosine triphosphate phosphohydrolase
Hyb domain	Hybrid domain
IL	Interleukin
JNK	C-Jun N-terminal kinase
LAP	Latency associated peptide
LLC	Large latent complex
LTBP	Latent transforming growth factor beta-binding protein
MAPK	Mitogen-activated protein kinase
MicroRNA	Micro ribonucleic acid
MMP	Matrix metalloproteinase
NF-κB	Nuclear factor-κB
p-	phosphorylated
SMAD	Sma and Mad related
SLC	Small latent complex
TAK	Transforming growth factor-beta-activated kinase
TB domain	Transforming growth factor-beta-binding protein-like domain
TGF-β	Transforming growth factor-beta
Wnt	Wingless-type mouse mammary tumor virus integration site family

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Structure and function of fibrillins

In humans and other non-rodent mammalian species, three highly homologous ~350 kDa extracellular matrix (ECM)

proteins, fibrillin-1, −2 and −3 constitute the fibrillin protein family (Hubmacher and Reinhardt 2011). In rodents, the gene for fibrillin-3 is inactive due to chromosomal rearrangements (Corson et al. 2004). Fibrillin-1 is the main form in postnatal life, whereas fibrillin-2 and −3 are primarily expressed during development (Zhang et al. 1995; Corson et al. 2004; Sabatier et al. 2011). Human fibrillin-1, −2 and −3 are encoded by different genes on chromosomes 15, 5 and 19 (Lee et al. 1991; Magenis et al. 1991; Corson et al. 2004), and are highly conserved between species (Robertson et al. 2011; Piha-Gossack et al. 2012).

Domain organization of fibrillins

Fibrillins are multi-domain proteins mainly composed of epidermal growth factor-like (EGF) and some other domains (Corson et al. 1993; Pereira et al. 1993) (Fig. 1). EGF domains are present 47 times in fibrillin-1 and −2, and 46 times in fibrillin-3 due to alternative splicing. The majority of these EGF domains, 43 in fibrillin-1 and −2 and 42 in fibrillin-3, contain the calcium binding (cb) consensus sequence D/N-X-D/N-E/Q-X_m-D/N-X_n-Y/F where m and n represent variable numbers of amino acid residues (Rees et al. 1988; Handford et al. 1991; Mayhew et al. 1992). These residues are involved in either the direct ligation of calcium or the stabilization of the calcium binding site. Calcium binding is a key property which provides structural stabilization to the fibrillin proteins (Downing et al. 1996; Reinhardt et al. 1997a; Werner et al. 2000). Together with interdomain hydrophobic packing interactions and short linker regions between individual cbEGF domains, calcium binding contributes to the characteristic rigid rod-like shape of fibrillins (Downing et al. 1996; Reinhardt et al. 1997a). Calcium binding is also functionally important for the protection of fibrillins against proteolysis (Reinhardt et al. 1997b), the control of self-interaction (Lin et al. 2002; Marson et al. 2005), and the interaction with several different ECM components, such as fibulin-2, heparin/heparan sulfate and microfibril-associated glycoprotein-1 (Reinhardt et al. 1996; Tiedemann et al. 2001; Rock et al. 2004). Disulfide bond formation between the six cysteine residues in EGF and cbEGF domains occurs in a C1-C3, C2-C4 and C5-C6 pattern and further stabilizes the protein (Downing et al. 1996; Smallridge et al. 2003). The second most common type of domain in fibrillins is the transforming growth factor-beta-binding protein-like (TB) domain (Corson et al. 1993; Pereira et al. 1993). TB domains occur seven times in fibrillins, and their eight cysteine residues form four disulfide bonds in a C1-C3, C2-C6, C4-C7 and C5-C8 pattern (Yuan et al. 1997; Lee et al. 2004). Fibrillins further contain hybrid (hyb) domains which show sequence similarities with TB domains in their N-terminus and with EGF domains in their C-terminus (Corson et al. 1993; Pereira et al. 1993). Hyb domains occur twice in all mammalian fibrillins and are stabilized by four intradomain disulfide bonds in a C1-C3, C2-C5,

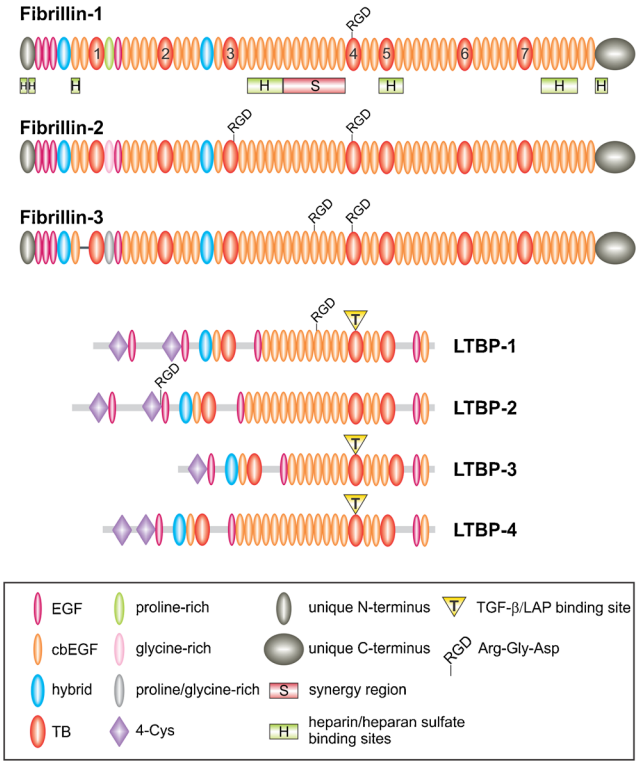

Fig. 1 Schematic of the fibrillin/LTBP superfamily. The domain organization of human fibrillins and LTBPs is shown. The most common type of domain are the cbEGF domains interspersed mainly by TB and hybrid domains. TB domains are numbered in fibrillin-1. The RGD cell binding sites, the synergy region (S) and heparin/heparan sulfate-binding sites (H) are shown for fibrillin-1. The latent small TGF-β/LAP complex covalently interacting with LTBP-1, −3 and −4 is indicated by *yellow triangles*. Only the longest splice variant is shown for each LTBP isoform and shorter splice variants are omitted for simplicity

C4-C6 and C7-C8 pattern (Jensen et al. 2009). Other protein domains in fibrillins include unique N- and C-terminal domains that are processed by furin convertases (Lönnqvist et al. 1998; Raghunath et al. 1999; Ritty et al. 1999; Kettle et al. 2000; Wallis et al. 2003). A distinguishing feature is a proline-rich domain close to the N-terminus of fibrillin-1, a glycine-rich domain in fibrillin-2, and a domain rich in proline and glycine in fibrillin-3 (Corson et al. 1993; Zhang et al. 1994; Nagase et al. 2001).

In addition to fibrillins, cbEGF domains can be found in many other ECM and serum proteins. TB domains and hyb domains, however, are unique to fibrillins and a related family of ECM proteins, the latent transforming growth factor beta-binding proteins (LTBPs) (Robertson et al. 2015). Fibrillins and LTBPs together constitute the fibrillin/LTBP superfamily (Fig. 1). In humans, four members of the LTBP family are known, LTBP-1, −2, −3 and −4. Similar to fibrillins, LTBPs consist of tandem cbEGF repeats that are interspersed with three TB domains and one hyb domain (Hyytiäinen et al. 2004). LTBPs are important for the sequestration and activation of transforming growth factor-beta (TGF-β)1, −2 and −3 as outlined in detail below.

RGD and heparin/heparan sulfate binding sites

The RGD (arginine-glycine-aspartic acid) cell binding site is a three amino acid motif that occurs in many ECM proteins, including fibrillins (Ruoslahti 1996). It mediates the interaction between ECM proteins and transmembrane receptors, the integrins (see paragraph Integrins). All fibrillins contain an RGD sequence in their TB4 domain (Fig. 1). Fibrillin-2 and −3 have an additional RGD cell binding site in the TB3 or the cbEGF18 domain, respectively (Robertson et al. 2011; Piha-Gossack et al. 2012). X-ray crystallographic structural analysis of the TB4 domain in a cbEGF22-TB4-cbEGF23 recombinant fibrillin-1 fragment demonstrated a tetragonal pyramid shape for this fragment (Lee et al. 2004). The RGD site is located on an exposed flexible loop in the TB4 domain.

Integrins $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_6$ were shown to interact with fibrillin-1 (Pfaff et al. 1996; Sakamoto et al. 1996; Bax et al. 2003; Jovanovic et al. 2007). Fibrillin-1 adhesion and migration on $\alpha_5\beta_1$ is strongly enhanced by an upstream tandem array of cbEGF domains, the synergy site (Bax et al. 2007). This study demonstrated that the strongest cell adhesion to $\alpha_5\beta_1$ integrin was promoted in the presence of seven upstream cbEGF domains, and to $\alpha_v\beta_3$ in the presence of four upstream domains. This indicates slightly different requirements for these integrins in terms of the complementary synergy site(s) present in fibrillin-1.

In addition, close to the RGD site in the TB4 domain, a downstream heparin/heparan sulfate binding site was identified in TB5-cbEGF25 that stimulates focal adhesion formation (Tiedemann et al. 2001; Ritty et al. 2003; Cain et al. 2005, 2008) (Fig. 1). It is possible that this site can modulate the integrin interaction with fibrillins, but this aspect is not explored. Six other heparin/heparan sulfate binding sites were identified throughout the fibrillin-1 molecule (Tiedemann et al. 2001; Ritty et al. 2003; Cain et al. 2005, 2008; Yadin et al. 2013) (Fig. 1). Some, or possibly all, of them may be relevant for fibrillin-mediated cell signaling. The addition of heparin/heparan sulfate to cultured human skin fibroblasts impaired the formation of a fibrillin network (Tiedemann et al. 2001; Ritty et al. 2003). Moreover, the inhibition of heparan sulfate sulfation or the biosynthesis of heparan sulfate inhibited fibrillin network formation (Trask et al. 2000a; Tiedemann et al. 2001). These data show that heparin/heparan sulfate interactions with fibrillins play a critical role in fibrillin network assembly. Other cell signaling functions of these heparin/heparan sulfate sites in fibrillins require future clarification.

Fibrillin-containing microfibrils

Fibrillins multimerize in the ECM of elastic and non-elastic tissues to form the core of supramolecular structures, the 10 nm in diameter microfibrils (Low 1962). Fibrillin-containing microfibrils (referred to as "microfibrils" throughout this review) provide mechanical stability in some tissues where they typically tether basement membranes to the underlying stroma, for example in superficial regions of the skin, the kidney and the ciliary zonules of the eye (Raviola 1971; Kriz et al. 1990). In elastic tissues, microfibrils provide the key scaffold machinery for the biogenesis of elastic fibers (Wagenseil and Mecham 2007). The importance of fibrillin-1 for the deposition of elastic fibers is highlighted by fibrillin-1 deficient *Fbn1*$^{-/-}$ mice (Carta et al. 2006). These mice die within two weeks after birth due to impaired lung function and ruptured aortic aneurysms as a result of disorganized elastic fibers in these tissues. Microfibrils also represent the principal units that mediate fibrillin-guided cell signaling as outlined in detail below throughout this review.

Microfibrils are associated with more than 20 other proteins to fulfill their highly specialized role in different tissue contexts (Baldwin et al. 2013). The interacting partners of fibrillins can be divided into functional subgroups: 1) microfibril biogenesis, 2) elastic fiber assembly, 3) proteoglycan interaction, and 4) growth factor regulation. Self-interaction sites, heparin-binding sites and the interaction with fibronectin are important for microfibril biogenesis (Ashworth et al. 1999a; Trask et al. 1999; Tiedemann et al. 2001; Lin et al. 2002; Marson et al. 2005; Kuo et al. 2007; Hubmacher et al. 2008; Sabatier et al. 2009). Crucial fibrillin interaction partners relevant for elastic fiber assembly are, among others, tropoelastin (Trask et al. 2000b; Rock et al. 2004), fibulin-4 and −5, and lysyl oxidase (Freeman et al. 2005; El-Hallous et al. 2007; Kobayashi et al. 2007; Choudhury et al. 2009; Ono et al. 2009). Fibrillin-1 interacting proteoglycans include decorin, versican and perlecan (Trask et al. 2000a; Isogai et al. 2002; Tiedemann et al. 2005). The interaction with growth factors can be either indirect via LTBPs in the case of TGF-βs (Isogai et al. 2003; Hirani et al. 2007; Ono et al. 2009), or direct for bone morphogenetic protein (BMP)-2, −4, −5, −7 and −10 and growth and differentiation factor (GDF)-5 (Gregory et al. 2005; Sengle et al. 2008a, 2011). This aspect will be discussed in detail below.

Fibrillinopathies

Mutations in fibrillin-1 and −2 can lead to a wide variety of heritable autosomal-dominant connective tissue disorders, collectively termed fibrillinopathies. Mutations in fibrillin-1 most commonly cause Marfan syndrome (Dietz et al. 1991), but also other disorders such as stiff skin syndrome (Loeys et al. 2010), acromicric and geleophysic dysplasia (Le Goff et al. 2011), dominant Weill-Marchesani syndrome (Faivre et al. 2003), and dominant ectopia lentis (Tsipouras et al. 1992).

Marfan syndrome affects two to three in 10,000 individuals and manifests in various organ systems, most importantly in large blood vessels, bones and eyes (Pyeritz and Dietz 2002).

The clinical symptoms range from long bone overgrowth, scoliosis, dural ectasia and ectopia lentis to life-threatening aortic aneuryms and dissections. Marfan syndrome was initially described in a 5-year old girl in 1896 by Antoine-Bernard Marfan (Marfan 1896). However, the link between Marfan syndrome and mutations in the fibrillin-1 gene (FBN1) on chromosome 15 was not established until 1991 (Kainulainen et al. 1990; Dietz et al. 1991). Until today, more than 3000 mutations have been identified in the fibrillin-1 gene (http://www.umd.be/FBN1) (Collod-Beroud et al. 2003). Most of these mutations cause different forms of Marfan syndrome, ranging from the common classical form to a more severe neonatal form with early disease onset to the progeroid form which is associated with premature aging (Dietz et al. 1991; Kainulainen et al. 1994; Graul-Neumann et al. 2010). Marfan syndrome is characterized by a wide inter- and intra-familial phenotypic variability and severity of the disease, which complicates genotype-phenotype correlations.

Mutations in the RGD-containing TB4 domain of fibrillin-1 cause an autosomal-dominant form of congenital scleroderma, known as stiff skin syndrome (Loeys et al. 2010). These patients present with diffuse skin fibrosis leading to hard, thick skin, which imposes major limitations on joint mobility and causes flexion contractures. The patients also are characterized by short body stature. Electron microscopy of microfibrils from stiff skin syndrome patients displayed accumulation of structurally altered microfibrils containing shorter and denser fibrils in addition to collagen accumulation (Loeys et al. 2010).

Acromicric and geleophysic dysplasias are caused by mutations in the TB5 domain of fibrillin-1 (Le Goff et al. 2011). Stiff joints, thick skin, short stature and extremities characterize both dysplasias. These authors also demonstrated that fibroblasts from these patients form a disorganized and reduced microfibril network. An in-frame deletion of eight amino acid residues in the region of fibrillin-1 that encodes the TB5 domain causes the autosomal dominant form of Weill-Marchesani syndrome (Faivre et al. 2003). Similar to acromicric and geleophysic dysplasias, clinical symptoms include a short stature and joint stiffness. Additionally, those patients often display spherophakia associated with glaucoma.

In summary, mutations within the same gene (*FBN1*) lead to a range of connective tissue disorders spanning a wide clinical spectrum. The current pressing question in the field is how mutations within *FBN1* can cause such a wide spectrum of clinical features. It is likely that these pleotropic disease manifestations are caused by alterations of the fine-tuning of growth factors and other fibrillin-guided cell signaling pathways, as summarized in the following paragraphs.

In addition to mutations in *FBN1*, mutations in *FBN2* give rise to congenital contractural arachnodactyly characterized by multiple joint contractures in the elbows, knees, ankles and fingers as well as a crumpled appearance of the ears (Viljoen 1994). No pathogenic mutation has yet been identified in *FBN3*, although some studies suggest its involvement in polycystic ovary syndrome (Jordan et al. 2010; Raja-Khan et al. 2010; Hatzirodos et al. 2011).

Growth factor regulation by fibrillins

Matrix sequestration of growth factors is an essential aspect in the regulation of their activity and signaling properties. The interaction of microfibrils and growth factors can be either indirect through LTBPs in case of TGF-β or direct in case of several BMPs. Perturbation of the growth factor matrix sequestration frequently leads to disease pathogenesis.

TGF-β superfamily in relation to fibrillins

The TGF-β superfamily comprises TGF-β1, −2 and −3, BMPs, activins, inhibins and GDFs (Massague 1998). The members of this superfamily include potent cytokines that regulate a variety of processes in development, homeostasis and tissue repair including cellular differentiation, migration, and ECM remodeling.

Synthesis, secretion and activation of TGF-βs

TGF-β1, −2 and −3 are synthesized as 55 kDa precursor proteins composed of the mature growth factor domain at the C-terminus and an N-terminal prodomain, the latency associated peptide (LAP) (Lawrence et al. 1984; Gentry et al. 1988) (Fig. 2). The precursor proteins homodimerize through disulfide bond formation. The homodimers are proteolytically processed by furin-like endoproteases in the trans-Golgi network, forming the small latent complex (SLC) with the LAP dimer non-covalently shielding the active TGF-β dimer (Shi et al. 2011). The SLC covalently interacts with several LTBPs forming the large latent complex (LLC) (Hyytiäinen et al. 2004; Rifkin 2005). LTBP-1 and −3 bind to the LAPs of all three TGF-βs, whereas LTBP-4 only interacts with the LAP of TGF-β1 (Saharinen and Keski-Oja 2000). Most cell types secrete TGF-β as latent complexes (Miyazono et al. 1991; Taipale et al. 1994). LTBP-1 colocalized with fibrillin-1 and fibronectin in cell culture (Taipale et al. 1996; Dallas et al. 2000, 2005; Klingberg et al. 2014). LTBP-1 was also found associated with microfibrils in different tissues, including skin, developing heart and the cardiovascular system, as well as the periosteum of developing bone (Nakajima et al. 1997; Raghunath et al. 1998; Dallas et al. 2000; Isogai et al. 2003). Direct biochemical interactions were shown between fibrillin-1 and the C-termini of LTBP-1, −2 and −4 (Isogai et al. 2003; Hirani et al. 2007; Ono et al. 2009), whereas the N-termini of LTBP-1 and −4 associate with fibronectin (Dallas et al. 2005; Fontana et al. 2005; Kantola et al. 2008). All LTBPs localize to microfibrils in tissues, even LTBP-3 despite the lack of biochemical interaction with fibrillin-1 (Zilberberg et al. 2012). Through this mechanism, inactive TGF-βs are targeted to microfibrils in

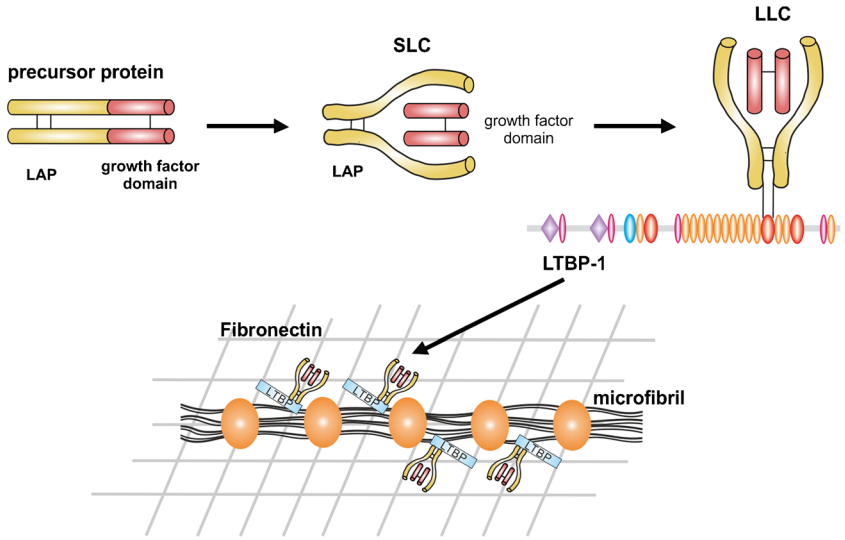

Fig. 2 Biosynthesis of TGF-β and targeting to microfibrils. TGF-βs are synthesized as precursor proteins consisting of a growth factor domain at the C-terminus (*red*) and the LAP at the N-terminus (*yellow*). Two precursor proteins homodimerize and are proteolytically cleaved to form the SLC where LAP is non-covalently bound to the active TGF-β dimer. The SLC can covalently bind to the penultimate TB domain in LTBP-1, −3 and −4 (shown here for LTBP-1), constituting together the LLC. The C-terminal region of LTBP-1 and −4 interact non-covalently with the N-terminal region of fibrillin-1 present in the core of beaded microfibrils. The N-terminal regions of LTBP-1 and −4 interact with fibronectin. LTBP-3 localizes to microfibrils via a different mechanism. In this way, TGF-βs are targeted to microfibrils, which directly or indirectly regulate their release and activation

the ECM, which regulate release and activation of the active growth factors. The formation of the LLC is schematically depicted in Fig. 2.

In its latent state, TGF-β is not accessible to its receptor and requires release from the ECM either through conformational changes in the SLC or through other mechanisms including proteolytic degradation. The LAPs of TGF-β1 and −3 contain an RGD sequence by which they can interact with $\alpha_v\beta_6$, $\alpha_v\beta_8$ or $\alpha_v\beta_5$ integrins expressed by different cell types (Munger et al. 1999; Mu et al. 2002; Wipff and Hinz 2008). Upon binding of LAPs to cell surface integrins and cell-mediated force transmission, a conformational change is induced in the SLC which leads to the release of active TGF-β (Annes et al. 2004; Wipff et al. 2007; Shi et al. 2011). Matrix metalloproteinase (MMP)-2 and −9 and the serine protease plasmin can either directly target LAPs or alternatively target the LTBPs to induce TGF-β activation (Sato and Rifkin 1989; Yu and Stamenkovic 2000). Interaction between thrombospondin-1 and LAP can also release active TGF-β through a conformational change in LAP (Schultz-Cherry et al. 1995). TGF-β can also be released from the SLC by reactive oxygen species that are generated during inflammation or by irradiation (Barcellos-Hoff et al. 1994). In vitro, TGF-β can be activated by heat or acid treatment, which leads to denaturation of LAP, and in turn to the release of active TGF-β (Lyons et al. 1988).

BMPs in relation to fibrillins

BMPs are also expressed as precursor proteins with a prodomain and a mature C-terminal growth factor. Unlike TGF-β, most BMPs are active when overexpressed as recombinant proteins due to an open armed conformation of the prodomain that allows interaction of the growth factor dimer with its receptor (Mi et al. 2015). BMP-7 is secreted as a stable complex with the growth factor dimer non-covalently associated with its two prodomains (Gregory et al. 2005). Due to the structural similarity to the TGF-β SLC, the prodomain of BMP-7 was tested by these authors for binding to LTBP-1 or fibrillin-1. Interactions were observed between the BMP-7 prodomain or the BMP-7 prodomain/growth factor complex and N-terminal regions of fibrillin-1, whereas the growth factor dimer alone did not interact. It is possible that this interaction sequesters BMP-7 complexes to microfibrils in the ECM. Binding assays with fibrillins were extended to BMP-2, −4, −5, −10 and GDF-5 and −8 using the recombinant prodomains without their respective growth factor dimers (Sengle et al. 2008a, 2011). All prodomains, except the GDF-8 prodomain, interacted with fibrillins. BMPs and GDFs bind to a region between the N-terminus and the proline-rich domain in fibrillin-1. The downstream region between EGF4 and TB3 also interacts with various affinities with GDF-5 and BMP-2, −4, and −10. Thus, the N-terminal region of fibrillins appears to act as a high affinity binding site and concentrator for BMP prodomains. However, other mechanisms for targeting some of these growth factors to the ECM exist. For example, the GDF-8 prodomain interacts with a glycosaminoglycan chain at the C-terminus of perlecan, a core basement membrane protein (Sengle et al. 2011). Given that perlecan has been shown to interact directly with fibrillin-1, GDF-8 might be indirectly targeted to microfibrils (Tiedemann et al. 2005).

Unlike the latent TGF-β prodomain/growth factor complex, the BMP-7 prodomain/growth factor complex was able to bind to its receptors and induce BMP signaling in cell culture (Sengle et al. 2008b). The growth factor dimers and the BMP-7 prodomain/growth factor complex did not show any overt differences in their signaling capabilities, and BMP type II receptors could displace the BMP-7 prodomains in solution and bind to the growth factor dimer. In vitro studies showed that capturing the BMP-7 complexes by binding to a fibrillin-2 scaffold impaired BMP signaling (Sengle et al. 2015). This inhibitory effect of fibrillin binding is most likely the result of a conformational change in the prodomain structure, which impedes the interaction of BMP receptors and growth factor. Thus, fibrillins might act as a latency factor for at least some BMPs.

TGF-β and BMP signaling

Active TGF-β family members signal via specific complexes of homodimeric type I and type II transmembrane serine/threonine kinase receptors (Wrana et al. 1992). Upon ligand-induced formation of heterotetrameric receptor complexes, the type II receptor transphosphorylates and activates the type I receptor. The activated type I receptors propagate the growth factor signal by phosphorylating specific Sma and Mad related (SMAD) transcription factors, the receptor-regulated (R-)SMADs (Feng and Derynck 2005). Upon activation, two R-SMADs form heteromeric complexes with the co-SMAD, followed by translocation into the nucleus, where the complex participates in the transcriptional regulation of target genes.

TGF-β can also activate non-canonical signaling through the serine/threonine kinase receptors by activating Ras guanosine triphosphate phosphohydrolases (GTPases) (Derynck and Zhang 2003; Lee et al. 2007; Yamashita et al. 2008). The Ras GTPases phosphorylate and thus activate the mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38. TGF-β can also activate TGF-β-activated kinase (TAK1), which is a MAP kinase kinase kinase activating the MAP kinase cascade. Non-canonical TGF-β signaling can either occur independently or in parallel to canonical signaling via SMADs.

Regulation of TGF-β/BMP activation and signaling in fibrillinopathies

TGF-β and BMP signaling is highly dependent on the cellular and tissue context. The generation of mouse models has significantly extended knowledge of how fibrillin-1 and -2 guide and modulate TGF-β/BMP signaling. Mice heterozygous for a missense mutation in fibrillin-1 (*Fbn1*$^{C1039G/+}$) are frequently used as a model for Marfan syndrome (Judge et al. 2004). These mice display aberrant thickening of the aortic media with fragmented and disorganized elastic fibers, and excessive TGF-β activity leads to the formation of aortic aneurysms (Habashi et al. 2006). This study also showed that postnatal administration of TGF-β-neutralizing antibodies improved aortic wall architecture and decreased elastic fiber fragmentation. *Fbn1*$^{C1039G/+}$ mice are further characterized by myxomatous changes of the atrioventricular valves, which include a postnatally acquired increase in leaflet length and thickness (Ng et al. 2004). The alterations in mitral valve architecture correlate with excess TGF-β activation and signaling, which could be rescued in vivo by TGF-β antagonism. *Fbn1*$^{C1039G/+}$ mice also display architectural abnormalities in the skeletal muscle, which was characterized by decreased muscle fiber sizes and numbers and an increase in the amount of interstitial tissue and fat between muscle fiber bundles (Cohn et al. 2007). These phenotypes also were generally correlated with elevated TGF-β activity as evidenced by the normalization of muscle architecture upon systemic antagonism of TGF-β. Mice that homozygously express a centrally deleted *Fbn1* allele at about 10 % of the wild-type level (*Fbn1*$^{mg\Delta/mg\Delta}$) present in addition to aortic dilation and rupture with lung abnormalities, which manifested as impaired distal alveolar septation (Pereira et al. 1997; Neptune et al. 2003). These mice are again characterized by enhanced TGF-β activation and signaling. Perinatal administration of a TGF-β-neutralizing antibody rescued the failed distal alveolar septation in vivo.

Fbn1$^{C1039G/+}$ mice show activation of both the canonical TGF-β signaling pathway via SMAD2 and the non-canonical pathway via ERK1/2 (Holm et al. 2011). This study clarified that it is the non-canonical pathway through ERK1/2 that drives aortic aneurysm progression, and hence inhibition of ERK1/2 pathways appears as a potential novel therapeutic strategy. Further evidence for the importance of non-canonical TGF-β signaling comes from studies with vascular smooth muscle cells explanted from the thoracic aortas of *Fbn1*$^{-/-}$ mice (Carta et al. 2009). These cells displayed abnormal accumulation of TAK1 and the phosphorylated MAPK p38. This study further demonstrated that phosphorylated p38 accumulated earlier than phosphorylated (p-)SMAD2 in the aortic wall of *Fbn1*$^{-/-}$ mice, and activated signaling via SMAD2. In addition, systemic inhibition of p38 activity lowered SMAD2 phosphorylation.

TGF-β antagonism proved as a valuable therapeutic approach in preclinical mouse models and is also the basis for treatment with losartan, an angiotensin II type 1 (AT1) receptor antagonist. Like beta-blockers, losartan lowers the blood pressure, albeit through a different molecular mechanism. Beta-blockers emerged as standard care for Marfan patients over the years (Shores et al. 1994). In addition to its beneficial effects on blood pressure, losartan was shown to antagonize TGF-β in animal models of chronic renal insufficiency and

cardiomyopathy (Lim et al. 2001; Lavoie et al. 2005). The interaction between angiotensin II and AT1 mediates the progression of aortic aneurysms and AT1 blockade abrogated aneurysm progression in *Fbn1*$^{C1039G/+}$ mice (Habashi et al. 2011). Loss of AT2 expression, in contrast, accelerates aortic aneurysm formation in these mice. The authors further demonstrate that losartan inhibits TGF-β-mediated activation of ERK1/2 by shunting signaling through the AT2 receptors, which in turn inhibits ERK1/2 activation. This lowers the expression of TGF-β ligands, receptors, and activators as their expression is stimulated through the ERK1/2 pathway.

Postnatal treatment with the beta-blocker propranolol or losartan in comparison demonstrated the advantages of losartan, which fully rescued the abnormalities in the aortic wall and also partially reversed the impaired alveolar septation (Habashi et al. 2006). Administration of propranolol, in contrast, reduced the aortic growth rate, but did not improve the aortic wall architecture. Angiotensin II type 1 receptor blockade through losartan also normalized the architectural abnormalities in the skeletal muscle, similar to TGF-β antagonism (Cohn et al. 2007).

The beneficial effects of losartan for patients with Marfan syndrome was assessed in various clinical trials (Lacro et al. 2007; Detaint et al. 2010). One of the largest recent studies with over 600 children and young adults with Marfan syndrome, however, did not show significantly different beneficial effects of losartan and the beta-blocker atenolol on the aortic growth rate over a period of three years (Lacro et al. 2014). These data exemplify differences that may exist in terms of drug efficiencies between preclinical mouse models and humans.

Deregulation of TGF-β activation and signaling is also observed in other fibrillinopathies. Fibroblasts from patients with acromicric or geleophysic dysplasia displayed enhanced TGF-β signaling (Le Goff et al. 2011). Increased TGF-β signaling was also observed in the dermis of patients with stiff skin syndrome, which can be partly explained by the increased expression of p-SMAD2 and the accumulation of LLCs which causes an elevated concentration of TGF-β in the ECM (Loeys et al. 2010).

It is still not completely understood how mutations in fibrillin-1 lead to enhanced TGF-β activation and signaling, either as a primary or a secondary cause. One explanation is a reduced number of functional microfibrils present in the ECM, another one is the inability of LTBPs to mediate TGF-β sequestration to microfibrils in the disease state. Residues within the hyb1 domain mediate binding of fibrillin-1 to LTBP-1 and LTBP-4 (Ono et al. 2009). This study showed that LTBP-1 and LTBP-4 were not incorporated into microfibrils produced by *Fbn1*$^{-/-}$ fibroblast cultures, demonstrating that fibrillin-1 is required for correct matrix incorporation of LTBPs. The authors of this study further showed that deletion of the hyb1 domain caused impaired interaction between fibrillin-1 and LTBP-1 and abrogated binding to LTBP-4 in vitro. Homozygous in vivo deletion of the hyb1 domain in *Fbn1*$^{H1\Delta/H1\Delta}$ mice did not show any gross phenotype (Charbonneau et al. 2010). Both, homozygous *Fbn1*$^{H1\Delta/H1\Delta}$ and heterozygous *Fbn1*$^{H1\Delta/+}$ mice did not develop aortic aneurysms or dissections and displayed normal assembly of microfibrils. Therefore, it can not only be a compromised interaction between LTBPs and fibrillin-1 that causes the spectrum of diseases associated with fibrillin-1 mutations. Likely, it is an integrated interplay of context specific factors and events that trigger the pathological cascade.

Global deletion of the fibrillin-2 gene (*Fbn2*$^{-/-}$) in mice causes a relatively mild phenotype including bilateral syndactyly and temporary joint contractures (Arteaga-Solis et al. 2001). This study showed that fibrillin-2 regulates BMP-7 signaling in the developing autopod. Upon deletion of fibrillin-2, BMP-7 does not localize to the interdigit region, where it normally mediates apoptosis, thus leading to syndactyly. A study analyzing *Fbn2*$^{-/-}$ null mice on a 129/Sv background described newborn mice with reduced muscle mass, abnormal muscle histology and activated BMP-7 signaling in skeletal muscle. These data indicate that fibrillin-2 mediated BMP signaling is important for muscle differentiation (Sengle et al. 2015).

The crosstalk between TGF-β and BMPs can exemplarily be described in bone remodeling. TGF-β1 stimulates matrix degradation through the induction of osteoclastogenesis (Nistala et al. 2010b). BMPs have an osteoinductive effect during bone maturation, mineralization and homeostasis. Primary cultured osteoblasts from both *Fbn1*$^{-/-}$ and *Fbn2*$^{-/-}$ mice showed enhanced activation of TGF-β signaling (Nistala et al. 2010a). *Fbn2*$^{-/-}$ osteoblasts showed impaired osteoblast maturation and bone formation as a result of enhanced TGF-β activation. Osteoblasts from *Fbn1*$^{-/-}$ displayed evidence for activated BMP signaling. Osteoblast maturation and mineralization occurred faster despite enhanced TGF-β signaling at similar levels as in *Fbn2*$^{-/-}$ osteoblasts. The increased availability of normally matrix-bound BMPs enhances osteoinductive BMP signaling and counteracts the anabolic effects of TGF-β signaling.

Figure 3 provides an overview of growth factor sequestration by fibrillin microfibrils in normal and in disease state.

Other growth factors relevant to fibrillins

Wnts (Wingless-type mouse mammary tumor virus integration site family) are important growth factors required for cellular differentiation, tissue morphogenesis and homeostasis with critical roles in cardiac development and differentiation, angiogenesis, cardiac hypertrophy, cardiac failure, and aging (Rao and Kuhl 2010). The Wnt signaling pathway is still largely unstudied in the context of its regulation by the ECM and in regard to its involvement in fibrillinopathies. Wnts propagate signaling through the stabilization of β-

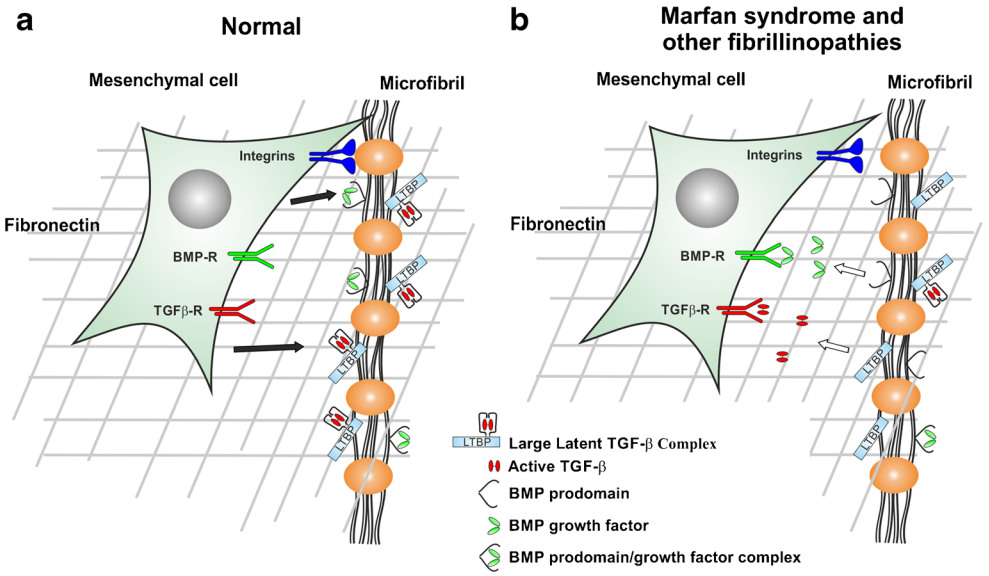

Fig. 3 Microfibrils as growth factor and cell signalling relay stations in normal and pathological situations. **a** In a normal physiological situation, cell surface located beaded microfibrils interact with smooth muscle cells and fibroblasts through integrins (*blue*). The TGF-βs LLC and BMP prodomain/growth factor complexes are secreted by cells and deposited onto microfibrils (*black arrows*). **b** In pathological situations, the release regulation of active TGF-β and BMP growth factors from microfibrils is disturbed. Mutations in fibrillin-1 lead to enhanced release of TGF-β, and potentially BMPs. The growth factors can interact with their respective cell surface receptors and trigger intracellular signaling events (*white arrows*). The interaction of integrins with microfibrils might also be dysregulated in pathological situations. Cells, microfibrils and growth factors are not drawn to scale

catenin, which is otherwise targeted for destruction. β-catenin can then mediate the regulation of gene expression in the nucleus with other transcription factors. Wnt signaling has been shown to play a role in skin fibrosis where it crosstalks with fibrillin. The tight skin (Tsk) mouse model displays certain features that are overlapping with stiff skin syndrome. It is caused by a large in-frame duplication of exons 17–40 in fibrillin-1, thus resulting in a larger ~420 kDa fibrillin-1 protein (Siracusa et al. 1996; Saito et al. 1999). Heterozygous *Fbn1*$^{Tsk/+}$ mice are characterized by tight skin and accumulation of microfibrils in the loose connective tissue (Green et al. 1976). mRNA microarray analysis of the Tsk mouse skin showed increased levels of several genes integrated with the Wnt signaling pathway, including Wnt2, -9a, -10b, and −11, inhibitors such as secreted frizzled-related protein 2 and −4, as well as Wnt-induced secreted protein 2 (Bayle et al. 2008). Furthermore, the authors demonstrated that Wnt3a increased fibrillin matrix formation in vitro, which further indicates that Wnts contribute to increased microfibril accumulation in the skin of *Fbn1*$^{Tsk/+}$ mice.

The CCN family is a group of six secreted proteins that are named after three of its members: **c**ysteine-rich protein 61 (CYR61, also called CCN1), **c**onnective tissue growth factor (CTGF, also called CCN2) and nephroblastoma overexpressed protein (NOV, also called CCN3) (Leask and Abraham 2006). CCNs are generally induced by growth factors and cytokines and are overexpressed in pathological conditions such as fibrosis. The CCNs act as adaptor molecules connecting the ECM with the cell surface. It has been shown that CCN3 plays a role in skin matrix remodeling of *Fbn1*$^{Tsk/+}$ mice (Lemaire et al. 2010). These authors showed that TGF-β and Wnt worked synergistically to stimulate fibrillin matrix assembly and CCN3 expression in the skin. CCN3 overexpression markedly impaired fibrillin assembly, as it blocked the effects of both cytokines. Fibrillin matrix in turn upregulated CCN3 expression, and thus provided a counter-regulatory mechanism to the stimulating effects of TGF-β and Wnt on fibrillin assembly.

Cellular sensing of fibrillin microfibrils

Fibrillin microfibrils are sensed by transmembrane receptors which transduce signals to the interior of the cells that influence cell shape, gene expression and function. Integrins and potentially other receptors mediate those effects.

Integrins

Numerous ECM proteins contain an RGD sequence, which mediates cell-matrix interactions by binding to integrins. In addition, many other amino acid sequences in extracellular ligands can mediate interaction with integrins. Each integrin heterodimer consists of an α and a β single-pass transmembrane protein that associate non-covalently (Hynes 2002). The interaction between RGD cell binding sites and integrins mediates cell attachment, focal adhesion formation and integrin signaling. This interaction is essential for cells to sense and

react to their extracellular microenvironment. Important intracellular components of this signaling complex are talin, Src-family kinases, focal adhesion kinase, vinculin and paxillin (Harburger and Calderwood 2009). Talin is a structural adaptor that links integrins directly to the cytoskeleton. Scaffolding adaptors (e.g., paxillin, vinculin) form bridges between focal adhesion proteins. Focal adhesion kinas and Src-family kinases are catalytic adaptors that propagate signal transduction.

Relevant integrins that mediate cell attachment to fibrillin-1 are $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_6$ (Pfaff et al. 1996; Sakamoto et al. 1996; Bax et al. 2003; Jovanovic et al. 2007). Mutations in the TB4 domain of fibrillin-1 that harbors the RGD sequence lead to stiff skin syndrome characterized by diffuse skin fibrosis as outlined in section Fibrillinopathies (Loeys et al. 2010). Interestingly, no mutation that directly affects the RGD cell binding site is listed in the fibrillin-1 mutation database (http://www.umd.be/FBN1) (Collod-Beroud et al. 2003). All known mutations that cause stiff skin syndrome are located across the entire TB4 domain in close vicinity of the RGD sequence. In vitro analyses of two stiff skin syndrome mutations, p.W1570C and p.C1564S, displayed significant loss of both, cell attachment and spreading, suggesting that the fibrillin-1 interaction with integrins $\alpha_5\beta_1$ and/or $\alpha_v\beta_3$ was affected. However, keratinocytes expressing integrin $\alpha_v\beta_6$ attached and spread normally on mutated fibrillin-1. Moreover, studies with cultured dermal fibroblasts from patients with stiff skin syndrome display reduced amounts of active focal adhesion kinase. This underlines the importance of integrin ligation and the resulting downstream signaling. In vivo studies showed that heterozygous mice with an RGD to RGE substitution in fibrillin-1 ($Fbn1^{RGE/+}$) phenocopied the diffuse skin fibrosis observed in patients with stiff skin syndrome (Gerber et al. 2013). The mice further mimicked the typical autoantibody production and dermal infiltration of pro-inflammatory immune cells including plasmacytoid dendritic cells, T helper cells and plasma cells. Homozygous inactivation of the RGD sequence in mice ($Fbn1^{RGE/RGE}$) resulted in early embryonic lethality. The in vivo studies of heterozygous $Fbn1^{RGE/+}$ mice further emphasize the importance of correct integrin ligation (Gerber et al. 2013). In this study, integrin-modulating therapies mimicking the integrin-matrix ligand interaction, which is impaired by the mutation, normalized the skin architecture and dermal infiltration of proinflammatory immune cells.

Other transmembrane receptors

The AT1 receptor is another possible cellular sensor. It can be activated by mechanical stress, independent of angiotensin II, in cardiac hypertrophy (Zou et al. 2004). Mice homozygous for a hypomorphic *Fbn1* allele ($Fbn1^{mgR/mgR}$) present with dilated cardiomyopathy (Cook et al. 2014). This study further showed that crossing these mice with AT1-deficient mice restored normal cardiac size and function, which was also normalized upon treatment with losartan, but not with TGF-β-neutralizing antibodies. This suggests that the activation of TGF-β signaling does not account for cardiomyopathy, but rather the abnormal muscle mechanosignaling via AT1.

Pericellular heparan-sulfate-containing proteoglycans (e.g., syndecans) might also be important in sensing the fibrillin matrix. The importance of the interaction between heparan sulfate and the TB5 domain in fibrillin-1 is highlighted by the disruption of heparin binding in acromicric and geleophysic dysplasias caused by mutations in the TB5 domain (Cain et al. 2012). However, the full spectrum of functional contributions of how cells sense their microenvironment needs to be clarified.

Fibrillin-associated ECM remodeling and inflammation

Regulation of matrix metalloproteinases

MMPs are a large family of endopeptidases, which are involved in degradation and remodeling of the ECM (Sekhon 2010). Several lines of evidence demonstrate that the regulation of MMPs is involved in the pathogenesis of Marfan syndrome and other fibrillinopathies. Several MMPs, including MMP-1, -2, −3 and −9, were present at increased concentrations in aortic specimens obtained from Marfan patients (Segura et al. 1998; Ikonomidis et al. 2006). Additionally, these aortic specimens displayed fibrillin fragmentation (Fleischer et al. 1997), and it has been shown that fibrillin is susceptible to proteolysis by several MMPs (Ashworth et al. 1999b; Hindson et al. 1999; Kirschner et al. 2011). Increased MMP expression was also found in lens specimens from individuals affected with Marfan syndrome (Sachdev et al. 2002).

Fibrillin-1 fragments that contain the RGD cell binding site were capable of inducing MMP-1 and −3 expression at the mRNA and protein level in human skin fibroblasts, whereas MMP-2 and −9 levels remained unaltered (Booms et al. 2005). Furthermore, this study also showed that fibrillin-1 fragments without the RGD cell binding site did not cause an increase in MMP-1 expression, but in MMP-3 expression. This indicates that both, RGD-dependent and independent mechanism exist for fibrillin-1 to regulate the expression of MMPs. One RGD-independent sequence that mediates MMP expression is the XGXXPG elastin-binding protein (EBP) consensus sequence present three times in fibrillin-1 (Brassart et al. 2001). The EBP is one of three subunits of the elastin-laminin receptor which has been implicated in different cell signaling processes by binding to XGXXPG motifs (Mecham et al. 1989). It acts as a mechanotransducer in vascular smooth muscle cells (Spofford and Chilian 2001), and it induces proliferation of arterial smooth muscle cells

(Mochizuki et al. 2002). A fibrillin-1 fragment containing a XGXXPG (EGFEPG) sequence in cbEGF33 upregulated the MMP-1 protein production in skin fibroblasts (Booms et al. 2006). This fragment was also capable of inducing monocyte chemotaxis as outlined in the section below Inflammatory infiltration of immune cells.

In aortic aneurysms of *Fbn1*$^{C1039G/+}$ mice, increasing MMP-2 and −9 mRNA and protein expression was detected between three to six months of age (Chung et al. 2007). Altered MMP expression was accompanied by elastic fiber degradation and deterioration of mechanical properties and aortic contraction. Treatment with doxycycline, a broad-spectrum inhibitor of all MMPs, significantly improved life expectancy in *Fbn1*$^{mgR/mgR}$ mice (Xiong et al. 2008). Doxycycline reduced elastic fiber degradation by decreasing MMP-2 and −9 levels. Similar results were obtained for *Fbn1*$^{C1039G/+}$ mice. Whereas aneurysm formation was prevented upon doxycycline treatment in *Fbn1*$^{C1039G/+}$ mice, there was still evidence of mild aneurysm formation after administration of atenolol (Chung et al. 2008). In this study, doxycyline improved elastic fiber integrity, normalized vasomotor function and reduced TGF-β activation. After establishment of aortic aneurysms at four months of age, neither doxycycline nor losartan treatment completely restored aortic wall integrity and function in *Fbn1*$^{C1039G/+}$ mice (Yang et al. 2010). The combination of doxycycline and losartan in this study led to a better outcome compared to single-drug treatment, as it improved elastic fiber organization, decreased MMP-2, −9 and TGF-β expression and normalized aortic mechanical properties. In another study, losartan and doxycycline were found to be equally effective in delaying, but not preventing aneurysm formation and rupture (Xiong et al. 2012). These authors further demonstrated that MMP-2 plays a role in the release of active TGF-β from the LLC, which then results in downstream signaling via ERK1/2. A combination therapy with losartan and doxycycline might prove more effective than a single-drug therapy, as these compounds additively reduce ERK1/2 signaling, and thus delay aneurysm progression and rupture.

In summary, these findings illustrate that proteolytic degradation, which generates fibrillin-1 fragments, triggers a vicious cycle. The generated fibrillin-1 fragments upregulate MMP expression, which in turn causes more proteolytic degradation of fibrillin, eventually impairing the sequestration of growth factors on microfibrils.

Inflammatory infiltration of immune cells

Monocyte infiltration in the medial layer of the aorta can be observed as early as eight weeks of age in *Fbn1*$^{mgR/mgR}$ mice (Pereira et al. 1999). The inflammatory infiltration is followed by adventitial inflammation, elastolysis of the media and a fibroproliferative response. Macrophages in inflammatory foci originate from blood monocytes that are attracted by a plethora of chemotactic factors (Brömme et al. 1993). There are also some ECM-derived components harboring a XGXXPG sequence, that mediate chemotaxis of fibroblasts and monocytes by binding to the EBP present on the surface of monocytes (Senior et al. 1984).

In line with the finding that aortic extracts from *Fbn1*$^{mgR/mgR}$ mice induced macrophage chemotaxis, recombinant fibrillin-1 fragments containing a XGXXPG EBP recognition sequence acted as chemotactic stimuli for macrophages (Guo et al. 2006). This response was significantly reduced by pre-treatment with monoclonal antibodies directed against an EBP recognition sequence VGVAPG, or by a mutation of the EBP sequence in fibrillin-1 fragments. These results show the involvement of the EBP sequence in the stimulation of macrophage chemotaxis in *Fbn1*$^{mgR/mgR}$ mice. Macrophage infiltration was also observed in aortic specimens obtained from individuals with Marfan syndrome (He et al. 2008). Treatment of *Fbn1*$^{mgR/mgR}$ mice with a monoclonal antibody directed against EBP recognition sequence XGXXPG in fibrillin-1 and elastin rescued elastin degradation and reduced macrophage infiltration in the aorta (Guo et al. 2013). Moreover, the treatment had beneficial effects on MMP expression and TGF-β signaling. The upregulation of MMP-2 and −9 was inhibited, and the upregulation of p-SMAD2, TGF-β1 and LTBP-1 was decreased. In addition, monoclonal antibody treatment prevented the development of pulmonary emphysema in this study.

The proinflammatory cytokine interleukin (IL)-6 plays a key role in vascular inflammation. Hypomorphic *Fbn1*$^{mgR/mgR}$ mice displayed significantly enhanced IL-6 signaling, which contributed to the aortic ECM degeneration and increased activity of MMP-9 (Ju et al. 2014). However, IL-6 deficiency delayed aneurysm formation, but did not prevent rupture or improve the survival time. This indicates the involvement of other pathways in the early stages of disease progression.

MicroRNA-dependent regulation in relation to fibrillins

MicroRNAs have been identified during the past decade as important regulators of gene expression (Bartel 2004). They represent single-stranded short non-coding RNAs that are about 21–23 nucleotides in length. MicroRNAs regulate gene expression on the mRNA level by binding directly to the 3′ untranslated regions of specific target mRNAs. This results in the repression of mRNA translation and thus protein expression.

Overall, the relationship between microRNAs, ECM and disease pathogenesis is very little explored. However, emerging evidence demonstrates that microRNAs are important regulators of the ECM as it relates to fibrillinopathies. MicroRNAs regulate the expression of critical extracellular

proteins including fibrillin, elastin, fibronectin and CTGF (van Rooij et al. 2008; Duisters et al. 2009; Shan et al. 2009), as well as vascular tissue integrity (Fish et al. 2008). MicroRNA-dependent regulation further controls smooth muscle fate and plasticity (Cordes et al. 2009), contractility (Boettger et al. 2009), and cytoskeletal dynamics (Xin et al. 2009). Moreover, microRNAs can integrate with TGF-β signaling in vessel stabilization (Climent et al. 2015).

Specific targets for members of the miR-29 family include fibrillin-1, tropoelastin and several collagens (van Rooij et al. 2008). Overexpression of miR-29a/b/c mimics repressed, whereas miR-29 inhibitors increased elastin mRNA and protein levels in cell culture (Zhang et al. 2012). Interestingly, transfection of smooth muscle cells obtained from a patient with elastin deficiency (supravalvular aortic stenosis) with an inhibitor for miR-29a increased tropoelastin mRNA and protein expression levels. It was shown that miR-29b regulates aortic wall apoptosis and ECM abnormalities with significantly increased levels in *Fbn1*$^{C1039G/+}$ mice compared to control animals (Merk et al. 2012). Increased apoptosis was found in the aorta of the *Fbn1*$^{C1039G/+}$ mice as evidenced by increased caspase-3 activity and decreased levels of anti-apoptotic proteins, Mcl-1 and Bcl-2. This study also showed evidence for an involvement of the nuclear factor (NF)-κB pathway. NF-κB is known to repress miR-29b and is itself suppressed by TGF-β. As expected, decreased activation of NF-κB was observed as a result of enhanced TGF-β signaling, and administration of an NF-κB inhibitor increased miR-29b levels. There was an effective reduction in miR-29b levels upon TGF-β blockade or losartan administration. Direct miR-29b blockade by antisense oligonucleotides significantly ameliorated the outcome by preventing early aneurysm development, aortic wall apoptosis, and ECM deficiencies. miR-29b was also shown as a critical player in abdominal aortic aneurysm development (Maegdefessel et al. 2012). The authors showed in this study that aortic aneurysm development in mouse models was accompanied by decreased aortic expression of miR-29b, correlating with increased expression of miR-29b targets including tropoelastin. miR-29b inhibition led to a significant reduction of the abdominal aneurysms.

microRNAs were also shown to be deregulated in systemic sclerosis, a disorder similar to stiff skin syndrome with unclear etiology. Fibroblasts from patients with systemic sclerosis showed decreased levels of miR-29 in vitro (Gerber et al. 2013). miR-29 is known to be repressed by TGF-β and inhibits the expression of multiple ECM components and suppresses fibrosis (van Rooij et al. 2008; Maurer et al. 2010). Integrin-modulating therapies and TGF-β antagonism restored miR-29 levels (Gerber et al. 2013).

In conclusion, these emerging results indicate the need to explore microRNA pathways in relation to fibrillinopathies. They may hold future potential for the design of novel therapies.

Conclusions

In summary, evidence accumulate demonstrating that fibrillin-containing microfibrils represent key relay stations for the transmission of extracellular information into cellular signaling and function. Microfibrils store growth factors including TGF-β and several BMPs and regulate their bioavailability and activity. Microfibrils directly or indirectly interact with cell surface receptors to sense normal and pathologically altered ECM. The integrity of microfibrils and associated elastic fibers determine expression of matrix degrading proteases, recruitment of inflammatory cells, and regulation of microRNAs. Loss of microfibril integrity and abundance leads to a number of connective tissue diseases through defects in cellular signaling mechanisms. The challenge for the next years is to understand and integrate all cell signaling functions mediated by fibrillin-containing microfibrils, which will ultimately aid in the design of novel preventative and therapeutic strategies for the associated fibrillinopathies.

Acknowledgments We are grateful for the financial support of the Canadian Institutes of Health Research (MOP-106494), the Canada Research Chair program, the Quebec Network of Oral and Bone Health Research, and the German Studienstiftung des deutschen Volkes.

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