Cell. Mol. Life Sci. 66 (2009) 1890 – 1902
1420-682X/09/111890-13
DOI 10.1007/s00018-009-8632-6

Cellular and Molecular Life Sciences

Review

Fibulins: Multiple roles in matrix structures and tissue functions

S. de Vega[a], T. Iwamoto[a, b] and Y. Yamada[a, *]

[a] Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial Research, Bldg. 30, Rm. 407, National Institutes of Health, DHHS, 30 Convent Drive MSC 4370, Bethesda, MD 20892 – 4370 (USA), Fax: +1-301-402-0897, e-mail: yoshi.yamada@nih.gov
[b] Department of Pediatric Dentistry, Kyusyu University School of Dentistry, Fukuoka 812 – 8582 (Japan)

Received 3 October 2008; received after revision 9 January 2009; accepted 16 January 2009
Online First 5 February 2009

Abstract. The fibulins are a family of secreted glycoproteins associated with basement membranes, elastic fibers, and other matrices. They are expressed in a variety of tissues. Association with these matrix structures is mediated by their ability to interact with many extracellular matrix constituents. The seven members of the family are defined by the presence of two structural modules, a tandem repeat of epidermal growth factor-like modules and a unique C-terminal fibulin-type module. They act not only as intermolecular bridges within the extracellular matrix to form supramolecular structures, but also as mediators for cellular processes and tissue remodeling. These important functions of fibulins in a wide range of biological processes have been shown in *in vitro* systems, gene knockout mice, and human genetic disorders. In this review, we describe the structure and function of these proteins and discuss the implication of fibulins in development and diseases.

Keywords. Fibulins, protein interactions, extracellular matrix network, embryonic development, elastogenesis, angiogenesis, genetic disorders.

Introduction

The extracellular matrix (ECM) is very diverse in nature and composition. This characteristic helps it serve many functions such as supporting cells and regulating intercellular communications. In addition to controlling cell motility, it is essential for growth, development, wound healing, and fibrosis. ECM components are secreted into and often aggregate with the existing matrix. Many proteins in the ECM are glycoproteins, which include relatively large molecules such as laminins, fibronectin, and elastins. Other smaller ECM proteins serve to modulate cellular behavior and functions. One such group of ECM proteins is the fibulin protein family. Fibulin-1 was the first member discovered two decades ago by affinity chromatography in an effort to elucidate the cytoplasmic interactions of the β subunit of the fibronectin receptor. In that study, fibulin-1 extracted from human placenta was found to bind to a synthetic peptide derived from the cytoplasmic domain of the fibronectin receptor β subunit. Fibulin-1 was also shown to bind to the native receptor in an *in vitro* binding assay [1]. Although the fibulin-1 protein was thought to be an intracellular molecule, serving as a bridge between β integrins and cytoskeletal components, sequencing and immunohistology revealed that fibulin-1 was an ECM protein present in the fibril matrix deposited by fibroblasts in culture and in the blood [2]. A separate group also isolated fibulin-1 from a mouse EHS (Engelbreth-Holm-Swarm)

* Corresponding author.

tumor, which produces a large amount of basement membrane components [3]. Since then, six more members have been identified. Fibulin-2 was identified by comparative sequence analysis of mouse fibulin-1 and was isolated from a mouse fibroblasts cDNA library [4]. Fibulin-3 (also known as S1–5, Efemp1) was isolated by subtractive screening of a cDNA library from senescent human diploid fibroblasts (HDF) established from a patient with Werner syndrome and was found to regulate DNA synthesis [5]. Fibulin-4 (also known as Efemp2) was cloned during a search for new members of the fibulin family by sequence homology [6]. Fibulin-5 (also known as EVEC, DANCE) was isolated by subtraction hybridization to identify genes that regulate the transition from quiescent vascular smooth muscle cells to the proliferative state [7, 8]. Fibulin-6 (also known as Hemicentin-1) was identified as the gene product of the *him-4* locus in *Caenorhabditis elegans* [9]. Lastly, fibulin-7 (also known as TM14) was identified by differential hybridization using tooth germ cDNA microarrays [10].

Fibulins share a common multimodular organization with tandem repeats of an epidermal growth factor (EGF)-like module and a unique C-terminal fibulin-type module. The fibulin-type module defines the fibulin family of proteins, which includes seven members in mammals, but has limited members in *C. elegans,* chicken, and zebrafish [11]. They have roles in the assembly and stabilization of supramolecular ECM complexes. Due to their involvement in the elaboration and stabilization of the ECM, the fibulins have been implicated in tissue organogenesis, vasculogenesis, fibrogenesis, and tumorigenesis. Fibulins have been found in association with ECM structures such as connective tissue fibers, basement membranes, and blood clots. Recent studies with *in vitro* systems, mouse models, and human genetic disorders have led to a better understanding of the functions of fibulins [12–14]. The aim of this review is to summarize the current knowledge about the roles of fibulins in development and disease as well as their interactions with other molecules.

Fibulin protein structure

Fibulins consist of modules grouped in domains I, II, and III. Domain I represents the N terminus and is variable among the family members. Domain II represents the central portion and contains a variable number of EGF-like modules in a tandem array. Most of the EGF-like modules contain a consensus sequence for calcium binding and are known as calcium-binding EGF (cbEGF)-like modules. The C-terminal portion is the domain III, called the fibulin-type module, specific to the fibulins and fibrillins [6] (Fig. 1).

Alternative splicing in the fibulin-1 gene produces two splice variants (C and D), which differ in domain III, in mice, chickens, zebrafish, and nematodes. In humans, two additional variants (A and B) exist, but at very low levels [11, 15, 16]. Variant A lacks the complete domain III, and variant B has smallest domain III compared to variants C and D. In fibulin-2, the third EGF-like module can be present or absent as a result of alternative splicing in an exon. Both variants are found in humans and mice, but it is not known if these proteins have a different function [16]. Alternative splicing of fibulin-3 results in five variants, which have a partial or complete absence of domain I [5]. Two of these variants lack the signal peptide and their expression levels are low. An alternatively spliced variant of fibulin-4 lacking the signal peptide has also been reported [17]. All of the fibulins are glycoproteins and have several N-linked acceptor sites [10, 14].

The members of the fibulin family have been classified into two subgroups. The first subgroup consists of fibulin-1 (100 kDa) and fibulin-2 (195 kDa). They are larger than the other members because they have three anaphylatoxin (AT) modules in domain I, which are components of the complement system involved in inflammation and defense against parasites, and more EGF-like modules in the central portion. In addition, fibulin-2 contains an extra portion in the N-terminal domain with two cysteine-rich segments (Fig. 1). The second subgroup contains the rest of the members, fibulin-3 to fibulin-7. They are smaller proteins of 50–60 kDa, except for fibulin-6, which contains a larger N-terminal domain with nine immunoglobulin C-2 modules and six thrombospondin type I repeats. Fibulin-7 has a unique N-terminal domain I, containing a sushi domain, also known as complement control protein (CCP) domain or short consensus repeat (SCR), which is involved in protein-protein interactions and in the regulation of the complement system and blood coagulation. These fibulins (fibulin-3 to fibulin-7) were originally identified during searches for new proteins and have been given different acronyms. Because they contain the fibulin motif and tandem array of EGF domains, they have been recognized as members of the fibulin family [6, 7, 10].

Interactions of fibulins with ECM components

Assigning functions to novel proteins is one of the most challenging problems. One approach is to study protein-protein interactions. As part of the ECM, fibulin proteins interact and bind to other proteins in

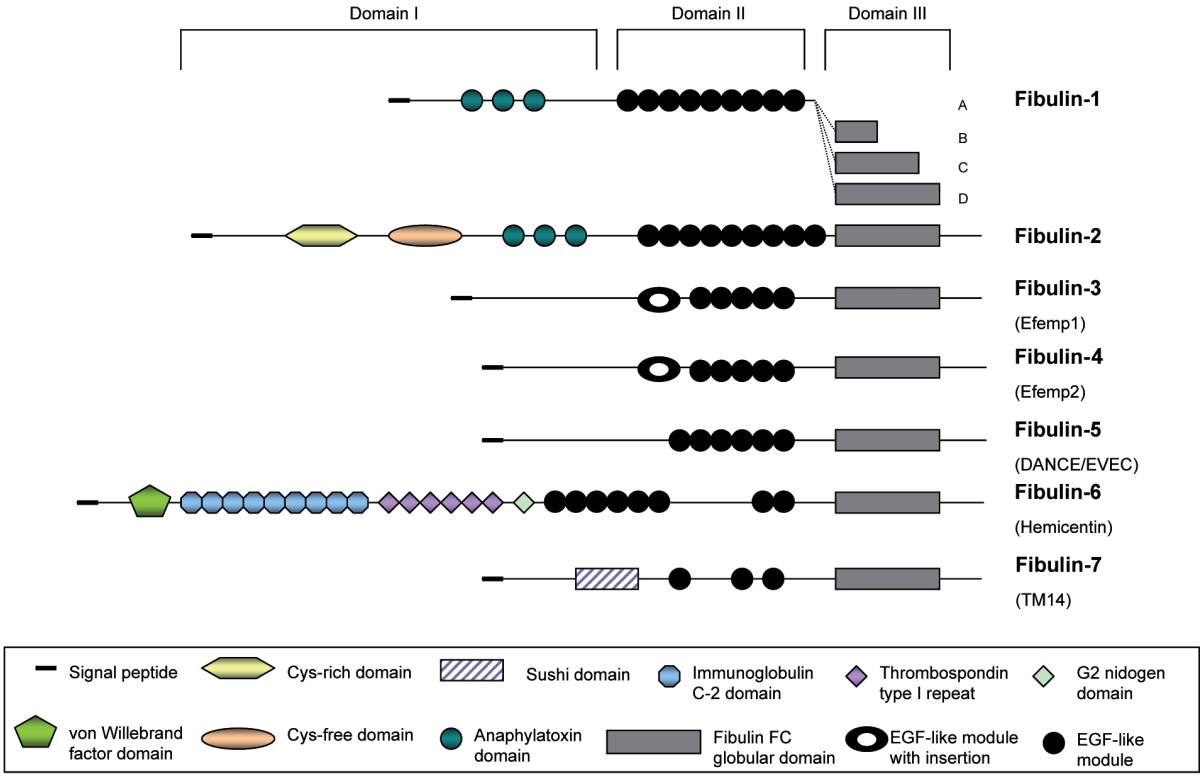

Figure 1. Domain structures of fibulin family proteins. The seven members of the family display similar modular arrangement, consisting of domains I, II and III. Domain III at the C terminus contains a sushi motif and domain II at the center consists of EGF-like motifs. These motifs in domains II and III are common to all fibulins. The N-terminal domain I varies in size and motifs among the fibulin family.

the matrix. Many studies have been done to identify the binding partners for fibulin proteins, which are summarized in Table 1 [14, 18]. Fibulin-1 and fibulin-2 are localized in basement membranes, elastic fibers, and other connective tissue structures [3, 19–21]. Both interact with many common binding partners, but with some specificity (Table 1). Cells produce fibronectin-based fibrillar matrices, which are essential for many biological processes including embryogenesis, morphogenesis, and tissue generation. Fibronectin fibrillogenesis is initiated by integrin binding to the RGD-containing cell-binding domain of fibronectin and facilitated by formation of focal and fibrillar adhesions [22, 23]. Fibulin-1 and fibulin-2 are associated with the fibronectin-based fibrils produced by fibroblasts and other cell types [2, 11, 24]. Fibulin-1 binds to the type III repeats of fibronectin, which has a heparin binding site, through EGF-like modules [25, 26]. Recombinant mouse fibulin-2, but not fibulin-1, binds strongly to purified αIIβ3 integrin and with a lesser extent to αvβ3 integrin through the RGD motif in the N-terminal domain [27].

Fibulin-1 and fibulin-2 are associated with basement membranes in various tissues. Basement membranes are thin ECMs that separate epithelial and mesenchymal cells and surround cells, such as endothelial and muscle cells. They provide the scaffolding for cells and tissues and have an essential role in tissue morphogenesis that affects cell adhesion, migration, proliferation, and differentiation. Basement membranes consist of collagen IV, laminin, perlecan, nidogen/entactin, and other molecules, which interact with each other to form the supramolecular structure and also bind cell surface receptors such as integrins and syndecans [28]. Fibulin-1 and fibulin-2 bind to several basement membrane components. Fibulin-1 binds to the C-terminal globular domain (G domain) of the laminin α1 and α2 chains [28, 29]. The G domain of laminin α chains has a role in many biological activities such as cell adhesion and migration and binding to α-dystroglycan and integrins [30, 31]. Fibulin-2 also binds to the G domain of the laminin α2 chain [32]. Fibulin-1 and fibulin-2 bind to the short arm of the laminin γ2 chain. The laminin γ2 chain is a subunit of epithelial cell-specific laminin-5, which is a component of the anchoring filament that functions to link these cells to the basement membrane. The interaction of these fibulins with laminin-5 may enhance the anchorage force between epidermal cells and the basement membrane.

Fibulin-1 and fibulin-2 bind to the globular domains G2 and G3 of nidogen-1, which have been identified as

Table 1. Molecular interactions of fibulin family members.

Protein	Interacting protein	References
Fibulin-1	Fibronectin	[25, 26]
	Aggrecan	[37]
	Versican	[37]
	Nidogen	[33]
	HB-EGF	[44]
	Laminin-α1 and laminin-α2 chains	[28,29]
	ECMI (extracellular matrix protein-1)	[47]
	Angiogenin	[46]
	NOVH (Nephrobalstoma overexpressed)	[43]
	Tropoelastin	[18]
	Fibulin-7	[10]
	Fibrinogen	[41]
	ADAMTS-1 (disintegrin-like and metalloprotease with thrombospondin motifs)	[39]
	Sex hormone-binding globulin (SHBG)	[40]
	β-amyloid precursor protein	[45]
Fibulin-2	αIIβ3 integrin; αvβ3 integrin	[27]
	Laminin-α2 chain	[32]
	Sex hormone-binding globulin (SHBG)	[40]
	Fibrillin	[21]
	Aggrecan	[38]
	Versican	[38]
	Fibronectin	[30]
	Nidogen	[34]
	Perlecan	[36]
	Tropoelastin	[18]
Fibulin-3 (Efemp1)	Tropoelastin	[18]
Fibulin-4 (Efemp2)	Tropoelastin	[18]
Fibulin-5 (DANCE; EVEC)	α5β1, α4β1	[102]
	Tropoelastin	[18]
	Elastin monomers	[48, 49]
	LOXL-1 (Lysyl oxidase-like 1)	[50,51]
	LTBP-2 (latent TGF-β-binding protein-2)	[52]
Fibulin-6 (Hemicentin)	Not described	
Fibulin-7 (TM14)	Fibronectin	[10]
	Heparin	[10]
	Fibulin-1	[10]
	Dsp (Dentin sialoprotein)	[10]

a binding region for perlecan and laminin γ1, a component of laminin-1, respectively [33, 34]. The fibulin-1-nidogen-1 interaction varies depending on fibulin domain III isoforms, and fibulin-1C (a splice variant of fibulin-1) shows the strongest binding to nidogen-1 compared to another splicing variant fibulin-1D [33]. In addition, EGF-like modules in domain II of fibulin-1 are also involved in the binding to nidogen-1 [35]. Fibulin-2, but not fibulin-1, binds to domain IV of perlecan, a large heparan sulfate proteoglycan, which is present in basement membranes and other tissues such as cartilage. Domain IV of perlecan consists of multiple Ig-like modules and has binding sites for nidogens and fibronectin [36]. The interaction of fibulin-1 and fibulin-2 with these basement membrane components may provide the scaffold to support tissues such as capillaries integrity and functions.

Fibulin-1 and fibulin-2 are localized in elastic fibers. In elastic fiber assembly, tropoelastins (elastin precursors) are deposited on microfibrils in an orderly manner and cross-linked by lysyl oxidases (LOXs). Elastic fibers provide the scaffold for connective tissues and they are essential for the function of the skin, lungs, arteries and other organs. Fibulin-1 and fibulin-2 bind to tropoelastin 2, with fibulin-2 having a much higher affinity to tropoelastin compared to fibulin-1. Fibulin-2, but not fibulin-1, binds to fibrillin-

1, a microfibril-associated protein [21], suggesting that fibulin-2 may function to anchor elastin to microfibrils.

The large chondroitin sulfate proteoglycans aggrecan and versican (PG-M) bind to both fibulin-1 and fibulin-2 through their C-terminal type-2 lectin domains [37, 38]. Aggrecan has a large number of chondroitin sulfate chains and forms large aggregates with hyaluronan and link protein in the cartilage matrix, which provides mechanical strength to resist compression in joints. Versican is expressed in articular cartilage and other mesenchyme tissues. It has been shown that fibulin-2 forms a network with aggrecan and versican. These results suggest that fibulin-1, fibulin-2, and other fibulins expressed in cartilage may play a role in stabilization and function of the cartilage matrix. A yeast two-hybrid-screen identified a specific interaction between fibulin-1 and ADAMTS-1, a member of the ADAMTS (a disintegrin-like and metalloprotease with thrombospondin motifs) family proteases [39]. Fibulin-1 enhances ADAMTS-mediated proteolysis of aggrecan, a known substrate for ADMTS-1 and a binding partner for fibulin-1 [39]. Although fibulin-1 is not a substrate for ADAMTS-1, it may form a ternary complex with ADAMTS-1 and aggrecan to promote aggrecan turnover and plays a role in tissue remodeling. Fibulin-1D and fibulin-2 interact with sex hormone-binding globulin (SHBG) [40], suggesting that these fibulins regulate steroid hormone action by sequestering SHBG in the ECM.

Fibulin-1 is also a binding partner for fibrinogen and it can be incorporated into fibrin clots, suggesting a role for fibulin-1 in thrombi formation [41]. Indeed, it has been shown that fibulin-1 mediates platelet adhesion by forming a bridge between cells and fibrinogen, a characteristic similar to collagens I and IV, and fibronectin [42]. Fibulin-1 also interacts with NOVH (nephroblastoma overexpressed), a member of the CCN family of growth regulators [43]. The isoform fibulin-1C interacts with the heparin-binding EGF-like growth factor (HB-EGF), which is implicated in tumor formation, cell migration, ECM formation, wound healing, and cell adhesion [44]. Fibulin-1 binds to the N-terminal portion of the secreted form of β-amyloid precursor protein (sAPP) [45]. This interaction is mediated through EGF modules of fibulin-1 in a Ca^{2+}-dependent manner and blocks sAPP-mediated proliferation of neural stem cells [45]. Since fibulin-1 in brain is expressed primarily by neurons, these results suggest that fibulin-1 may modulate neurotrophic activities of APP. Other ECM proteins such as angiogenin, fibulin-7, and ECM-1 also interact with fibulin-1 [10, 18, 46, 47].

Fibulin-1 and fibulin-2 present a wide repertoire of interactions. However, for the other members of the family, fewer binding partners have been found to date. No protein interactions have been reported for fibulin-6. Fibulin-3, fibulin-4, and fibulin-5 bind to tropoelastin and play important roles in the assembly of elastic fibers during development, but they do not interact with fibronectin [18]. Fibulin-5 binds monomer elastin through the cbEGF modules [48, 49]. Fibulin-5 interacts with LOXL-1 (lysyl oxidase-like 1), -2 and -4, which are essential for the initial step for the polymerization of tropoelastin monomers, the process called coacervation [50, 51]. This interaction is mediated through the C-terminal part of fibulin-5. Recent study suggests that fibulin-5 acts as an organizer for elastic-fiber formation by inducing elastic fiber assembly and promoting coacervation of tropoelastin through tethering LOXs [51]. Fibulin-5 also binds to LTBP-2, a member of the latent TGF-β-binding family, through cbEGF modules [52]. Unlike other members of LTBPs, LTBP-2 cannot bind to TGF-β [53] and it is localized to the elastin-associated microfibrils [54]. It was shown in cell culture that deposition of fibulin-5 and elastin is dependent on fibrillin-1 but not either fibrillin-2 or LTBP-2. On the other hand, suppression of LTBP-2 promotes deposition of fibulin-5 and elastin onto fibrillin-2 microfibrils. These results may suggest that LTBP-2 regulates which microfibrils fibulin-5 should be deposited on for elastic fiber assembly. In fibulin-5-transfected cells, a portion of recombinant fibulin-5 is specifically cleaved to yield a C-terminal truncated fragment by a serine protease [51]. The truncated fragment cannot deposit on microfibrils and causes inactivation of the elastogenic activities of the full-length fibulin-5. Interestingly, in mouse skin, the amount of the full-length fibulin-5 is reduced and that of a C-terminal truncated fibulin-5 fragment increases with age [51]. Since elasticity in tissue is thought be reduced with aging, proteolysis of fibulin-5 may be involved in the deterioration of tissue elasticity during aging. Fibulin-7 binds to dentin sialoprotein (Dsp), fibronectin, and heparin. It also interacts with fibulin-1 [10]. Table 1 summarizes the known binding proteins for each fibulin member. All of these interaction data indicate that fibulins are versatile proteins that associate with ECM proteins for the formation of supramolecular structures and cellular processes.

Expression of fibulins

The expression pattern of fibulins is summarized in Table 2. Fibulin-1 and fibulin-2, the first 2 members identified in the family, are present in some basement

Table 2. Expression pattern.

Fibulin member	Expression
Fibulin-1	Vessels walls, basement membranes, microfibrils and elastic fibers. Cartilage
Fibulin-2	Basement membranes, heart, placenta and ovary. Cartilage
Fibulin-3 (Efemp1)	Cartilage, bone and retina.
Fibulin-4 (Efemp2)	Cardiac valve, heart, lung, kidney and skeletal muscle. Cartilage.
Fibulin-5 (DANCE; EVEC)	Great vessel and cardiac valves. Lung, uterus, cartilage.
Fibulin-6 (Hemicentin-1)	Skin fibroblasts and retinal pigment epithelium.
Fibulin-7 (TM14)	Incisors and molars. Cartilage, hair follicles and extraembryonic tissues.

membranes and many other connective tissues [1, 4, 15]. Fibulin-1 expression is particularly prominent in areas undergoing epithelial-mesenchymal transitions such as the endocardial cushion tissue, developing myotomes, neural crest, tooth, and hair follicle during development [19]. Fibulin-1 exists in the great vessels, coronary artery, brain, choroid plexus and the meninges, and other connective tissues such as kidney, lung and liver [19]. Fibulin-1 is expressed in the ECM in association with the digits in the developing limb. It has also been found in perichondrial structures in human embryos [55]. The chondrocytes do not express fibulin-1 at gestational week 6.5 and 8, but by gestational week 10, the interterritorial matrix of hypertrophic chondrocytes is very rich in fibulin-1 and fibulin-2. Fibulin-1 is observed in cartilage during chondrogenesis in mice [18, 19].

Fibulin-2 expression partially overlaps with that of fibulin-1 but is more restricted to certain tissues. Similar to fibulin-1, fibulin-2 is expressed at sites where polarized epithelial cells convert into mesenchyme during development of endocardial cushion tissue and in neural crest cells. Fibulin-2 expression is particularly high in the developing heart [56]. Fibulin-2 is expressed by the smooth muscle precursor cells of developing aortic arch vessels [57]. Fibulin-2 is synthesized by the endothelial cells of coronary arteries and veins but not by the capillary endothelial cells in the myocardium. Fibulin-2 is expressed in developing cartilage, especially in the perichondrium [18, 19, 57].

Fibulin-3 is found in condensing mesenchyme, which gives rise to cartilage and bone [58]. It resides in developing bone structures of the cranial and axial skeleton [58]. In E12.5 mouse, the expression of fibulin-3 is localized in the regions of developing filamentous bones and in the primordial cartilage of limb buds. From this stage on, the signal becomes intense in the developing vertebral cartilage. By E13.5, expression can be seen in the cartilage anlagen of developing forelimbs and hindlimbs as well as in the face, where the ossification of the membranous bone of the facial skeleton occurs [58]. Fibulin-3 expression is detected in structures in the developing cranial area, rib cage, vertebrae, and appendicular skeleton. This expression pattern suggests that fibulin-3 may play a role in regulating the shaping of the skeletal elements in the body. Indeed, fibulin-3 knockout mice have reduced bone density [59]. Fibulin-3 is also highly expressed by epithelial and endothelial cells throughout the body [6, 18, 58].

Fibulin-4 is strongly expressed in the heart, moderately in skeletal muscle, and weakly in the placenta, brain, lungs, kidneys and pancreas [6]. It is expressed by adult human fibroblasts and is located in vessel walls and basement membranes. Fibulin-4 is expressed in articular chondrocytes and cultured chondrocytes [60]. A recent study reported that fibulin-4 is an osteoarthritis (OA)-specific autoantigen because the protein is present in sera obtained from patients with OA [60].

Fibulin-5 is expressed prominently in regions of epithelial-mesenchymal interactions during development of the artery, endothelial cushion tissue, neural crest, and mesenchymal tissue [7, 8, 12, 14]. Fibulin-5 is first detected in neural crest cells at E9.5 and subsequently expressed in the dorsal aortae, the vascular smooth muscle of the great vessels, the endocardial cushion and epicardium in rodent embryos. In adult tissues, it is localized in the heart, ovaries, and colon as well as in the kidneys, pancreas, testis, and lungs.

Fibulin-6 is expressed in skin fibroblasts, retinal pigment epithelial cells, and retinal endothelial cells [61, 62].

Fibulin-7 mRNA is highly expressed in teeth as well as the placenta, hair follicles, and cartilage. Expression of fibulin-7 mRNA is seen in preodontoblasts and in odontoblasts during molar and incisor development. The protein is deposited in predentin and dentin matrices. In dentin, fibulin-7 is located along the dentinal tubes. In addition, fibulin-7 mRNA is expressed in spongiotrophoblasts of the placenta, the articular cartilage, and proliferative and prehypertrophic chondrocytes of cartilage as well as in the perichondrium [10].

Dysregulation of fibulin-1 and fibulin-5 has been reported in cancer [13, 63]. Fibulin-1 expression is increased in ovarian and breast carcinomas [64–67]. However, overexpression of fibulin-1D reduces tumor formation [68], whereas the ratio of fibulin-1C to fubulin-1D is increased in ovarian carcinoma [69]. Fibulin-1 variants may function as a modulator for tumor formation. Fibulin-5 expression is decreased in a variety of human cancers, including those of the breast, kidney, ovary, and colon [70], suggesting that fibulin-5 may function as a tumor suppressor.

Mutations of fibulins in human diseases

Mutations of several fibulin genes have been identified in human genetic disorders [12]. It was reported that the fibulin-1 gene is disrupted in patients from one family with complex synpolydactyly (SPD), a rare dominantly inherited malformation of the distal limbs [54]. They have metacarpal and metatarsal synostoses (bone fusion), which is different from that of HOXD13 mutations, the most common causes of SPD [71]. A chromosomal translocation occurs in the last intron (intron 19) of the fibulin-1 gene that results in the deficiency of alternative splicing variant fibulin-1D, but not fibulin-1C. Since no fusion transcript is detectable, the translocation likely results in a haploinsufficiency for fibulin-1D that may be responsible for the digit malformation. Fibulin-1D may be required for cell migration and apoptosis for proper digit formation. Mutations in other ECM molecules such as laminin-α5 [72, 73] and fibrillin-2 [73] are also known to cause malformed digits. A defect in fibulin-1D expression is associated with the autosomal dominant giant platelet syndromes, which represent a group of disorders characterized by variable degrees of macrothrombocytopenia with combinations of deafness, renal disease, and eye abnormalities. In this case, a heterozygous mutation in the splice acceptor site of fibulin-1 exon 19 causes lack of fibulin-1D expression and overexpression of the antisense RNA [74]. These results indicate distinct functional roles between fibulin-1C and fibulin-1D isoforms, which are consistent with those from biochemical data [33].

Several findings indicate the involvement of fibulins in inherited eye disorders such as retinopathies or macular degeneration. Missense mutation R345W in the EGF domain of fibulin-3 is thought to cause Doyne honeycomb retinal dystrophy, also known as malattia leventinese (ML), which is a dominant macular degenerative disease characterized by yellow-white deposits known as drusen that accumulate beneath the retinal pigment epithelium (RPE) [75]. Patients with ML develop symptoms comparable to those age-related macular degeneration (AMD), the most common cause of incurable blindness.

Defects in fibulin-4, such as the missense mutation G169A [76], cause autosomal recessive cutis laxa, which is a heterogeneous group of connective tissue disorders characterized by cutaneous abnormalities and variable systemic manifestations such as loose skin. In addition to skin, internal organs enriched in elastic fibers, such as the lung and the arteries, are also affected.

Defects in the fibulin-5 gene cause cutis laxa and AMD. In most subjects, the disease manifests as yellowish accumulations of drusen beneath the RPE and within the elastin-containing structure (known as Bruch membrane). In cutis laxa, the missense mutations in fibulin-5 gene S227P and C217R result in misfolding and decreased secretion and interactions of fibulin-5 with elastin and fibrillin-1 [77, 78]. There is impaired elastic fiber development, suggesting that fibulin-5 is necessary for the proper elastic fiber formation [79]. In AMD, missense mutations G412E, G267S, I169T and Q124P lead to decreased fibulin-5 secretion [80]. A heterozygous gene duplication resulting in a tandem duplication of the first to the fourth cbEGF modules of fibulin-5 was found in a sporadic cutis patient [81]. The mutant fibulin-5 is secreted and causes a dominant negative effect.

Although the Q5346R mutation in fibulin-6 was thought to be a causal mutation for AMD pedigree, a recent study suggests that the mutation may not contribute to the disease [82]. No human diseases associated with fibulin-7 gene have been described to date.

Fibulin animal models

Creation of animal models provides an insight on the roles of fibulins in development, tissues functions, and disease. Fibulin-1, -2, -3, -4, and -5 have been knocked out in mice and fibulin-1 and fibulin-6 in *C. elegans.* All of them, except fibulin-2, display marked phenotypic defects in multiple organ systems (Table 3). Creation of knock-in mice that have a missense mutation in the fibulin-3 locus confirmed that the R345W fibulin-3 mutation causes the pathogenic phenotype.

Fibulin-1 knockout mice and fibulin-1 mutant *C. elegans.* Two studies have analyzed mice deficient of fibulin-1. In the first report [83], mice lacking the fibulin-1 gene were created by homologous recombination in embryonic stem (ES) cells. These mice develop bleeding in the cranial mesenchyme, skin and skeletal muscles, and most of them die within 24–48 h after birth. These mice also have reduced loop

Table 3. Fibulin knockout mice and human diseases.

Fibulin member	M**ouse** KO **phenotype**	H**uman disorders**	
		Mutation	Phenotype
Fibulin-1	Die perinatally as a result of hemorrhages, due to defects associated with capillary endothelial cells. Profound morphological abnormalities of the heart, pharyngeal glands and bones of the skull.	Translocation Defect of the D variant haploinsuficinecy	Limb defects. Giant platelet syndrome. Vitroretinal dystrophy.
Fibulin-2	No phenotype. Viable and fertile. No anatomical abnormalities.	Unknown	Unknown.
Fibulin-3 (Efemp1)	Reduced reproductiviy, early aging and herniation.	Missense	Malattia leventinese. Doyne honeycomb retinal dystrophy. Age-related macular degeneration.
Fibulin-4 (Efemp2)	Die perinatally. Lung and vascular defects: emphysema, artery tortuosity, aneurysm, and hemorrhages.	Missense	Neonatal lethal pulmonary artery occlusion, aortic aneurysm, arachnodactyly, and mild cutis laxa.
Fibulin-5 (DANCE, EVEC)	Disorganized elastic-fiber networks, resulting in loose skin, aortic abnormalities, and lung defects.	Missense	Cutis laxa. Age-related macular degeneration.
Fibulin-6 (Hemicentin-1)	Unknown.	Missense (uncertain)	Age-related macular degeneration (uncertain).
Fibulin-7 (TM14)	Unknown.	Unknown	Unknown.

formation in renal glomeruli and a delay in the proper formation of lung alveoli. The endothelial compartments show irregular cell shape, suggesting that fibulin-1 may interact with endothelial cells. More recently, knockout mice for the fibulin-1 gene were created by gene trap insertion [84]. These mice also show bleeding, lung abnormalities and perinatal lethality as reported by Kostka and coworkers [83], but in the gene-trap fibulin-1 null mice there are also abnormalities of the outflow tract, arch arteries, pharyngeal glands, cranial nerves, and cephalic skeleton, which were not previously reported. One of the reasons for these differences is variation in the genetic background [84].

Fibulin-1 knockout *C. elegans* are smaller in overall body size than the wild type and they had severe defects in gonad morphogenesis [85–87]. Matrix metalloproteases, the major family of proteolytic enzymes responsible for degrading ECM components, have a key role in remodeling the ECM during cell migration. In *C. elegans,* two secreted ADAMTS proteases MIG-17 and GON-1 are required for gonad morphogenesis by regulating migration of the distal tip cell (DTC) that guides the developing gonad as it extends [88, 89]. GON-1 is required for DTC migration and general expansion of the gonad rudiment. On the other hand, MIG-17 regulates the direction of DTC migration. Fibulin-1 has been shown to work cooperatively with these ADAMTS proteases in this process [86, 87]. Fibulin-1 and GON-1 have antagonistic roles in gonad formation; GON-1 promotes expansion and elongation, whereas fibulin-1 blocks these processes [87]. It is proposed that proteolysis by MIG-17 recruits fibulin-1 to the gonad basement membrane for guiding DTC migration [86]. However, the molecular mechanisms and signaling pathways in these processes are not clear.

C. elegans fibulin-1C and fibulin-1D isoforms have distinct second EGF and fibulin-like modules as a result of alternative splicing variants [11, 85]. Studies using a transgene approach in rescue experiments and loss-of function analysis have identified the roles of fibulin-1 isoforms and domain functions. Fibulin-1C has specific roles for pharynx, intestine, gonad and muscle morphogenesis and is required to regulate cell shape and adhesion. Fibulin-1D assembles in the flexible polymers that connect the pharynx and body wall muscle. Both isoforms requires fibulin-6 (hemicentin) for assembly at hemidesmosomes-mediated mechanosensory neuron and uterine attachments to the epidermis [90]. In addition, assembly of fibulin-1C at uterine and mechanosensory neuron attachments is depend on perlecan, whereas assembly of fibulin-1D at mechanosensory neuron attachments is dependent on laminin [90]. These results indicate that these isoforms have distinct functional roles in matrix networks. Fibulin-1C regulates both gonadal width and growth [91]. The N-terminal two complete EGF repeats are critical for gonadal growth, whereas both the EGF and fibulin-like domains are required for constraining gonadal width [91]. These studies suggest that these modules of fibulin-1 have distinct functions

in nematode development and tissue functions. Whether mammal fibulins have similar activities of their modules remain to be elucidated.

Fibulin-2 knockout mice. The fibulin-2 knockout mice [92] are viable, fertile, and free of anatomic abnormalities. Fibulin-2 may have a functional redundancy with other matrix proteins and/or play a role in pathological conditions.

Fibulin-3 knockout and knock-in mice. The knockout mice for fibulin-3 [59] show reduced reproduction and display early aging-related phenotypes including reduced lifespan, body mass, hair growth, fat, and muscle as well as organ atrophy. Although the missense mutation R345W in the fibulin-3 gene is a likely cause of ML, an inherited AMD [59], the fibulin-3 knockout mice do not have macular degeneration [49]. However, knock-in mice containing the R345W mutation in fibulin-3 develop early onset of macular degeneration in both heterozygous and homozygous mice [93, 94]. The phenotype and haploinsufficiency of the knock-in mice mimics those of AMD patients. The mutant mice develop deposits of membranous materials between Bruch's membrane and the RPE. This basal deposition increases with age and is considered to be a major cause of macular degeneration. In transfection experiments, the R345W mutant fibulin-3 is misfolded in cells and secreted insufficiently, which causes endoplasmic reticulum (ER) stress and may lead to dysfunction of RPE cells [95]. The ER stress may explain in part extensive vacuolization and loss of basolateral infoldings of RPE cells. The secreted mutant fibulin-3 might cause an altered structure of Bruch's membrane, which may lead to abnormal cellular signaling in RPE cells that induces excess matrix production. Unlike fibulin-3 deficiency, mutant fibulin-3 causes only macular degeneration but no other obvious systemic defects. The function of fibulin-3 in Bruch's membrane is unique to RPE cells, which may be more susceptible to subtle changes in the matrix structure. Missense mutations in fibulin-4 and fibulin-5 have been reported for association with AMD [61, 96]. These mutations may cause AMD by a mechanism similar to that of the fibulin-3 mutation. Although the reason why mutant fibulin-3 leads to the basal deposits is still not clear, the knock-in mice are a useful model for the development of prevention and treatment strategies for AMD.

Fibulin-4 knockout mice. The fibulin-4 knockout mice die in the perinatal period [97]. They exhibit lung and vascular defects such as emphysema, artery tortuosity, irregularity, aneurysm, and hemorrhages. They do not form elastic fibers, indicating a key role of fibulin-4 in vascular homeostasis. Cardiac abnormalities have been further supported by a mouse model of underexpression of fibulin-4 [98] where they generated a fibulin-4 allele with reduced expression by transcriptional interference through the placement of a TKneo targeting construct in a downstream gene (Mus81). The phenotype of these mice resembles connective tissue disorders such as Marfan syndrome.

Fibulin-5 knockout mice. Fibulin-5 knockout mice survive to adulthood and develop elastinopathy with disorganization of elastic fibers, resulting in loose skin, tortuous and extended blood vessels and emphysematous lungs [99, 100], resembling the cutis laxa syndrome in humans. Because fibulin-5 interacts with elastic fibers and with cells through integrins, it may anchor elastic fibers to cells, which may stabilize elastic fibers in the skin, lung and vasculature. In mutant mice, cutaneous blood vessels are increased, and excess vascular sprouting from larger systemic vessels is also observed, suggesting that fibulin-5 not only plays a role in elastic fiber development, but also modulates angiogenesis. In vascular injury induced by carotid artery ligation, fibulin-5 knockout mice show an exaggerated vascular remodeling response including neointima formation and thickened adventitia. Altered extensibility of the vessel wall alone cannot explain these abnormalities. An increase in vascular smooth muscle cell proliferation and migration in the absence of fibulin-5 may, in part, cause the abnormal vascular remodeling as supported by the analysis in cell culture analysis [99, 100]. These results suggest that fibulin-5 may modulate signaling pathways to inhibit vascular smooth muscle cell proliferation and migration.

Fibulin-6 knockout *C. elegans.* *C. elegans* deficient in fibulin-6 (hemicentin-1) display defective cell-cell and cell-matrix interactions [9]. In these nematodes the uterine and intestinal cells do not affix to the body wall. There are also failures in the assembly of hemidesmosomes and intermediate filaments in the epidermis.

Cell adhesion and migration activities of fibulins

Some of the fibulin proteins are involved in cell adhesion and migration. Fibulin-2, fibulin-5, and fibulin-7 bind cells through integrin receptors. Mouse fibulin-2 binds to αIIbβ3 and αvβ3 integrins through the RGD motif in the N-terminal domain [27]. Fibulin-1 has no activity in cell adhesion in the majority of cell lines tested [27, 101]. However, A431

epidermal carcinoma cells moderately adhered to fibulin-1 [101]. Fibulin-1 inhibits cell adhesion to and migration on fibronectin. It was proposed that the binding of fibulin-1 to fibronectin generates a new anti-adhesive site [101].

Fibulin-5 binds to human umbilical vein endothelial cells in an RGD-dependent manner [8]. A bacterially expressed fibulin-5 binds to Chinese-hamster ovary cells expressing recombinant αvβ3, αvβ5 and α9β1 integrins [100]. Fibulin-5 also interacts with primary human smooth muscle cells through α5β1 and α4β1, but the cells show low spreading and low migration and proliferation on fibulin-5 [102]. Proliferation and migration of smooth muscle cells from fibulin-5 knockout mice are enhanced in response to platelet-derived growth factor and inhibited by overexpression of fibulin-5 [103]. Fibronectin promotes attachment, spreading and migration of smooth muscle cells through the same integrins used for fibulin-5. However, fibulin-5 inhibits fibronectin-mediated cellular activities [102]. These results suggest that fibulin-5 modulates fibronectin-mediated cell adhesion and migration. Fibulin-5 is an antagonist of angiogenesis and endothelial cell activities [104]. It abrogates angiogenic sprouting by endothelial cells, and inhibits their proliferation and invasion through Matrigel.

Fibulin-7 has been shown to have cell-type specific adhesion activity. The binding activity is specific for dental mesenchyme cells and odontoblasts. Data obtained from inhibition assays suggest that heparan-sulfate receptors and β1-integrin are responsible for the fibulin-7–cell interaction [10]. When odontoblasts differentiate from dental mesenchyme, the basement membrane separating the dental mesenchyme and epithelium is replaced by dentin. However, the matrix molecules responsible for odontoblasts attachment to predentin are unknown. Since fibulin-7 is localized at the apical pericellular regions of preodontoblasts, fibulin-7 may serve this role.

Conclusions

The fibulin family consists of seven members in mammals but fewer members in *C. elegans,* chicken, and zebrafish. Fibulins interact with ECM molecules, stabilize the supramolecular structures, and in some case bridge cells and ECM structures and play roles in tissue functions. Genetic studies of inherited diseases and animal models have demonstrated the importance of fibulins in multiple developmental and pathogenic processes, such as proper elastogenesis, vascular formation and vision. Fibulins function not only as a structural ECM component but also as a modulator for various cellular processes, such as cell growth, differentiation, angiogenesis, and tumor growth. The molecular mechanisms of fibulin actions in those cellular processes need to be further explored. Many fibulin members are expressed in cartilage, especially in articular cartilage and the perichondrium during development. However, their roles in skeletal development and diseases are still unclear.

Acknowledgement. This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Dental and Craniofacial Research, USA.

1 Argraves, W. S., Dickerson, K., Burgess, W. H. and Ruoslahti, E. (1989) Fibulin, a novel protein that interacts with the fibronectin receptor beta subunit cytoplasmic domain. Cell 58, 623–629
2 Godyna, S., Mann, D. M. and Argraves, W. S. (1995) A quantitative analysis of the incorporation of fibulin-1 into extracellular matrix indicates that fibronectin assembly is required. Matrix Biol. 14, 467–477
3 Kluge, M., Mann, K., Dziadek, M. and Timpl, R. (1990) Characterization of a novel calcium-binding 90-kDa glycoprotein (BM-90) shared by basement membranes and serum. Eur. J. Biochem. 193, 651–659
4 Pan, T. C., Kluge, M., Zhang, R. Z., Mayer, U., Timpl, R. and Chu, M. L. (1993) Sequence of extracellular mouse protein BM-90/fibulin and its calcium-dependent binding to other basement-membrane ligands. Eur. J. Biochem. 215, 733–740
5 Lecka-Czernik, B., Lumpkin, C. K. Jr. and Goldstein, S. (1995) An overexpressed gene transcript in senescent and quiescent human fibroblasts encoding a novel protein in the epidermal growth factor-like repeat family stimulates DNA synthesis. Mol. Cell. Biol. 15, 120–128
6 Giltay, R., Timpl, R. and Kostka, G. (1999) Sequence, recombinant expression and tissue localization of two novel extracellular matrix proteins, fibulin-3 and fibulin-4. Matrix Biol. 18, 469–480
7 Kowal, R. C., Richardson, J. A., Miano, J. M. and Olson, E. N. (1999) EVEC, a novel epidermal growth factor-like repeat-containing protein upregulated in embryonic and diseased adult vasculature. Circ. Res. 84, 1166–1176
8 Nakamura, T., Ruiz-Lozano, P., Lindner, V., Yabe, D., Taniwaki, M., Furukawa, Y., Kobuke, K., Tashiro, K., Lu, Z., Andon, N. L., Schaub, R., Matsumori, A., Sasayama, S., Chien, K. R. and Honjo, T. (1999) DANCE, a novel secreted RGD protein expressed in developing, atherosclerotic, and balloon-injured arteries. J. Biol. Chem. 274, 22476–22483
9 Vogel, B. E. and Hedgecock, E. M. (2001) Hemicentin, a conserved extracellular member of the immunoglobulin superfamily, organizes epithelial and other cell attachments into oriented line-shaped junctions. Development 128, 883–894
10 de Vega, S., Iwamoto, T., Nakamura, T., Hozumi, K., McKnight, D. A., Fisher, L. W., Fukumoto, S. and Yamada, Y. (2007) TM14 is a new member of the fibulin family (fibulin-7) that interacts with extracellular matrix molecules and is active for cell binding. J. Biol. Chem. 282, 30878–30888
11 Barth, J. L., Argraves, K. M., Roark, E. F., Little, C. D. and Argraves, W. S. (1998) Identification of chicken and *C. elegans* fibulin-1 homologs and characterization of the *C. elegans* fibulin-1 gene. Matrix Biol. 17, 635–646
12 Chu, M. L. and Tsuda, T. (2004) Fibulins in development and heritable disease. Birth Defects Res. C Embryo Today 72, 25–36
13 Argraves, W. S., Greene, L. M., Cooley, M. A. and Gallagher, W. M. (2003) Fibulins: Physiological and disease perspectives. EMBO Rep. 4, 1127–1131

14 Timpl, R., Sasaki, T., Kostka, G. and Chu, M. L. (2003) Fibulins: A versatile family of extracellular matrix proteins. Nat. Rev. Mol. Cell Biol. 4, 479–489
15 Argraves, W. S., Tran, H., Burgess, W. H. and Dickerson, K. (1990) Fibulin is an extracellular matrix and plasma glycoprotein with repeated domain structure. J. Cell Biol. 111, 3155–3164
16 Grassel, S., Sicot, F. X., Gotta, S. and Chu, M. L. (1999) Mouse fibulin-2 gene. Complete exon-intron organization and promoter characterization. Eur. J. Biochem. 263, 471–477
17 Gallagher, W. M., Greene, L. M., Ryan, M. P., Sierra, V., Berger, A., Laurent-Puig, P. and Conseiller, E. (2001) Human fibulin-4: Analysis of its biosynthetic processing and mRNA expression in normal and tumour tissues. FEBS Lett. 489, 59–66
18 Kobayashi, N., Kostka, G., Garbe, J. H., Keene, D. R., Bachinger, H. P., Hanisch, F. G., Markova, D., Tsuda, T., Timpl, R., Chu, M. L. and Sasaki, T. (2007) A comparative analysis of the fibulin protein family. Biochemical characterization, binding interactions, and tissue localization. J. Biol. Chem. 282, 11805–11816
19 Zhang, H. Y., Timpl, R., Sasaki, T., Chu, M. L. and Ekblom, P. (1996) Fibulin-1 and fibulin-2 expression during organogenesis in the developing mouse embryo. Dev. Dyn. 205, 348–364
20 Roark, E. F., Keene, D. R., Haudenschild, C. C., Godyna, S., Little, C. D. and Argraves, W. S. (1995) The association of human fibulin-1 with elastic fibers: An immunohistological, ultrastructural, and RNA study. J. Histochem. Cytochem. 43, 401–411
21 Reinhardt, D. P., Sasaki, T., Dzamba, B. J., Keene, D. R., Chu, M. L., Gohring, W., Timpl, R. and Sakai, L. Y. (1996) Fibrillin-1 and fibulin-2 interact and are colocalized in some tissues. J. Biol. Chem. 271, 19489–19496
22 Mao, Y. and Schwarzbauer, J. E. (2005) Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. Matrix Biol. 24, 389–399
23 Larsen, M., Artym, V. V., Green, J. A. and Yamada, K. M. (2006) The matrix reorganized: Extracellular matrix remodeling and integrin signaling. Curr. Opin. Cell Biol. 18, 463–471
24 Sasaki, T., Wiedemann, H., Matzner, M., Chu, M. L. and Timpl, R. (1996) Expression of fibulin-2 by fibroblasts and deposition with fibronectin into a fibrillar matrix. J. Cell Sci. 109, 2895–2904
25 Balbona, K., Tran, H., Godyna, S., Ingham, K. C., Strickland, D. K. and Argraves, W. S. (1992) Fibulin binds to itself and to the carboxyl-terminal heparin-binding region of fibronectin. J. Biol. Chem. 267, 20120–20125
26 Tran, H., VanDusen, W. J. and Argraves, W. S. (1997) The self-association and fibronectin-binding sites of fibulin-1 map to calcium-binding epidermal growth factor-like domains. J. Biol. Chem. 272, 22600–22606
27 Pfaff, M., Sasaki, T., Tangemann, K., Chu, M. L. and Timpl, R. (1995) Integrin-binding and cell-adhesion studies of fibulins reveal a particular affinity for alpha IIb beta 3. Exp. Cell Res. 219, 87–92
28 Miner, J. H. and Yurchenco, P. D. (2004) Laminin functions in tissue morphogenesis. Annu. Rev. Cell Dev. Biol. 20, 255–284
29 Timpl, R., Tisi, D., Talts, J. F., Andac, Z., Sasaki, T. and Hohenester, E. (2000) Structure and function of laminin LG modules. Matrix Biol. 19, 309–317
30 Utani, A., Nomizu, M. and Yamada, Y. (1997) Fibulin-2 binds to the short arms of laminin-5 and laminin-1 *via* conserved amino acid sequences. J. Biol. Chem. 272, 2814–2820
31 Sasaki, T., Gohring, W., Mann, K., Brakebusch, C., Yamada, Y., Fassler, R. and Timpl, R. (2001) Short arm region of laminin-5 gamma2 chain: Structure, mechanism of processing and binding to heparin and proteins. J. Mol. Biol. 314, 751–763
32 Talts, J. F., Andac, Z., Gohring, W., Brancaccio, A. and Timpl, R. (1999) Binding of the G domains of laminin alpha1 and alpha2 chains and perlecan to heparin, sulfatides, alpha-dystroglycan and several extracellular matrix proteins. EMBO J. 18, 863–870
33 Sasaki, T., Kostka, G., Gohring, W., Wiedemann, H., Mann, K., Chu, M. L. and Timpl, R. (1995) Structural characterization of two variants of fibulin-1 that differ in nidogen affinity. J. Mol. Biol. 245, 241–250
34 Sasaki, T., Gohring, W., Pan, T. C., Chu, M. L. and Timpl, R. (1995) Binding of mouse and human fibulin-2 to extracellular matrix ligands. J. Mol. Biol. 254, 892–899
35 Adam, S., Gohring, W., Wiedemann, H., Chu, M. L., Timpl, R. and Kostka, G. (1997) Binding of fibulin-1 to nidogen depends on its C-terminal globular domain and a specific array of calcium-binding epidermal growth factor-like (EG) modules. J. Mol. Biol. 272, 226–236
36 Hopf, M., Gohring, W., Kohfeldt, E., Yamada, Y. and Timpl, R. (1999) Recombinant domain IV of perlecan binds to nidogens, laminin-nidogen complex, fibronectin, fibulin-2 and heparin. Eur. J. Biochem. 259, 917–925
37 Aspberg, A., Adam, S., Kostka, G., Timpl, R. and Heinegard, D. (1999) Fibulin-1 is a ligand for the C-type lectin domains of aggrecan and versican. J. Biol. Chem. 274, 20444–20449
38 Olin, A. I., Morgelin, M., Sasaki, T., Timpl, R., Heinegard, D. and Aspberg, A. (2001) The proteoglycans aggrecan and Versican form networks with fibulin-2 through their lectin domain binding. J. Biol. Chem. 276, 1253–1261
39 Lee, N. V., Rodriguez-Manzaneque, J. C., Thai, S. N., Twal, W. O., Luque, A., Lyons, K. M., Argraves, W. S. and Iruela-Arispe, M. L. (2005) Fibulin-1 acts as a cofactor for the matrix metalloprotease ADAMTS-1. J. Biol. Chem. 280, 34796–34804
40 Ng, K. M., Catalano, M. G., Pinos, T., Selva, D. M., Avvakumov, G. V., Munell, F. and Hammond, G. L. (2006) Evidence that fibulin family members contribute to the steroid-dependent extravascular sequestration of sex hormone-binding globulin. J. Biol. Chem. 281, 15853–15861
41 Tran, H., Tanaka, A., Litvinovich, S. V., Medved, L. V., Haudenschild, C. C. and Argraves, W. S. (1995) The interaction of fibulin-1 with fibrinogen. A potential role in hemostasis and thrombosis. J. Biol. Chem. 270, 19458–19464
42 Godyna, S., Diaz-Ricart, M. and Argraves, W. S. (1996) Fibulin-1 mediates platelet adhesion *via* a bridge of fibrinogen. Blood 88, 2569–2577
43 Perbal, B., Martinerie, C., Sainson, R., Werner, M., He, B. and Roizman, B. (1999) The C-terminal domain of the regulatory protein NOVH is sufficient to promote interaction with fibulin 1C: A clue for a role of NOVH in cell-adhesion signaling. Proc. Natl. Acad. Sci. USA 96, 869–874
44 Brooke, J. S., Cha, J. H. and Eidels, L. (2002) Latent transforming growth factor beta-binding protein-3 and fibulin-1C interact with the extracellular domain of the heparin-binding EGF-like growth factor precursor. BMC Cell Biol. 3, 2
45 Ohsawa, I., Takamura, C. and Kohsaka, S. (2001) Fibulin-1 binds the amino-terminal head of beta-amyloid precursor protein and modulates its physiological function. J. Neurochem. 76, 1411–1420
46 Zhang, H., Gao, X., Weng, C. and Xu, Z. (2008) Interaction between angiogenin and fibulin 1: Evidence and implication. Acta Biochim. Biophys. Sin. (Shanghai) 40, 375–380
47 Fujimoto, N., Terlizzi, J., Brittingham, R., Fertala, A., McGrath, J. A. and Uitto, J. (2005) Extracellular matrix protein 1 interacts with the domain III of fibulin-1C and 1D variants through its central tandem repeat 2. Biochem. Biophys. Res. Commun. 333, 1327–1333
48 Zheng, Q., Davis, E. C., Richardson, J. A., Starcher, B. C., Li, T., Gerard, R. D. and Yanagisawa, H. (2007) Molecular analysis of fibulin-5 function during *de novo* synthesis of elastic fibers. Mol. Cell. Biol. 27, 1083–1095
49 Wachi, H., Nonaka, R., Sato, F., Shibata-Sato, K., Ishida, M., Iketani, S., Maeda, I., Okamoto, K., Urban, Z., Onoue, S. and Seyama, Y. (2008) Characterization of the molecular inter-

action between tropoelastin and DANCE/fibulin-5. J. Biochem. 143, 633–639
50 Liu, X., Zhao, Y., Gao, J., Pawlyk, B., Starcher, B., Spencer, J. A., Yanagisawa, H., Zuo, J. and Li, T. (2004) Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. Nat. Genet. 36, 178–182
51 Hirai, M., Ohbayashi, T., Horiguchi, M., Okawa, K., Hagiwara, A., Chien, K. R., Kita, T. and Nakamura, T. (2007) Fibulin-5/DANCE has an elastogenic organizer activity that is abrogated by proteolytic cleavage *in vivo*. J. Cell Biol. 176, 1061–1071
52 Hirai, M., Horiguchi, M., Ohbayashi, T., Kita, T., Chien, K. R. and Nakamura, T. (2007) Latent TGF-beta-binding protein 2 binds to DANCE/fibulin-5 and regulates elastic fiber assembly. EMBO J. 26, 3283–3295
53 Koski, C., Saharinen, J. and Keski-Oja, J. (1999) Independent promoters regulate the expression of two amino terminally distinct forms of latent transforming growth factor-beta binding protein-1 (LTBP-1) in a cell type-specific manner. J. Biol. Chem. 274, 32619–32630
54 Gibson, M. A., Hatzinikolas, G., Davis, E. C., Baker, E., Sutherland, G. R. and Mecham, R. P. (1995) Bovine latent transforming growth factor beta 1-binding protein 2: Molecular cloning, identification of tissue isoforms, and immunolocalization to elastin-associated microfibrils. Mol. Cell. Biol. 15, 6932–6942
55 Miosge, N., Gotz, W., Sasaki, T., Chu, M. L., Timpl, R. and Herken, R. (1996) The extracellular matrix proteins fibulin-1 and fibulin-2 in the early human embryo. Histochem. J. 28, 109–116
56 Zhang, H. Y., Chu, M. L., Pan, T. C., Sasaki, T., Timpl, R. and Ekblom, P. (1995) Extracellular matrix protein fibulin-2 is expressed in the embryonic endocardial cushion tissue and is a prominent component of valves in adult heart. Dev. Biol. 167, 18–26
57 Tsuda, T., Wang, H., Timpl, R. and Chu, M. L. (2001) Fibulin-2 expression marks transformed mesenchymal cells in developing cardiac valves, aortic arch vessels, and coronary vessels. Dev. Dyn. 222, 89–100
58 Ehlermann, J., Weber, S., Pfisterer, P. and Schorle, H. (2003) Cloning, expression and characterization of the murine Efemp1, a gene mutated in Doyne-Honeycomb retinal dystrophy. Gene Expr. Patterns 3, 441–447
59 McLaughlin, P. J., Bakall, B., Choi, J., Liu, Z., Sasaki, T., Davis, E. C., Marmorstein, A. D. and Marmorstein, L. Y. (2007) Lack of fibulin-3 causes early aging and herniation, but not macular degeneration in mice. Hum. Mol. Genet.16, 3059–3070
60 Xiang, Y., Sekine, T., Nakamura, H., Imajoh-Ohmi, S., Fukuda, H., Yudoh, K., Masuko-Hongo, K., Nishioka, K. and Kato, T. (2006) Fibulin-4 is a target of autoimmunity predominantly in patients with osteoarthritis. J. Immunol. 176, 3196–3204
61 Schultz, D. W., Klein, M. L., Humpert, A. J., Luzier, C. W., Persun, V., Schain, M., Mahan, A., Runckel, C., Cassera, M., Vittal, V., Doyle, T. M., Martin, T. M., Weleber, R. G., Francis, P. J. and Acott, T. S. (2003) Analysis of the ARMD1 locus: Evidence that a mutation in HEMICENTIN-1 is associated with age-related macular degeneration in a large family. Hum. Mol. Genet. 12, 3315–3323
62 Schultz, D. W., Weleber, R. G., Lawrence, G., Barral, S., Majewski, J., Acott, T. S. and Klein, M. L. (2005) HEMICENTIN-1 (FIBULIN-6) and the 1q31 AMD locus in the context of complex disease: Review and perspective. Ophthalmic Genet. 26, 101–105
63 Gallagher, W. M., Currid, C. A. and Whelan, L. C. (2005) Fibulins and cancer: Friend or foe? Trends Mol. Med. 11, 336–340
64 Roger, P., Pujol, P., Lucas, A., Baldet, P. and Rochefort, H. (1998) Increased immunostaining of fibulin-1, an estrogen-regulated protein in the stroma of human ovarian epithelial tumors. Am. J. Pathol. 153, 1579–1588
65 Forti, S., Scanlan, M. J., Invernizzi, A., Castiglioni, F., Pupa, S., Agresti, R., Fontanelli, R., Morelli, D., Old, L. J., Pupa, S. M. and Menard, S. (2002) Identification of breast cancer-restricted antigens by antibody screening of SKBR3 cDNA library using a preselected patient's serum. Breast Cancer Res. Treat. 73, 245–256
66 Greene, L. M., Twal, W. O., Duffy, M. J., McDermott, E. W., Hill, A. D., O'Higgins, N. J., McCann, A. H., Dervan, P. A., Argraves, W. S. and Gallagher, W. M. (2003) Elevated expression and altered processing of fibulin-1 protein in human breast cancer. Br. J. Cancer 88, 871–878
67 Pupa, S. M., Argraves, W. S., Forti, S., Casalini, P., Berno, V., Agresti, R., Aiello, P., Invernizzi, A., Baldassari, P., Twal, W. O., Mortarini, R., Anichini, A. and Menard, S. (2004) Immunological and pathobiological roles of fibulin-1 in breast cancer. Oncogene 23, 2153–2160
68 Qing, J., Maher, V. M., Tran, H., Argraves, W. S., Dunstan, R. W. and McCormick, J. J. (1997) Suppression of anchorage-independent growth and matrigel invasion and delayed tumor formation by elevated expression of fibulin-1D in human fibrosarcoma-derived cell lines. Oncogene 15, 2159–2168
69 Moll, F., Katsaros, D., Lazennec, G., Hellio, N., Roger, P., Giacalone, P. L., Chalbos, D., Maudelonde, T., Rochefort, H. and Pujol, P. (2002) Estrogen induction and overexpression of fibulin-1C mRNA in ovarian cancer cells. Oncogene 21, 1097–1107
70 Schiemann, W. P., Blobe, G. C., Kalume, D. E., Pandey, A. and Lodish, H. F. (2002) Context-specific effects of fibulin-5 (DANCE/EVEC) on cell proliferation, motility, and invasion. Fibulin-5 is induced by transforming growth factor-beta and affects protein kinase cascades. J. Biol. Chem. 277, 27367–27377
71 Debeer, P., Schoenmakers, E. F., Twal, W. O., Argraves, W. S., De Smet, L., Fryns, J. P. and Van De Ven, W. J. (2002) The fibulin-1 gene (FBLN1) is disrupted in a t(12;22) associated with a complex type of synpolydactyly. J. Med. Genet. 39, 98–104
72 Miner, J. H., Cunningham, J. and Sanes, J. R. (1998) Roles for laminin in embryogenesis: Exencephaly, syndactyly, and placentopathy in mice lacking the laminin alpha5 chain. J. Cell Biol. 143, 1713–1723
73 Putnam, E. A., Zhang, H., Ramirez, F. and Milewicz, D. M. (1995) Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. Nat. Genet. 11, 456–458
74 Toren, A., Rozenfeld-Granot, G., Heath, K. E., Amariglio, N., Rocca, B., Crosson, J., Epstein, C. J., Laghi, F., Landolfi, R., Carlsson, L. E., Argraves, S., Bizzaro, N., Moxey-Mims, M., Brok-Simoni, F., Martignetti, J. A., Greinacher, A. and Rechavi, G. (2003) MYH9 spectrum of autosomal-dominant giant platelet syndromes: Unexpected association with fibulin-1 variant-D inactivation. Am. J. Hematol. 74, 254–262
75 Michaelides, M., Jenkins, S. A., Brantley, M. A. Jr., Andrews, R. M., Waseem, N., Luong, V., Gregory-Evans, K., Bhattacharya, S. S., Fitzke, F. W. and Webster, A. R. (2006) Maculopathy due to the R345W substitution in fibulin-3: Distinct clinical features, disease variability, and extent of retinal dysfunction. Invest. Ophthalmol. Vis. Sci. 47, 3085–3097
76 Hucthagowder, V., Sausgruber, N., Kim, K. H., Angle, B., Marmorstein, L. Y. and Urban, Z. (2006) Fibulin-4: A novel gene for an autosomal recessive cutis laxa syndrome. Am. J. Hum. Genet. 78, 1075–1080
77 Loeys, B., Van Maldergem, L., Mortier, G., Coucke, P., Gerniers, S., Naeyaert, J. M. and De Paepe, A. (2002) Homozygosity for a missense mutation in fibulin-5 (FBLN5) results in a severe form of cutis laxa. Hum. Mol. Genet. 11, 2113–2118
78 Claus, S., Fischer, J., Megarbane, H., Megarbane, A., Jobard, F., Debret, R., Peyrol, S., Saker, S., Devillers, M., Sommer, P. and Damour, O. (2008) A p.C217R mutation in fibulin-5 from cutis laxa patients is associated with incomplete extracellular

matrix formation in a skin equivalent model. J. Invest. Dermatol. 128, 1442–1450
79 Hu, Q., Loeys, B. L., Coucke, P. J., De Paepe, A., Mecham, R. P., Choi, J., Davis, E. C. and Urban, Z. (2006) Fibulin-5 mutations: Mechanisms of impaired elastic fiber formation in recessive cutis laxa. Hum. Mol. Genet. 15, 3379–3386
80 Lotery, A. J., Baas, D., Ridley, C., Jones, R. P., Klaver, C. C., Stone, E., Nakamura, T., Luff, A., Griffiths, H., Wang, T., Bergen, A. A. and Trump, D. (2006) Reduced secretion of fibulin 5 in age-related macular degeneration and cutis laxa. Hum. Mutat. 27, 568–574
81 Markova, D., Zou, Y., Ringpfeil, F., Sasaki, T., Kostka, G., Timpl, R., Uitto, J. and Chu, M. L. (2003) Genetic heterogeneity of cutis laxa: A heterozygous tandem duplication within the fibulin-5 (FBLN5) gene. Am. J. Hum. Genet. 72, 998–1004
82 Fisher, S. A., Rivera, A., Fritsche, L. G., Keilhauer, C. N., Lichtner, P., Meitinger, T., Rudolph, G. and Weber, B. H. (2007) Case-control genetic association study of fibulin-6 (FBLN6 or HMCN1) variants in age-related macular degeneration (AMD). Hum. Mutat. 28, 406–413
83 Kostka, G., Giltay, R., Bloch, W., Addicks, K., Timpl, R., Fassler, R. and Chu, M. L. (2001) Perinatal lethality and endothelial cell abnormalities in several vessel compartments of fibulin-1-deficient mice. Mol. Cell. Biol. 21, 7025–7034
84 Cooley, M. A., Kern, C. B., Fresco, V. M., Wessels, A., Thompson, R. P., McQuinn, T. C., Twal, W. O., Mjaatvedt, C. H., Drake, C. J. and Argraves, W. S. (2008) Fibulin-1 is required for morphogenesis of neural crest-derived structures. Dev. Biol. 319, 336–345
85 Muriel, J. M., Dong, C., Hutter, H. and Vogel, B. E. (2005) Fibulin-1C and Fibulin-1D splice variants have distinct functions and assemble in a hemicentin-dependent manner. Development 132, 4223–4234
86 Kubota, Y., Kuroki, R. and Nishiwaki, K. (2004) A fibulin-1 homolog interacts with an ADAM protease that controls cell migration in *C. elegans.* Curr. Biol.14, 2011–2018
87 Hesselson, D., Newman, C., Kim, K. W. and Kimble, J. (2004) GON-1 and fibulin have antagonistic roles in control of organ shape. Curr. Biol. 14, 2005–2010
88 Nishiwaki, K., Hisamoto, N. and Matsumoto, K. (2000) A metalloprotease disintegrin that controls cell migration in *Caenorhabditis elegans.* Science 288, 2205–2208
89 Blelloch, R. and Kimble, J. (1999) Control of organ shape by a secreted metalloprotease in the nematode *Caenorhabditis elegans.* Nature 399, 586–590
90 Muriel, J. M., Xu, X., Kramer, J. M. and Vogel, B. E. (2006) Selective assembly of fibulin-1 splice variants reveals distinct extracellular matrix networks and novel functions for perlecan/UNC-52 splice variants. Dev. Dyn. 235, 2632–2640
91 Hesselson, D. and Kimble, J. (2006) Growth control by EGF repeats of the *C. elegans* Fibulin-1C isoform. J. Cell Biol. 175, 217–223
92 Sicot, F. X., Tsuda, T., Markova, D., Klement, J. F., Arita, M., Zhang, R. Z., Pan, T. C., Mecham, R. P., Birk, D. E. and Chu, M. L. (2008) Fibulin-2 is dispensable for mouse development and elastic fiber formation. Mol. Cell. Biol. 28, 1061–1067
93 Marmorstein, L. Y., McLaughlin, P. J., Peachey, N. S., Sasaki, T. and Marmorstein, A. D. (2007) Formation and progression of sub-retinal pigment epithelium deposits in Efemp1 mutation knock-in mice: A model for the early pathogenic course of macular degeneration. Hum. Mol. Genet. 16, 2423–2432
94 Fu, L., Garland, D., Yang, Z., Shukla, D., Rajendran, A., Pearson, E., Stone, E. M., Zhang, K. and Pierce, E. A. (2007) The R345W mutation in EFEMP1 is pathogenic and causes AMD-like deposits in mice. Hum. Mol. Genet. 16, 2411–2422
95 Roybal, C. N., Marmorstein, L. Y., Vander Jagt, D. L. and Abcouwer, S. F. (2005) Aberrant accumulation of fibulin-3 in the endoplasmic reticulum leads to activation of the unfolded protein response and VEGF expression. Invest. Ophthalmol. Vis. Sci. 46, 3973–3979
96 Stone, E. M., Braun, T. A., Russell, S. R., Kuehn, M. H., Lotery, A. J., Moore, P. A., Eastman, C. G., Casavant, T. L. and Sheffield, V. C. (2004) Missense variations in the fibulin 5 gene and age-related macular degeneration. N. Engl. J. Med. 351, 346–353
97 McLaughlin, P. J., Chen, Q., Horiguchi, M., Starcher, B. C., Stanton, J. B., Broekelmann, T. J., Marmorstein, A. D., McKay, B., Mecham, R., Nakamura, T. and Marmorstein, L. Y. (2006) Targeted disruption of fibulin-4 abolishes elastogenesis and causes perinatal lethality in mice. Mol. Cell. Biol. 26, 1700–1709
98 Hanada, K., Vermeij, M., Garinis, G. A., de Waard, M. C., Kunen, M. G., Myers, L., Maas, A., Duncker, D. J., Meijers, C., Dietz, H. C., Kanaar, R. and Essers, J. (2007) Perturbations of vascular homeostasis and aortic valve abnormalities in fibulin-4 deficient mice. Circ. Res. 100, 738–746
99 Yanagisawa, H., Davis, E. C., Starcher, B. C., Ouchi, T., Yanagisawa, M., Richardson, J. A. and Olson, E. N. (2002) Fibulin-5 is an elastin-binding protein essential for elastic fibre development *in vivo.* Nature 415, 168–171
100 Nakamura, T., Lozano, P. R., Ikeda, Y., Iwanaga, Y., Hinek, A., Minamisawa, S., Cheng, C. F., Kobuke, K., Dalton, N., Takada, Y., Tashiro, K., Ross Jr, J., Honjo, T. and Chien, K. R. (2002) Fibulin-5/DANCE is essential for elastogenesis *in vivo.* Nature 415, 171–175
101 Twal, W. O., Czirok, A., Hegedus, B., Knaak, C., Chintalapudi, M. R., Okagawa, H., Sugi, Y. and Argraves, W. S. (2001) Fibulin-1 suppression of fibronectin-regulated cell adhesion and motility. J. Cell Sci. 114, 4587–4598
102 Lomas, A. C., Mellody, K. T., Freeman, L. J., Bax, D. V., Shuttleworth, C. A. and Kielty, C. M. (2007) Fibulin-5 binds human smooth-muscle cells through alpha5beta1 and alpha4-beta1 integrins, but does not support receptor activation. Biochem. J. 405, 417–428
103 Spencer, J. A., Hacker, S. L., Davis, E. C., Mecham, R. P., Knutsen, R. H., Li, D. Y., Gerard, R. D., Richardson, J. A., Olson, E. N. and Yanagisawa, H. (2005) Altered vascular remodeling in fibulin-5-deficient mice reveals a role of fibulin-5 in smooth muscle cell proliferation and migration. Proc. Natl. Acad. Sci. USA 102, 2946–2951
104 Albig, A. R. and Schiemann, W. P. (2004) Fibulin-5 antagonizes vascular endothelial growth factor (VEGF) signaling and angiogenic sprouting by endothelial cells. DNA Cell Biol. 23, 367–379