

Fibulins: physiological and disease perspectives

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The fibulins are a family of proteins that are associated with basement membranes and elastic extracellular matrix fibres. This review summarizes findings from studies of animal models of fibulin deficiency, human fibulin gene mutations, human tumours and injury models that have advanced our understanding of the normal and pathological roles of members of this formerly obscure family.

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Introduction

In little more than a decade since the discovery of the first fibulin (Argraves *et al.*, 1989), a six-member family of extracellular-matrix (ECM) proteins has emerged (Table 1; Fig. 1). The fibulins are minimally defined as having a series of epidermal growth factor (EGF)-like modules, followed by a carboxy-terminal fibulin-type module (Fig. 2). It is evident that the fibulins are an ancient family of proteins, which are highly conserved in species as evolutionarily distant as worms and humans. Fibulins have a diverse array of protein ligands (Timpl *et al.*, 2003; and see supplementary information online). As a consequence of these widespread interactions, fibulins are hypothesized to function as intramolecular bridges that stabilize the organization of supramolecular ECM structures, such as elastic fibres and basement membranes. Indeed, the family name originates from the Latin word *fibula*, which means clasp or buckle. The biophysical features of the fibulins have been well described in a recent article (Timpl *et al.*, 2003). Here, we place into perspective findings from many types of studies, including DNA microarray and gene-targeting experiments, that collectively provide new insights into the functions of the fibulins under physiological and pathological conditions.

Fibulins in elastic fibre biology

There is substantial evidence that implicates the fibulins in both elastic matrix fibre assembly and function. Fibulins 1, 2 and 5 all bind to tropoelastin (Nakamura *et al.*, 2002; Sasaki *et al.*, 1999; Yanagisawa *et al.*, 2002). Early in development, fibulin 1 is associated with ECM

fibres that contain both elastin and the microfibril-associated proteins fibrillins 1 and 2 (Visconti *et al.*, 2003). The expression of fibulin 1 during murine lung development is coordinately expressed with tropoelastin and another elastin-associated microfibril protein, latent TGF- β -binding protein 2 (LTBP2; Mariani *et al.*, 2002). Fibulin 1 also colocalizes with elastin in the core of mature elastin-containing fibres in skin and blood vessels (Roark *et al.*, 1995). Unlike fibulin 1, fibulin 2 is found at the interface between microfibrils and the elastin core (Reinhardt *et al.*, 1996). The ability of fibulin 2 to bind elastin and fibrillin 1 may indicate that it anchors fibrillin-containing microfibrils to elastin fibres. Fibulin 5 binding to both integrins and elastin implicates it as a connector of elastin fibres to cells (Nakamura *et al.*, 1999). The interaction of these fibres with the cell surface might be an integral part of elastic fibre assembly, as for other ECM fibres, such as fibronectin (FN). In support of this, mice deficient in fibulin 5 have defective assemblages of elastic fibres (Nakamura *et al.*, 2002; Yanagisawa *et al.*, 2002).

Fibulins in cardiovascular biology

Fibulins 1 and 2 are highly expressed during cardiac valvuloseptal formation. Both are produced by migratory cardiac mesenchymal cells that have transdifferentiated from endocardial cells (Bouchev *et al.*, 1996; Tsuda *et al.*, 2001; Zhang *et al.*, 1995). In developing and adult heart valves, fibulins 1 and 2 are prominently expressed and fibulin 4 is moderately expressed (Giltay *et al.*, 1999; Zhang *et al.*, 1995). Relatively little fibulin 3 is found in adult heart valves (Giltay *et al.*, 1999). The fact that fibulin 1 deficiency does not result

Table 1 | Fibulin family nomenclature

| Name | Synonymous names | Gene symbol | Human chromosome location | References |
|-----------|--------------------------|---------------|---------------------------|---|
| Fibulin 1 | BM90 | <i>FBLN1</i> | 22q13.31 | (Argraves <i>et al.</i> , 1990; Kluge <i>et al.</i> , 1990) |
| Fibulin 2 | – | <i>FBLN2</i> | 3p24–p25 | (Pan <i>et al.</i> , 1993) |
| Fibulin 3 | S15, T16, EFEMP1 | <i>FBNL</i> | 2p16 | (Tran <i>et al.</i> , 1997) |
| Fibulin 4 | MBP1, EFEMP2, UPH1, H411 | <i>EFEMP2</i> | 11q13 | (Gallagher <i>et al.</i> , 1999; Giltay <i>et al.</i> , 1999) |
| Fibulin 5 | DANCE, EVEC, UP50 | <i>FBLN5</i> | 14q32.1 | (Kowal <i>et al.</i> , 1999; Nakamura <i>et al.</i> , 1999) |
| Fibulin 6 | Hemicentin, him4 | <i>FBLN6</i> | 1q25.3 | (Vogel & Hedgecock, 2001) |

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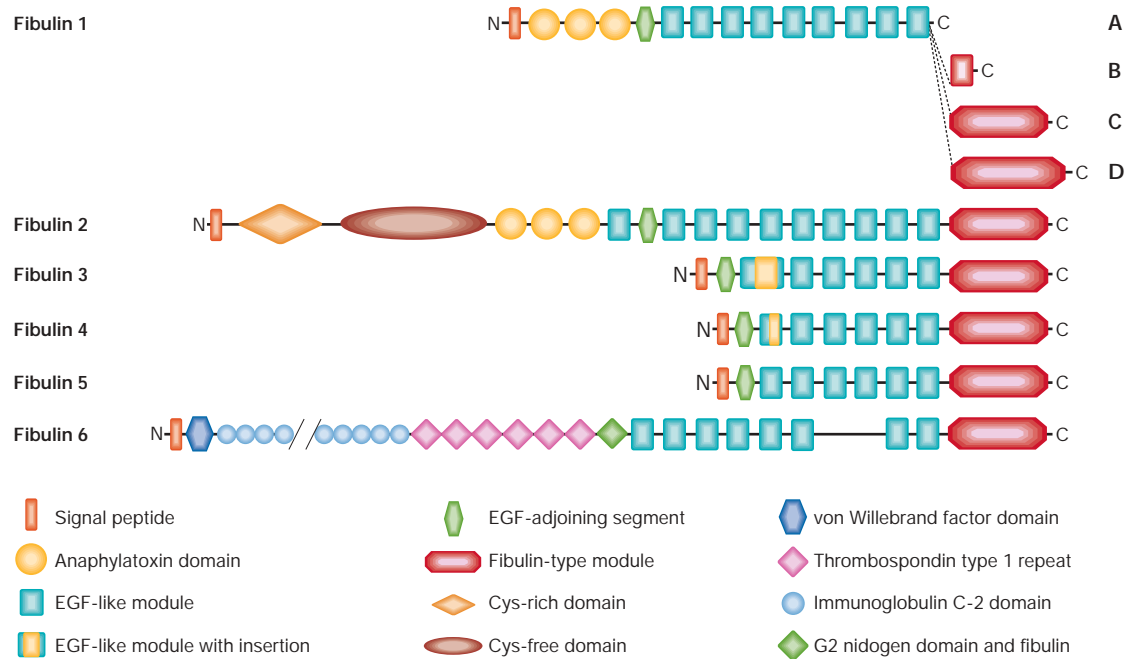


Fig 1 | Modular structures of the fibulins. The most recent addition to the family, fibulin 6 (hemicentin), was originally identified in the nematode (Vogel & Hedgecock, 2001), with orthologues in other species (human, mouse and rat) having now been identified. Nine of the 48 immunoglobulin domains in fibulin 6 are shown (double slashes indicate where the omitted domains occur). Alternative splice variants are known for fibulins 1–4, albeit only variants for fibulin 1 (designated A–D) are displayed.

in overt valvuloseptal defects (Kostka *et al.*, 2001) could indicate compensation by other fibulins.

Fibulins are prominently expressed in blood vessels. During development, fibulin 1 is expressed by the primordial vascular smooth muscle cells (VSMCs) that associate with the ventral endothelium of the dorsal aortae (Hungerford *et al.*, 1997). Primordial VSMCs of the developing aortic-arch vessels also synthesize fibulin 2. In addition, fibulin 2 is expressed by coronary endothelial cells (ECs) that originate from epicardial cells, but it is not expressed by capillary ECs (Tsuda *et al.*, 2001). In adult blood vessels, pronounced fibulin 1 deposition occurs in the matrix that surrounds VSMCs and in the elastic laminae of arteries (Roark *et al.*, 1995). Fibulin 3 expression is prominent in some capillaries, but not in large blood vessels (Giltay *et al.*, 1999). Fibulin 3 is highly expressed in human umbilical vein ECs (on the basis of a GeneAtlas analysis; <http://expression.gnf.org>), but its expression is repressed during *in vitro* human capillary tube formation (Bell *et al.*, 2001). Fibulin 4 is found in the medial layers of large veins and arteries and in some small capillaries (Giltay *et al.*, 1999). Fibulin 5 seems to be restricted to the arterial vasculature and is expressed predominantly by VSMCs of developing arteries and at low levels by VSMCs of adult blood vessels (Kowal *et al.*, 1999). ECs also express fibulin 5, especially the pulmonary artery endothelium (Jean *et al.*, 2002; Kowal *et al.*, 1999). Fibulin 1 is not generally expressed in ECs (Roark *et al.*, 1995).

Knockout animal models and heritable diseases in humans

The importance of the fibulins in development and disease has been highlighted by gene-targeting experiments in animal models and the identification of spontaneous mutations in humans. Fibulin 1 deficiency in mice causes extensive haemorrhaging and

perinatal death (Kostka *et al.*, 2001). The bleeding observed in this case was not due to defective coagulation, but rather to abnormal EC morphology that included hypertrophy, peculiar apical processes and increased intracellular vacuoles (Kostka *et al.*, 2001). In humans, a type of synpolydactyly (congenital malformation of the hand) involves a chromosomal translocation between the fibulin 1 gene and *C12orf2* (Debeer *et al.*, 2002). A haploinsufficiency in the level of fibulin 1D is hypothesized to account for the limb abnormalities seen (Debeer *et al.*, 2002). In this regard, mutation of fibrillin 2, a fibulin 1-associated protein (Visconti *et al.*, 2003), also leads to syndactyly (Chaudhry *et al.*, 2001).

Several recent findings indicate the involvement of fibulins in inherited eye disorders. Fibulins 1 and 4 are candidate genes for retinopathies that map to chromosomes 22 and 11, respectively (Weigell-Weber *et al.*, 2003). A mutation (Arg345Trp) in the fibulin 3 gene has been linked to Malattia Leventinese (ML), a macular dystrophy (Stone *et al.*, 1999). During the development of this disease, as well as in age-related macular degeneration (AMD), an amorphous material known as drusen accumulates between the retinal-pigment epithelium (RPE) and the Bruch's membrane. In ML, fibulin 3 is not found in the drusen, but accumulates within cells of the RPE (Marmorstein *et al.*, 2002). In AMD, which has no associated fibulin 3 mutation, fibulin 3 nonetheless accumulates between the RPE and the drusen (Marmorstein *et al.*, 2002). The expression of fibulins 3 and 1 are also elevated in a murine retinopathy model that primarily involves degeneration of rod photoreceptors (Kennan *et al.*, 2002). Ectopic expression of fibulin 1 also disrupts *Xenopus* eye morphogenesis (Grammer *et al.*, 2000).

Knockout experiments emphasize the essential role that fibulin 5 has in elastic fibre assembly. Mice deficient in the expression of fibulin 5, an elastin-binding protein, are viable but show symptoms of

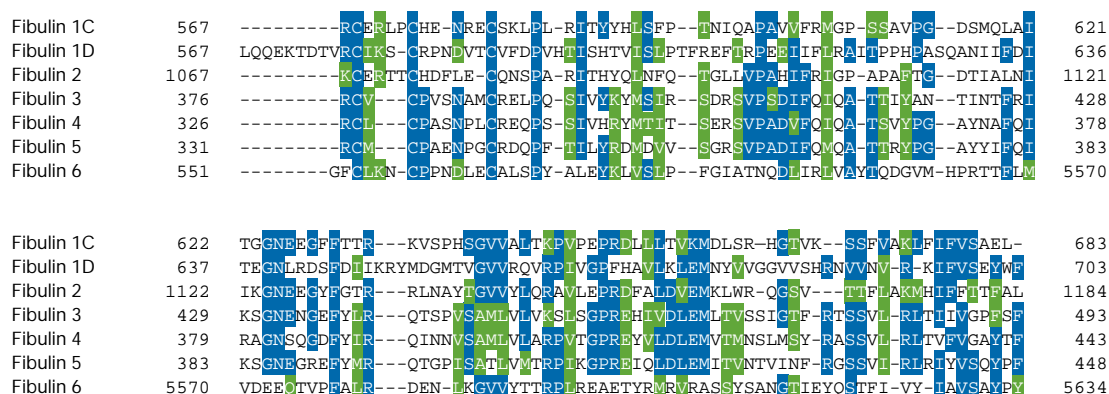


Fig. 2 | Alignment of fibulin-type module sequences from the human fibulins. The carboxy-terminal regions of the fibulins were aligned using ClustalW 1.82, and Boxshade 3.21 was used to highlight conserved amino acids. Identical residues are indicated in the blue background and chemically similar residues with green shading. The GenBank accession numbers for the sequences depicted are as follows: fibulin 1C, CAA37772.1; fibulin 1D, AAB17099.1; fibulin 2, CAA57876.1; fibulin 3, NP_004096.2; fibulin 4, CAA10791.2; fibulin 5, NP_006320.2; fibulin 6, NP_114141.1.

defective elastic fibre formation, including a tortuous aorta, severe emphysema and loose skin (*cutis laxa*; Nakamura *et al.*, 2002; Yanagisawa *et al.*, 2002). In humans, homozygosity for a missense mutation in fibulin 5 is also associated with a severe form of *cutis laxa* (Loeys *et al.*, 2002) and a scarcity of elastic fibres.

Nematodes deficient in hemicentin (a homologue of fibulin 6) display defective cell–cell and cell–matrix interactions (Vogel & Hedgecock, 2001). Uterine and intestinal cells fail to affix stably to the body wall, and cells of the vas deferens fail to join the cloaca. There is also a failure in the assembly of hemidesmosomes and intermediate filaments in the epidermis.

Fibulins and cancer

Human fibrosarcoma tumour cell lines show a trend towards a reduction or absence of fibulin 1D expression (Qing *et al.*, 1997). Fibrosarcoma cells that express fibulin 1D show reduced growth *in vivo*, as well as a lowered growth capacity in soft agar and a reduced ability to invade reconstituted basement membranes (Qing *et al.*, 1997). Similarly, the ectopic expression of fibulin 1D inhibits the motility of breast carcinoma cells on FN (Twal *et al.*, 2001). The motility suppressive effects of fibulin 1D are attributed to a reduction in the cell adhesion and migration-promoting activity of FN (Twal *et al.*, 2001). Ectopic expression of fibulin 1D also inhibits transformation by the papillomavirus E6 gene (Du *et al.*, 2002). The mechanism by which fibulin 1D regulates E6-mediated oncogenic activities might relate to the fact that these two proteins interact (Du *et al.*, 2002). These findings support the conclusion that fibulin 1D acts as a tumour suppressor.

Elevated expression of fibulin 1 is associated with human breast tumours (Forti *et al.*, 2002; Greene *et al.*, 2003). Also suggestive of fibulin 1 overexpression in breast carcinoma is the fact that breast-cancer patients produce antibodies against fibulin 1 (Forti *et al.*, 2002). These observations seem paradoxical in light of the evidence that fibulin 1D is a tumour suppressor. An explanation may come from findings that there is a trend towards increased expression of fibulin 1C compared with the D variant in ovarian carcinomas (Moll *et al.*, 2002). Levels of fibulin 1 splice variants have not been quantified in breast cancer but if fibulin 1C levels are elevated in breast tumours as in ovarian tumours, it would suggest that fibulin 1C

opposes the action of fibulin 1D and promotes tumorigenesis. It is also possible that humoral immunity to fibulin 1 in breast cancer reflects the breakdown of fibulin 1D and concomitant loss of tumour suppression. In support of this, increased levels of fibulin 1 fragments have been reported in human breast tumours (Greene *et al.*, 2003). Furthermore, findings from DNA microarray studies of lung adenocarcinomas show that fibulins 1 and 2 are consistently associated with matrix metalloproteinase 2 expression, a protein that promotes tumour invasion and metastasis (Creighton & Hanash, 2003).

Fibulin 2 has been identified as one of 64 overexpressed metastasis-associated genes in solid tumours of diverse types (Ramaswamy *et al.*, 2003). Fibulin 4 expression is elevated in human colon tumours (Gallagher *et al.*, 2001), whereas cancers in other tissues tend to show downregulation of fibulin 5 (Schiemann *et al.*, 2002). In contrast to the motility suppressive effects of fibulin 1D on fibrosarcoma cells, overexpression of fibulin 5 increases fibrosarcoma cell migration (Schiemann *et al.*, 2002).

Fibulins in injury

The expression of several of the fibulins is induced in response to injury. Fibulin 1 expression is increased in a murine model of cardiomyopathy that is caused by increased G_i-receptor signalling (Redfern *et al.*, 2000). In sun-damaged skin elastosis, fibulin 2 deposition in association with elastic fibres is greatly increased (Hunzelmann *et al.*, 2001). Fibulin 2 expression is also increased in the early phase of streptozotocin-induced diabetic glomerulosclerosis (Wada *et al.*, 2001). In elastase-induced emphysema in mice, fibulin 5 expression is increased in the alveolar wall (Kuang *et al.*, 2003). The expression of fibulin 5, which is low in adult arteries, is activated in medial and neointimal VSMCs in response to vascular injury (Kowal *et al.*, 1999), as well as in lung vasculature in response to hyperoxia (Jean *et al.*, 2002) and in atherosclerotic plaques (Kowal *et al.*, 1999). Transforming growth factor- β (TGF- β), which has a key role in vascular injury response, stimulates fibulin 5 expression (Schiemann *et al.*, 2002). Overexpression of fibulin 5 enhances basal and TGF- β -mediated activation of p38 mitogen-activated protein kinase and ERK1/ERK2 (Schiemann *et al.*, 2002). A similar profile of protein kinase activation has also been observed in response to fibulin 1 stimuli (Twal *et al.*, 2001). Overexpression of

fibulins 5 and 3 increase fibroblast DNA synthesis (Lecka-Czernik *et al.*, 1996; Schiemann *et al.*, 2002). Overexpression of fibulin 4 in macrophages also promotes DNA synthesis (Heine *et al.*, 1999), and fibulin 4 expression is augmented in macrophages by lipopolysaccharide treatment, which suggests a role in response to sepsis (Heine *et al.*, 1999).

A recent study has shown that patients with unstable angina pectoris and acute myocardial infarction have significantly reduced levels of plasma fibulin 1 (Kawata *et al.*, 2001). This has led to speculation that plasma fibulin 1 may be transferred to or consumed in or around the atherosclerotic lesion. Indeed, fibulin 1 is incorporated into fibrin clots that are associated with atherosclerotic lesions (Tran *et al.*, 1995). The significance of fibulin 1 in the development of atherosclerosis is not yet known, but plasma fibulin 1 could be important as a risk factor for cardiovascular diseases and atherosclerosis progression.

Regulation of fibulin expression

Information is gradually emerging concerning the mechanisms that regulate the expression of the fibulins during development or disease. Evidence indicates that steroids regulate the expression of fibulins 1, 2 and 3. Oestradiol stimulates fibulin 1C expression in ovarian tumour cells (Clinton *et al.*, 1996; Hayashido *et al.*, 1998), and dexamethasone increases fibulin 1C expression in human eye trabecular meshwork cells (Ishibashi *et al.*, 2002). Progesterone has been shown to stimulate the expression of fibulins 1 and 2 in human endometrial stromal cells (Okada *et al.*, 2003). In a mouse Wilms' tumour model, the expression of fibulin 2, but not fibulin 1, is increased by dexamethasone (Talts *et al.*, 1995). Glucocorticoids downregulate the expression of fibulins 1 and 2 in bone marrow stroma (Gu *et al.*, 2001) and oestrogen represses the expression of fibulin 3 in MCF7 breast cancer cells (Hayashi *et al.*, 2003).

Sp transcription factors are important in fibulin 1 expression (Castoldi & Chu, 2002; Grassel *et al.*, 1999). Fibulin 1 transcription is activated by the ubiquitous Sp1 and Sp3, but not by the more tissue-restricted Sp4 (Castoldi & Chu, 2002). Fibulin 2 may be similarly regulated (Grassel *et al.*, 1999). The fibulin 2 gene also contains two consensus cAMP-negative response elements. Interaction of cAMP-activated liver X receptor- α with these enhancer elements results in increased fibulin 2 expression (Anderson *et al.*, 2003).

Future directions

The studies cited herein implicate the fibulins in an array of physiological and pathological processes and open many new avenues for investigation. For example, the finding that mutation of the fibulin 5 gene is linked to *cutis laxa* highlights the possibility that mutations in genes for the other family members might contribute to disorders that involve elastic fibres. Given the circumstantial evidence that fibulin 1C and D variants might have opposing effects on tumorigenesis, studies that directly test this possibility and evaluate the expression of fibulin 1 variants in human tumours are warranted. The finding that plasma fibulin 1 levels are reduced in coronary heart disease patients raises questions as to the molecular basis for this and whether this protein is a useful diagnostic serum marker. Just as our understanding of the function of fibulins 1 and 5 has benefited from the study of mice that are genetically deficient in the expression of these proteins, similar benefit can be expected from the creation and study of mice that are deficient in the other fibulin family members.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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