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REVIEWS

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Diverse Functions of Fibulin-5 in Tumor[1]

J.-C. Tang, A.-Y. Xie, and X.-J. Cai*

Key Laboratory of Surgery of Zhejiang Province, Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou, 310016 P. R. China;

**e-mail: cxjzu@hotmail.com*

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Abstract—Altered interactions between the extracellular matrix and cells play an important part in tumorigenesis and metastasis. As a member of a matricellular glycoprotein, fibulin-5 is expressed in elastin-rich tissues and organizes the matrix structures by interacting with many extracellular proteins. Fibulin-5 expression is closely associated with normal embryonic development and organogenesis. Mice deficient for the fibulin-5 gene exhibit systemic elastic fiber defects with manifestation of loose skin, emphysematous lungs and tortuous vessels. Additionally, fibulin-5 null mice exhibited increased angiogenesis after wound healing or PVA sponge implantation and matrigel implantation experiments show that fibulin-5 inhibits vessel formation, suggesting its function as an angiogenesis inhibitor. Fibulin-5 also plays critical roles in proliferation, migration and invasion of certain tumors, and the effect of fibulin-5 on tumorigenesis appears to be largely context-dependent. This effect might involve the inhibiting action of fibulin-5 on angiogenesis. This review focuses on recent advances in our understanding of the roles of fibulin-5 in tumorigenesis: both tumor promoting and suppressing activities of fibulin-5 are reviewed, and the emerging evidence of its promising potential for therapeutic options and/or targets in the treatment of cancer is also highlighted.

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The extracellular matrix (ECM) is composed of structural proteins, glycosaminoglycans, growth factors, cytokines, and glycoproteins (also called matricellular proteins). It provides structural support and directly affects cell behavior, including adhesion, migration, proliferation and survival [1]. Moreover, matricellular proteins participate in matrix-cell interactions and exert regulatory roles via a variety of molecular mechanisms [2]. Although an increasing number of ECM proteins have emerged as matricellular proteins, it is still difficult to accurately draw a line between structural proteins and matricellular proteins. The fibulin family of ECM proteins is essential for the formation of elastic fibers and basement membranes, and plays diverse cellular and biological functions both in matrix and neighboring cells [3], which are supposedly included as part of the matricellular family.

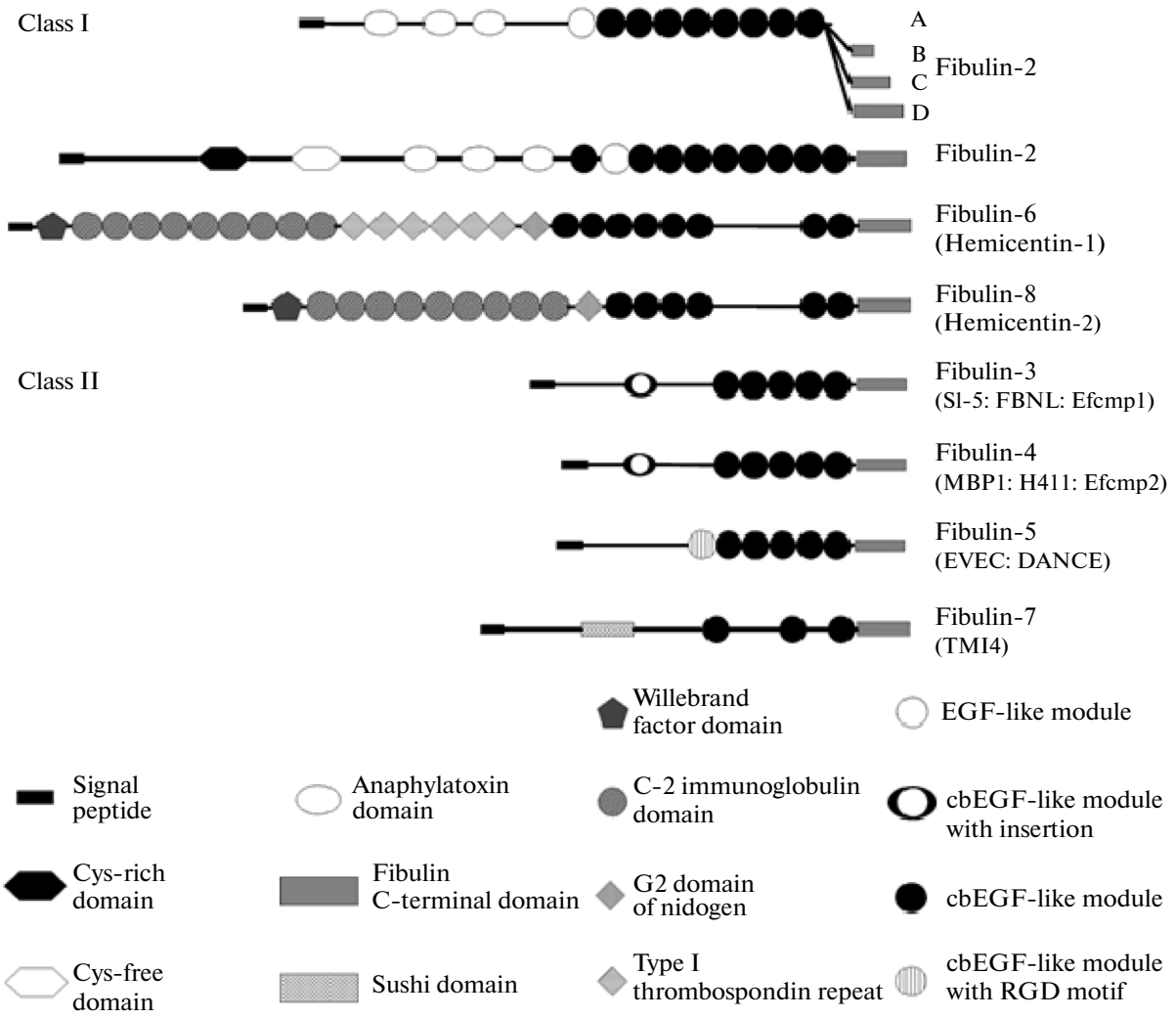
Fibulin-5 (FBLN-5; also known as DANCE or EVEC) is a member of Class II short fibulins and has molecular weight of 66Kda. This matricellular protein, identified by two research groups in an attempt to isolate a novel regulator of vascular growth, is expressed in elastin-rich tissues, such as aorta, kidney, lung, uterus, adult heart, ovary and colon [4, 5], and functions as a mediator of cell-cell and cell-matrix communications. Moreover, FBLN-5 is an endogenous angiogenesis inhibitor and is associated with the development of cutis laxa (a genetically heritable disorder) or age-related macular degeneration. Although the roles of FBLN-5 in tumorigenesis remain to be fully elucidated, recent research has revealed that FBLN-5 regulates cancer cell proliferation, migration and invasion in a cell- and context-specific manner. *FBLN-5* has reduced expression in certain metastatic human tumors such as lung cancer and prostate cancer [6, 7], but is overexpressed in fibrosarcoma and nasopharyngeal carcinoma [8, 9]. Here, the role of FBLN-5 in regulating cancer cell activities is summarized, and the potential use of FBLN-5 as a target to prevent the growth and metastasis of human malignancies is discussed.

[1] The article is published in the original.

Abbreviations: ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transitions; ERK, extracellular signal-regulated kinase; LTBP-2, latent TGF-β-binding protein 2; LOXL-1, lysyl oxidase-like enzyme 1; MAPK, mitogen-activated protein kinases; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; RGD, arginine-glycine-aspartic acid; ROS, reactive oxygen species; TGF-β, transforming growth factor β; TSP-1, thrombospondin 1; VEGF, vascular endothelial growth factor.

FBLN-5 STRUCTURE AND MOLECULAR INTERACTIONS

FBLN-5, a 448 amino acid protein, belongs to the fibulin family defined by the presence of two structural modules, namely EGF-like domain repeats and a unique C-terminal fibulin-type module [3] (figure).



Structural features of the fibulin family. The fibulin family currently has eight members characterized by a tandem array of EGF-like module repeats and the Fibulin-type C-terminal domain. Class I fibulins include long fibulins (fibulin-1, -2, -6 and -8) and Class II fibulins include short fibulins (fibulin-3, -4, -5, and -7). Four different alternative splice variants of the human fibulin-1 are designated -1A to -1D as shown.

FBLN-5 interacts physically with a considerable number of ECM and secreted proteins through the top domains. However, unlike the other fibulins, FBLN-5 contains an integrin-binding RGD motif, which binds certain integrins and mediates cell adhesion [5] (Table 1).

The RGD motif is relatively conserved in chicken, mouse and rat FBLN-5 proteins [10], suggesting that it may play an important role in the biological functions of FBLN-5. FBLN-5 supports the attachment of human umbilical vein cells [5] and inhibits endothelial cell sprouting and invasion[11] in an RGD-dependent manner. The N-terminal half of FBLN-5 mediates cell attachment via $\alpha v\beta 3$, $\alpha v\beta 5$ and $\alpha 9\beta 1$ integrins [12], and binds primary aortic smooth-muscle cells through fibronectin receptors $\alpha 5\beta 1$ and $\alpha 4\beta 1$, but not to $\alpha v\beta 3$ [13]. Furthermore, direct protein interaction assays have revealed that FBLN-5 is only able to bind to $\alpha v\beta 3$ after reduction and alkylation, which unmask the RGD sequence [14]. In addition to the RGD motif, the N-terminus is necessary for FBLN-5 to maintain its elastogenic organizer activity by interacting with lysyl oxidase-like enzyme 1 (LOXL-1) [15], and to select suitable microfibrils to form elastic fibers by interacting with latent TGF-β-binding protein 2 (LTBP-2) [16]. All together, these findings reveal that changes in the FBLN-5 structure are directly associated with its function in certain physiological or pathological conditions.

FBLN-5 AND EPITHELIAL-MESENCHYMAL TRANSITIONS

Epithelial cells normally undergo epithelial-mesenchymal transitions (EMT) as a necessary process during numerous developmental processes, wound healing and tissue remodeling. Pathological EMT is

Table 1. Molecular interactions of fibulin-5 (EVEC, DANCE)

Interacting protein	Experimental context	Elastin monomers
α5β1 integrin, α4β1 integrin	Human smooth-muscle cells	[13]
αvβ3 integrin, αvβ5 integrin, α9β1 integrin	Normal mouse skin fibroblasts	[12]
Apolipoprotein A	In vitro binding assay	[27]
LTBP-2 (latent TGF-β-binding protein-2)	In vitro and lung extracts from mice (in vivo)	[16, 28]
LTBP-4 (latent TGF-β-binding protein-4)	In vitro binding assay	[29]
Fibrillin-1	In vitro binding assay	[30]
LOXL-1 (Lysyl oxidase-like 1)	In vitro and uterine tissues from mouse (in vivo)	[31]
LOXL-2, -4 (Lysyl oxidase-like 2, 4)	In vitro binding assay	[15]
Tropoelastin	In vitro binding assay	[14, 32]
Elastin monomers	In vitro and Chinese hamster ovary cells (in vivo)	[33]
Nogo-B	In vitro and Hela cells (in vivo)	[34]
SOD3 (Superoxide dismutase 3)	In vitro and *Drosophila* schneider cells, chinese hamster ovary cells (in vivo)	[35]
EMILIN-1	In vitro and 293T cells (in vivo)	[36]
Fibulin-5	In vitro experiment	[37]

however believed as an aberrant process essential in organ fibrosis, rheumatoid arthritis and cancer cell metastasis. Recent evidence suggests that FBLN-5 enhances EMT induced by transforming growth factor β (TGF-β). It has also been shown that FBLN-5 plays a novel EMT promoting role in development and progress of breast cancers [17].

TGF-β is a major inducer of both physiological and pathological EMT [18]. It has been reported that TGF-β significantly induces FBLN-5 expression in mammary epithelial cells. Overexpression of FBLN-5 enhances EMT induced by TGF-β by up-regulating MMPs expression and activity, and enhances the growth of a 4T1 tumor implanted into normal wild-type mice [17], leading to the conclusion that FBLN-5 plays an EMT- and cancer-promoting functional role in development and progress of breast cancers. Interestingly, a RGE mutant that loses the integrin binding activity of FBLN5 fails to promote EMT and elevate MMP expression. Since integrin β1 adhesion is necessary for the complex process of TGF-β-induced EMT [19], integrin αvβ3 signaling was found to promote EMT in response to TGF-β in mammary epithelial cells [20]. This suggests that part of FBLN-5's function may be to promote EMT through interaction with integrins. Thus, it is important to determine the mechanism by which FBLN-5 regulates EMT via integrins.

FBLN-5 AND TUMOR METASTASIS

FBLN-5 expression is associated with metastasis in various human tumors in a context-specific manner (Table 2). Initial reports have shown that *FBLN-5* mRNA expression is down-regulated in 62% of tumors (kidney, breast, prostate, lung and gastrointestinal organs) in comparison to normal tissues [8]. In contrast, a recent clinicopathological study has shown that *FBLN-5* is overexpressed in nasopharyngeal carcinoma and this overexpression might be critical for the invasiveness of malignant tumor cells [9].

Accordingly, several studies have also indicated that the effects of FBLN-5 on cancer cell proliferation, migration and invasion are complex and appear to be largely context-dependent. For instance, *FBLN-5* overexpression augments the tumorigenicity of HT1080 fibrosarcoma cells by increasing their DNA synthesis, and enhances their invasion through synthetic basement membranes [8]. In contrast, when *FBLN-5*-overexpressing HT1080 fibrosarcoma cells were injected in BALB/c SCID mice, tumor growth was inhibited and tumor blood vessel formation was significantly decreased [21]. Furthermore, FBLN-5 inhibits MMP-7 expression in lung cancer cells through the ERK pathway by binding integrin in an RGD-dependent manner, and epigenetic silencing of *FBLN-5* promotes lung cancer invasion and metastasis [6]. FBLN-5 also modulates the expression of MMP-2, MMP-3, TIMP-1 and TIMP-3 by inhibiting the angiogenesis of fibrosarcoma [22], suppresses

Table 2. The context-specific expression of FBLN-5 in human malignancies

Reduced expression of FBLN-5 in human cancer cells	Refs
Lung cancer	[6]
Prostate cancer	[7]
Calcified thoracic aortic aneurysms	[38]
Urothelial carcinomas of bladder	[39]
Overexpression of FBLN-5 in human cancer cells	**Refs**
Fibrosarcoma	[8]
Nasopharyngeal carcinoma	[9]
Breast cancer	[17]
Oxidative stress-induced renal carcinogenesis	[40]

MMP-9 expression in fibroblasts, and reduces metastasis formation [23], indicating at a broad relevance of FBLN-5 in ECM proteolysis and remodeling by regulating the MMP/TIMP families.

FBLN-5 AND ANGIOGENESIS

Lethal tumor progression cannot occur without angiogenesis, which facilitates tumor growth and survival. Direct evidence has been reported linking FBLN-5 to angiogenesis and regarding FBLN-5 as an endogenous inhibitor of angiogenesis and endothelial cell activities [22, 24]. VEGF, a key regulator of vasculogenesis and angiogenesis, dramatically down-regulates FBLN-5 expression, suggesting that reduced FBLN-5 expression is necessary for angiogenesis activation [11]. FBLN-5-null smooth muscle cells display enhanced proliferation and migration in response to serum and PDGF, whereas this enhancement is inhibited by over-expression of FBLN-5 [25]. Additionally, FBLN-5$^{-/-}$ mice display an apparent increase in in situ sprouting from vessels and vascular invasion [26], and a stable expression level of FBLN-5 reduces tumor angiogenesis and tumor growth in mice [21, 22], further underlining the role of FBLN-5 in the process of angiogenesis.

By mediating the anti-angiogenic function, FBLN-5 targets many cell types and uses different mechanisms, direct or indirect. In endothelial cells, FBLN-5 expression prevents the activation of ERK1/2 and p38 MAPK, but promotes angiogenesis resolution by targeting TSP-1, a natural inhibitor of neovascularization and tumorigenesis [11]. FBLN-5 binds to β1 integrins to activate integrin-induced ROS production, representing the underlying cause for the reduction in angiogenesis and tumor growth [24]. On the other hand, FBLN-5 antagonizes fibronectin-induced stress fiber formation and focal adhesion in smooth muscle cell by binding to α5β1 and α4β integrins, but fails to activate downstream signaling [13], suggesting that FBLN-5 acts in a dominant-negative role to inhibit fibronectin receptor-mediated signaling. Furthermore, animal experiments have indicated that antagonization of angiogenic activities of FBLN-5 is associated with angiopoietin 1 [26] in smooth muscle cell. Given these facts, future studies need to dissect the molecular determinants underlying the various activities of FBLN-5 in normal and cancer cells.

CONCLUDING REMARKS

Metastatic spread always represents the most lethal characteristic of cancer and the leading cause of cancer-related death. Both tumor angiogenesis and EMT comprise complex cascades of gene expression and enhance cancer morbidity by establishing an escape route for metastatic cancer cells. Given the aforementioned functions of FBLN-5 in various tumor microenvironments, FBLN-5 might be a good diagnostic target for the clinic. For example, a population-based study has demonstrated that fibulin-5 expression coincides with advanced nasopharyngeal tumor metastasis [9]; thus, fibulin-5 expression might be an important indicator of poor survival for nasopharyngeal carcinoma patients.

Although more and more evidence implicates the role of FBLN-5 in tumor development and progression, there remain several questions to be addressed in future studies. First of all, the exact pathological mechanisms leading to altered expression of FBLN-5 in diverse tumors need to be clarified. Secondly, further studies should determine whether the subcellular and extracellular localized FBLN-5 functions differently since the distribution of FBLN-5 is different in different cell lines [9]. Thirdly, FBLN-5 ligates several integrins in a cell-type specific manner, but the concrete mechanism of selection of the specific kind of integrins remains to be further elucidated. Finally, FBLN-5 mediates its adhesion to endothelial cells in an RGD-dependent manner [12], whereas the angiostatic activities of FBLN-5 are independent of its integrin-bound RGD motif [22]. Therefore, the receptors and cellular signals involved need to be further investigated.

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