Fibulin-5 function during tumorigenesis

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Tumorigenesis is the process by which normal cells evolve the capacity to evade and overcome the constraints normally placed upon their growth and survival. During cancer progression, indolent tumors experience an array of genetic and epigenetic events that ultimately coordinate the development of tumor metastasis, which is the most lethal facet of cancer and the leading cause of cancer-related death. The therapeutic necessity to combat tumor metastasis continues to drive investigations aimed at identifying novel regulators of this deadly process. Fibulin-5 is a newly described extracellular matrix protein that is important for normal embryonic development and organogenesis. Fibulin-5 expression may also be associated with the suppression of tumor formation through its control of cell proliferation, motility and angiogenic sprouting. Here, the tumor suppressing activities of fibulin-5 are reviewed, and the potential use and targeting of fibulin-5 to combat growth and metastasis of human malignancies is discussed.

Accurate and faithful progression through the cell cycle is regulated by an intricate balance between positive and negative growth signals. Cells do not grow autonomously; their commitment to proliferate depends upon environmental cues or messages [1]. These messages exist in three forms: as extracellular matrix (ECM) components, soluble factors, and cell–cell contacts, each interacting with a specific cell surface receptor [2]. Genetic or epigenetic events which alter any form of cellular communication can liberate cells from the restraints normally placed upon their growth, thus allowing them to proliferate autonomously. Such rogue cells contribute to the formation of human diseases, particularly cancer.

Fibulin (FBLN)-5 is a recently described ECM protein belonging to the fibulin family, whose members figure prominently in the formation and stabilization of basement membranes, elastic fibers, and loose connective tissues [3,4]. In addition to their structural functions, evidence now implicates fibulins as mediators of cell–cell and cell–matrix communication. Although the biology of FBLN-5 remains to be fully elucidated, genetic events resulting in the production of aberrant FBLN-5 proteins in humans is associated with the development of cutis laxa, a connective tissue disorder that results in loose skin and internal organ abnormalities due to aberrant elastic fiber organization [5–7]. Human FBLN-5 missense mutations are also associated with the development of age-related macular degeneration, which is the most common form of irreversible vision loss due to

drusen formation within macula of the neural retina [8]. FBLN-5 ablation in mice results in severe emphysema [9,10], suggesting that the loss or inactivation of FBLN-5 may be instrumental during emphysema development in humans. More recently, the aberrant absence of FBLN-5 mRNA in a variety of human malignancies suggests that reduced FBLN-5 expression may enhance cancer formation and progression in humans [11]. Here, the multifunctional nature of FBLN-5 in regulating epithelial [11], endothelial [12], and cancer cell activities [11] is summarized, and the potential use and targeting of FBLN-5 to prevent the growth and spread of human malignancies is discussed.

FBLN-5 structure & binding partners

FBLN-5 is the newest member of the fibulin family of ECM proteins that collectively play important roles in the biology of organogenesis and vasculogenesis, as well as in the pathology of fibrogenesis, tumorigenesis and other heritable diseases (Table 1). Fibulins arise as products from five distinct genes, each housed on a separate chromosome. Although fibulins represent a small gene family, their genetic and biological diversity is increased significantly via the use of alternative mRNA splicing, which greatly increases the number of potential fibulin proteins produced by cells.

Structurally, fibulins contain sequential repeats of calcium-binding epidermal growth factor (EGF)-like (cbEGF) modules, followed by a globular C-terminal fibulin-type module (Figure 1A–C) [3,4,13]. The fibulin family can be

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Table 1. Fibulins and human pathologies.						
Fibulin gene	Synonyms	Alternative splice variants	Human chromosome	Heritable disease (underlying mutation)	Cancer association	Refs
<i>FBLN-1</i>	BM-90	4	22q12.31	Synpolydactyly [FBLN-1D inactivation by reciprocal chromosomal translocation t(12:22)] Autosomal-dominant Giant Platelet Syndrome [FBLN-1D inactivation by splice acceptor mutation & antisense mRNA expression]	Yes	[30,33,35, 36,70–73]
<i>FBLN-2</i>	–	2	3p25.1	–	Yes	[74]
<i>FBLN-3</i>	EFEMP-1 S1-5, T16	5	2p16	Malattia Leventinese/Doyme Honeycomb retinal dystrophy [Arg345Trp]	–	[28,75–77]
<i>FBLN-4</i>	EFEMP-2 MBP-1, H411 UPH1	2	11q13	–	Yes	[39,40,75]
<i>FBLN-5</i>	DANCE EVEC, UP50	None	14q32.1	Cutis laxa [Ser227Pro; internal gene duplication] Age-related macular degeneration [7 distinct missense mutations]	Yes	[5,7,8,11,12, 14,23]

[§]Chromosomal locations determined at National Center for Biotechnology (www.ncbi.nlm.nih.gov/genome/guide/human/).
EFEMP: EGF-containing fibulin-like extracellular matrix protein; MBP: Mutant p53 binding protein; UP50: Urine p50 protein.

further subdivided into two distinct groups based on their size and the presence or absence of additional functional domains. Thus, the larger and more structurally complex fibulins 1 and 2 comprise subgroup 1, while the smaller and structurally simpler fibulins 3, 4, and 5 comprise subgroup 2 (Figure 1A). Recently, hemicentin-1 has been designated as FBLN-6 based on its cbEGF-like domains and C-terminal fibulin-type module [4]. This designation is somewhat tenuous because hemicentin-1 is significantly larger and contains additional protein modules not found in fibulins (e.g., thrombospondin Type 1 repeats, immunoglobulin C-2 domains, and a von Willebrand factor domain). Thus, while hemicentin-1 is clearly fibulin-like, this protein likely belongs to a class of ECM molecules distinct from fibulins. Whether hemicentin-1 is subject to proteolytic processing that releases biologically active FBLN-6 into cell microenvironments, remains to be determined.

FBLN-5 is a 448 amino acid glycoprotein that contains six cbEGF-like modules, which are followed by the requisite C-terminal fibulin-type module. Similar to FBLN-3 and -4, the first cbEGF-like domain in FBLN-5 is interrupted by a Proline-rich insert (Figures 1A & B). Figure 1B shows a hypothetical structure of

FBLN-5 cbEGF-like domains 1–4 that was generated by molecular modeling conserved and unique features of FBLN-5 cbEGF-like domains 1 and 2 onto the nuclear magnetic resonance (NMR) structure of two tandem fibrillin-1 cbEGF-like domains [69]. This process was repeated and extended to include FBLN-5 cbEGF-like domains 3 and 4, resulting in an extended linear conformation. Although the biological significance of the Proline-rich insert remains unknown, this *de novo* structural analysis localizes this helical peptide sequence and the integrin-binding RGD motif to exposed FBLN-5 surfaces (Figure 1B). If verified, this finding raises the possibility that the Proline-rich insert plays an important regulatory role in FBLN-5 biology, and as such, may be an ideal target for genetically manipulating and modifying various FBLN-5 activities.

FBLN-5 interacts physically with a growing list of ECM and secreted proteins through three distinct mechanisms. First, FBLN-5 is distinguished from other fibulins by the presence of an integrin-binding RGD motif (Figure 1B), which mediates endothelial cell adhesion [14], and binds $\alpha v \beta 3$, $\alpha v \beta 5$, and $\alpha 9 \beta 1$ integrins [9]. The integrin-binding RGD motif is conserved in chicken, mouse, and rat FBLN-5 proteins, suggesting an important function in

Figure 1A. Modular structural elements of fibulins and FBLN-5.

FBLN-5 biology. Thus, abrogating integrin binding by FBLN-5 provides a means to manipulate FBLN-5 function. Indeed, this approach significantly enhanced the anti-angiogenic activity of FBLN-5 in endothelial cells [12]. Second, cbEGF-like domains bind calcium with moderate-to-high affinity, which aids in maintaining protein stability, and in mediating protein–protein interactions. These domains form rigid, elongated rod-like structures that are stabilized by disulfide bridges arrayed in a characteristic pattern (Figure 1B) [3,15]. Presently, only FBLN-1 has been shown to bind calcium directly [16,17]; however, FBLN-5 binds tropoelastin in a calcium-dependent manner [10], suggesting that FBLN-5 structure and function also are dependent on calcium binding by its cbEGF-like domains. FBLN-5 also binds the elastin-binding protein, elastin microfibril interface-located protein (EMILIN)-1, whose genetic ablation in mice elicits mild elastinopathy reminiscent of that observed in FBLN-5-deficient mice [18]. Whether FBLN-5 interacts physically with tropoelastin and EMILIN-1 via its cbEGF-like domains remains to be

determined. Finally, FBLN-5 interacts with apolipoprotein A [19] and lysyl oxidase-like 1 (LOXL1) via its C-terminal fibulin-type module [20]. Although the biological significance of FBLN-5 binding apolipoprotein A remains unknown, binding of FBLN-5 to LOXL1 appears essential for normal elastogenesis of the uterus, lung, skin, and vasculature [20].

Collectively, these findings highlight the modular nature of FBLN-5 in mediating its physical interactions with a currently growing number of ECM and secreted proteins. Future studies need to identify the structural determinants operant in mediating FBLN-5 protein binding, and to determine how these determinants participate in regulation of cell adhesion, proliferation and motility mediated by FBLN-5. Moreover, the modular structure of FBLN-5 is especially attractive for the synthesis of designer FBLN-5 molecules or peptide (modular) mimetics capable of manipulating select FBLN-5 functions to control the growth and spread of human malignancies (e.g., enhancing anti-proliferative, -invasive, or -angiogenic activities).

Figure 1B. Consensus sequence of cbEGF-like domains and alignment of FBLN-5 cbEGF-like domains.

Upper panel. Blue text, amino acids involved in calcium-binding. Pink text, integrin-binding RGD motif. Green text, Pro-rich insert that interrupts cbEGF-like domain 1. Disulfide bridges pair in a Cys1:Cys3 (red text and line), Cys2:Cys4 (green text and line), and Cys5:Cys6 (purple text and line). Lower panel. Hypothetical structure of FBLN-5 cbEGF-like domains 1–4. Conserved and unique features of FBLN-5 cbEGF-like were identified by sequence alignment and mapped onto the NMR structure of two tandem fibrillin-1 cbEGF-like domains (PDB code 1EMN; [69]). This process was repeated on FBLN-5 cbEGF-like domains 3 and 4, resulting in a possible linear conformation. This hypothetical FBLN-5 structure localizes the integrin-binding RGD motif and Pro-rich insert to exposed FBLN-5 surfaces. The molecular analysis and figure were kindly provided by Dr S Johnston.

FBLN-5 & tumorigenesis

Recent studies have implicated involvement of FBLN-5 in carcinogenesis where it is believed to suppress or promote tumorigenesis in a context-specific manner [11,12]. For instance, FBLN-5 was identified independently as a novel fibroblast and endothelial cell gene target of the tumor suppressor, transforming growth factor (TGF)- β [11]. When compared to corresponding normal tissue, FBLN-5 mRNA expression is downregulated dramatically in the majority of surveyed human tumors, particularly metastatic malignancies of the kidney, breast, ovary and colon (Figure 2) [11]. These findings suggest that the loss or inactivation of FBLN-5 may participate in cancer progression. Moreover, these findings are intriguing because FBLN-5 is a fibroblast and endothelial cell gene target of TGF- β [11], whose expression is upregulated in developing tumors [21]; and 90% of all human tumors derive from epithelial tissues [22], which do not express FBLN-5 [14,23]. Thus, despite increases in tumor TGF- β expression, FBLN-5 expression is not correspondingly upregulated. The loss of FBLN-5 from tumor microenvironments may result from the actions

of molecules which repress FBLN-5 expression, including oncogenic *Myc* [24,25], vascular endothelial growth factor (VEGF) [12], and interleukin (IL)-1 β [26]. The latter observation indicates that prolonged chronic inflammation, which can promote cancer development [27], will also downregulate FBLN-5 expression, thereby potentially linking chronic cellular damage and FBLN-5 expression to tumorigenesis in humans. Future studies need to address these issues, as well as identify the FBLN-5-producing cells negatively affected by tumorigenesis.

FBLN-5 is a multifunctional signaling molecule that regulates cell proliferation and motility in a context-specific manner [11]. For instance, FBLN-5 stimulates DNA synthesis and motility in fibroblasts and fibrosarcoma cells, but inhibits these processes in epithelial [11] and endothelial [12] cells (Table 2). Other fibulins also regulate normal and cancer cell activities in a context-specific manner. For example, FBLN-3 expression both positively and negatively regulates cell proliferation, and is upregulated significantly in transformed cell lines [28]. FBLN 1 inhibits breast cancer cell motility [29,30],

Figure 1C. Alignment of C-terminal fibulin module present in human fibulins.

reduces fibrosarcoma anchorage-independent cell growth and invasion [31], and interacts physically with oncogenic papillomavirus E6 protein, thereby antagonizing E6-mediated transformation of murine C127 and NIH 3T3 cells [32]. Thus, FBLN-1 suppresses cancer cell activities associated with tumor progression. Accordingly, re-expression of FBLN-1 in FBLN-1-deficient fibrosarcoma cells significantly reduces their growth and formation of tumors after implantation in mice [31]. In stark contrast, breast cancers exhibit elevated FBLN-1 expression compared with surrounding normal tissue [33,34].

Indeed, the sera of breast cancer patients contains antibodies against FBLN-1 unlike healthy volunteers [34]. Ovarian cancer cells also upregulate FBLN-1 expression [35,36], which localizes extensively to proximal tumor stroma [37]. Moreover, ovarian cancer cells preferentially upregulate FBLN-1C expression compared with FBLN-1D [35], which differs in its unique C-terminal fibulin-type module. Thus, FBLN-1C appears to promote tumorigenesis, while FBLN-1D suppresses tumorigenesis. If so, then the diagnostic detection of FBLN-1C may serve as a prognostic indicator for disease risk and

Figure 2. Tumorigenesis dramatically alters FBLN-5 expression.

Radiolabeled cDNA probes corresponding to either human FBLN-5 (left panel) or ubiquitin (middle panel) were hybridized to matched human normal/tumor cDNA array. Normal (upper spot) FBLN-5 expression is altered dramatically in adjacent tumor tissue (lower spot) by tumorigenesis. Tumor type and metastasis status are indicated by: (i) open boxes, no information; (ii) red-filled boxes, metastasis observed; and (iii) pink boxes, metastasis not observed (right panel). K: Kidney; S.I.: Small intestine. Reprinted with kind permission from [11].

aggressiveness in patients with ovarian cancers [35,36], as well as those with breast cancers. More recently, elevated FBLN-2 expression has been associated with the acquisition of a metastatic phenotype during adenocarcinoma progression [38], while upregulated FBLN-4 expression has been associated with human colon cancer [39]. Although clearly a secreted protein, a FBLN-4 signal sequence polymorphism, i.e., Ala5Thr has been identified that may function in preventing its efficient secretion from cells [39], raising the possibility that aberrant subcellular localization of FBLN-4 contributes to tumorigenesis in humans. Accordingly, FBLN-4 enhances cellular transformation and cancer cell proliferation by interacting physically with mutant p53 proteins [40]. Thus, given the context-specific functions of fibulins, including FBLN-5, future studies need to determine which cell types are predominantly targeted by FBLN-5 in tumor microenvironments and, more importantly, which FBLN-5 functions predominate within these same microenvironments.

Table 2 summarizes the context-specific activities of FBLN-5 on responsive cells. Mechanistically, FBLN-5 couples to cell cycle machinery in fibroblasts and epithelial cells (e.g., cyclin A), as well as to extracellular signal regulated kinases (ERK)1 and 2, and p38

mitogen-activated protein kinase (MAPK) activation, which synergize with TGF-β during stimulation of activator protein (AP)-1 activity [11]. Interestingly, FBLN-5 stimulates a conserved signaling system minimally comprised of MAPKs (i.e., ERK1/ERK2 and p38 MAPK) and AP-1 transcription factors, whose ultimate biological outcome reflects the genetic makeup of a particular cell (i.e., cell type-specific activation and formation of distinct AP-1 dimers by FBLN-5 leads to context-specific gene expression and biological function). Unlike fibroblasts and epithelial cells, FBLN-5 stimulation of endothelial cells fails to activate ERK1/ERK2 and p38 MAPK, but instead inhibits their stimulation by VEGF [12]. The mechanisms underlying this reversal in FBLN-5 signaling remain unknown, but may reflect differences in FBLN-5 binding to integrins, which govern cell adhesion, migration and invasion, as well as cell proliferation and survival [41,42]. Although FBLN-5 clearly mediates endothelial cell adhesion by binding integrins [9,14], the ability of FBLN-5 to antagonize endothelial cell activities is enhanced significantly by ablation of its integrin-binding RGD motif [12]. Thus, future studies need to determine the contribution of integrins to various FBLN-5 functions in responsive cells, as well as identify which FBLN-5 signaling systems are activated

Table 2. Context-specific effects of FBLN-5.				
Cell type	Regulator of FBLN-5 expression	Cellular function	Targeted signaling system/pathway	Refs
Fibroblast	TGF-β (↑) Myc (↓) Interleukin-1β (↓) Tropoelastin (↑) Lung Injury (↑)	Proliferation (↑)	ERK1/ERK2 (↑) p38 MAPK (↑)	[11,24–26,50,78]
Epithelial		Proliferation (↓)	ERK1/ERK2 (↑) p38 MAPK (↑) AP-1 activity (↑) Cyclin A expression(↓) TGF-β synergy	[11]
Endothelial	TGF-β (↑) VEGF (↓) Vascular Injury (↑)	Adhesion (↑) Proliferation (↓) Invasion (↓) Angiogenic sprouting (↓)	ERK1/ERK2 (↓) p38 MAPK (↓) TSP expression (↑) VEGF signaling (↓)	[12,14,23]
Fibrosarcoma		Proliferation (↑) Migration (↑) Invasion (↑)		[11]

Arrows indicate an increase or decrease in a described activity. AP: Activator protein; ERK: Extracellular signal regulated kinase; MAPK: Mitogen-activated protein kinase; TGF: Transforming growth factor; TSP: Thrombospondin; VEGF: Vascular endothelial growth factor.

via integrin-dependent and -independent mechanisms.

Collectively, these studies suggest that measures designed to augment FBLN-5 expression or concentration within tumor micro-environments may suppress the growth and progression of developing tumors. Moreover, because FBLN-5 expression is altered by tumorigenesis [11], changes in FBLN-5 expression may prove useful in diagnosing and predicting the clinical course of cancers of the kidney, breast, colon and ovary. Equally important will be the performance of population-based studies designed to more thoroughly examine the linkage between FBLN-5 and cancer development in humans.

FBLN-5 & angiogenesis

The growth and progression of small, innocuous tumors is restricted by their reliance on simple diffusion to acquire nutrients. Indolent tumors overcome this growth impediment by stimulating angiogenesis, a complex cascade of gene expression and repression resulting in the construction of new vasculature networks (Box 1) [43–46]. Besides facilitating tumor growth and survival, angiogenesis enhances cancer morbidity by providing malignant cells a means for their metastatic spread, which clinically is the most lethal aspect of cancer and the leading cause of cancer-related death [47,48]. Thus, identifying novel anti-angiogenic molecules and deciphering

their mechanisms of action may one day be exploited to limit and/or prevent tumor neovascularization, and consequently, the proliferation, survival, and metastasis of malignant cells.

Several recent observations have implicated fibulins, particularly FBLN-5, as potential regulators of angiogenesis and endothelial cell activities. For instance, fibulins are expressed in a spatiotemporal manner during cardiovascular development and, based on their expression profiles, may regulate the formation of endocardial and epicardial structures, as well as that of the aortic arch [3,4,13]. In adults, endothelial and vascular smooth muscle cells synthesize fibulins, which associate with and incorporate into vascular basement membranes, elastic laminae, and vessel walls [3,4,13]. Murine FBLN-1 deficiency elicits abnormal endothelial cell morphologies that result in aberrantly dilated and irregularly shaped capillaries, whose rupture ultimately elicits fatal hemorrhaging and perinatal lethality [49]. Similarly, FBLN-5 deficiency also manifests vascular malformations in the form of aortic torsion with loss of compliance [9,10]. Although FBLN-5 expression is low in adult vasculature, its expression is abundant in developing or injured blood vessels [14,23,26,50]; uterine myometrial arteries undergoing cyclic angiogenesis [23]; and endothelial cells stimulated with the angiogenic cytokine, TGF- β [11,12]. FBLN-5 also mediates endothelial cell adhesion via its integrin-binding RGD motif [14], which is bound by the angiogenesis-associated integrin $\alpha v \beta 3$ [9]. Collectively, these observations implicate FBLN-5 as a prominent regulator of endothelial cell activities coupled to vessel development and repair, as well as those to angiogenesis.

Direct evidence linking FBLN-5 to angiogenesis has now been described [12]. Indeed, tubulating murine endothelial cells or those stimulated with the pro-angiogenic cytokine, VEGF, dramatically downregulate FBLN-5 expression, suggesting that reduced FBLN-5 expression is necessary for angiogenesis activation. Accordingly, supplying endothelial cells with a continual source of FBLN-5 antagonizes their ability to undergo angiogenic sprouting *in vitro* in response to pro-angiogenic signals (Figure 3A). Mechanistically, FBLN-5 might inhibit angiogenesis by binding endothelial cell integrin receptors via its RGD motif. However, engineered FBLN-5 molecules which lack the RGD motif exhibit increased anti-angiogenic

Box 1. Angiogenesis.

Angiogenesis is the process whereby new blood vessels sprout and develop from pre-existing vessels. Angiogenesis is accomplished via two distinct phases, activation and resolution. The activation phase encompasses the initiation and development of forming vessels, and comprises at least five distinct events which must occur in order to form a new, functional blood vessel:

- Degradation of the extracellular matrix of existing tubules
- Disruption of cell–cell contacts and cell adhesion
- Augmentation of cell permeability
- Proliferation of endothelial cells
- Invasion and migration of endothelial cells towards the site of new blood vessel formation [43–45]

After the activation phase is complete, the resolution phase essentially completes or matures the newly formed vessel by:

- Cessation of cell migration
- Inhibition of cell division
- Reconstitution of basement membranes
- Reformation of cell–cell contacts [43–46]

Angiogenesis is essential to many biological processes, including embryonic development, wound repair and the female reproductive cycle [43]. In addition, the pathogenicity of many human disease states such as arthritis, diabetic retinopathy and cancer depends on their ability to stimulate angiogenesis [44].

Figure 3. FBLN-5 inhibits angiogenic sprouting by tubulating MB114 endothelial cells.

activity (Figure 3B). This finding suggests that FBLN-5 may mediate both pro- and anti-angiogenic activities by interacting with two distinct receptor systems in a concentration-dependent manner (i.e., pro-angiogenic function via moderately high-affinity, low occupancy of integrins and anti-angiogenic function via low-affinity, high occupancy of an unknown anti-angiogenic receptor). Alternatively, integrin-mediated RGD binding may tether and sequester FBLN-5 from its anti-angiogenic receptor. Distinguishing between these and other possible mechanisms needs to be addressed in greater detail.

In mediating its anti-angiogenic function, FBLN-5 targets multiple endothelial cell activities operant in mediating angiogenic sprouting, most likely via direct and indirect mechanisms. For instance, by antagonizing VEGF stimulation of ERK1/ERK2 [12], which couples to matrix metalloproteinase (MMP) expression [43,51,52], and p38 MAPK [12], which couples to actin cytoskeletal reorganization [53], FBLN-5 reduces endothelial cell invasion and migration requisite for angiogenesis activation. Interestingly, VEGF and thrombospondin (TSP)-1, which inhibits angiogenesis [54,55], exhibit reciprocal expression patterns during angiogenesis activation (i.e., high VEGF, low TSP-1) and resolution (i.e., low VEGF, high TSP-1). Consistent with its inhibitory effects on VEGF signaling, FBLN-5 also significantly

stimulates TSP-1 expression in endothelial cells [12], thereby enhancing angiogenesis resolution. FBLN-5 stimulation of TSP-1 expression will also antagonize angiogenesis via TSP-1-mediated induction of endothelial cell apoptosis [56] and the inhibition of MMP-9 activation [57]. Accordingly, FBLN-5 reduces endothelial cell MMP-2 and -3 expression, while simultaneously enhancing that of tissue inhibitor of matrix metalloproteinase (TIMP)-1 and -3 [Manuscript in Preparation].

Collectively, these studies establish FBLN-5 as a novel angiogenesis antagonist, as well as implicate additional fibulins, particularly FBLN-1 [49] and FBLN-3 [Manuscript in Preparation], as novel regulators of endothelial cell activities coupled to angiogenesis. Future studies need to address this issue, as well as to determine whether the loss of FBLN-5 within tumor microenvironments *in vivo* promotes cancer progression by alleviating the constraints to angiogenesis. Future studies also need to determine which FBLN-5 anti-angiogenic activities are mediated directly by FBLN-5 and which are mediated indirectly by downstream factors such as TSP-1. Detailed structure-function studies also are needed to dissect the molecular determinants underlying the various activities of FBLN-5 on normal and cancer cells. Indeed, the results of these studies may enable the engineering of FBLN-5 molecules or peptides whose growth and motility promoting activities are fully separated from their growth, motility, and angiogenic suppressing activities. Such

Box 2. Epithelial-mesenchymal transition.

Epithelial–mesenchymal transition (EMT) is a metastable process whereby polarized epithelial cells acquire a motile fibroblastoid phenotype. EMT comprises:

- Loss of cell polarity due to downregulated expression of epithelial cell markers (e.g., E-cadherin, zona occluden-1, and $\beta 4$ integrin)
- Cytoskeletal architecture reorganization and intracellular organelle redistribution
- Upregulated expression of fibroblast markers (e.g., vimentin, N-cadherin, α -smooth muscle actin)
- Elevated cell invasion and migration

EMT is a normal and important component of early embryonic development, wound healing, and tissue remodeling and repair. Inappropriate reactivation of EMT in adult tissues is associated with chronic inflammation, chronic obstructive pulmonary disease and lung fibrosis, rheumatoid arthritis and the acquisition of metastatic phenotypes by dedifferentiated tumors [58,59].

inhibitory FBLN-5 molecules would be attractive cancer therapeutics due to their potent tumor starving activities.

FBLN-5 & epithelial-mesenchymal transitions

Epithelial cells normally undergo epithelial–mesenchymal transitions (EMT) as a necessary process during embryonic development, and again in adulthood during wound healing, tissue

remodeling, and repair (Box 2). Oncogenic EMT however, is increasingly recognized as an aberrant process essential in promoting cancer progression via a complex array of gene expression and repression that elicits tumor metastasis in localized carcinomas [58,59]. Indeed, the acquisition of metastatic phenotypes by indolent tumors represents the most lethal component of cancer and the leading cause of cancer-related death [47,48].

A function for fibulins in regulating EMT has yet to be addressed directly. However, during embryonic development and organogenesis, FBLN-1 and -2 are prominently expressed in active EMT regions, particularly during formation of the neural tube and crest, skeletal muscle, and the epicardium, endocardial cushion tissue, and cardiac valves and septa [60–65]. Analogs to FBLN-1 and -2, FBLN-5 is widely expressed throughout human and murine tissues in a developmentally-regulated manner [11,14,23]. Moreover, FBLN-5 expression localizes to regions of EMT during arterial, endocardial cushion tissue, neural crest, and mesenchymal tissue development [14,23]. Thus, FBLN-5 may be an important regulator of normal EMT during embryonic development, as well as abnormal EMT during cancer development. Indeed, because cancer progression is characterized by cell de-differentiation [66], it is plausible that under certain conditions, FBLN-5 may actually enhance the motility and invasion of dedifferentiated epithelial cells (i.e., fibroblastoids). Future studies need to test this hypothesis, as well as the corresponding hypothesis that FBLN-5 mediates tumor suppression (e.g., anti-proliferative, -invasive, and -angiogenic activities) and promotion (e.g., proliferative, motility, and EMT) in tumor grade-specific manner.

Conclusion & future perspective

Cancer mortality rates have declined in recent years due in large part to improvements in early detection. Unfortunately, the continued failure to diagnose and treat localized cancers (i.e., carcinoma *in situ*) prior to their metastasis to distant locales, significantly reduces patient survival rates. Clinically, metastatic spread represents the most lethal characteristic of cancer and is the leading cause of cancer-related death. Tumor angiogenesis facilitates tumor metastasis by providing cancer cells a route for their dissemination. Both processes are extremely complex and involve a highly ordered and coordinated cascade of gene expression and repression.

Figure 4. Schematic summary of FBLN-5 biology and pathology.

FBLN-5 is a multifunctional ECM protein that plays important roles during embryonic development and organogenesis. Aberrant FBLN-5 expression or protein production has been linked to human disease formation, including cancer. Signaling molecules or physiological conditions that regulate FBLN-5 expression are shown, as are FBLN-5 binding partners and signaling systems whose activation are regulated by FBLN-5. See text for details.

Executive summary***Fibulin-5 biology & pathology***

- Fibulin (FBLN)-5 mediates cell–cell and cell–matrix communication during embryonic development, organogenesis and vasculogenesis. FBLN-5 expression regulates tissue remodeling and repair in injured arteries and lung.
- FBLN-5 regulates elastic fiber formation and stabilizes basement membranes and loose connective tissues.
- FBLN-5 mutants are associated with human cutis laxa and age-related macular degeneration development. Loss or inactivation of FBLN-5 leads to emphysema-like structures in lung.

FBLN-5 structure & binding partners

- FBLN-5 is a 448 amino acid secreted glycoprotein.
- Structure comprises six calcium-binding epidermal growth factor (EGF)-like modules, followed by a globular C-terminal fibulin-type module. First calcium-binding EGF-like module is interrupted with a Proline-rich sequence of unknown function.
- FBLN-5 integrin-binding RGD motif mediates endothelial cell adhesion and binds $\alpha v\beta 3$, $\alpha v\beta 5$, and $\alpha 9\beta 1$ integrins. Loss of integrin-binding significantly improves anti-angiogenic signaling by FBLN-5.
- FBLN-5 binds tropoelastin in a calcium-dependent manner.
- FBLN-5 C-terminal fibulin-type module binds apolipoprotein A and lysyl oxidase-like 1, which is essential for normal elastogenesis of the uterus, lung, skin and vasculature.
- Modular nature of FBLN-5 is amenable for the synthesis of designer FBLN-5 molecules or peptide mimetics capable of manipulating select FBLN-5 functions to control cancer progression.

FBLN-5 & tumorigenesis

- FBLN-5 expression is stimulated by the tumor suppressor, transforming growth factor (TGF)- β .
- FBLN-5 inhibits the proliferation and motility of epithelial and endothelial cells, but stimulates that of fibroblasts and fibrosarcoma cells.
- FBLN-5 regulates cell cycle machinery by coupling to mitogen-activated protein kinases (MAPKs), and by synergizing with TGF- β during stimulation of activator protein (AP)-1 activity.
- FBLN-5 transcript expression is altered aberrantly in 65% of human tumors, of which 95% exhibit downregulated FBLN-5 expression. FBLN-5 mRNA expression is altered aberrantly in 68% of metastatic tumors, of which 100% exhibit downregulated FBLN-5 expression. Findings suggest that reduced FBLN-5 expression enables cancer formation and progression.
- FBLN-5 transcript expression is downregulated disproportionately in cancers of the kidney, breast, colon and ovary.

FBLN-5 & angiogenesis

- Angiogenesis is the process whereby new blood vessels develop from pre-existing vessels. Without angiogenesis, tumors would remain small and innocuous. Angiogenesis also provides cancer cells a route for their metastatic spread.
- TGF- β regulates angiogenesis and induces endothelial cell expression of FBLN-5, whereas pro-angiogenic signals downregulate FBLN-5 expression in endothelial cells. Findings implicate FBLN-5 as an angiogenesis antagonist.
- FBLN-5 prevents endothelial cell angiogenic sprouting by inhibiting their proliferation and invasion. Effects on endothelial cell motility are mediated via downregulated matrix metalloproteinase (MMP) expression and upregulated tissue inhibitor of matrix metalloproteinase expression (TIMP).
- FBLN-5 reduces endothelial cell response to the pro-angiogenic factor, vascular endothelial growth factor (VEGF), and enhances endothelial cell expression of the anti-angiogenic factor, thrombospondin (TSP)-1.
- Abrogating integrin binding significantly improves the anti-angiogenic function of FBLN-5. Developing designer FBLN-5 molecules may prove effective in preventing tumor angiogenesis, thereby improving the prognosis and treatment of cancer patient.

FBLN-5 & epithelial–mesenchymal transitions

- Epithelial–mesenchymal transition (EMT) is a metastable process whereby polarized epithelial cells acquire a motile fibroblastoid phenotype.
- EMT is a necessary component of normal embryonic development, and of wound healing, tissue remodeling and repair.
- Oncogenic EMT is essential for the development of tumor metastasis.
- FBLN-5 expression is widely distributed during development and localizes to regions of EMT. FBLN-5 may be a novel regulator of oncogenic EMT.

Interference at any step along these cascades inhibits their completion, thereby preventing tumor metastasis. Thus, thoroughly combating cancer progression necessitates that science and medicine identify and validate the effectiveness of novel antagonists of tumor angiogenesis and metastasis.

Although our understanding of FBLN-5 function is in its infancy, FBLN-5 does possess activities that one day may be exploited to prevent the formation and progression of human cancers. A summary of FBLN-5 biology and pathology is presented in Figure 4. FBLN-5 clearly regulates cell proliferation and motility in a cell- and context-specific manner, as well as tissue development, remodeling and repair. The loss or inactivation of FBLN-5 in human malignancies implicates FBLN-5 in mediating tumor suppression, a function supported by its ability to inhibit angiogenic sprouting in endothelial cells. Interestingly, tumors are analogous to wounds that will not heal [67] and, quite paradoxically, FBLN-5 stimulates wound healing *in vivo* [68]. Thus, similar to its context-specific regulation of cell growth and motility, FBLN-5 may actually promote tumorigenesis in some circumstances. Deciphering this FBLN-5 paradox remains the most important question concerning the biological and pathological

actions of this multifunctional ECM protein, as well as its potential for use and targeting to prevent cancer progression. A vital step in this process will be the performance of *in vivo* models of tumor progression to ascertain the anti-angiogenic and tumor suppressing functions of FBLN-5. Equally important will be the identification of FBLN-5 determinants operant in mediating its tumor suppressing and anti-angiogenic activities, and the pharmacological characterization of these determinants in alleviating cancer progression. Finally, population-based studies are needed to assess the usefulness of FBLN-5 as a diagnostic and/or prognostic indicator for cancer patient survival, and to identify and determine the contribution of FBLN-5 polymorphisms to cancer development in humans.

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