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## Roles of Fibulin-2 in Carcinogenesis

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Fibulin-2, an extracellular matrix (ECM) protein expressed in normal epithelia, is a kind of fibulin which is associated with basement membranes (BM) and elastic ECM fibers. The role of fibulin-2 has been recognized as an oncogene. The upregulation of fibulin-2 correlates with cancer development and progression. Furthermore, the upregulation of fibulin has been detected in ovarian cancer and stomach adenocarcinoma. However, the downregulation of fibulin has been detected in different intestinal and respiratory tumor cells. Additional studies have revealed that the role of fibulin-2 in carcinogenesis is context dependent and is caused by the interaction of fibulin proteins such as cell surface receptors and other ECM proteins, including integrins and syndecans. The present study summarizes the role of fibulin in carcinogenesis and its underlying molecular mechanism.

**MeSH Keywords:** **Carcinogenesis • Extracellular Matrix Proteins • Fibula • Molecular Mechanisms of Pharmacological Action**

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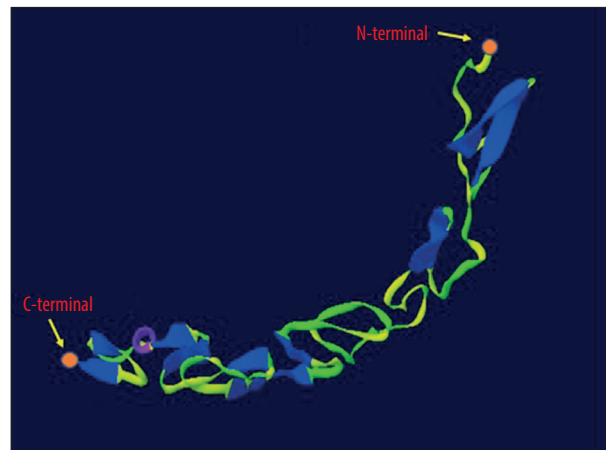


## Background

Fibulins are in the family of extracellular matrix (ECM) proteins. There are 7 isomer glycoproteins in the fibulin family (fibulin 1–7), and these are localized to the ECM. These proteins have the fibulin module, which can secrete glycoproteins and co-own a globular domain at the carboxy-terminus. These are preceded by tandem calcium-binding epithelial growth factor (cb-EGF). The study conducted by Argraves et al. revealed that fibulins are multidomain proteins with 2 types of repeat motifs. One is homologous to anaphylatoxins C3a, C4a, and C5, and the albumin gene family, while the other is homologous to EGF [1]. In the fibulin family, these are grouped into 2 subgroups [2]. The first group includes fibulin-1 and fibulin-2, and these have 3 anaphylatoxin (AT) modules in their domain I. Fibulin-1 is the first member discovered in 2 decades. Its isoforms size range 50 kDa to 200 kDa, and it has an elongated multidomain structure dominated by numerous calcium-binding EGF-like modules. The second group includes fibulin-3–7, and the molecular weights of the first 3 members range within 50–60 kDa. On the structure, these members do not contain any module in their amino-terminal region. Compared with other members, fibulin-6 (615 kDa) is the largest member of the fibulin family, because it has 44 tandem immunoglobulin and 6 thrombospondins type-I modules in its domain I [3]. Finally, fibulin-7 (50 kDa) is distinguished by the presence of a module called “sushi”, which is correlated to protein-protein interactions [4].

Fibulin is severed as a scaffold for the ECM, which comprises of several macromolecules bound together in a complex network. This characteristic allows it to have a number of functions, including support for the tissue structure and intercellular communications. Furthermore, the ECM is a well-organized complex network that secretes a significant number of proteins, glycoproteins, proteoglycans and polysaccharides [5]. The dysfunction of the ECM affects the development and progression of multiple and different diseases, including cancer. The abnormal process of the ECM affects cell activities and results in dysfunctions in cell adhesion, shape, migration, proliferation, and differentiation. The ECM influences almost all cellular behaviors, and it is essential for a variety of biological processes and regulatory mechanisms [6,7].

Fibulin is widely spread in various ECMs, and based on its widespread distribution feature, fibulin is well correlated with their broad binding repertoire for fibronectin [8,9], collagens, basement membrane (BM) proteins, laminin [10,11], and proteoglycans. These structures comprise of the BM, which has been widely considered as one of the barriers of the body against cancer progression [12–14]. More importantly, multiple studies have been indicated that these not only serve as intermolecular bridges which could form supramolecular structures, but



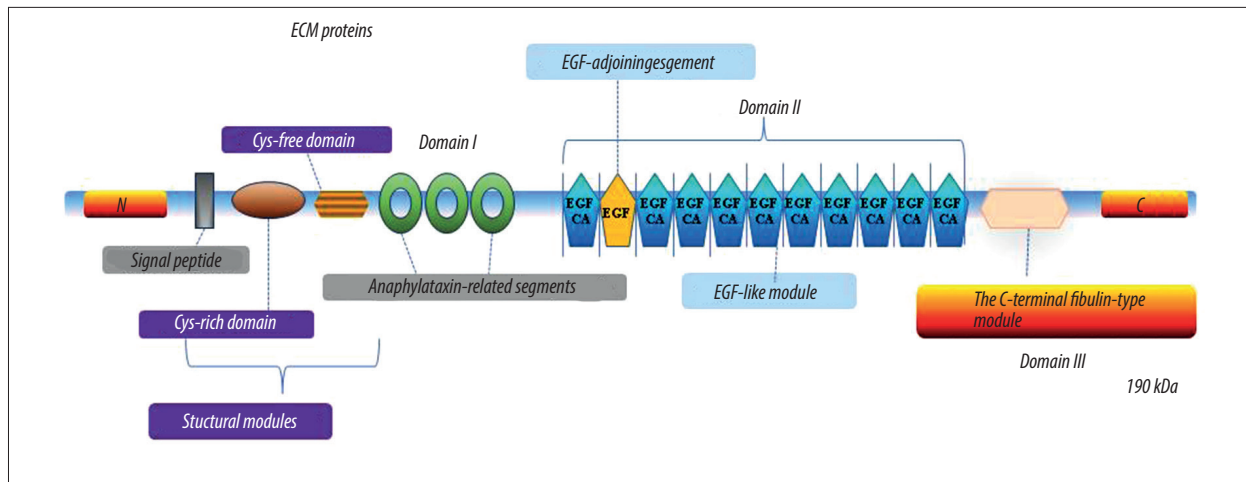
**Figure 1.** Crystal structure of the human fibulin-2 protein characterized by x-ray diffraction.

also serve as a mediator for cumbersome biological processes, including cell migration, adhesion, proliferation, as well as mediator for intercellular signaling pathways [15].

Fibulin-2, a member of the fibulin family, is known to interact with a various of ECM ligands, and adjusts the contact between cells and their surroundings. Fibulin-2 was confirmed by the comparative sequence analysis of mouse fibulin-1, and it was picked out from a mouse fibroblast cDNA library [16]. Fibulin-2 may serve as a matrix organizer in the ECM to maintain the tissue architecture. Recently, an increasing number of studies have highlighted the involvement of fibulin-2 in tumorigenesis. In this regard, fibulin-2 can display both tumor-promoting and tumor-protective properties in various types of neoplasia. The present findings indicate the significance of fibulin-2 in tumor development. The mechanisms underlying these opposing effects are complex and not fully understood. The aim of this study was to summarize the present knowledge on the roles of fibulin-2, to explore the potential contributions of fibulin-2 to tumor progression, and to further explore the mechanism of fibulin-2.

## The Structure and Function of Fibulin-2

Fibulin-2, an ECM protein, was originally identified in 1990 by predicting from the sequence analysis of cDNA clones which was obtained from a mouse fibroblast library. Fbln2, the gene symbol of fibulin-2, is located at chromosome 3p24-p25, and is correlated to tumor development through its important interaction with several ECM proteins such as laminin, collagen IV, etc. Fibulin-2 is a large 190-kDa protein, and it is determined by the existence of 2 structural modules, the tandem repeat of epidermal growth factor-like (EGF-like) modules, and unique C-terminal and N-terminal fibulin-type modules (Figure 1). It has been proven that fibulin-2 consists of 1195-residue polypeptide and 26-residue



**Figure 2.** An illustration of the fibulin-2 domain structure.

signal peptide. The C-terminal region with 787 amino acids had 3 motifs related to anaphylatoxins (domain I), domain II, which represents the 11 EGF-like repeats (10 had a consensus motif for calcium binding), and 115-residue globular domain III. For fibulin-2, the third EGF-like domain in the calcium-binding EGF-like repeated modules of Fbln2, is either present or absent due to the alternative splicing of exon 9. This suggests that both isoforms may be protein-coded, since only a single deleted EGF-like domain [17,18]. In addition, research evidence has suggested that this C-terminal region revealed a 43% sequence similar to fibulin-1. The N-terminal region of the 408-residue is unique to fibulin-2, showing no similar sequence to other fibulins in the fibulin family. Furthermore, research evidence has shown that fibulin-2 strongly binds to  $\alpha 1 \beta 3$  integrin but binds less to  $\alpha v \beta 3$  integrin through the RGD motif in the N-terminal region [19]. Moreover, fibulin-2 contains an extra protein in the N-terminal domain with 2 cysteine-rich segments. Briefly, fibulin-2 is made up of 4 domains: N-terminal domain, 3 anaphylatoxin-related segments, tandem of EGF-like repeats, and C-terminal fibulin-type module (Figure 2) [20]. At present, fibulin-2 is known as the second largest member of the fibulin family. Evidence has shown that fibulin-2 has 2 isoforms: short fibulin-2 (fibulin-2s) and long fibulin-2 (fibulin-2l). Both the long and short Fbln2 isoforms are present in humans and mice, however, there are no reports on the comparative functional studies of these isoforms [17]. It was reported that fibulin-2s is expressed at high levels both at the protein and mRNA level in several nasopharyngeal carcinoma (NPC) cell lines. In contrast, fibulin-2l is expressed at low levels, or is not detectable in normal and tumor tissues. Overall, fibulin-2s is the dominant expression isoform in normal tissues and immortalized NPC cell lines [21].

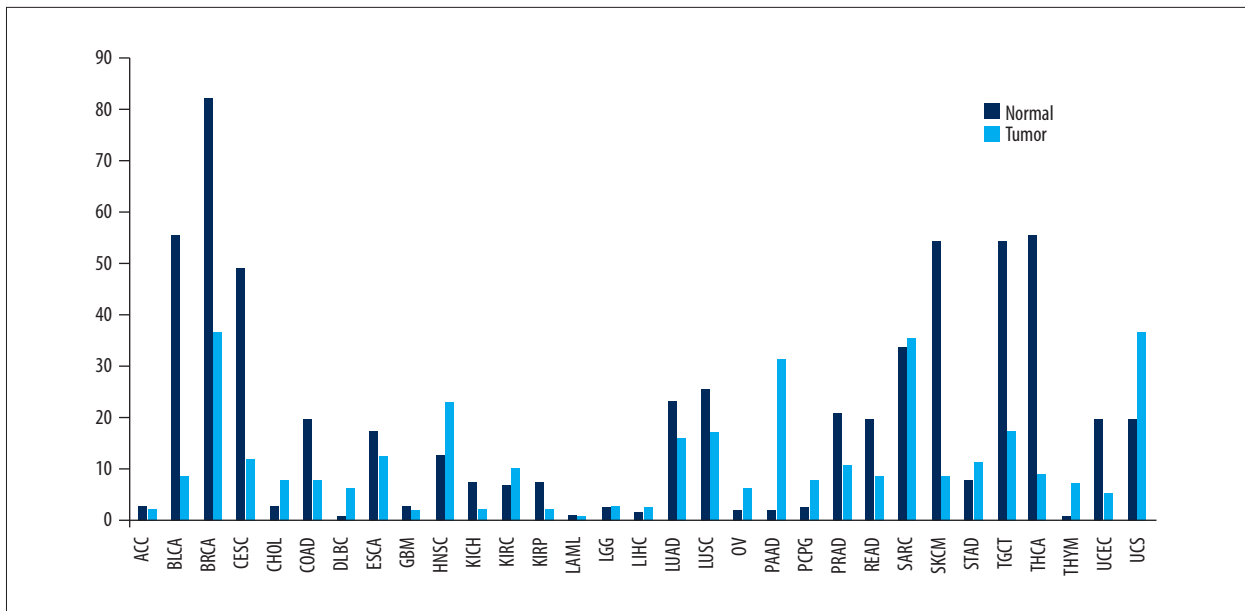
Fibulin-2 can form homodimeric complexes that fold into 3–4 armed structures to stabilize the matrix macromolecules. For its structure, fibulin-2 can bind to several molecular interactions, including  $\alpha 1 \beta 3$  integrin,  $\alpha v \beta 3$ , and  $\alpha 5 \beta 1$  integrins [18],

laminin- $\alpha 2$  chain [22,23], fibronectin [24], sex hormone-binding globulin (SHBG) [25], aggrecan, versican [26], nidogen [27], perlecan [28,29], and tropoelastin [30].

Specifically, fibulin-2 has the specific sequence in the N-terminal globular domain, which is responsible for  $\alpha 1 \beta 3$  binding [19]. Studies have shown that the C-terminal G domain of the mouse laminin  $\alpha 2$  chain includes 5 lamin-type G domain (LG) modules ( $\alpha 2 \text{LG}1$  to  $\alpha 2 \text{LG}5$ ), and the fragments of these LG modules are also bound to fibulin-2 and nidogen-2 through kinetic analysis [22]. In addition, perlecan domain V consists of 3 LG modules and 4 epidermal-growth-factor-like (EG) modules, which have been identified to bind to fibulin-2 and nidogen [28,29]. The yeast 2-hybrid screens indicated that the carboxyl-terminal domains of fibulin-2 interacts with the amino-terminal laminin G domain of SHBG in a steroid-dependent manner, and that estradiol is the most potent ligand. Moreover, aggrecan and versican interact through their lectin domains with 2 different binding sites in domain II of fibulin-2. In addition, fibulin-2 competes for the tenascin-R on the aggrecan and versican C-type lectin-like domain. Therefore, fibulin-2 is a potential mediator of interaction with adhesion receptors or the cytoskeleton [31,32].

## The Role of Fibulin-2 in Carcinogenesis

The dysregulation of fibulin-2 has been revealed as a potential therapeutic target in the development of cancer such as breast cancer, NPC, Kaposi's sarcoma, brain cancer, etc. Interestingly, in lung cancer, acute myeloid leukemia, and pancreatic cancer, it exhibits a pro-tumor effort (Figure 3, Table 1). In these diseases, fibulin-2 could modulate many factors, including vascular endothelial growth factor (VEGF), the secreted metalloproteases ADAMTS-4 and ADAMTS-5, the laminin-binding integrin  $\alpha 3 \beta 1$ , transforming growth factor (TGF)- $\beta$ , and so on (Figure 4).



**Figure 3.** The height of the bar represents the median expression of certain tumor types or normal tissues. (Gene Expression Profiling Interactive Analysis (GEPIA)). The tumor types and English abbreviations are as follows: ACC – adrenocortical carcinoma; BLCA – bladder urothelial carcinoma; BRCA – breast cancer; CESC – cervical squamous cell carcinoma; CHOL – cholangiocarcinoma; COAD – colon adenocarcinoma; DLBC – lymphoid neoplasm diffuse large B-cell lymphoma; ESCA – esophageal carcinoma; GBM – glioblastoma multiforme; HNSC – head-neck squamous cell carcinoma; KICH – kidney chromophobe; KIRC – kidney renal clear cell carcinoma; KIRP – kidney renal papillary cell carcinoma; LAML – acute myeloid leukemia; LGG – brain lower grade glioma; LIHC – liver hepatocellular carcinoma; LUAD – lung adenocarcinoma; LUSC – lung squamous cell carcinoma; OV – ovarian cancer; PAAD – pancreatic adenocarcinoma; PCPG – adrenocortical carcinoma; PRAD – prostate adenocarcinoma; READ – rectum adenocarcinoma; SARC – sarcoma; SKCM – skin cutaneous melanoma; STAD – stomach adenocarcinoma; TGCT – testicular germ cell tumors; THCA – thyroid carcinoma; THYM – thymoma; UCEC – uterine corpus endometrial carcinoma; UCS – uterine carcinosarcoma.

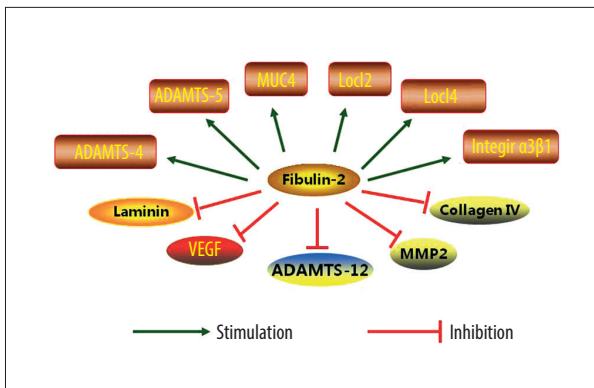
**Table 1.** Biological function of fibulin-2 in tumors.

Number	Tumor types	Function of fibulin-2	Reference
1	Breast cancer	Migration, invasion	[44–46]
		Differentiation	[47]
2	Nasopharyngeal carcinoma	Proliferation, migration, invasion, angiogenesis	[21]
3	Kaposi’s sarcoma	Infection KSHV	[15]
4	Lung adenocarcinoma	Intracellular	[33]
5	Pancreatic cancer	Metastasis, migration, invasion	[34–36]
6	Skin cancer	Invasion, matrix remodeling, metastasis	[41–43]
7	Brain cancer	Proliferation, migration, invasion	[51]

### The Oncogenic Role of Fibulin-2

A number of studies have clearly shown that fibulin-2 has a functional role in various cancers (Table 1). In lung adenocarcinoma, the study conducted by Brandi et al. revealed that the expression of fibulin-2 is overexpressed in lung adenocarcinoma cell lines, which was obtained from the co-expression of mutant K-ras and p53 mice (KP mice) [33]. Furthermore, they showed

that the co-expression of mutant K-ras and p53 cells (KP cells) can express mucoprotein 4 (MUC4), in which it was noted that the nidogen-like domain of MUC4 binds to fibulin-2, and that this relationship would promote the breaching of BM integrity, contributing to the spreading of pancreatic cancer cells [34]. In addition, this combination is shown to enhance the aggressiveness of pancreatic cancer cells. Interestingly, in lung adenocarcinoma, they did not determine whether fibulin-2 has the

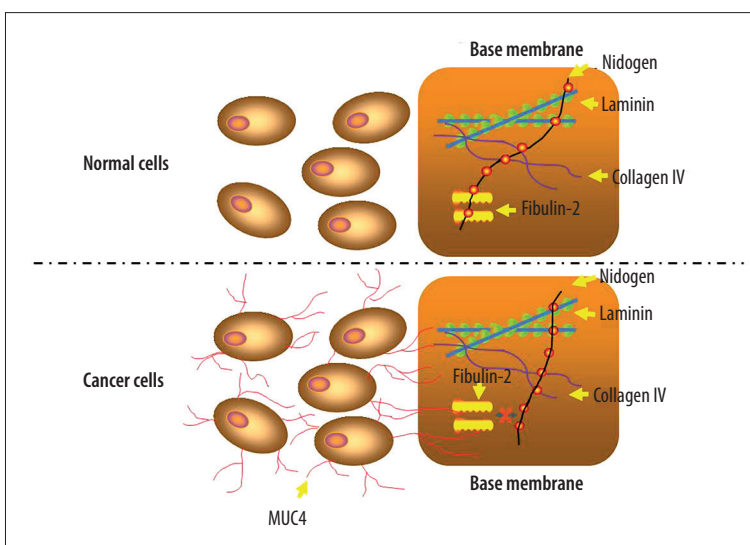


**Figure 4.** Upstream regulating factors and downstream effectors of fibulin-2.

ability to interact with MUC4. Furthermore, Brandi et al. discovered that the deficiency of fibulin-2 resulted in the increase in lysyl oxidase-like 2 (Loxl2) expression and the reduction in lysyl oxidase-like 4 (Loxl4) expression by stably transfecting Fbln2 shRNA into 344SQ cells. This result may infer that fibulin-2 has intracellular roles, which have not yet been uncovered.

The study conducted by Senapati et al. found that fibulin-2 co-localized with the MUC4-NIDO domain in the BM. MUC4 is a large transmembrane type I glycoprotein, which has been indicated to be associated with cancer cell progression and metastasis in pancreatic cancer. The expression of MUC4 is obviously higher in PC tissues, when compared to adjacent normal tissues. In addition, the high level of MUC4 is significantly associated with the progression of PC, and this function may have been induced through the activation of the MAPK pathway, which is associated with HER2 [35]. Another expectation verified in the study conducted by Senapati et al. was the domain-mediated role of MUC4. In an *in vivo* study, mice implanted with MiaPaCa-MUC4 PC cells that express MUC4

were detected to metastasize to the liver, while liver metastasis was not observed in the MiaPaCa-MUC4-NIDOΔ group, which was expressed in MiaPaCa PC cells as lacking the endogenous MUC4 protein [34]. In addition, the colocalization of fibulin-2 and MUC4 was detected in the BM in PC tissue samples. Furthermore, fibulin-2 has been confirmed to be a major component of murine liver blood vessels [36]. MUC4 interacts with fibulin-2, which hinders the normal interaction between fibulin-2 and nidogen. This relationship potentially disrupts the integrity of the BM. Nidogen is widely found in the BM, and its globular structure domains interact with laminin and collagen IV (Figure 5). Overall, these findings imply that in pancreatic cancer, MUC4 hinders the interaction between fibulin-2 and laminin, in addition to the link between fibulin-2 and collagen IV. This function contributes to the BM imperfection, which is the result of promoting PC cell migration and invasion. The study conducted by Missan et al. revealed that the knockdown of fibulin-2 in immortalized/transformed keratinocyte cells reduced cell invasion. This experiment revealed that laminin-binding integrin  $\alpha3\beta1$  has the ability to regulate fibulin-2 expression to promote matrix remodeling and invasion [37]. Integrin  $\alpha3\beta1$  is a main receptor for adhesion to laminin-332 in the epidermis and plays important role in regulating cell-autonomous and paracrine functions in skin tumorigenesis [38–40]. Furthermore, it promotes the tumor growth, invasion, and metastasis in squamous cell carcinoma (SCC) [41,42]. The findings reported by Longmate et al. also indicated that in both neonatal skin and adult wounds,  $\alpha3\beta1$  increases the stability of the BM by adjusting fibulin-2. This conclusion is consistent with aforementioned viewpoints [43].



**Figure 5.** Schematic representation for the proposed action of MUC4-NIDO domain in disrupting the basement membrane integrity.

## Antitumor Activity of Fibulin-2

### The function of fibulin-2 in breast cancer

Fibulin-2 has the ability to regulate cancer processes, such as breast cancer, NPC, Kaposi's sarcoma and astrocytoma (Table 1). Hence, this may implicate fibulin-2 as a potential therapeutic target in tumor treatment. It is noteworthy that the mechanism of fibulin-2 in breast cancer has been very thorough. Recent evidences have revealed that the expression level of fibulin-2. Fibulin-2 has the ability to modulate the cancer processes, such as breast cancer, NPC, Kaposi's sarcoma and Astrocytoma (Table 1). Therefore, this may indicate that fibulin-2 is a potential therapeutic target in tumor therapy. It is worth noting that the mechanism of fibulin-2 in breast cancer is closely related to the progression of breast cancer. Reduced fibulin-2 contributes to loss of basement membrane integrity and skin blistering in mice lacking integrin $\alpha$ 3 $\beta$ 1 in the epidermis. Fibulin-2 has the ability to regulate cancer processes, such as breast cancer, NPC, Kaposi's sarcoma, and astrocytoma (Table 1). Hence, this may implicate fibulin-2 as a potential therapeutic target in tumor treatment. It is noteworthy that the mechanism of fibulin-2 in breast cancer. To our knowledge, the study conducted by Fontanil et al. has proven that different fragments corresponding to A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-12 exosites are employed as bait to screen for molecular partners of the metalloprotease using yeast 2-hybrid assays. Furthermore, they tested the presence of the co-immunoprecipitation of both fibulin-2 and ADAMTS-12. In the end, they speculated that ADAMTS-12 may act as the fibulin-2 potential interacting protein, and that this combination effect could promote anti-tumor effects in breast cancer cells [44]. Furthermore, they found that ADAMTS-12 blocks the process of ADAMTS-5 to cleave fibulin-2 by binding to fibulin-2, contributing to cancer cell retention [45]. Strikingly, according to the BLAST analysis at National Center for Biotechnology Information (NCBI) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)), it was found that spacer-1 of ADAMTS-12, which can select different clones that contain inserts, matched with the carboxyl-end region of fibulin-2, including the final 88 amino acids of the fibulin module. Moreover, it has been confirmed that the secreted metalloproteases ADAMTS-4 and ADAMTS-5 have the ability to digest fibulin-2, and that fibulin-2 can better combine with the latter [21,46]. ADAMTS-1 was also found to degrade fibulin-2. However, fibulin-2 is not cleaved by ADAMTS-1, which is different from ADAMTS-4 and ADAMTS-5. The migration and invasion of breast cancer cells are associated with the fibulin-2 protein digestion. Overall, these direct evidences indicated that fibulin-2 acts as a substrate of ADAMTS, and that this proteolysis could change the cellular microenvironment, and adjust the balance between pro-tumor and antitumor effects. In the meantime,

degradation or protection of fibulin-2 provides novel insights into the development of breast cancer.

The study conducted by Tan et al. suggested that fibulin-2 expression can be observed around collagen IV, when compared to adjacent non-tumor tissues. However, the expression of fibulin-2 is significantly incomplete in malignant breast cancer tissues. For the detailed mechanism, they proposed that fibulin-2 forms a novel protective barrier that is different from the BM, and that this new barrier performs an important role in inhibiting cancer cell penetration and metastasis [47]. Liu et al. collected specimens from 30 cases of breast cancer and the corresponding adjacent normal tissues, and specimen from 32 cases of breast fibroadenoma and the corresponding adjacent normal tissues. In determining the expression difference of the fibulin-2 gene and protein among these tissues, they found that the expression of mRNA and protein of fibulin-2 was significantly decreased in breast cancer, compared with the adjacent normal tissues, and this was related to the degree of tumor differentiation. Furthermore, the expression of fibulin-2 in poorly differentiated tumors was significantly lower, when compared to that in moderately differentiated breast cancer tissues [48]. Therefore, these results suggest that fibulin-2 may have an impact on the invasion, metastasis and proliferation of breast cancer.

### The antitumor functions of fibulin-2 in other cancers

In addition to breast cancer, the anti-tumor effect of fibulin-2 has also been verified in NPC. The knockdown of VEGF and matrix metalloproteinase (MMP)-2 expression has been found to contribute to angiogenesis inhibition. In Fbln2S transfectants, angiogenesis-related genes, and VEGF-165 and VEGF-189 are downregulated. At the same time, the downregulation of MMP-2 was also observed, but there was no significant difference in MMP-9 in NPC. In addition, fibulin-2 stable transfectants exhibited at least a 2-fold downregulation of these proteins. Hence, these ensures that the decrease in MMP-2 and VEGF may provide an explanation to the function of Fbln2S in cancer progression. In subsequent studies, the re-expression of Fbln2S inhibits its proliferation, migration, invasion and angiogenesis *in vivo*, and colony formation *in vitro*. Through their experiment, it was revealed that the long isoform (fibulin-2l) was barely detectable in both normal and tumor tissues, compared with fibulin-2s [49], which is consistent with a previous report [17].

Similar to other cancers, fibulin-2 downregulates the expression of VEGF, which was also confirmed in Kaposi's sarcoma. One study revealed that Kaposi's sarcoma-associated herpesvirus (KSHV) may be linked to the development of tumors by regulating fibulin-2 protein. They also found that the protein and mRNA expression of fibulin-2 decreased by 50-fold and

26-fold in dermal microvascular endothelial cells (DMVECs) infected by KSHV [40]. This shows that fibulin-2 has antitumor functions of inhibiting glioma cell proliferation, migration and invasion in brain cancer [50].

### The association between fibulin-2 and non-cancerous disorders

The study conducted by Schaeffer et al. revealed that in spinal nerve tissues, fibulin-2 was observed in the posterior half-sclerotome, and both the mRNA and protein were also found. In detail, they found the increase in fibulin-2 protein levels in reactive astrocytes at the lesion site of the adult central nervous system (CNS) injury mouse model. This confirms that fibulin-2 is involved in the contact-repulsion in the posterior half-sclerotome. Overall, these results suggest that fibulin-2 may be a possible novel therapeutic target for brain and spinal cord injury [51].

The study conducted by Radice et al. revealed that transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), an anti-inflammatory cytokine, has pro-neurogenic effects on adult neural stem cells, and that this is induced by regulating the major mediator, fibulin-2. In detail, it was identified that fibulin-2 increased by 477-fold in the functional genomic analysis through microarray analysis and qRT-PCR analysis [52]. Various studies have shown that transforming growth factor (TGF)- $\beta$  is a multifunctional growth factor, and it has a great important role in adjusting cell growth, differentiation and repair in various tissues. TGF- $\beta$  has been confirmed to be strongly associated with many pathological changes, such as the progression of cancer, fibrosis and autoimmune disease [53]. The study conducted by Zhang et al. revealed that fibulin-2 can enhance TGF- $\beta$  activation to inhibit Ang II-induced cardiac remodeling [54]. For the detailed mechanism in myocardial infarction (MI), fibulin-2 could significantly upregulate both the mRNA and protein levels of TGF- $\beta$ , when compared to Fbln2 null mice. Furthermore, compared with Fbln2 null mice, the phosphorylation of Smad2, TAK1, and p38 MAPK significantly increased in wild-type (WT) mice. The study conducted by Tsuda et al. revealed that in the MI model, the decrease in fibulin-2 contributes to the downregulation of TGF- $\beta$ , which is against ventricular dysfunction [55]. Unexpectedly, they found that the loss of fibulin-2 can improve the survival of mice after MI by attenuating ventricular dysfunction. In detail, the mRNA level for collagen I, collagen III and MMP-2 significantly decreased in Fbln2 null mice, when compared to WT mice. Furthermore, changes in cleaved caspase-3 were also found in both WT mice and Fbln2 null mice. The study conducted by Khan et al. also confirmed that fibulin-2 is a positive modulator of TGF- $\beta$  [56]. It can be concluded that these findings indicate that fibulin-2 interacts with TGF- $\beta$ , and that this may be an attractive potential therapeutic target for preventing tissue remodeling, providing a new approach in curing tumors

by interfering with the TGF- $\beta$  signaling pathway. Fibulin-2 may be competing in TGF- $\beta$ 1 binding sites with other ECM proteins, especially fibulin-1, in order to release TGF- $\beta$ 1 from its latent complex [57]. The study conducted by Kanan et al. revealed that in retinal detachment, fibulin-2 can enhance the cellular adhesion between the retina and retinal pigment epithelium, and prevent cell migration [58]. Furthermore, this conclusion, which demonstrates that fibulin-2 is necessary for retinal development, was also confirmed in another study [59].

In the vascular wall and atherosclerotic plaques, simvastatin can induce the significant increase of both the mRNA and protein level of fibulin-2 through a RhoA and Rho-kinase-mediated mechanism. This result indicates that fibulin-2 has an important role maintaining plaque stability [60,61]. Fibulin-2 is associated with arterial stiffness, and different levels of fibulin-2 and variations in the Fbln2 gene (rs3732666 and rs1061376) may account for the development of hypertension [62]. It was also found that fibulin-2 protein is significantly elevated in hypertension [63].

The internal elastic lamina (IEL), which is located beneath the endothelium of blood vessels, provides elasticity and recoil to the vessel wall, resists chemical and mechanical stresses, and prevents the plasma components from touching the smooth muscle cells (SMCs). Chapman et al. used the DKO mouse model, double knockout mice of fibulin-2 gene (Fbln2) and fibulin-5 gene (Fbln5), to test the vessel integrity during a variety of pathological insults. Interestingly, they found that fibulin-2 and fibulin-5 could cooperate to maintain vessel integrity by forming an IEL. Furthermore, they found that the luminal surface of IEL is maintained in the adult Fbln5<sup>-/-</sup> aorta. Moreover, a knockout study revealed that fibulin-2 is dispensable for elastic fiber development. However, the IEL is strongly disrupted in the DKO aorta. Thus, they speculated that fibulin-2 may have a similar molecular structure with fibulin-5, and it can compensate for fibulin-5 [64].

The study conducted by Perez-Rico et al. confirmed that fibulin-2 increased around the blood and lymphatic vessels in subepithelial connective tissues. In this immunohistochemical study, they found that fibulin-3 and fibulin-2 expression was co-localized in the pathologic samples [30]. The study conducted by Gerhard et al. revealed that Fbln2 null mice exhibited severe defects in musculature and accumulated white fat, both *in vivo* and *in vitro*. This result may be associated with the bone morphogenetic protein (BMP) signaling pathway [65].

### Conclusions

Multiple groups have independently demonstrated the critical role of fibulin-2 in carcinogenesis. It is well-known that

metastasis is an important biological feature of malignant tumors. The prognosis of patients with cancer is cancer type dependent. The mortality of some patients is caused by primary cancer directly, but some caused by tumor metastasis. Therefore, the death caused by cancer metastasis is an urgent problem. Tumor development is the result of multi-factor and multi-gene coordination. As it is known, the ECM serves as a bridge to regulate organogenesis and tissue homeostasis, which in turn affects the progression of inflammation and other diseases [66–68]. Fibulin-2 protein provides a potential therapeutic target for cancer treatment. In human cancer, tumor initiation, proliferation, migration and metastasis are linked to the composition of the ECM [69–71]. Fibulin-2 has the ability to interact with the ECM, such as integrin  $\alpha 3\beta 1$ , collagen IV, and laminin-332, thereby providing cells with physical and chemical cues that act in connection with growth factors to support survival and proliferation. The upregulation or downregulation of fibulin-2 is vital to either the promotion or inhibition of cancer invasion and metastasis.

It was indicated that fibulin-2 is closely correlated to the occurrence and development of many tumors. Furthermore, the role of fibulin-2 in promoting cancer or suppressing cancer has been identified in various cancers, but the specific

mechanisms for the progression of tumors remains to be determined. In some tumors, fibulin-2 has a clear mechanism, such as in breast cancer and pancreatic cancer. And fibulin-2 binds to the proteins of the ADAMTS family to affect tumor progression. This can be used as an indicator of molecular biology malignancy. Moreover, the BMs secrete MUC4, which interacts with fibulin-2 to affect tumor progression. In addition, fibulin-2 can adjust the expression of VEGF and MMP family proteins to inhibit migration and invasion. Besides, fibulin-2 also plays a vital role in other non-neoplastic diseases, and its effects are closely correlated to cell membranes or blood vessels. More importantly, fibulin-2 can influence the development of disease by affecting the TGF- $\beta$  signaling pathway. However, the mechanism associated with the TGF- $\beta$  signaling pathway has not been confirmed in tumors. In conclusion, regardless of whether fibulin-2 can promote cancer or suppress cancer, this would open a new research perspective for tumor detection and diagnosis, and hopefully, this could serve as a potential molecular target for cancer treatment in the near future.

### Conflicts of interest

None.

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