Potential of Fibulin2 as a therapeutic target against cancer and as a diagnostic marker (Review)

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Abstract. Cancers are not merely composed of tumor cells; rather, they constitute a complex tumor microenvironment (TME) comprising diverse cell types and noncellular factors. Extracellular matrix (ECM) represents a critical component of the TME. Fibulin2 participates in ECM formation in various tumors, and its altered expression in multiple malignancies can affect tumor cell proliferation and invasiveness. Additionally, Fibulin2 has emerged as a potential biomarker in various cancer types and serves a pivotal role in tumor progression. Consequently, therapeutic strategies targeting Fibulin2 hold considerable promise. However, the research and development of Fibulin2-targeted therapeutics has progressed at a relatively slow pace. Therefore, the roles and mechanisms of Fibulin2 in various malignancies, along with investigations into its utility as a biomarker, are comprehensively discussed in the present review. This may provide valuable guidance for the clinical translation and application of Fibulin2-targeted therapies, and the utilization of Fibulin2 as a predictive biomarker.

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Abbreviations: TME, tumor microenvironment; CAF, cancer-associated fibroblast; ECM, extracellular matrix; BM, basement membrane; TIMP, tissue inhibitor of metalloproteinases; WB, western blotting; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AI, artificial intelligence; HCM, hypertrophic cardiomyopathy; CRC, colorectal cancer

Key words: tumor, Fibulin2, role, mechanism, biomarker

Contents

1. Introduction

Cancer is a paramount societal, public health and economic challenge in the twenty-first century, accounting for nearly 16.8% of global deaths (one in six deaths) and 22.8% of deaths attributable to non-communicable diseases (1). The disease is responsible for 3 in 10 premature deaths from non-communicable diseases worldwide (30.3% among individuals aged 30-69 years), ranking amongst the three principal causes of mortality within this demographic across 177 of 183 nations (2). In the United States in 2025, there will be ~2,041,910 new cancer diagnoses, equating to ~5,600 cases daily (3). Oncological research has predominantly focused on intrinsic characteristics of neoplastic cells, with comparatively limited attention to extracellular stromal components. The extracellular matrix (ECM) constitutes a crucial element of tumor stroma. Alterations in ECM protein expression represent significant mechanisms influencing tumor cell proliferation, migration and invasion, with Fibulin2 serving as a typical protein. Even intracellular modifications in Fibulin2 expression may have a substantial impact on the ECM due to the secretory properties of Fibulin2. Furthermore, Fibulin2 is considered to be a promising biomarker in multi-tumor research contexts (4-7).

Fibulin2 was initially characterized by Pan *et al* (8), who identified it in a murine fibroblast cDNA library using the Fibulin1 cDNA probe. Fibulin2 is expressed in humans and mice (Fig. 1A and B). In human physiology, Fibulin2 is highly expressed in cardiac tissue, placenta, testes and the urinary

bladder, but expressed at low levels in brain tissue, splenic parenchyma and bone marrow (Fig. 1A). Fibulin2 exhibits aberrant expression across malignancies of diverse organs (9), such as colorectal cancer (4) and lung cancer (10). The protein structures of human and murine Fibulin2 exhibit remarkable homology (8,11) (Fig. 2A and B), providing a robust theoretical basis for using murine cellular systems and animal models as surrogates for human studies in oncological research. In the Na, I, II and III domains, the amino acid sequence identity is ~90%, whereas the Nb domain shows only 62% sequence identity (8,11). Human Fibulin2 contains 18 exons (11) (Fig. 3), while mouse Fibulin2 contains 17 exons (8). Surface plasmon resonance and/or solid-phase microplate analyses have shown that Fibulin2 has high binding affinity for Perlecan (12,13), laminin5 (14,15), Fibronectin (16), collagen XVIII (17), Aggrecan, Versican, Brevican lectin domains (18), the C-terminal region V of Perlecan (19), and the short arms of Laminin-5 and Laminin-1 (20), which helps maintain the ECM (21). Consequently, ECM stabilization mediated by Fibulin2 represents a pivotal mechanism underlying tumor suppression.

Nevertheless, Fibulin2 does not universally exert inhibitory effects across all malignancies; rather, it acts as a promoter in certain neoplastic contexts. For instance, increased Fibulin2 expression inhibits cell proliferation in nasopharyngeal carcinoma (22), Kaposi's sarcoma (23) and breast cancer (24). Conversely, suppression of Fibulin2 expression effectively attenuates lung adenocarcinoma progression (10). Therefore, the present review summarizes the diverse roles of Fibulin2 across various malignancies, while examining underlying mechanisms, alongside comprehensive analysis of the potential of Fibulin2 as a biomarker. Regarding the mechanistic influence of Fibulin2 on tumor development and its utility as a tumor marker, several critical issues persist. The mechanisms governing Fibulin2 function across numerous malignancies require further elucidation. The role and mechanistic basis of the effects of Fibulin2 on tumor stroma remain poorly understood. Furthermore, its clinical application as a biomarker is not yet mature. Therefore, the present overview provides guidance for the clinical translation of Fibulin2 as both a therapeutic target and predictive marker in oncological diseases.

2. Summary of the mechanisms and roles of Fibulin2

Fibulin2 acts as an upstream regulator of the Ras-MEK-ERK1/2 pathway in hepatocellular carcinoma and the TGF-β/Smad2/TGFB induced factor homeobox 2 (TGIF2) pathway or β-catenin in gastric cancer (25-27). Differences in Fibulin2 expression between tumor tissues and adjacent normal tissues lead to the activation of these pathways (25,26). However, to the best of our knowledge, the factors causing altered Fibulin2 expression in liver and gastric cancer, as well as its upstream regulators, remain unclear. In p53-null immortalized mouse keratinocytes and RasV12-transformed mouse keratinocytes, which are skin cancer models, Fibulin2 is regulated by the upstream molecule Integrin α3β1 (28). Changes in *Fbln2* expression in lung adenocarcinoma cells alter the expression of downstream Integrin genes, including *Itga1*, *Itga2*, *Itga10* and *Itgb1* (10). A reciprocal regulatory relationship may exist between Fibulin2 and certain Integrin proteins, which warrants further investigation. In nasopharyngeal carcinoma, *Fbln2* acts as an upstream inhibitor of *VEGF-165*, *VEGF-189* and *MMP-2*. The upstream factors regulating *Fbln2* expression in nasopharyngeal carcinoma remain to be elucidated (22). Given that Fibulin2 is a secreted protein, it likely exerts autocrine or paracrine effects on its producing cells or neighboring cells, potentially binding to receptors such as Integrin proteins to establish feedback loops. However, such feedback mechanisms have not received sufficient attention in current tumor-related studies, and further research is needed to demonstrate their existence across various tumor types. In the extracellular space, Fibulin2 interacts with multiple proteins: Integrin β1 in non-small cell lung cancer (29), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-5 and ADAMTS-12 in breast cancer (30), Nidogen1 in colorectal cancer (CRC) (31), and mucin 4 (MUC4) in pancreatic cancer (32). These are protein-protein interactions rather than upstream-downstream regulatory relationships (Fig. 4). In breast cancer, ADAMTS-5 cleaves Fibulin2, and this role can be inhibited by ADAMTS-12 (30). Differences in mechanisms of Fibulin2 regulating tumor development across tumor types are shown in Table I.

3. Roles and mechanisms of Fibulin2 in promoting various tumor types

In the enteric nervous system of patients with colorectal carcinoma, Ndrg4 expression is absent or markedly reduced (33), while enteric nerve cells concurrently secrete higher levels of Fibulin2. Fibulin2 and Nidogen1 promote human colorectal carcinoma cell proliferation and migration. A study substantiated its conclusions by stimulating cells with Fibulin2 and Nidogen1 proteins, then assessing cell proliferation and migration (31). However, the study failed to demonstrate the isolated effects of Fibulin2 on CRC cells and mechanisms (31). In an experiment investigating subcutaneous tumor formation in lung adenocarcinoma, tumors were generated in mice by injecting tumor cells in PBS subcutaneously into the right flank. Tumors formed by *Fbln2*-knockdown cells exhibited reduced size, weight and tissue consistency compared with those formed by normal cells (10). *In vitro* experiments revealed that *Fbln2*-knockdown cells, compared with normal cells, exhibited markedly attenuated type I collagen adhesion capacity alongside markedly reduced tumor cellular pseudopodia (10). *Itga1*, *Itga2*, *Itga10* and *Itgb1* were notably reduced following *Fbln2* depletion in lung adenocarcinoma cells (10). These genes encode Integrin proteins α1β1, α2β1, α10β1 and α11β1, respectively, which can bind to collagen (34). In summary, Fibulin2 is closely associated with other ECM proteins in lung adenocarcinoma. Aberrant Fibulin2 expression leads to abnormal changes of other ECM proteins such as integrin proteins and collagen (10). Immunohistochemistry revealed Fibulin2 upregulation in lung cancer tissues compared with adjacent normal tissues. *In vitro* validation relied exclusively on *Fbln2* knockdown. Incorporating overexpression experiments would enhance methodological rigor (10). In p53-null immortalized mouse keratinocytes and RasV12-transformed mouse keratinocytes, knockdown of *Fbln2* diminished cell invasion; however, subcutaneous injection of *Fbln2*-knockdown cells failed to produce a notable reduction in tumor growth volume

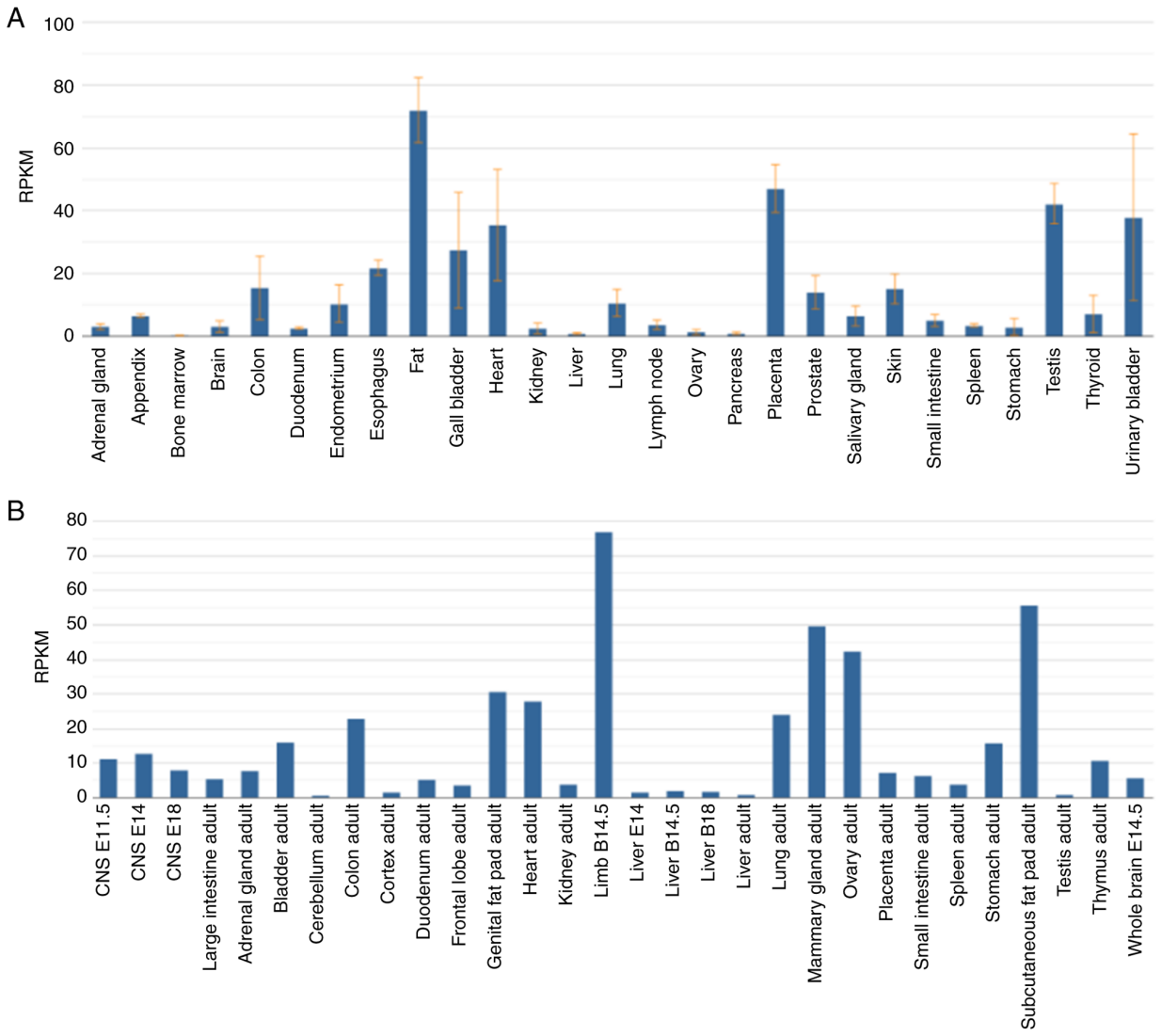

Figure 1. Distribution of *Fbln2* mRNA in (A) human and (B) mouse tissues. RNA-sequencing data from the National Center for Biotechnology Information were employed for the generation of these figures. Data sources for human and mouse: https://www.ncbi.nlm.nih.gov/gene/2199 and https://www.ncbi.nlm.nih.gov/gene/14115. E, embryo; CNS, central nervous system; RPKM, reads per kilobase per million mapped reads.

compared with controls (28). Fibulin2 expression was regulated by Integrin α3β1 and promoted the invasion of immortalized/transformed keratinocytes (28). The research was limited by overly simplistic mechanistic exploration, necessitating further investigation of more specific mechanisms. In mouse non-small cell lung cancer tissue sections, ECM-expressed Fibulin2 interacts with cell membrane-expressed Integrin β1. The interaction between Integrin β1 and Fibulin2 mediates tumor cellular adhesion to the ECM while conferring chemotherapy drug resistance (29). The study employed immunofluorescence double-labeling experiments to demonstrate partial colocalization of Integrin β1 and Fibulin2, subsequently inferring disease mechanisms (29). Additional experiments are necessary, including experiments verifying whether Integrin β1 is a receptor of Fibulin2 and assessing its impact on tumors. In human hepatocellular carcinoma cells, Fibulin2 promotes carcinoma cell proliferation while inhibiting apoptosis through Ras-MEK-ERK1/2 signaling pathway activation (25). In *in vitro* experiments on liver cancer cells, *Fbln2* knockdown and overexpression were performed, and the expression changes of key proteins in the Ras-MEK-ERK1/2 signaling pathway were detected by western blotting (WB) (25). Fibulin2 exerts tumor-promoting effects in both *in vitro* and *in vivo* experiments (25). However, this merely establishes an association between Fibulin2 and the Ras-MEK-ERK1/2 signaling pathway rather than a direct regulatory relationship, as indirect mechanisms may exist. Future research could use glutathione S-transferase (GST) pull-down to further verify the direct interactions between Fibulin2 and these pathway proteins. Fibulin2 expression alterations within neoplastic tissues and corresponding modifications in tumor cell proliferation, migration and invasion (demonstrating the pro-carcinogenic properties of Fibulin2) are shown in Table II.

Fibulin2 is associated with Integrin proteins in mechanistic investigations of lung adenocarcinoma (10), non-small cell lung carcinoma (29), p53-null immortalized murine keratinocytes and RasV12-transformed murine keratinocytes (28). In a hepatocellular carcinoma study, the authors did not study Integrin proteins (25), the Ras-MEK-ERK1/2 signaling

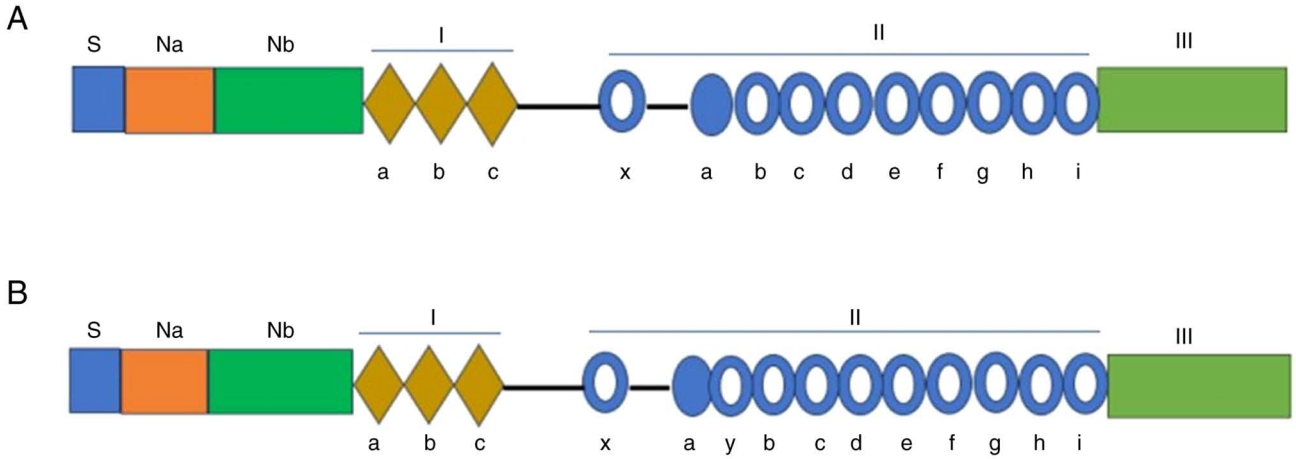

Figure 2. Protein structure of human Fibulin2 and mouse Fibulin2. (A) Diagram of the protein domains of human Fibulin2 starting with the S, Na and Nb subdomains, followed by domain I, II and III. Diamonds indicate anaphylatoxin-like motifs in domain I. Solid circles represent EGF-like repeats, while those depicted as hollow rounds contain the consensus sequence for calcium binding. (B) Diagram of the protein domains of mouse Fibulin2 showing that the Fibulin2 protein comprises S, Na, Nb, I, II and III domains. At the N-terminus are domains Na and Nb, which span 408 amino acids, and include the 'Na' subdomain containing 22 cysteines and the 'Nb' subdomain devoid of cysteine. Domain I consists of three anaphylatoxin-like motifs. Domain II encompasses 11 EGF-like repeats, all of which except IIa possess a consensus sequence for calcium binding. Domain I is connected via a 50-amino-acid linker segment to the first EGF module (IIx) of domain II, which is followed by another 34-amino-acid linker segment and then 10 EGF modules. Domain III is depicted as a light green box. Na, N-terminal cysteine-rich; Nb, N-terminal cysteine-free; S, signal peptide.

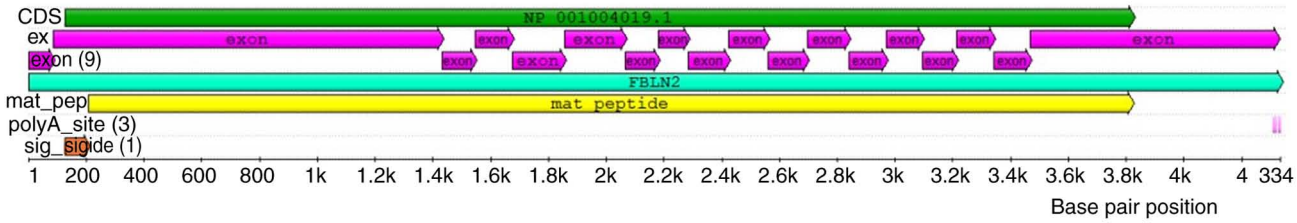

Figure 3. Structure of human *Fbln2* transcripts. The figure shows the CDS, 18 exons, the positions of poly(A) signals and sites, and distinct domains. CDS, coding sequence; sig, signal peptide. The diagram was generated by the software UGENE 36.0 (http://ugene.net/ugene/).

pathway constitutes a crucial downstream pathway of Integrin according to previous investigations (35-37). Integrins, as principal ECM cell-surface receptors, represent essential mediators of cellular communication with the tumor microenvironment (TME) (35,38,39). Integrins comprise a family of 24 heterodimeric receptors (40). Integrins consist of two subunits: α and β subunits, which have extensive extracellular domains and short cytoplasmic domains (41,42). These interact with ECM proteins outside the cell membrane while connecting to downstream pathways, including the Ras-MEK-ERK1/2 and FAK/SFK pathways in the cytoplasm (35). When ECM protein, including Fibulin2, Nidogen, Collagen and Laminin, or Integrin expression is altered, fibrosis or remodeling can change tissue stiffness, and downstream Integrin pathways such as the Ras-MEK-ERK1/2 and FAK/SFK pathways may be activated, affecting tumor migration, invasion and proliferation (35,43,44) (Fig. 5).

Limitations. Most studies examining the tumor-promoting functions of Fibulin2 are simplistic. Future research may include additional experiments to enhance persuasiveness. Furthermore, other factors impact future drug development. For example, studies have used immortalized cell lines such as the HCT116 and Caco-2 human CRC cell lines (31), and mouse models such as nude mice subcutaneously injected with SNU398 cells (25). These models differ substantially from primary cells and the human body, potentially leading to the failure of related drugs in human applications. Inferring disease mechanisms solely from protein spatial associations, exemplified by partial Integrin β1 and Fibulin2 colocalization in lung carcinoma tissue sections (29), may fail to establish causative relationships, resulting in ineffective therapeutic targets. Focusing on single signaling pathways, such as the Ras-MEK-ERK1/2 signaling cascade in hepatocellular carcinoma (25), while disregarding other potential pathways, may lead to compensatory pathway activation and therapeutic resistance after single-target inhibition, thereby limiting the applicability of conclusions. To the best of our knowledge, the impact of changes in Fibulin2 expression on tumor stroma activity [which depends on cancer-associated fibroblasts (CAFs)] has not been investigated. Tumor stromal activity and changes in CAF properties substantially influence tumor development. In advanced-stage malignancies, activated stroma promotes further genetic and epigenetic alterations in carcinoma cells (45-47) while supporting carcinoma progression (48). CAFs are one of the cell types in the TME. CAFs exhibit proliferative, migratory and high secretory activities (49,50). Due to their altered morphology

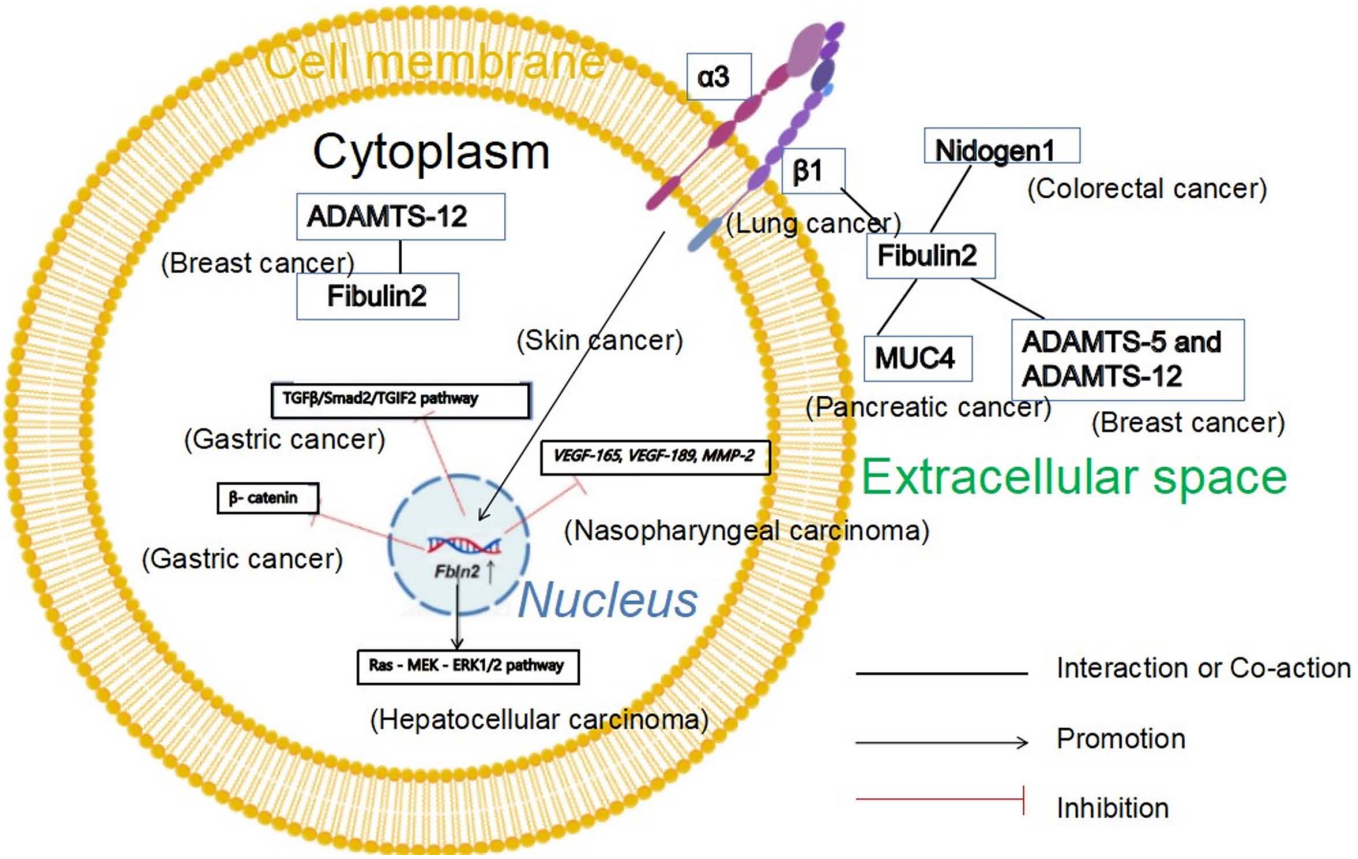

Figure 4. Mechanistic roles of Fibulin2 in various tumors. The yellow circle denotes the phospholipid bilayer, and the small blue circle inside represents the nucleus. Solid lines indicate an interaction or co-action relationship between molecules. Arrow-headed lines represent a positive promoting relationship between molecules, and red lines denote an inhibitory relationship between molecules. Overexpression of *Fbln2* in gastric cancer cells results in diminished expression of β-catenin, phosphorylated-Smad2 and TGIF2 in the TGF-β/Smad2/TGIF2 pathway. In liver cancer cells, overexpression of *Fbln2* activates the Ras-MEK-ERK1/2 pathway. In nasopharyngeal carcinoma cells, overexpression of *Fbln2* leads to decreased expression of *VEGF-165*, *VEGF-189* and *MMP-2*. Overexpression of ADAMTS-12 and Fibulin2 in breast cancer cells can inhibit tumor development. Fibulin2 and Integrin β1 exhibit partial colocalization near the cell membrane in non-small cell lung cancer tissue sections. As secreted proteins, Fibulin2 and Nidogen1 collectively promote the development of CRC. The interaction between MUC4 and Fibulin2 promotes the development of pancreatic cancer. ADAMTS-5, ADAMTS-12 and Fibulin2 are secreted proteins. In breast cancer, ADAMTS-5 cleaves Fibulin2, and this role can be inhibited by ADAMTS-12. BioGDP.com was used to generate figures (https://biogdp.com/?tg=CFXL). ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; MUC4, mucin 4; TGIF2, TGFB induced factor homeobox 2.

[multispindled rather than single-spindled (51)] and *Acta2* and prolyl endopeptidase expression, CAFs are frequently referred to as myofibroblasts (50). CAFs secrete ECM factors, including tenascin, periostin, secreted protein acidic and rich in cysteine, and collagens (51-58). The expression levels of MMPs and other enzymes that degrade and metabolize the ECM are increased in CAFs, enabling cell penetration through the ECM (59-61). CAFs also secrete high levels of growth factors, cytokines and chemokines, promoting intrinsic malignant hallmarks of carcinoma cells through autocrine and paracrine mechanisms (62). In these studies where Fibulin2 had a tumor-promoting effect (10,25,28,31), while Fibulin2 was secreted by tumor cells and acted on the stroma, it remained unclear how the stroma reciprocally affects tumor cells. Whether tumor cell secretion of Fibulin2 impacts CAFs requires further exploration.

4. Roles and mechanisms of Fibulin2 in inhibiting various tumor types

Alterations in Fibulin2 expression in tumor tissues and corresponding changes in tumor cell proliferation, migration and invasion (demonstrating the anti-carcinogenic properties of Fibulin2) are summarized in Table III. In human gastric cancer, Fibulin2 expression in malignant tissues is reduced compared with that in adjacent normal tissues, and is negatively associated with β-catenin (26). β-catenin is a bifunctional protein with both cell adhesion and signal transduction activities, and was initially identified through a study of the cell adhesion molecule E-cadherin (63). β-catenin is an essential component of the Wnt/β-catenin signaling pathway, serving a pivotal role in gastric carcinoma pathogenesis and progression (64). A study has established direct or indirect regulatory relationships between the Fibulin family and β-catenin, with both substantially influencing gastric carcinoma development (65). β-catenin is typically localized in the cytoplasm, with its expression suppressed through ubiquitin/proteasome-mediated protein degradation (66). Following construction of *Fbln2* overexpression plasmids and transfection of them into AGS and SGC-790 human gastric cancer cell lines, WB analysis has confirmed that Fibulin2 overexpression downregulated β-catenin and its downstream c-myc and cyclin D1, thereby inhibiting the proliferation of gastric cancer cells (26). Immunohistochemistry demonstrated downregulation of Fibulin2 in gastric cancer

Table I. Differences in mechanisms of Fibulin2 regulating tumor development across tumor types.

A, Pro-carcinogenic

First author/s, year	Tumor type	Mechanism	(Refs.)
Baird *et al*, 2013	Lung adenocarcinoma	*Fbln2* promotes the adhesion of tumor cells to type I collagen and the expression of *Itga1*, *Itga2*, *Itga10* and *Itgb1*	(10)
Vaes *et al*, 2021	CRC	Fibulin2 and Nidogen1 jointly promote tumor development	(31)
Hu *et al*, 2023	Hepatocellular carcinoma	Fibulin2 facilitates the activation of the Ras-MEK-ERK1/2 signaling pathway	(25)
Missan *et al*, 2014	Immortalized/transformed keratinocytes	Fibulin2 expression is regulated by Integrin α3β1	(28)

B, Anti-carcinogenic

First author/s, year	Tumor type	Mechanism	(Refs.)
Law *et al*, 2012	Nasopharyngeal carcinoma	*Fbln2* inhibits the expression of *VEGF-165*, *VEGF-189* and *MMP-2*	(22)
Ma *et al*, 2019	Gastric cancer	Fibulin2 downregulates β-catenin and its downstream c-myc and cyclin D1	(26)
Zhou *et al*, 2025	Gastric cancer	Fibulin2 inhibits the TGF-β/Smad2-TGIF2 pathway	(27)
Senapati *et al*, 2012	Pancreatic cancer	MUC4 interacts with Fibulin2, disrupting the intact BM and promoting tumor development	(32)
Fontanil *et al*, 2017; Fontanil *et al*, 2014	Breast cancer	Fibulin2 and ADAMTS-12 jointly inhibit tumor development; ADAMTS-5 cleaves Fibulin2, promoting tumor development	(30,80)

ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BM, basement membrane; CRC, colorectal cancer; MUC4, mucin 4; TGIF2, TGFB induced factor homeobox 2.

tissues compared with adjacent normal tissues (26). *In vitro* validation relied exclusively on *Fbln2* overexpression, and incorporation of knockdown experiments would enhance methodological rigor (26). Another study has confirmed that reduced Fibulin2 expression in gastric carcinoma tissues promotes tumor cell proliferation and metastasis through activation of the TGF-β/Smad2/TGIF2 pathway (27). The study used normal and *Fbln2*-knockdown human gastric cancer cell lines for RNA sequencing, demonstrating upregulation of the TGF-β signaling pathway in *Fbln2*-knockdown gastric cancer cells (27). Following *Fbln2* knockdown and overexpression in gastric carcinoma cells, WB analysis revealed corresponding increases and decreases in Smad2 and TGIF2 phosphorylation (27). Addition of TGF-β signaling pathway inhibitor reversed these expression changes (27). The specific mechanism by which decreased *Fbln2* expression in cells alters the activity of the TGF-β pathway requires further exploration. This establishes an association rather than a direct regulatory relationship between Fibulin2 and the TGF-β/Smad2/TGIF2 pathway, as indirect mechanisms cannot be excluded. Future research could use GST pull-down to demonstrate direct interactions between Fibulin2 and pathway proteins.

Kaposi's sarcoma, which originates from human vascular endothelial cells, is characterized by vascular proliferation caused by HIV infection of endothelial cells (23). In cutaneous microvascular endothelial cells after 10 days of infection, Fibulin2 protein and mRNA expression are decreased by 50- and 26-fold, respectively. Simultaneously, mRNA levels of the ECM-binding partners fibronectin and tropoelastin are decreased 5- and 25-fold, respectively. This weakens the binding of Fibulin2 to ECM proteins, including fibronectin and tropoelastin, compromising basement membrane (BM) stability and promoting tumor progression (23). Fibronectin is crucial for numerous cell functions, including migration, proliferation and differentiation (67). Transcriptional downregulation of fibronectin has been associated with highly metastatic breast carcinoma cells in murine models (68). Tropoelastin is the soluble precursor of elastin, inducible by ultraviolet irradiation and degradable by MMP-12 (69). Loss of tropoelastin causes developmental tissue disorders, including aneurysms, atherosclerosis and reduced skin elasticity (70). Tropoelastin and fibronectin are essential ECM proteins critical for wound healing (71). The study investigating Kaposi's sarcoma failed to establish causative relationships

Table II. Changes in Fibulin2 expression in tumor tissue and changes in tumor cell proliferation, migration and invasion (Fibulin2 exerts a pro-carcinogenic effect).

First author/s, year	Tumor	Tumor vs. non-tumor tissue	Fibulin2 expression	Proliferation	Migration	Invasion	(Refs.)
Vaes *et al*, 2021	CRC	Carcinoma vs. paracarcinoma	Upregulated	Increased after Nidogen1 and Fibulin2 stimulation	Increased after Nidogen1 and Fibulin2 stimulation		(31)
Baird *et al*, 2013	Lung adenocarcinoma	Carcinoma vs. paracarcinoma	Upregulated	Decreased after knockdown of *Fbln2*	Decreased after knockdown of *Fbln2*	Decreased after knockdown of *Fbln2*	(10)
Hu *et al*, 2023	Hepatocellular carcinoma	Carcinoma vs. paracarcinoma	Upregulated	Decreased after knockdown of *Fbln2*; increased after overexpression of *Fbln2*			(25)

CRC, colorectal cancer.

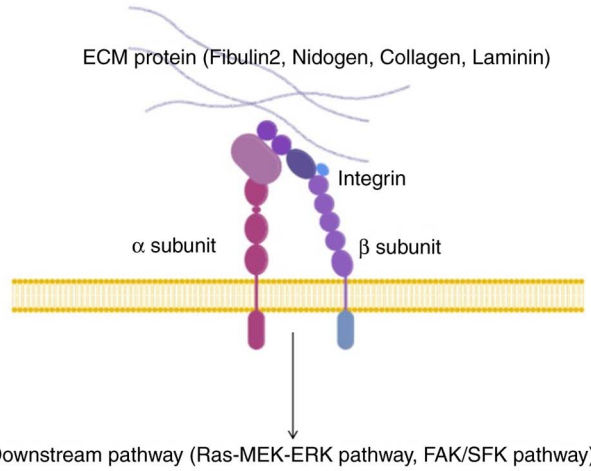

Figure 5. Changes in the ECM activate Integrin-mediated downstream signaling pathways. Integrin is a membrane protein comprising α and β subunits. Extracellularly, Integrin is influenced by the expression of ECM proteins. Alterations in the expression of ECM proteins such as Fibulin2 may activate Integrin-mediated downstream signaling pathways, such as the Ras-MEK-ERK1/2 signaling pathway, thus affecting tumor cell proliferation. ECM, extracellular matrix; FAK, focal adhesion kinase; SFK, src family kinases.

between Fibulin2 alterations and changes in fibronectin or tropoelastin (23). Future research should include cellular *Fbln2* knockdown and overexpression for validation.

In astrocytoma research, U251 cells (a human glioma cell line) exhibited reduced migration and invasion following Fibulin2 overexpression. The conclusion that Fibulin2 inhibits astrocytoma was confirmed by comparing *Fbln2*-overexpressing U251 cells with negative controls transfected with empty vectors (7). A previous study revealed that across different astrocytoma grades, Fibulin2 inhibits tumor development (7). The authors suggested an ECM-stabilizing function of Fibulin2; however, experimental verification is required (7). During pancreatic carcinoma development, MUC4 protein expression is markedly upregulated on pancreatic carcinoma cell surfaces and MUC4 binds to Fibulin2 (32). This binding interferes with the normal interaction between Fibulin2 and the G1 domain of Nidogen (NIDO), disrupting BM integrity (32) (Fig. 6). Tumor cells consequently breach the BM more readily, facilitating invasion and metastasis. In pancreatic cancer tissue sections, immunofluorescence double-labeling experiments have shown colocalization of MUC4 and Fibulin2 (32). However, immunofluorescence double-labeling experiments merely suggest potential connections between these proteins and do not convincingly demonstrate the roles of MUC4 and Fibulin2 in pancreatic carcinoma (32). Future studies may use conditional knockout of MUC4 and Fibulin2 in murine models for further validation. Additionally, numerous previous articles and reviews have erroneously characterized Fibulin2 as an oncogenic protein in pancreatic carcinoma when citing the conclusions of this study about pancreatic carcinoma. The original study provides no evidence to suggest that Fibulin2 promotes pancreatic carcinoma metastasis and invasion (32).

Breast carcinoma is a prevalent malignancy in women with a high incidence (72). In a breast carcinoma study, transfection of *Fbln2* plasmids into human breast carcinoma cell lines reduced tumor cell migration and invasion compared

Table III. Changes in Fibulin2 expression in tumor tissue and changes in tumor cell proliferation, migration and invasion (Fibulin2 exerts an anti-carcinogenic effect).

First author/s, year	Tumor	Tumor vs. non-tumor tissue	Fibulin2 expression	Proliferation	Migration	Invasion	(Refs.)
Law *et al*, 2012	Nasopharyngeal carcinoma	Nasopharyngeal carcinoma biopsy tissue vs. non-tumor tissue	Downregulated	Decreased after *Fbln2s* overexpression	Decreased after *Fbln2s* overexpression	Decreased after *Fbln2s* overexpression	(22)
Ren *et al*, 2016	Glioma			Decreased after Fibulin2 overexpression	Decreased after Fibulin2 overexpression	Decreased after Fibulin2 overexpression	(7)
Zhang *et al*, 2020	Breast cancer			Decreased after Fibulin2 overexpression	Decreased after Fibulin2 overexpression	Decreased after Fibulin2 overexpression	(24)
Alcendor *et al*, 2011	Kaposi's sarcoma	Carcinoma tissue vs. paracarcinoma tissue	Downregulated				(23)
Ma *et al*, 2019	Gastric cancer	Carcinoma tissue vs. paracarcinoma tissue	Downregulated	Decreased after Fibulin2 overexpression			(26)

Figure 6. Interaction between MUC4 and Fibulin2 prevents Fibulin2 from binding to NIDO, thereby disrupting the integrity of the pancreatic tissue BM. In normal pancreatic tissues, Fibulin2 and NIDO constitute integral components of the BM, maintaining its integrity. Following malignant transformation of pancreatic epithelial cells, MUC4 expression is markedly upregulated. The MUC4 hinders the interaction between Fibulin2 and NIDO by interacting with Fibulin2. This disrupts the integrity of the BM and facilitates the invasion and extravasation of pancreatic cancer cells. BioGDP.com was used to generate figures (https://biogdp.com/?tg=CFXL). BM, basement membrane; ECM, extracellular matrix; MUC4, mucin 4; NIDO, G1 domain of Nidogen.

with those of negative control lentiviral vector groups (24). Fibulin2 expression is reduced in breast carcinoma tissues compared with adjacent normal tissues (73). Knockdown of *Fbln2 is* associated with disruption of the type IV collagen sheath surrounding breast cells *in vitro* (74); Ibrahim *et al* (74) hypothesized that its downregulation may be associated with BM disruption and early invasion. However, the BM composition is complex, precluding exclusion of compensation by other Fibulin family members (such as Fibulin1 and Fibulin5) or ECM proteins, which requires demonstration (75). A study involving 272 patients with breast carcinoma from a Norwegian hospital found a positive association between elevated perivascular Fibulin2 expression in breast carcinoma and survival rates (76). Elevated Fibulin2 expression was associated with luminal breast carcinoma, pronounced elastic tissue hyperplasia in the tumor stroma and favorable prognosis, while reduced expression was associated with the basal-like phenotype, triple-negative breast carcinoma, interval breast carcinoma, vascular invasion and poor prognosis (76). The study lacked mechanistic exploration and only reported observed associations.

The TME is currently being investigated as a novel therapeutic target for cancer. ADAMTS is secreted by carcinoma and stromal cells, potentially modifying the TME through various mechanisms, thereby promoting or inhibiting tumors (77). ADAMTS-12 is a secreted metalloproteinase that has functions in tissue remodeling and cell migration or adhesion (78,79). In 293-EBNA cells, yeast two-hybrid screening and co-immunoprecipitation experiments demonstrated that the carboxyl-terminal region of Fibulin2 interacts with the spacer region of ADAMTS-12 (80). When Fibulin2 and ADAMTS-12 were concurrently overexpressed in breast cancer cells, they reduced cell invasion and migration *in vitro*, inhibited cell migration on relevant matrices, decreased mammosphere unit formation, and suppressed tumor growth *in vivo* (80). Fibulin2 and ADAMTS-12 expression in breast cancer tissue was negatively associated with histopathological tumor stage, with patients exhibiting the best prognosis when both were highly expressed (80). ADAMTS-4 and ADAMTS-5 are members

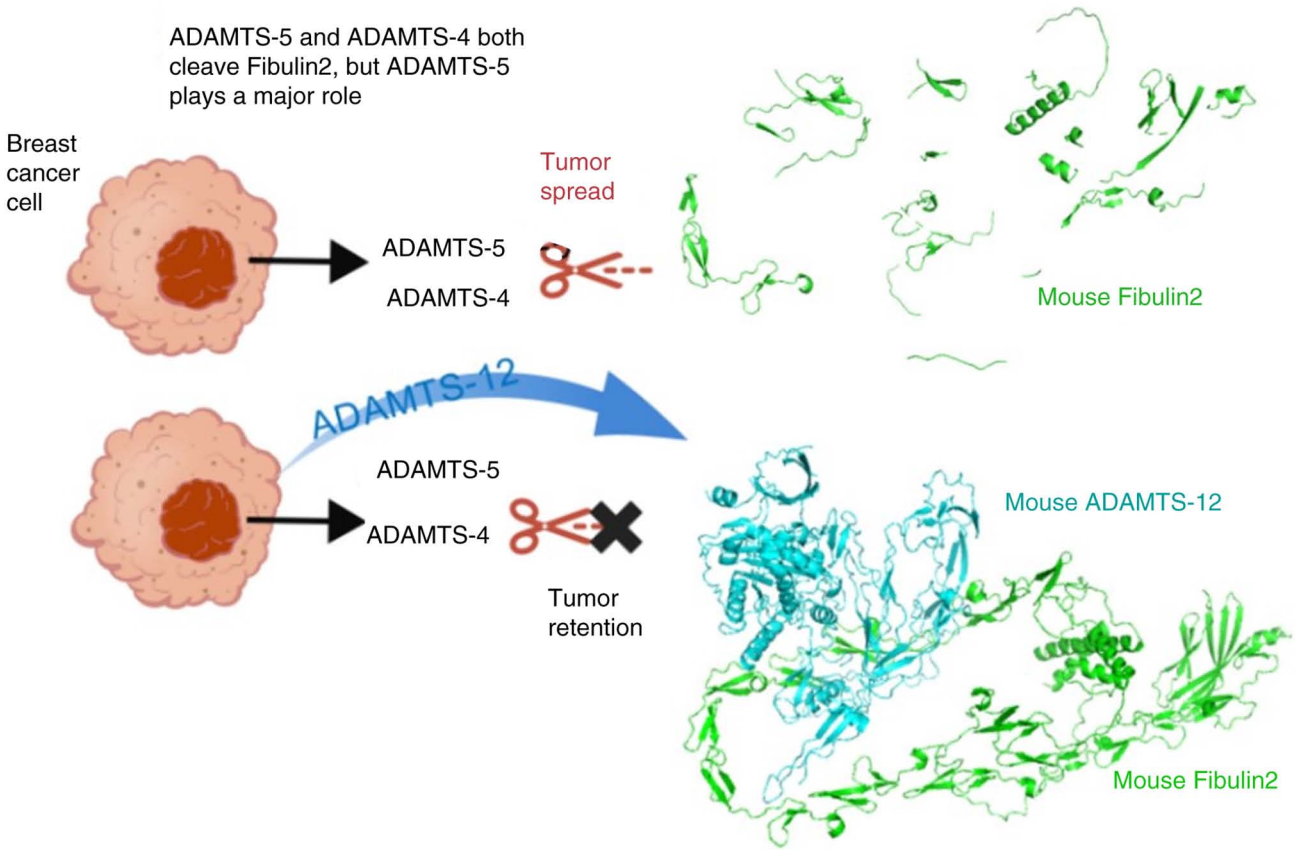

Figure 7. ADAMTS-12 blocks digestion of Fibulin2 by ADAMTS-5. Tumor cells secrete ADAMTS-4 and ADAMTS-5, which possess the capacity to cleave Fibulin2, especially ADAMTS-5, facilitating cancer cell dissemination. ADAMTS-12 can interact with Fibulin2 in breast cancer cells. This interaction impedes the cleavage of Fibulin2 by ADAMTS-5, consequently inhibiting tumor cell progression. BioGDP.com was used to generate figures (https://biogdp.com/?tg=CFXL). ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs.

of the ADAMTS secreted metalloproteinase family (81). A previous study has indicated that both ADAMTS-5 and Fibulin2 are expressed in the stromal and epithelial components of breast cancer tissue, with immunofluorescence double staining showing partial colocalization (30). ADAMTS-4 and ADAMTS-5, predominantly ADAMTS-5, can specifically cleave Fibulin2, enhancing breast carcinoma cell migration and invasion, while increasing the tumorigenic potential (30) (Fig. 7). The study compared the invasion of breast cancer cell lines with simultaneous overexpression of ADAMTS-5 and Fibulin2, breast cancer cell lines with overexpression of ADAMTS-5 or Fibulin2 alone and the control group (30). The invasion capacity of MCF-7 cells was increased by the simultaneous overexpression of Fibulin-2 and ADAMTS-5 compared with the control group and overexpression of Fibulin2 alone (30). However, the invasion capacity of MCF-7 cells was decreased by the simultaneous overexpression of Fibulin-2 and ADAMTS-5 compared with overexpression of ADAMTS-5 alone (30). ADAMTS-5-mediated Fibulin2 degradation is inhibited by ADAMTS-12 (Fig. 7) (30). However, *in vivo* experiments only showed partial colocalization of ADAMTS-5 or ADAMTS-12 with Fibulin2 via immunofluorescence or immunohistochemistry, which is insufficient to establish their relationship with breast cancer (30). Future research using conditional gene-knockout mice to substantiate the relationship between these proteins and breast cancer would be more compelling. When mammary fibroblasts are cultured in medium containing excess Fibulin2 and ADAMTS-5, the expression levels of α-smooth muscle actin (α-SMA) increase. Stimulation with excess Fibulin2 alone can also increase α-SMA expression levels, as observed by WB (the authors did not conduct statistical analysis), indicating that Fibulin2 promotes breast fibroblast activation (30). Breast fibroblasts are a key component of the tumor stroma (82). This indicates that changes in Fibulin2 expression in breast cancer cells can activate stromal fibroblasts and potentially drive their differentiation into CAFs, a phenomenon closely related to the secretory properties of Fibulin2 (30). Similar mechanisms may occur in other tumor types; however, they have received limited attention to date and warrant further investigation.

Due to the opposing roles of different ADAMTS subtypes in breast cancer, developing highly specific inhibitors that selectively block the tumor-promoting activities of ADAMTS subtypes without affecting their potential tumor-suppressive effects remains challenging. Small-molecule inhibitors targeting aggrecanases, including ADAMTS-4 and ADAMTS-5, have advanced to a phase III clinical trial for orthopedic applications (83). This provides valuable insights for the development of ADAMTS-targeted therapeutics for breast cancer treatment.

Cancer progression is accompanied by uncontrolled tumor growth, local invasion and metastasis; processes that depend heavily on the proteolytic activities of multiple MMPs. These enzymes affect tissue integrity by degrading ECM components

such as Fibulin2 (84). In a nasopharyngeal carcinoma study involving samples from 30 patients, *Fbln2* levels in carcinoma tissues substantially exceeded those in normal tissues, as detected by gene chip technology (22). In nasopharyngeal cancer cell lines, overexpression of *Fbln2s*, which encodes the short isoform of Fibulin2, downregulated the expression levels of *VEGF-165*, *VEGF-189* and *MMP-2* compared with those in the control group (22). Sustained angiogenesis is indispensable for both tumor growth and metastasis (85). MMP-2 is an effective gelatinase capable of cleaving protein components of the ECM and is involved in the invasion and metastasis process of tumor cells (86). Evidence has indicated that Fibulin2 is cleaved by MMP-2 (87), and inhibits nasopharyngeal carcinoma cell migration and invasion by strictly regulating *MMP-2* expression (22). However, the study could not establish a direct regulatory relationship between *Fbln2* and *VEGF-165*, *VEGF-189* or *MMP-2*, because there may be unknown molecules or signaling pathways mediating the interaction between *Fbln2* and *VEGF-165*, *VEGF-189* or *MMP-2*. WB analysis of osteosarcoma cell line lysates has indicated multiple Fibulin2 fragments (87). Gelatinase MMP-2 facilitates Fibulin2 degradation through MMP-dependent mechanisms in osteosarcoma cell lines (87). Following addition of an MMP-2 inhibitor to the cell culture medium, WB indicated no change in the cleaved Fibulin2 fragments, indicating that unidentified mechanisms require exploration (87). This study using an osteosarcoma cell line relied entirely on *in vitro* experiments, and *in vivo* experiments are required for verification, as the *in vivo* environment is more complex than the *in vitro* cell culture environment (87).

Most documented MMP inhibitors exhibit non-specific binding and have diminished efficacy, attributable to their pronounced sequence homology with other MMPs (88). To date, no effective MMP inhibitor has successfully completed clinical trials and secured regulatory approval from the Food and Drug Administration for tumor treatment (84,88).

Limitations. Studies on the tumor-inhibitory effect of Fibulin2 predominantly focus on preserving BM structural integrity (7,32,74). Few studies have investigated the mechanisms of interaction between Fibulin2 and classical signaling pathways. Only the association among Fibulin2, β-catenin and the TGF-β/Smad2/TGIF2 pathways has been demonstrated in gastric cancer studies (26,27). An intricate association exists between the β-catenin and TGF-β signaling pathways and the aforementioned Integrins (89,90). Whether Fibulin2 indirectly regulates the β-catenin and TGF-β/Smad2/TGIF2 pathways through Integrins warrants comprehensive investigation. Some methodological considerations profoundly impact future pharmaceutical development efforts. For example, the use of established cell lines, such as the HGC27 and MKN28 cell lines, to study gastric cancer fails to replicate the complexity of primary cells isolated from living tissues (27). Inferring causal relationships in disease mechanisms based solely on gene or protein expression associations or simultaneous alterations, exemplified by MUC4 and Fibulin2 in pancreatic cancer (32), and simultaneous changes in Fibulin2, fibronectin and tropoelastin in Kaposi's sarcoma (23), may result in ineffective drug targets. Focusing on signaling pathways, such as the TGF-β/TGIF2 pathway in gastric cancer (27), while disregarding other potential pathways, may lead to therapeutic resistance due to the activation of compensatory pathways following single-target inhibition. The role of tumor stroma in inhibiting or promoting tumors is associated not only with signaling pathway activation but is also closely associated with stromal cells, such as the activity or senescence state of fibroblasts (91). Secretion of growth factors with inhibitory functions by non-activated fibroblasts (92) or upregulation of the tissue inhibitor of metalloproteinases (TIMP) family are potential mechanisms by which tumor stroma suppresses neoplastic progression (93,94). Notably, both MMPs and the previously discussed ADAMTS are strictly regulated by TIMPs in normal tissues (95,96). Expression of TIMP family proteins in fibroblasts governs ECM structural organization and stromal cell architecture (97). These proteins function as endogenous negative regulators of MMP activity, and numerous malignancies exhibit aberrant TIMP and/or MMP expression patterns (93,98-99). Loss or reduction of TIMP expression leads to enhanced MMP functionality, facilitating stromal activation and subsequent tumor progression (100,101). TIMP overexpression attenuates tumorigenesis, growth, angiogenesis and metastasis in some cancer types, such as pancreatic cancer (94). Whether altered Fibulin2 expression in tumor cells influences fibroblast activity and stromal TIMP family expression requires further elucidation.

5. Fibulin2 as a biomarker for tumors

Studies on Fibulin2 as a biomarker are summarized in Table IV. For case-control studies involving human populations in Table IV, according to the 2011 Evidence Hierarchy of the Oxford Centre for Evidence-based Medicine, the evidence level of most studies was 4 (102).

Fibulin2 as a marker to distinguish patients with tumors from normal populations. In breast cancer mouse models, Fibulin2 expression in plasma was substantially higher than that in normal mice, indicating its potential as a plasma biomarker (103). The study used mice rather than humans as research subjects. Due to species differences, the effectiveness of the marker in humans requires verification, limiting the applicability of the conclusions. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and label-free quantitative methods, differences in Fibulin2 expression have been detected in the sera of patients with advanced colon cancer and healthy individuals. Fibulin2 is a diagnostic marker for advanced colon cancer (4). The sample sizes for the patient and normal control groups were only 8 and 10, respectively, in the study investigating advanced colon cancer, representing inadequate sample sizes (4). Consequently, the cohort lacks representativeness, predisposing it to false positives or false negatives. Future research should expand the sample size to enhance the robustness of the conclusions. In a study on patients with lung cancer, *Fbln2* gene expression levels of the enriched epithelial cells of peripheral blood lymphocytes were found to be decreased ~2-fold in patients with metastatic and non-metastatic lung cancer compared with healthy controls (104). *Fbln2* is a potential biomarker to distinguish patients with lung cancer from healthy controls (104).

Table IV. Fibulin2: A potential biomarker in tumors and other diseases.

First author/s, year	Disease	Source	Expression	Comparison/ evidence level	(Refs.)
Ren *et al*, 2016	Astrocytoma	Human astrocytomas	Downregulation	Grades II/III/IV vs. grade I/4	(7)
Whiteaker *et al*, 2007	Breast cancer	Mouse plasma	Upregulation	Breast cancer mouse vs. normal mice	(103)
Ibrahim *et al*, 2020	HCM	Human serum	Upregulation	Patients with HCM vs. healthy individuals/4	(107)
Sofela *et al*, 2021; Sofela *et al*, 2021	Meningioma	Human plasma	Upregulation	Grade II vs. grade I/4	(5,6)
Li *et al*, 2022	Infection	Human plasma	Upregulation	Patients with infection vs. healthy individuals/3	(108)
Knittel *et al*, 1999	Liver fibrosis	Rat liver myofibroblasts	Upregulation	Liver stellate cells vs. liver myofibroblasts	(109)
Setiawati *et al*, 2024	Meningioma	Human tumor tissue	Fibulin2 expression was elevated in patients <50 years old or exhibiting high histopathological grading	High vs. low Fibulin2 expression/4	(106)
Klingen *et al*, 2021	Breast cancer	Human breast cancer tissue	Diminished Fibulin2 expression in perivascular regions was associated with attenuated survival outcomes	High vs. low Fibulin2 expression/4	(76)
WalyEldeen *et al*, 2024	Breast cancer	Human breast cancer mRNA data	High *Fbln2* mRNA expression was associated with favorable prognosis in relatively early breast cancer, with contrary associations observed in relatively late lesions	High vs. low *Fbln2* expression/4	(105)
Takakura *et al*, 2023	Advanced CRC	Human serum	Fibulin2 expression was increased in recurrent and advanced CRC	Patients with advanced CRC vs. healthy individuals/4	(4)
Ibrahim *et al*, 2018	Breast cancer	Human breast cancer tissue	Higher *Fbln2* mRNA expression was associated with improved survival in low and intermediate grade breast cancer, exhibiting contrary associations in high-grade breast cancer	High vs. low *Fbln2* expression/4	(74)
Avsar *et al*, 2019	Lung cancer	Human blood	Downregulation	Patients with lung cancer vs. healthy individuals/4	(104)

CRC, colorectal cancer; HCM, hypertrophic cardiomyopathy.

Fibulin2 as a marker to distinguish different grades of tumors. Surgical specimens of astrocytomas have been analyzed using LC-MS/MS, WB and reverse transcription-quantitative PCR, revealing that Fibulin2 was downregulated in grade II/III/IV astrocytomas compared with grade I astrocytomas, with negative expression being associated with advanced clinical stages (7). The sample sizes of patients with different grades of glioma were only 5 each, representing small sample sizes that may reduce diagnostic accuracy. Fibulin2 expression in the plasma of patients with grade II meningioma is higher than that in grade I patients, indicating that Fibulin2 is a potential marker to distinguish between patients with grade II and I meningioma (5,6).

Fibulin2 as a marker to predict the prognosis of patients. In studies of Fibulin2 as a marker to predict the prognosis of patients (74,105), comprehensive analysis of human sample data from multiple databases yielded information on patients with breast cancer, including their stages, molecular subtypes and treatment conditions. Based on the Kaplan-Meier plotter dataset, after chemotherapy, high *Fbln2* expression was associated with improved overall survival in $Her2^-$ patients. In grade 2 patients (including those after chemotherapy/hormonal therapy), unstratified patients with breast cancer, $Her2^-$ patients, $Luminal^-$ patients and estrogen $receptor^+$ patients after chemotherapy, high *Fbln2* expression was associated with improved recurrence-free survival. Conversely, in grade 3 patients, low *Fbln2* expression was associated with improved overall survival. In $Her2^+$ patients, $estrogen\ receptor^-$ patients and grade 3 patients, low *Fbln2* expression was associated with improved recurrence-free survival. Analysis of sample data from patients with breast cancer with different molecular subtypes in The Cancer Genome Atlas dataset revealed that in Luminal B patients, low *Fbln2* expression was associated with improved overall survival. In $Her2^+$ patients, high *Fbln2* expression was associated with improved overall survival (105). Another study used the Kaplan-Meier plotter dataset to collect the mRNA expression and survival data of patients with breast cancer, analyzing the association between *Fbln2* expression and prognosis in different subgroups (74). In patients with lymph node-negative and intermediate-grade breast cancer, high *Fbln2* mRNA expression was associated with improved distant metastasis-free survival compared with that of patients with low expression. By contrast, in patients with high-grade breast cancer, the opposite was true: Elevated *Fbln2* mRNA expression was associated with poorer prognosis than low expression (74). The conclusions of both studies were derived from database analyses (74,105). However, samples from public databases are prone to heterogeneity, so clinical validation of their findings is necessary. In a study analyzing human meningioma tissue samples, high-grade histopathology was associated with elevated Ki-67 and Fibulin2 expression. The higher the histological grade of meningioma was based on histopathology, the poorer the prognosis of patients. Higher grades were associated with increased risks of recurrence, progression and mortality. The younger age group (<50 years) exhibited higher Fibulin2 expression than the older age group (>50 years). Fibulin2 expression exhibited an association with age and histopathological grading (106). The sample sizes for low-grade and high-grade gliomas were both 25, representing relatively small cohorts. Furthermore, as the study was a cross-sectional study, it could not determine whether elevated Fibulin2 expression was the cause or consequence of increased meningioma grading.

Fibulin2 as a marker in non-tumor diseases. In a study examining patients with hypertrophic cardiomyopathy (HCM), serum Fibulin2 expression in patients with HCM markedly exceeded that of normal individuals, suggesting that Fibulin2 may be beneficial for HCM diagnosis (107). This retrospective study included 95 consecutive patients with obstructive HCM eligible for septal myectomy surgery. However, HCM has numerous subtypes, with sampling limited to specific subtypes, introducing bias that affects the generalizability of the conclusions to HCM overall. In a study examining patients with infections, plasma Fibulin2 expression in patients with infections was higher than that in normal individuals (108). All patients in the study were from the emergency department, and the disease types included were limited, leading to selection bias in the study population. The applicability of the conclusions supporting Fibulin2 as a clinical diagnostic marker for infection remains limited. Immunohistochemical expression of Fibulin2 distinguishes liver stellate cells from liver myofibroblasts after liver injury, suggesting that liver myofibroblasts are a unique cell population involved in matrix production during liver fibrosis caused by chronic liver injury (109). Differences in Fibulin2 expression have been observed between liver stellate cells and liver myofibroblasts in normal livers, livers with acute damage and livers with chronic damage (109). This study provides a novel marker for researchers to label liver myofibroblasts in related research, although its application remains limited to basic research. Future research should explore whether Fibulin2 can be translated into a clinical diagnostic marker for liver-related diseases based on this finding.

6. Challenges and limitations of clinical translation

Although Fibulin2 shows considerable potential as a therapeutic target and biomarker, several challenges and limitations remain. Addressing these impediments is crucial for successful clinical translation.

Mechanisms of Fibulin2 in tumors are not fully understood. Currently, studies on Fibulin2 receptors remain limited. In infected bone marrow mesenchymal stem cells, Fibulin2 binds to the transmembrane receptor Notch2 (110). In a rat model of neuropathic pain, Fibulin2 expression in the spinal dorsal horn was upregulated (111). Fibulin2 specifically binds to the B1a subunit of γ-aminobutyric acid B receptor, inhibiting its activity and thereby exacerbating pain (111). Following multiple sclerosis, Fibulin2 suppresses oligodendrocyte generation through Notch pathway activation (112). In tumor research, Integrin proteins have been demonstrated to be receptors of Fibulin2 (29). The mechanistic evidence in numerous studies exhibits oversimplification (10,22,28,29). These studies predominantly involve *Fbln2* knockdown or overexpression, followed by detection of expression changes in specific key proteins *in vitro*. Other studies primarily encompass influencing the alteration of the BM (7,32,74). The impact of Fibulin2 on neoplastic processes is complex, requiring further investigation into more comprehensive mechanistic pathways. This would facilitate the design of precision-targeted pharmaceuticals against disease-associated molecular targets.

Side effects and toxicities. Due to the complexity of the function of Fibulin2, targeting Fibulin2 may induce various adverse effects and toxicities. Beyond its role in tumors, Fibulin2 serves a pivotal role in maintaining tissue integrity (74,75,113), regulating fibrosis and immunity (114-120), and supporting tissue development and repair (121-126). For example, Fibulin2-deficient mouse pups developed blisters, demonstrating that Fibulin2 deficiency during development could cause BM rupture. However, adult Fibulin2-deficient mice exhibited no blisters, likely due to compensatory mechanisms

mediated by other ECM components (75,113). Reduced *Fbln2* expression disrupts sheath formation in the mammary epithelium and downregulates Integrin β1 expression, compromising BM integrity. Fibulin2 serves a crucial role in stabilizing the BM structure of the mammary epithelium (74). The Fibulin2 protein exhibits close associations with fibrosis. By establishing cardiac hypertrophy models in Fibulin2 homozygous and wild-type mice via angiotensin II infusion, Fibulin2 knockout has been shown to inhibit myocardial fibrosis via H&E staining, Masson staining and immunohistochemistry analysis (114). This finding was corroborated by observations that *Fbln2* reduced fibrosis markers in primary cardiomyocyte fibroblasts from Fibulin2 homozygous and wild-type mice stimulated with TGF-β1 (114). Consistent results were obtained when establishing myocardial infarction models in Fibulin2 homozygous and wild-type mice: The survival rate of Fibulin2 homozygous mice was higher than that of wild-type mice (115). A 5-day mouse model of skin injury revealed markedly elevated Fibulin2 expression in mice with skin injuries compared with normal mice, as detected by nucleic acid analysis and immunofluorescence (116). Unilateral ureteral obstruction leads to renal fibrosis. Immunofluorescence staining in mice after 7 days of unilateral ureteral obstruction showed higher Fibulin2 expression compared with that in normal mice (117). After establishing a liver fibrosis model in rats, immunofluorescence revealed higher Fibulin2 expression in rats with liver fibrosis compared with normal rats (118). Fibulin2 is upregulated in patients with idiopathic pulmonary fibrosis (119). Fibulin2 inhibition suppresses α-SMA, collagen type Iα1 and fibronectin expression in human lung fibroblast-derived MRC-5 cells (119). Fibulin2 influences immune function, with reduced levels being associated with immune impairment after bone trauma (120). In terms of tissue development and repair, Fibulin2 is a key mediator of the neurogenic effect of TGF-β1 on adult neural stem cells (121). Fibulin2-mediated TGF-β signaling in astrocyte extracellular vesicles promotes synapse formation (122). During myoblast differentiation, *Fbln2* expression is upregulated, and *Fbln2* is indispensable for myoblast differentiation (123-125). Fibulin2 regulates smooth muscle cell migration during blood vessel wall repair (126).

Consequently, the functions of Fibulin2 exhibit extraordinary complexity. Although targeting of Fibulin2 has therapeutic potential in tumor treatment, it may cause immunosuppression (120), disrupt the balance between ECM production and degradation, and induce severe complications such as excessive collagen deposition and fibrosis (114-119).

Clinical translation challenges. To the best of our knowledge, currently, there are no clinical studies on Fibulin2 as a therapeutic target. Existing clinical studies related to Fibulin2 only focus on its use as a marker (5,6,7,107,108). The clinical translation of Fibulin2-targeted drugs for cancer treatment faces numerous obstacles, although current research has not yet reached this stage. Beyond the aforementioned incomplete understanding of mechanisms, there are substantial challenges in clinical drug trials themselves. The following issues are similarly prevalent in the research and development of oncology drugs. For example, determining the optimal dosing and regimens for targeted drugs is challenging due to their narrow therapeutic window and potential toxicity (127). Careful dose escalation studies and monitoring of adverse reactions are necessary. Additionally, for combination therapies involving targeted drugs and other anticancer drugs, synergistic dose regimens need to be determined. Establishing appropriate clinical trial endpoints and efficacy measures is quite complex, as traditional endpoints such as overall survival and progression-free survival may inadequately reflect the therapeutic effects and potential long-term implications of targeted drugs (128). Determining the optimal combination, sequence and potential interactions between treatments is challenging. Extensive preclinical and clinical studies are necessary to evaluate synergistic effects, additional benefits or potential antagonistic interactions of treatments to maximize therapeutic efficacy.

Challenges in the clinical application of markers. Biomarkers should meet strict criteria to ensure concordance between measured and actual physiological values, including accuracy, precision, sensitivity, reproducibility, stability (129,130), specificity, dynamics, detectability and minimal invasiveness (131). Fibulin2 exhibits significant expression differences across distinct stages of tumor development and between normal and diseased conditions (4,5,6,7,103). Fibulin2 is also a potentially effective biomarker in studies of infection and fibrosis (108,109). However, numerous studies are limited by inadequate sample sizes. Furthermore, Fibulin2 can serve as a marker in studies on different diseases, and thus, does not meet the specificity criteria for a marker. Its low specificity fundamentally hinders the clinical utility of Fibulin2 as a single biomarker. In breast cancer, meningioma, infection and HCM, Fibulin2 in human serum samples is detected using ELISA (5,103,107,108). ELISA has considerable advantages, including high sensitivity, high specificity (132) and the ability to analyze multiple samples simultaneously within short timeframes (133). This ensures practical feasibility and convenience for large-scale screening programs and facilitates high-throughput sample processing (133). However, its inherent limitations include lengthy sample pretreatment and purification procedures, unsuitability for rapid detection, high cost, lack of real-time detection capabilities (134), and the need for a relatively large sample volume (100-200 μl) (135). As a biomarker, Fibulin2 should not merely aid in staging disease progression or pathological exclusion but also provide therapeutic guidance. This requires that Fibulin2 demonstrates systematic changes corresponding to dynamic disease progression. Most studies so far have not conducted comprehensive dynamic monitoring.

7. Future perspectives of clinical translation

Emerging Fibulin2-targeted therapies. At present, there is a lack of pharmaceutical investigations targeting Fibulin2 for tumor treatment. A key contributing factor to this scarcity is the multifaceted complexity of its biological functions as aforementioned. Fibulin2-directed therapeutic interventions may induce considerable toxicity and adverse sequelae (74,75,113-126). Next-generation small-molecule inhibitors, novel monoclonal antibodies, ligand traps, gene editing, RNA interference technologies, nanomaterials and peptide-based therapies are emerging targeted treatments in cancer. Beyond the need to further elucidate the mechanistic

basis of the role of Fibulin2 in tumors, the strategic application of these emerging technologies in prospective Fibulin2-targed drugs will prove instrumental in enhancing therapeutic specificity and potency while reducing adverse effects.

Combining other therapies with Fibulin2-targeted therapies. Integrating Fibulin2-targeted treatment with other modalities is a strategic approach to enhance antitumor efficacy and circumvent resistance mechanisms. For example, synergistic combinations with immunotherapeutic agents, particularly immune checkpoint inhibitors (such as anti-programmed cell death protein 1 and anti-cytotoxic T-lymphocyte-associated protein 4), show promise by reactivating antitumor immune responses by reducing immunosuppression (136,137). Combining Fibulin2-targeted treatment with adoptive cell therapy, such as chimeric antigen receptor-T cells, may enhance cellular persistence and effectiveness (138). Co-administering signaling pathway inhibitors with Fibulin2-targeted treatment helps attenuate parallel signaling pathways, thereby blocking the compensatory mechanisms exploited by malignant cells. This strategy aims to enhance comprehensive antitumor activity and delay drug resistance. Integrating Fibulin2-targeted treatment with traditional chemotherapy and radiotherapy increases tumor susceptibility to these treatments. Concomitant use of epigenetic modulators can enhance therapeutic responsiveness by systematically reprogramming the TME, which is effective in addressing epigenetic changes that promote cancer progression (139).

Combining Fibulin2 with other markers. Given the limited specificity of Fibulin2 as a single biomarker, integrating multiple biomarkers is advantageous to address this limitation. While there are currently no reports on the use of Fibulin2 in combination with other markers for tumor detection, the use of microRNAs (miRNAs) and other proteins as combined markers for early-stage cancer diagnosis has demonstrated the considerable potential of combined marker detection, offering valuable insights for combining Fibulin2 with other markers (140,141). For example, Yu *et al* (140) described a multi-marker diagnostic method for early-stage hepatocellular carcinoma, using α fetoprotein and miRNA-125b as combined markers to simultaneously improve diagnostic sensitivity and specificity. Yuan *et al* (141) proposed a new combination of circulating miRNAs and plasma protein biomarkers for pancreatic cancer diagnosis. These combined indicators exhibited higher specificity in distinguishing pancreatic cancer from other gastrointestinal cancers than CA19-9 and individual indicators (141). Combining Fibulin2 with other markers such as miRNAs for early cancer screening, diagnosis and prognosis may enhance specificity compared with using Fibulin2 alone. The introduction of artificial intelligence (AI) and machine learning can facilitate the identification of novel potential combined biomarkers. AI algorithms excel in analyzing and identifying unique combinations of gene mutations and using large-scale genomic databases to identify cancer-specific markers (142).

Improvements in traditional ELISA detection methods. The quantification of Fibulin2 in serum and plasma is performed using traditional ELISA. ELISA detection requires air-conditioned laboratories, refrigeration facilities for chemicals and reagents, a reliable power supply, precision-calibrated equipment, and highly trained personnel (135). Some laboratories or hospitals still lack access to affordable infrastructure for these complex diagnostic tests. To address these deficiencies, laboratories worldwide have evaluated and implemented various modified forms of ELISA. These innovative ELISA formats have greater potential for clinical translation due to their low cost, short detection time, portability and reduced reagent needs (143-146). For example, microfluidic-based ELISA, paper-ELISA and aptamer-ELISA can mitigate the limitations of traditional ELISA to some extent (143-146).

8. Conclusion

The oncogenic or suppressive effects of altered Fibulin2 expression in malignant cells vary markedly across diverse tumors. The contributing factors include distinct mechanisms, including the activation of different signaling pathways and the complex relationships with BM or other ECM proteins, tumor development stage, species and tumor origin. Although current mechanistic studies help clarify aspects of pathogenic processes, several outstanding issues remain to be resolved. These include validating the upstream and downstream molecules of Fibulin2, confirming the existence of feedback loops, exploring alternative pathways, and clarifying the relationship between Fibulin2 and stromal fibroblasts. These considerations are crucial to improve the safety and effectiveness of future relevant drug research. The use of Fibulin2 alone as a marker for tumor diagnosis or staging remains not well-established. Future research should further explore methods to enhance specificity, such as identifying novel and specific markers to be used in combination with Fibulin2 as a dual-marker system.

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Availability of data and materials

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Authors' contributions

YY completed most of the work, wrote the original draft and created illustrations. ZW wrote and revised the article, and collected the literature. LW revised the article and collected the literature. JF reviewed and edited the manuscript, provided resources, and acquired funding. ZL supervised the study, edited and reviewed the manuscript, was involved in project

administration, and acquired funding. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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