Exploring the mechanism of fibronectin extra domain B in the tumor microenvironment and implications for targeted immunotherapy and diagnostics (Review)

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Abbreviations: ^{124}I/^{131}I, Iodine-124//131 radioisotope; ^{131}I-L19-SIP ^{131}I-labeled L19 small immunoprotein; ^{18}F, Fluorine-18 radioisotope; ^{64}Cu, copper-64 radioisotope; ^{68}Ga, gallium-68 radioisotope; ^{76}Br, bromine-76 radioisotope; 99mTc, technetium-99m radioisotope; ADC, Antibody-Drug Conjugate; ADCC, Antibody-Dependent Cellular Cytotoxicity; ANG-1, Angiopoietin-1; ANG-2, Angiopoietin-2; APTEDB, aptamer targeting EDB; fibronectin extra domain B specific peptide; CAR-T, chimeric antigen receptor T; CD70, Cluster of Differentiation 70; CRISPR/Cas9, Clustered Regularly interspaced short palindromic repeats/CRISPR-associated protein 9; Cy7, near-infrared dye Cy7; DOTA, 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid; ECM, extracellular matrix; FAK, focal adhesion kinase; FN, fibronectin; FN-EDB, fibronectin extra domain B; GM-CSF, granulocyte-macrophage colony stimulating factor; HCC, hepatocellular carcinoma; HIF-1α/β, hypoxia-inducible factor-1 α/β; HP-DO3A, hydroxypropyl-dodecylamine chelating agent for Gd; MG, malignant glioma; MRI, magnetic resonance imaging; NJB2, alpaca-derived libraries of nanobodies; NK, natural killer; NOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid; NPs, nanoparticles; NSCLC, non-small cell lung cancer; OVs, oncolytic viruses; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PDCs, tumor-specific peptide-drug conjugates; PDGF, platelet-derived growth factor; PET, positron emission tomography; PI3K, phosphatidylinositol 3-kinase; RA, rheumatoid arthritis; RGD, Arg-Gly-Asp; rhFEB, recombinant fusion protein; RIT, radioimmunotherapy; rTCR, recombinant T cell receptor; TAMs, tumor-associated macrophages; TC, thyroid cancer; TME, tumor microenvironment; TNF-α, tumor necrosis factor-alpha

Key words: additional domain B, tumor microenvironment, fibronectin, extracellular matrix, integrin, targeted FN-EDB immunotherapy, targeted FN-EDB diagnostic method

Abstract. Fibronectin extra domain B (FN-EDB) is a unique domain of FN), whose expression is significantly upregulated in the tumor microenvironment (TME). FN-EDB plays a key role in tumor cell adhesion, angiogenesis and invasion, and is closely related to tumor malignancy and poor prognosis. Moreover, the high expression of FN-EDB in multiple cancer types makes it a potential therapeutic target. However, comprehensive studies of the mechanism of FN-EDB in different cancer types and its potential as therapeutic targets are lacking. The present study aimed to explore the general role of FN-EDB in multiple types of cancer and to integrate the knowledge of cell biology, molecular biology and immunology, so as to give a comprehensive understanding of the role of FN-EDB in TME. Furthermore, by focusing on the use of FN-EDB in clinical diagnosis and treatment, the potential of targeting FN-EDB as a diagnostic and therapeutic target was evaluated and the progress in clinical trials of these drugs was discussed. By searching web sites such as PubMed and web of science, various high-quality studies including RNA sequencing, drug experiments, cell experiments, animal models, clinical randomized controlled experiments and large-scale cohort studies were collected, with sufficient evidence to support a comprehensive evaluation of the function and potential application of FN-EDB. The present study revealed the general role of FN-EDB in multiple types of cancer and evaluated its potential as a diagnostic and therapeutic target. It also provided a basis for future development of more effective and precise cancer therapies.

Contents

1. Introduction

The tumor microenvironment (TME) encompasses a complex network of tumor cells, surrounding tissue cells, extracellular matrix (ECM) and various immune cells and cytokines. The ECM, a 3D structure, plays a pivotal role in maintaining tissue and organ homeostasis (1). The non-cellular component of the TME is the extracellular matrix (ECM), which is a complex 3D structure, a universal scaffold for maintaining tissue and organ homeostasis and a complex network composed of fibrils or non-fibrillar collagen, elastin, proteoglycans, glycoproteins, laminin, fibronectin (FN) and other matrix proteins (2). FN, a key component of the ECM, with its extra domain B (FN-EDB), regulates tumor cell interactions with the ECM, influencing tumor cell adhesion, migration and proliferation (3). The role of FN-EDB in the TME includes promoting tumor cell adhesion, aiding in tumor angiogenesis and modulating the activity of tumor-associated immune cells.

FN-EDB provides an anchor effect for the survival and spread of tumor cells by promoting the adhesion of tumor cells and ECM (4); second, FN-EDB participates in the formation of tumor neovascularization, provides nutrition and oxygen for tumor and promotes its growth (5); in addition, FN-EDB can also regulate the activity of tumor-related immune cells and affect the anti-tumor immune response (6). FN-EDB has emerged as an important target for in targeted immunotherapy studies of tumors (7). Overexpression of FN-EDB is closely related to the malignancy, aggressiveness of tumors and the poor prognosis of patients (8). By inhibiting the function of FN-EDB, it is possible to effectively block the interaction between tumor cells and ECM and inhibit tumor growth and metastasis. Immunotherapy strategies against FN-EDB, such as antibody drug conjugates (ADC) and chimeric antigen receptor T cell therapy (CAR-T), have shown promising application in clinical trials (9). However, the study of the FN-EDB remains controversial. On one hand, the expression and mechanism of FN-EDB in different types of tumors may differ, which poses a challenge for the targeted therapy of FN-EDB. On the other hand, the role of FN-EDB in tumor development is not fully understood, especially when FN-EDB interacts with multiple integrins and signaling pathways, such as FAK/PI3K/AKT and MAPK/ERK to promote tumor cell survival and angiogenesis, making it difficult to design potent inhibitors that specifically target FN-EDB without affecting normal cell function (10). Thus, this may present potential difficulties in inhibiting the function of the tumor cells.

Therefore, the present review aimed to summarize the role of FN-EDB in tumor progression, including its molecular mechanisms, biological functions, and clinical implications, with a focus on elucidating potential mechanisms such as tumor angiogenesis and vascular cell signaling. It also summarized the latest targeted therapy and diagnostic strategies in the field to provide necessary guidance for researchers, clinicians and drug developers to facilitate informed decision making. The present study could provide important information for future research directions and treatment strategies that could guide the development of new diagnostic and treatment modalities in cancer treatment in the future.

2. Molecular structural properties and biological characteristics of FN and FN-EDB

Structural features of the FN-EDB molecules. FN is a large glycoprotein found in the ECM. It plays a key role in a variety of biological processes, including cell adhesion, migration, proliferation, differentiation and tissue repair and regeneration of (11). The versatility of FN is attributed in part to its complex molecular structure and multiple isoforms generated by alternative splicing. FN is composed of two similar subunits linked by disulfide bonds, each containing multiple domains such as the N-terminal domain, multiple types of repeats (such as type I, II, III and V repeats) and linker domain and C-terminal domain (EIIIA, EIIA, EIIIB and EDB) (12). The type I and II repeats are small and composed of 45 and 60 amino acids, respectively, which contain key cysteine residues that form disulfide bonds within the domain (13). These repeats are involved in the formation of stable domains in FN molecules and are essential for maintaining the overall structure of FN and function. Unlike type I and II repeats, type III repeats are large, each consisting of 90 amino acids and containing no cysteine (14). These repeats are organized into two antiparallel β folds, forming a sandwich conformation with a hydrophobic core. This structure of the FNIII repeats allows it to extend when subjected to mechanical strain, thus playing an important role in cell adhesion and migration (15). In addition to the standard type III repeat, two additional variant domains exist; EDB and EDA (16). EDB is an alternative splice variant of FN and its structure and function are particularly important in tumor biology. The EDB domain consists of about 90 amino acids, located between the sea urchin-derived domain (EIIIA) and the rod helical domain of the FN molecule (17). Compared with the full-length FN, the FN molecules containing EDB show an enhanced cell adhesion and proliferation activity, especially in tumor cells (Fig. 1).

Biological properties of FN-EDB. FN-EDB is an ECM protein upregulated in the TME, playing a multifaceted role in cancer progression. It interacts with integrin receptors αvβ3 and αvβ5, activating downstream signaling pathways including focal adhesion kinase (FAK), PI3K, AKT and MAPK/ERK, which enhance tumor cell adhesion and migration (18). FN-EDB influences cell cycle progression and gene expression, promoting cell proliferation and participating in differentiation processes such as skeletal muscle cells (19). It contributes to ECM remodeling, maintaining its structure and function through interaction with cells and matrix proteins (20). FN-EDB also promotes tumor angiogenesis by upregulating factors like VEGF and matrix metalloproteinases, providing necessary nutrients and oxygen for tumor growth (10). It may modulate the tumor immune microenvironment, potentially affecting immune checkpoint molecules like PD-L1 and cytotoxic T lymphocyte antigen 4, thereby facilitating immune evasion (21). FN-EDB can influence the expression of Cyclin D1 and apoptosis inhibitors like Bcl-2, contributing to tumor cell proliferation and apoptosis suppression (22). Additionally, it plays a role in the inflammatory response within the TME, interacting with cytokines such as TNF-α and IL-6 to promote tumor development (23). Given its involvement in critical biological processes of cancer cells, FN-EDB serves not

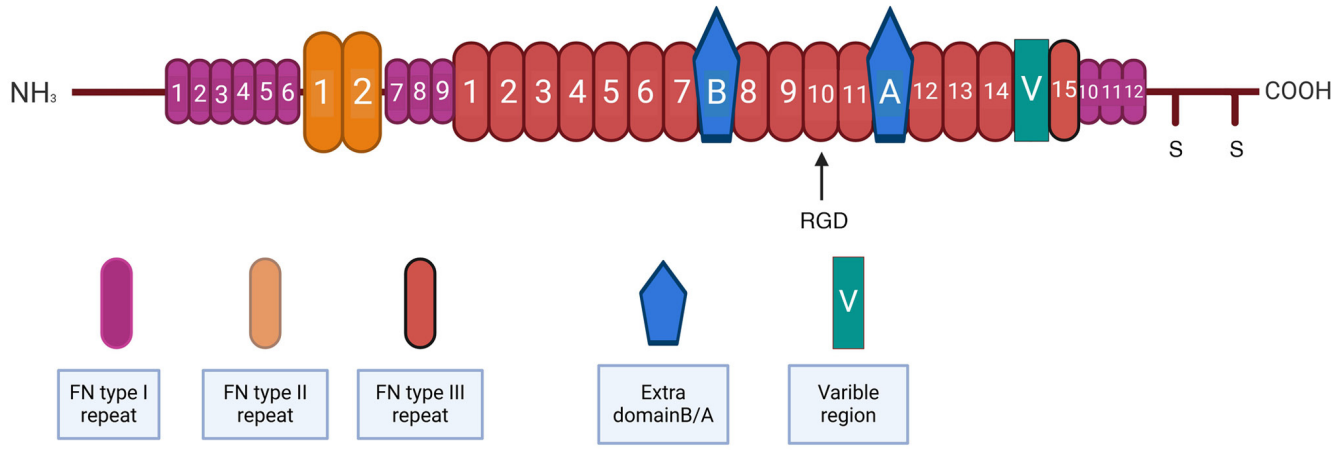

Figure 1. Molecular structure diagram of FN. Each FN subunit consists of three types of repetitive sequences: Type I (purple), Type II (orange) and Type III (brown). The domain (brown capsule) required for initiating assembly includes the cell binding domain (cooperative site 9 in RGD site 10 + III in III); the variable spliced additional domains EIIIA, EIIIB are shown in blue pentagons and the variable region in green. SS and NH are the disulfide bond and amino groups in proteins. Figure created with BioRender.com, the software was developed by Biorender, Inc. FN, fibronectin; V, variable region; SS, disulfide bond; NH, amino group.

only as a key factor in tumor biology but also as a potential biomarker for diagnosis and prognostic assessment, making it an attractive target for therapeutic intervention (24).

FN-EDB is highly expressed in the tumor tissues. RNA-Seq data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression projects reveal that FN and its splicing isoforms, particularly FN-EDB, are significantly overexpressed across various types of cancer (25). Bioinformatics analyses correlate FN-EDB expression patterns in tumor tissues with cancer cell biological behaviors, with FN-EDB levels notably higher in cancer cell lines than in normal solid tumor cells (26). Further comparative analysis shows that FN-EDB expression is markedly different from normal tissue in 15 out of 17 types of cancer, especially in head and neck squamous cell carcinoma and malignant glioma (MG) (8,12). Experimental validations at the tissue and cellular levels confirm FN-EDB overexpression in malignant glioma, suggesting its utility as a diagnostic and prognostic biomarker (27). In oral squamous cell carcinoma, high FN-EDB expression is associated with increased invasiveness (28). In breast cancer, EDB-FN overexpression correlates with poor overall survival and is upregulated in invasive cell populations with acquired chemotherapy resistance following long-term TGF-β treatment (29). These findings underscore the variable FN-EDB expression across types of cancer and its intimate link with tumor development and progression, warranting further investigation into its expression patterns and functions in different types of cancer.

3. FN-EDB and the TME

The TME includes all non-cancerous host cells in tumors, including fibroblasts, endothelial cells, adipocytes, adaptive and innate immune cells including T cells, macrophages and its noncellular components, including ECM and soluble products such as chemokines, cytokines, growth factors and extracellular vesicle (30). Dynamic remodeling of the ECM is crucial for influencing the TME. Fibronectin components in ECM play a role in tumor angiogenesis, cell invasion and metastasis development. In general, the role of FN-EDB in the TME is multi-dimensional, which not only directly promotes the malignant behavior of tumor cells, but also indirectly supports the tumor progression and metastasis of by affecting the tumor angiogenesis, immune escape and stem cell properties (31).

FN-EDB participates in the regulated and regulated signaling pathways in the TME. In the TME, FN-EDB as a key ECM protein, through a regulation of multiple signaling pathways, activated the series of signaling pathways closely related to tumor cell behavior (Fig. 2), including integrin signaling pathways, vascular formation signaling pathways, signaling pathways, immune regulatory signaling pathways, tumor stem cell signaling pathway, inflammatory signaling pathways and epithelial-stromal transformation (EMT) signaling pathway promote the occurrence and development of tumor and provides the theoretical basis for regulating targeted immunotherapy of tumor under various pathological conditions.

Integrin signaling pathway

Molecular characteristics of integrin. Integrins are heterodimeric cell surface adhesion molecules composed of 18α and 8β subunits, forming 24 distinct heterodimers with functional and tissue-specific properties. The high-affinity interaction of integrins with the central cell binding domain (CCBD) of fibronectin requires the Arg-Gly-Asp (RGD) sequence within the 10th III-type repeat and a second site within the adjacent 9th III-type repeat, which synergistically promote cell adhesion and signaling (18,32). Three β1 integrins (α4, α5, α8) and three αv integrins (β1, β3, β6) serve as receptors for fibronectin. A total of genes bind to the RGD sequence in fibronectin III-10 and one gene, α4β1, recognizes the Leu-Asp-Val-Pro-like sequence in fibronectin III-CS1 (33).

Despite the non-traditional RGD binding site of FN-EDB, it interacts with integrins through its unique amino acid sequence, displaying ligand recognition specificity (34). Conformational changes of integrins are also crucial for their binding with FN-EDB, as signaling events induced by cellular activation can lead to integrin conformational changes, increasing their

affinity for FN-EDB. Integrins bind to various ECM ligands with different affinities, inducing integrin clustering and significant conformational changes in their external domains, thereby activating intracellular signaling pathways (35).

Binding characteristics of different subtypes of integrins to FN-EDB. The interaction between integrins αvβ3 and αvβ5 with FN-EDB is a finely regulated process involving multiple layers of interaction. First, structural complementarity forms the basis of this interaction, with FN-EDB's specific three-dimensional structure complementing the binding sites of integrins, promoting tight molecular interactions (36). Secondly, the αv subunit of integrins contains specific domains responsible for recognizing FN-EDB, while the β3 or β5 subunits contribute to the overall structural stability of the integrin. FN-EDB can also interact with other integrin subtypes, albeit with differing binding characteristics (37). α5β1 integrin binds to the RGD sequence in fibronectin and participates in cell adhesion, while α8β1 integrin primarily interacts with other domains of fibronectin but can also interact with FN-EDB (38). αvβ1 integrin has the ability to bind to various ECM proteins, including FN-EDB (39) and αvβ6 and αvβ8 integrin subtypes, especially their interactions with epithelial and tumor cells, suggest their potential binding with FN-EDB under specific cell types and pathological conditions (40). Additionally, α4β1 (VLA-4) integrin, primarily interacting with VCAM-1 and the CS-1 region of fibronectin, may also interact with FN-EDB. α9β1 integrin, although less studied in its direct interaction with FN-EDB, can also bind to some domains of fibronectin (41).

The diversity of integrin subtypes and their interactions with FN-EDB highlight the complexity and importance of the ECM in cell function and pathology. The dynamic nature of the ECM, including its composition and physical state, such as rigidity or disassembly, can also regulate integrin activity and subsequently influence their interactions with FN-EDB (42). This intricate network of interactions between FN-EDB and integrins underscores the multifaceted role of the ECM in cellular behavior and disease progression.

Transmembrane signaling mode of integrins. Integrins are a bidirectional signaling molecule (Fig. 3) and integrins are transmembrane receptor that play a key role in cell-ECM interactions. On one hand, they transduce information from the extracellular environment to modulate cellular responses, a process often referred to as 'inside-out' signaling. On the other hand, the active state of integrins can also be modulated by intracellular signaling molecules that, when integrins are bound to their ligands, can trigger an intracellular signaling pathway, a process known as 'outside-in' signaling (33). Integrins mediate inside-in and inside-out signaling, required for various cellular processes such as cell adhesion, migration, survival, proliferation and gene expression (36).

In outside-in signaling inactive integrins are described as their basal state, waiting for the activation of ECM ligands, such as fibronectin, which contains the RGD sequence. Upon ligand binding, integrins undergo a conformational change to become active integrins, initiating a cascade of intracellular signaling events. FAK and Src homology 2 (SH2) domain-containing proteins (Src) are key components of the signaling complex, which assemble following integrin activation, leading to the recruitment of various signaling molecules and the subsequent activation of downstream signaling pathways, including the FAK/PI3K/AKT and MAPK/ERK pathways (35); for example, the monomeric form of talin protein, which then regulates actin polymerization and affects cell morphology and motility.

The inside-out signaling pathway involves the regulation of intracellular signaling molecules on the activation of integrins, thereby regulating the affinity of integrins for their ligands (43). Talin is a cytoskeletal protein considered a key regulator of integrin activation and is critical for integrin activation and the formation of focal adhesions that are essential for the transmission of mechanical and biochemical signals between the ECM and the cell interior (44). By interacting with integrin and its enhanced ability to bind to ECM, it participates in the transmission of signals, ultimately realizing the directed movement of cells and the remodeling of cellular structure (45). In addition, talin activation in turn affects the actin cytoskeleton assembly and the formation of focal adhesions. Actin polymerization is thought to be a critical process downstream of integrin activation, which contributes to cell polarity, survival, migration and the assembly of the ECM (46).

FN-EDB promotes cell adhesion to the ECM by binding to specific integrin isoforms such as α v β 3 and α v β 5. This adhesion is fundamental to cell stability and is critical for cellular sensing and response to external signals (47). The interaction of FN-EDB with integrins activates a series of downstream signaling events that triggers the activation of integrin signaling pathways, including cellular signaling cascade in FAK, PI3K, AKT and MAPK/ERK (48).

The reorganization of the cytoskeleton underlies cell migration and morphological changes; FN-EDB positively regulates this process through a signaling pathway activated by integrins. This is extremely critical for the physiological and pathological processes such as maintaining cell adhesion, cytoskeletal structure reorganization, gene expression, tumor cell invasion and metastasis (49).

FN-EDB modulation of classical integrin signaling pathways. FN-EDB plays a pivotal role in regulating classical integrin signaling pathways within the TME. This process is initiated by the binding of integrins to ECM components, particularly FN-EDB, leading to the activation of FAK at Tyr397, resulting in its autophosphorylation [phosphorylated (p-)FAK-Y397] (50). This event marks the initiation of intracellular signaling events crucial for tumor progression.

Abnormal activation of FAK signaling can be achieved through various mechanisms, including integrin-mediated autophosphorylation and interactions with kinase families such as Src and Grb2. FAK promotes activation of Ras, which in turn activates RAF, MEK and ultimately ERK1/2, initiating the ERK/MAPK signaling pathway (48,51). This phosphorylation ultimately leads to the translocation of ERK1/2 to the nucleus, where it regulates the expression of genes involved in cell proliferation, differentiation and survival. The activation state of ERK1/2 is closely associated with the invasiveness and metastatic potential of tumor cells, with its high expression often correlating with poor prognosis in various tumors (52).

Parallel to the ERK/MAPK pathway, FAK also participates in the PI3K-Akt pathway, a key regulator of cell survival, proliferation and metabolism. FAK activation of PI3K leads to the phosphorylation and activation of Akt, which then phosphorylates multiple downstream targets, influencing cellular

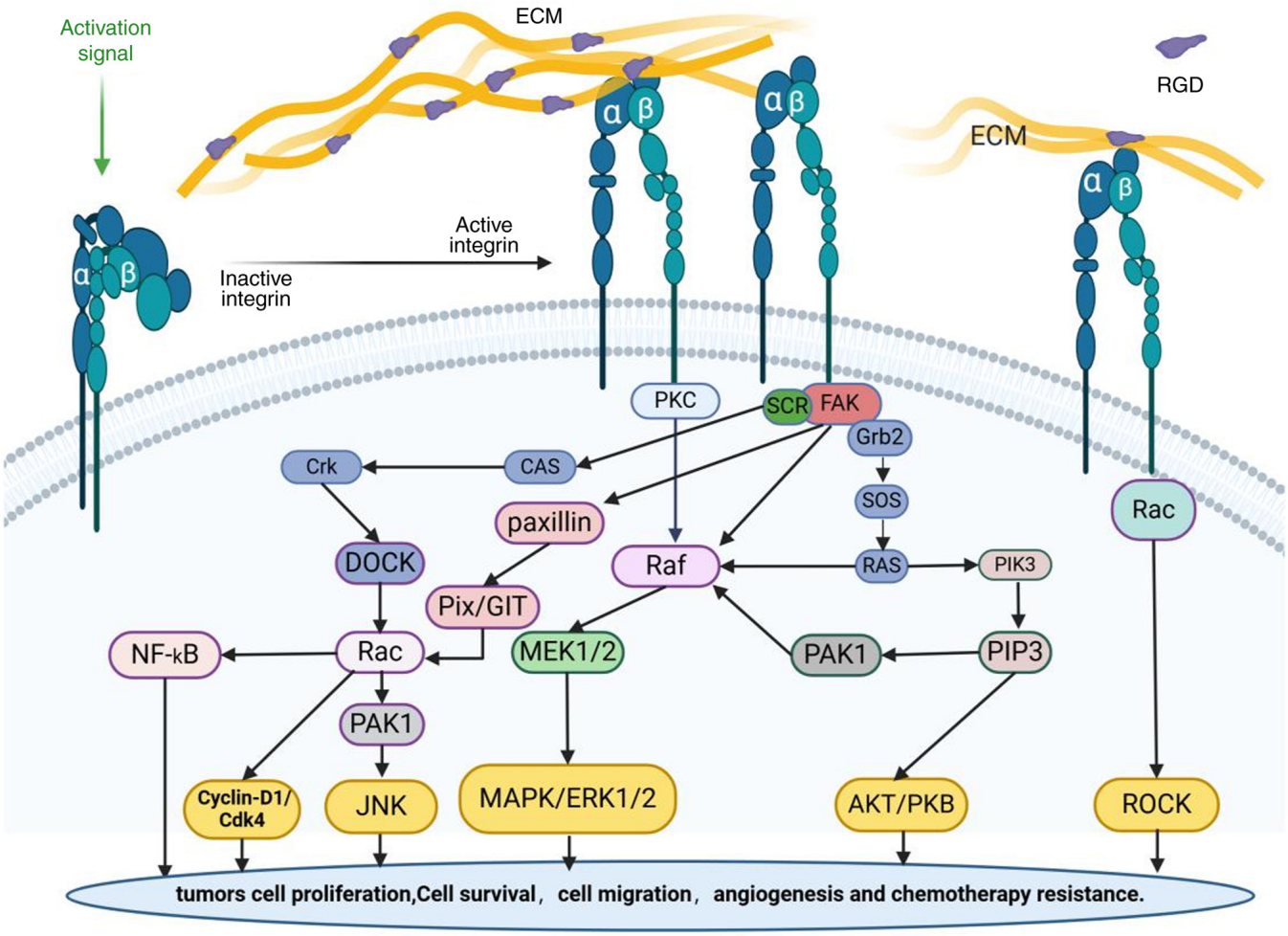

Figure 2. ECM signaling and integrin activation. There is complex interplay between the ECM and cell surface integrins, leading to intracellular signaling cascades that regulate various cellular processes. The central motif is the activation of integrins by specific ECM components, such as fibronectin, through the recognition of the RGD sequence by integrin receptors. *Integrin activation*; inactive integrins are depicted as receptors that, upon binding to ECM ligands, undergo a conformational change to become active integrins. This activation is crucial for the initiation of downstream signaling. The RGD motif is highlighted as a key recognition site for integrin binding, which is essential for mediating cell adhesion and signaling. *Signaling pathways;* PKC and FAK are early signaling molecules that become activated upon integrin engagement with the ECM. SH2 domain-containing proteins such as Grb2 and Crk, along with Cas, are involved in the propagation of signals from the cell membrane to the cytoplasm. SOS, a guanine nucleotide exchange factor, activates the small GTPase RAS, which in turn activates the Raf-MEK1/2-ERK1/2 (MAPK/ERK) pathway, leading to cell proliferation and survival. PI3K generates PIP3, which recruits AKT/PKB and PAK1 to the membrane, promoting cell survival and cytoskeletal rearrangements. Rac, a small GTPase, activates PAK1 and NF-kB, contributing to cell migration and inflammation. Cyclin-D1and JNK are also implicated in cell cycle progression and stress response, respectively. *Cell Functions;* Activation of these signaling pathways ultimately leads to a range of cellular responses, including cell proliferation, cell survival, cell migration, angiogenesis and chemotherapy resistance in tumor cells. The figure also includes a schematic representation of focal adhesion complexes, with key proteins such as paxillin, DOCK and Pix/GIT, which are involved in the formation and regulation of these structures. Figure created with BioRender.com, the software was developed by Biorender, Inc. ECM, extracellular matrix; RGD, arginine-glycine-aspartic acid; PKC, Protein Kinase C; FAK, focal adhesion kinase; SH2, Src Homology 2; SOS, Son of Sevenless.

metabolism and survival signals. The sustained activation of Akt is also associated with tumor invasiveness and chemoresistance, making it a significant target for cancer therapy (51-53).

In clinical research, the activation status of FAK, ERK1/2 and Akt signaling pathways has become an important biomarker for assessing tumor progression and prognosis. Targeted therapeutic strategies against these signaling pathways, such as the use of FAK inhibitors, MEK inhibitors and PI3K/Akt inhibitors, have shown potential therapeutic value in clinical trials for various types of cancer (54).

FN-EDB modulation of non-canonical β1-integrin signaling pathways. FN-EDB not only regulates classical integrin signaling pathways but also modulates atypical β1-integrin signaling pathways, which are critical for tumor cell plasticity and metastatic potential. Unlike the classical FAK-ERK/MAPK or PI3K-Akt signaling axes, the atypical β1-integrin signaling mechanism involves integrin-mediated endocytosis, leading to the internalization of integrin-ECM complexes into endosomes (55). This process allows for the continued signaling within the cell through the formation of endosomal vesicles.

In endosomes, activated integrins co-localize with phosphorylated FAK (p-FAK-Y397), indicating the sustained transmission of integrin signals within the cell (56). The presence of integrin ligands like fibronectin and integrin-activating proteins such as talin in endosomes further confirms the connection between functional receptor-ligand coupling and active FAK pools associated with endosomes (57).

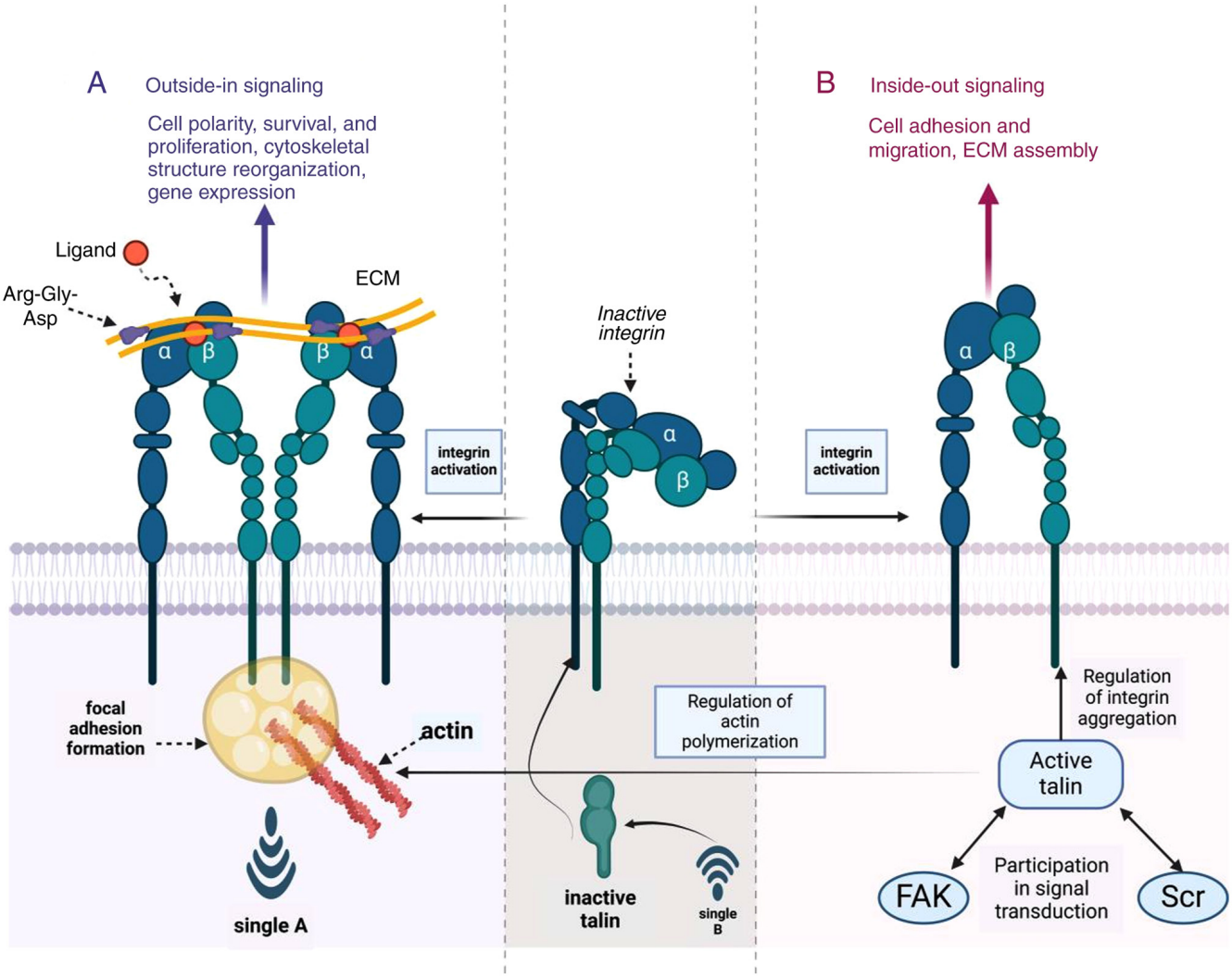

Figure 3. Signaling mechanisms of integrins in cell-matrix interactions. The schematic represents integrin's role in bidirectional signaling, influencing cell adhesion, migration, survival and proliferation. (A) Outside-in signaling is initiated by ECM ligands, such as fibronectin, binding to inactive integrins, triggering conformational changes and activation. Key players include talin, which facilitates integrin activation and FAK, crucial for signal transduction and cytoskeletal reorganization. (B) Inside-out signaling modulates integrin affinity for ECM, regulated by intracellular signaling and cytoskeletal dynamics. This bidirectional communication is essential for maintaining cell polarity and ECM assembly, underpinning cellular homeostasis. Figure created with BioRender.com (Biorender, Inc. ECM, extracellular matrix; FAK, focal adhesion kinase; Scr, Src homology 2 domain-containing protein.

In endosomal lumen, integrin signaling continues through the activation of FAK, which can occur independently of its typical activation at the plasma membrane. This atypical activation of FAK in endosomes is associated with the regulation of cytoskeletal dynamics and cell migration, processes essential for tumor invasion (58). Key regulators such as early endosome antigen 1 and Ras-related protein Rab21 play crucial roles in the endocytosis and signaling of integrins, potentially promoting the assembly of specific signal complexes on endosomes (59).

Inhibition of endocytosis, for example through dynamin inhibitors, significantly reduces the activation of FAK and ERK1/2, highlighting the central role of endocytosis in integrin-ECM signaling (60). Additionally, endocytosis is crucial for maintaining cell resistance to anoikis, revealing its protective role in cell survival signaling (61).

Integrin signaling on endosomes may involve different molecular mechanisms and regulatory proteins than those at focal adhesions on the cell membrane, such as direct interaction with integrins and the assembly of unique signaling complexes. Furthermore, FN-EDB-mediated integrin activation can activate Rho GTPases signaling pathways, leading to the activation of RhoA and its downstream effector ROCK, which are crucial for regulating cell contraction, migration and invasion (62).

Endosomal integrin signaling also intersects with the JNK signaling pathway, potentially affecting cell stress responses and apoptosis (63). Non-classical integrin signaling through FN-EDB can activate NF-κB, which is associated with inflammation and cell survival regulation, further highlighting the multifaceted role of FN-EDB in tumor biology (64).

In clinical research, atypical β1-integrin signaling mechanisms are related to the development and progression of various types of tumor. For example, in breast cancer, atypical β1-integrin signaling enhances tumor invasiveness and metastatic potential by promoting EMT and angiogenesis (65). In non-small cell lung cancer, atypical integrin signaling is associated with tumor cell proliferation, drug resistance and metastasis. Integrin-mediated signaling can enhance tumor

cell invasiveness and promote immune evasion within the TME (66). In glioblastoma (67), atypical β1-integrin signaling is linked to the maintenance of tumor stem cell properties, which may contribute to tumor recurrence and treatment resistance.

In summary, the regulation of both classical and atypical integrin signaling pathways by FN-EDB underlines its multifaceted role in tumor progression. Understanding the complex interactions between FN-EDB, integrins and their downstream signaling molecules is key for deciphering the intricate regulatory networks that control tumor angiogenesis, invasion and metastasis. Future research aimed at dissecting these pathways may reveal new therapeutic targets for cancer treatment, providing strategies to disrupt the complex signaling networks regulated by FN-EDB in the TME.

Angiogenic signaling pathways

FN-EDB is a key regulator of tumor angiogenesis. FN-EDB is a unique structural module of fibronectin involved in the pathogenesis of various malignant tumors, especially due to its ability to regulate the TME. The core of this action is the interaction of FN-EDB with integrin receptors, such as αVβ3 found on the surface of angiogenic endothelial cells, known for their key functions in cell adhesion and signal transduction. Immunohistochemical and histological analyses provide morphological evidence of FN-EDB's involvement in tumor angiogenesis, showing the co-localization of FN-EDB and integrin αvβ3 in tumor tissues and increased vascular density (68). After contact with integrins, FN-EDB initiates a series of intracellular signaling events that ultimately promote angiogenesis.

Mechanisms of the signaling pathways for angiogenesis

Integrins in the activation of signaling pathways that mediate angiogenesis. Binding of FN-EDB to integrins triggers a phosphorylation event involving FAK, a non-receptor tyrosine kinase that acts as a node of extracellular signaling and activates a plethora of downstream signaling molecules. Notably, p-FAK is involved and phosphorylated with other substrates, including members of MAPK pathway, such as ERK1/2 and the serine/threonine kinase Akt (51-53). These kinases contribute to the transduction of proangiogenic signals, thereby promoting the proliferation, migration and survival of endothelial cells. FN-EDB also affects tumor vascularization and maturation by regulating the DLL4/Notch and Wnt/β-catenin signaling pathways (69). The DLL4/Notch pathway is crucial for the regulation of vessel size and patterning during angiogenesis. It influences the balance between Tip and Stalk cells in the angiogenic sprouts. FN-EDB may promote the polarization of vascular endothelial cells through DLL4/Notch signaling, a key step in tip formation and trailing cells of vascular endothelial cells during angiogenesis (Fig. 4).

Transcriptional regulation in angiogenesis. The activation of ERK1/2 and Akt not only regulates immediate cellular responses but also extends to the regulation of gene expression. Specifically, these kinases promote the nuclear translocation of transcription factors such as NF-κB and hypoxia-inducible factor-1alpha (HIF-1α). In the hypoxic environment within tumor masses, the HIF-1α dimeric protein complex remains stable and activates the expression of numerous genes involved in angiogenic processes (70). HIF-1-induced proteins include VEGF and basic fibroblast growth factor (bFGF), which promotes vascular permeability, while the latter promotes endothelial cell growth (71). Other secreted factors such as platelet-derived growth factor (PDGF), angiopoietin-1 (ANG-1) and angiopoietin-2 (ANG-2) promote chemotaxis, while hepatocellular signaling controls migration and cell-cell adhesion, thereby guiding the formation and stabilization of newly formed blood vessels (72). Other HIF-1-induced gene products include MMP, where MMP resolves the ECM, promoting endothelial cell migration and releasing associated growth factors. Once activated, these factors bind to the promoter regions of genes encoding angiogenic factors, including VEGF, to amplify the angiogenic response (73).

Endothelial cell behavior and angiogenesis. Endothelial cells and pericytes are fundamental cellular components of new blood vessels and their interactions regulate angiogenesis (74). FN-EDB has been shown to disrupt the interaction between pericytes and endothelial cells, leading to pericyte detachment and vascular system instability, resulting in further angiogenesis and remodeling of the tumor vasculature (75). FN-EDB-induced signaling pathways enhance endothelial cell proliferation, migration and invasion, which are crucial for vascular sprout formation (76). Pericytes provide support by covering the basal surface of endothelial cells and regulate vascular contraction and relaxation under normal physiological conditions. Newly formed vessels often lack pericytes, but endothelial cells recruit these pericytes to provide additional structural support, enhancing tumor vascularization (77).

FN-EDB promotes formation of tubular structures by promoting the proliferation and organization of endothelial cells. It achieves this by activating FAK and subsequent downstream signaling molecules, including ERK/MAPK and PI3K-Akt pathways that enhance cell adhesion and cytoskeletal rearrangements, required for tube morphogenesis (78).

In the TME, in addition to endothelial cells, many other cell types contribute to angiogenesis. FN-EDB interacts with tumor-associated macrophages (TAMs) and other immune cells, promoting ECM remodeling and creating favorable physical and chemical conditions for angiogenesis (79). Factors secreted into the TME activate TAMs, producing VEGF and MMP, further promoting angiogenesis. Neutrophils, a significant component of immune cell infiltration, promote tumor angiogenesis through various mechanisms, including the release of MMP into the TME, triggering the release of VEGF and other angiogenic factors (80). Other immune cell types (such as B and T cells) secrete VEGF-A, bFGF, MMP9, interferon γ (IFNγ) and interleukin (IL)-17, indirectly affecting angiogenesis (81). Adipocytes release a variety of cytokines, chemokines and hormones (collectively referred to as adipokines), many of which are pro-angiogenic factors (82).

A positive feedback loop in angiogenesis. FN-EDB expression and activity can be further enhanced by tumor-secreted cytokines, forming a positive feedback loop that drives tumor angiogenesis. This positive feedback mechanism amplifies the impact of FN-EDB on tumor angiogenesis (83). VEGF and other growth factors respond to the upregulation of FN-EDB

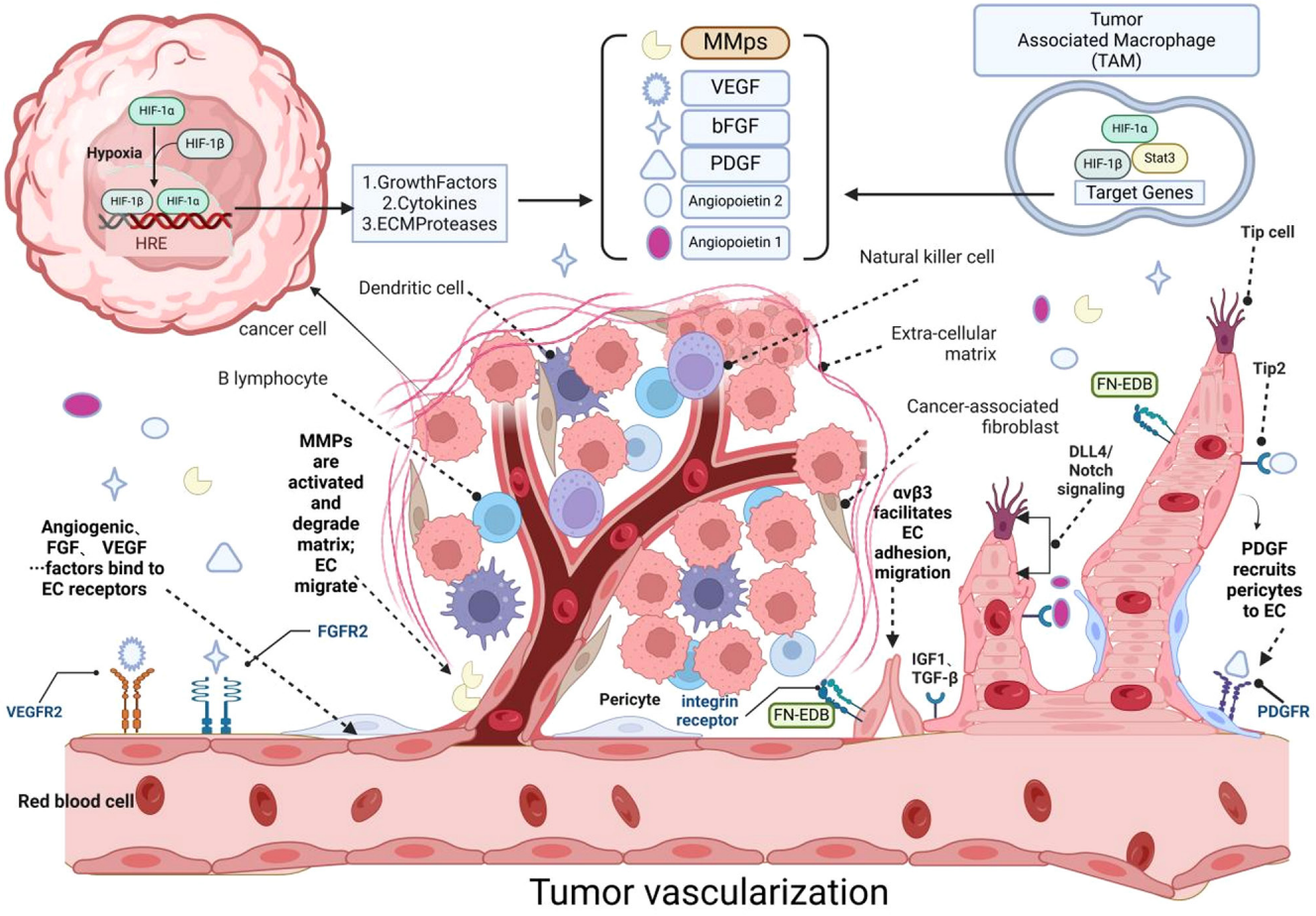

Figure 4. Molecular mechanisms of tumor. *Hypoxia and HIF-1α;* Hypoxia, a condition of low oxygen, activates HIF-1α, a master regulator of the cellular response to oxygen deprivation. HIF-1α, in collaboration with HIF-1β, forms the HIF-1 complex that binds to hypoxia response elements in the promoter regions of target genes, leading to their transcription. *Angiogenic factors;* VEGF and its receptor VEGFR2 are pivotal in angiogenesis. VEGF signaling promotes endothelial cell proliferation, migration and survival. Angiopoietin 1 and 2 regulate the maturation and stabilization of newly formed blood vessels, interacting with Tie-2 receptors on endothelial cells. *ECM and proteases;* MMPs facilitate tumor vascularization by degrading the basement membrane, allowing endothelial cells to migrate and form new vessels. ECM proteases, such as FN-EDB, are involved in the proteolytic processing of ECM components, promoting endothelial cell migration and tube formation. *Cytokines and growth factors;* Insulin-like Growth Factor 1 and TGF-β contribute to the angiogenic process by modulating endothelial cell function and ECM remodeling. Endothelial cells release PDGF to recruit pericytes to endothelial cells: This process contributes to the maturation and stabilization of new blood vessels. tumor-associated macrophages *TAMs;* TAMs secrete angiogenic factors, such as growth factors, cytokines and ECM proteases and contribute to the degradation of the ECM, thereby promoting tumor vascularization. *Notch signaling;* The DLL4/Notch pathway is crucial for the regulation of vessel size and patterning during angiogenesis. It influences the balance between Tip and Stalk cells in angiogenic sprouting. *Cell types and functions;* EPCs are mobilized and incorporated into the growing vasculature, contributing to vessel formation. Tip cells guide the direction of sprouting vessels, while Stalk cells proliferate to elongate the vessel. *Regulation of tumor vascularization;* The interplay between hypoxia, growth factors, ECM remodeling and cellular interactions is essential for the regulation of tumor vascularization, which supports tumor growth, invasion and resistance to therapy. Figure created with BioRender.com, the software was developed by Biorender, Inc. HIF-1α, hypoxia-inducible factor-1 alpha; ECM, extracellular matrix; FN, fibronectin EDB, extra domain B; PDGF, platelet-derived growth factor; TAMs, tumor-associated macrophages EPCs, endothelial progenitor cells.

signaling, creating a feedforward mechanism that strengthens the angiogenic process. This autocrine and paracrine signaling enhances the recruitment of endothelial cells, pericytes and smooth muscle cells, promoting the stability and maturation of newly formed blood vessels (84).

4. Targeting FN-EDB in tumor diagnosis

In the field of tumor diagnosis, FN-EDB is a key protein in the TME and its expression in tumor tissues is closely related to the occurrence and development of multiple types of cancer (85). Tissue-expression type FN-EDB is mainly found in tumor stroma and its high expression is correlated with tumor malignancy and poor prognosis (86). Therefore, examining the expression level of FN-EDB in tumor tissue can be used as a biomarker for to assess tumor aggressiveness and predict patient prognosis (87) (Table I).

Targeting of the FN-EDB aptamer polypeptide Thr-Val-Arg-Thr-Ser-Ala-Asp (ZD2). ZD2, a small peptide aptamer, selectively targets the TME-specific fibronectin extra domain B (FN-EDB), a protein overexpressed in various types of cancer. Its tumor-targeting capability positions ZD2 as a promising agent for diagnostic imaging and therapeutic applications (88,89). Recent advances include the development of a Tc-99m-labeled aptamer (EDB) as an effective tumor imaging agent, demonstrating high specificity for EDB-positive cell lines *in vitro* and favorable pharmacokinetics *in vivo*, with

rapid blood clearance and renal excretion. The potential of this agent to reflect tumor metastasis status offers a valuable tool for early cancer diagnosis and therapeutic response monitoring (90,91).

FN-EDB, a splice variant of fibronectin, has emerged as a promising target for the design of clinically translatable magnetic resonance imaging (MRI) and positron emission tomography (PET) contrast agents. A study investigated the MRI dose-response effect of ZD2-N3-Gd (HP-DO3A) (MT218), a targeted contrast agent, in a rat prostate cancer xenograft model and its detection potential in mouse lung and pancreatic cancer models (92). MT218 exhibited high relaxivities (r1 and r2), favorable physicochemical properties, pharmacokinetics and safety, leading to effective tumor enhancement at reduced contrast agent doses. In the realm of PET imaging, a ZD2-(^{68}Ga-NOTA) conjugate was developed for hepatocellular carcinoma (HCC) imaging, demonstrating high affinity for FN-EDB in a marmoset model, rapid tumor accumulation and a stable state within 5 min post-injection (93). Additionally, a ^{64}Cu-DOTA conjugated to the ZD2 peptide was created for sensitive detection and risk stratification of prostate cancer (94). These findings validate the feasibility of using ZD2 peptide-based radiopharmaceuticals for PET imaging, offering new perspectives for clinical management of patients with HCC and prostate cancer.

ZD2-Gd (HP-DO3A), a molecular MRI contrast agent targeting FN-EDB, has demonstrated significant contrast enhancement in primary and metastatic triple-negative breast cancer tumors, outperforming the nonspecific clinical agent Gd (HP-DO3A) (95-97). The high expression of this agent in the tumor ECM positions it as a promising tool for precise cancer diagnosis. Furthermore, Cy5-EDBp, (^{18}F)-EDBp and (^{177}Lu)-EDBp have emerged as potential candidates for surgical navigation, radionuclide imaging and targeted radionuclide therapy, respectively (98).

The development of novel targeted contrast agents, such as the EDB-FN-targeted imaging probe (ZD2-Gd-DOTA-Cy7), has shown good performance in dual-modality MRI and near-infrared fluorescence imaging for pancreatic cancer, as well as in the precise assessment of chemotherapy efficacy (99). This probe requires only half the dose of conventional clinical gadolinium contrast agents to achieve accurate cancer imaging and evaluation of therapeutic responses. By conjugating ZD2 with commonly used clinical MRI gadolinium contrast agents (Gd-DOTA) and the near-infrared fluorescence dye Cy7, researchers have created an EDB-FN-targeted imaging probe. This probe, combined with dual-modality imaging techniques, has been used to assess the efficacy of chemotherapy regimens, offering a new approach for personalized treatment of pancreatic cancer patients (100).

In summary, ZD2 peptide and its derived targeted probes have shown considerable potential and application prospects in the field of tumor diagnosis and therapy targeting FN-EDB. With continuing technological advances and clinical trials, ZD2 peptide and its associated targeted probes are expected to play an increasingly vital role in future cancer therapy.

Targeting peptide, APTEDB. APTEDB Peptide, a molecule designed based on structural features of FN-EDB, ensures high affinity and selective binding to the TME by recognition of key amino acid residues (101). Ranjbar *et al* (102) achieved specific binding and high tumor uptake in colorectal cancer models by Tc-APTEDB probe, demonstrating its potential as a diagnostic imaging probe. The high binding specificity of the APTEDB peptide, which selectively targets the fibronectin extra domain B (FN-EDB) overexpressed in the tumor microenvironment, enables the development of novel tumor imaging modalities. For example, APTEDB has been conjugated to imaging agents, such as fluorescent dyes, quantum dots, and radionuclides, to create targeted probes that enhance the detection and visualization of tumors with high contrast and resolution (103). These targeted imaging modalities have shown promise in preclinical models, providing a non-invasive approach to cancer detection and monitoring. In the field of optical imaging, the coupling of APTEDB with fluorescent labels or quantum dots enables the visualization of tumor regions with high contrast and resolution (104). In PET and MRI, the coupling of APTEDB to a radionuclide or contrast agent, respectively, enhances the detection of tumors in preclinical models (105). Using FN-EDB-specific peptide [APT (EDB)] Sun *et al* (106) conjugated thermal cross crosslinked superparamagnetic iron oxide nanoparticles [APT (EDB)-TCL-SPIONs] in a breast cancer stem-cell-like cell xenograft mouse model. The results of these studies confirm the broad application of APTEDB peptides in tumor-targeted therapy and imaging (107).

Other diagnostic methods for targeting the FN-EDB. Recent advances in nanotechnology have facilitated the development of FN-EDB-targeting nanoparticles for enhanced diagnostic and therapeutic capabilities in oncology (108). Surface modification of these nanoparticles has been instrumental in augmenting their accumulation and internalization within the TME. Jailkhani *et al* (109) identified a high-affinity nanobody, alpaca-derived libraries of nanobodies (NJB2), which selectively bind to FN-EDB expressed in the ECM of tumors and metastases, offering a non-invasive diagnostic tool for cancer detection and a targeted approach to fibrotic diseases. Using immunoPET/CT, ^{64}Cu-NJB2 nanobodies demonstrate the ability to detect primary and metastatic tumors across various cancer models, highlighting their potential as robust tools for oncological imaging (110). In parallel, Rossin *et al* (111) successfully radiolabeled an anti-ED-B fibronectin human antibody derivative, L19-SIP, using an enzymatic radiobromination method. *In vivo* studies in animal models revealed rapid and specific tumor targeting by (76) Br-L19-SIP, with subsequent validation of its potential in tumor imaging through small animal PET. Berndorff *et al* (112) developed 99 mtc-tagged L19 derivatives to target ED-B fibronectin for the angiogenesis imaging in tumors. Compounds 99 mTc-AP39,99 mTc-L19-His and 99 mTc-L19-Hi20, were synthesized and evaluated *in vivo*. 99mTc-AP39 shows high tumor uptake, rapid blood clearance and excellent imaging properties, providing a promising tool for visualizing tumor-expressing ED-B.

These studies underline the value of targeted molecular imaging with radiolabeled antibodies in enhancing the detection and monitoring of cancer, paving the way for more precise and personalized therapeutic strategies.

Table I. Molecular diagnostic applications targeting FN-EDB.

First author/s, year	Targeting EDB molecule	Molecule type	Conjugate	Full name	Detection method	Tumor application	(Refs.)
Rossin *et al*, 2007	L19	Monoclonal antibody	^{76}Br	^{76}Br-L19-SIP	PET	Dermoid tumor	(111)
Tijink *et al*, 2009			^{124}I	^{124}I-L19SIP	PET	Brain metastases from solid cancer	(180)
Petrini *et al*, 2022			^{131}I	^{131}I-L19-SIP	PET	TETs	(183)
Berndorff *et al*, 2006			^{99m}Tc	^{99m}Tc-AP39 ^{99m}Tc-L19-His ^{99m}Tc-L19-Hi20	PET	Teratocarcinoma	(112)
Ye *et al*, 2017	ZD2	Aptamer peptide	^{99m}Tc	^{99}mTc-HYNIC-ZD2	SPECT	Breast cancer	(98)
Qiao *et al*, 2020			Gd	ZD2-N3-Gd (HP-DO3A)	MRMI	Cancer of pancreas	(88)
Feng *et al*, 2023			GVs	ZD2-GVs	Unique molecular identifier	Carcinoma of urinary bladder	(89)
Lu *et al*, 2022			N3-Gd	ZD2-N3-Gd (HP-DO3A)	MRI	Prostatic carcinoma	(95)
Han *et al*, 2019			Gd	ZD2-Gd (HP-DO3A)	MRI	Prostate cancer	(94)
Sergeeva *et al*, 2023			^{68}Ga-NOTA	ZD2-(^{68}Ga-NOTA)	PET	Hepatocellular carcinoma	(93)
Zhang *et al*, 2024			Cys-TVRTSAD	ZD2-Gd-DOTA-Cy7	MRI/ fluorescence molecular imaging EDB	Ductal	(99)
Yang *et al*, 2024			Gd-DOTA	CREKA-Cy7-(Gd-DOTA)	MR/near-infrared fluorescence	GC	(100)
Han *et al*, 2019			^{64}Cu	ZD2-^{64}Cu-DOTA	PET	Prostatic carcinoma	(96)
Park *et al*, 2012	APTEDB	Nano antibodies	SPION	APT (EDB)-SPIONs	MRI	Mammary cancer	(108)
Sun *et al*, 2014			SPION	APTEDB-TCL-SPION	MRI	Mammary cancer	(106)
Jailkhani *et al*, 2019			NJB2	^{64}Cu-NJB2	SPECT	Ductal adenocarcinoma of pancreas	(109)
Ranjbar *et al*, 2020			^{99}mTc	^{99}mTc-APTEDB	Radio-isotope thin-layer chromatography scanner	Brain cancer and colorectal cancer	(102)
Li *et al*, 2023	EDBp	Aptamer peptide	^{18}F/^{177}Lu	^{18}F-NOTA-PEG4-EDBp ^{177}Lu-DOTA-P EG4-EDBp	PET	TC	(179)

FN, fibronectin EDB, extra domain B; PET, positron emission tomography; TET, thymic epithelial tumor; SPECT, Single Photon Emission Computed Tomography; TC, Thyroid Cancer; MRMI, Magnetic Resonance Molecular Imaging; MRI, Magnetic Resonance Imaging; DB, Dextran Binding; EDBp, Extracellular Domain B Peptide; SIP, Small Immunoprotein; HP-DO3A, Hydroxypropyl-dodecylamine; NOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid; PEG, polyethylene glycol; DOTA-P, DOTA-Probe; EG, ethylene glycol; TVRTSAD, thioValine-Arginine-Thr-Ser-Ala-Asp; CREKA, Cysteine-Arginine-Glutamic Acid-Lysine-Alanine; SPION, Superparamagnetic Iron Oxide Nanoparticles.

5. Application of targeted FN-EDB in tumor immunotherapy

Targeting FN-EDB, a tumor-specific marker, has emerged as a promising strategy in cancer immunotherapy. This approach exploits the unique expression pattern of FN-EDB in the TME to enhance the selective delivery of therapeutic agents, thereby improving treatment efficacy and minimizing damage to healthy tissues.

The rationale behind FN-EDB-targeted immunotherapy is to direct cancer treatments specifically to tumor cells, sparing healthy cells that do not express the target. This strategy encompasses a range of therapeutic modalities (Table II), including small molecule inhibitors, monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs) and gene therapy, all designed to exploit molecular characteristics or cell surface proteins associated with cancer cells (113).

Antibodies or antibody-like molecules that target FN-EDB can inhibit tumor cell adhesion and migration by binding specifically to FN-EDB, thereby suppressing tumor progression (7). Additionally, small molecule compounds can disrupt tumor cell interactions with the ECM, inhibiting tumor growth and metastasis by targeting FN-EDB's functional roles. These molecules can also interfere with signaling pathways within the TME, such as FAK/PI3K/AKT and MAPK/ERK, which are crucial for tumor cell proliferation, survival, angiogenesis and immune cell infiltration (114).

mAbs can induce antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) upon binding to FN-EDB, leading to the direct killing of tumor cells (115). Gene therapies, using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 and similar editing technologies, can knock out or downregulate FN-EDB expression, thus inhibiting the malignant behavior of tumor cells (116). Furthermore, gene-edited T cells with enhanced specificity for FN-EDB-positive tumor cells can improve immune clearance through approaches such as T cell receptor (TCR) or chimeric antigen receptor (CAR) modification.

Conjuome-mediated targeted therapy of FN-EDB tumor-specific carriers. At present, antibodies (L19) are often targeted with cytokines in FN-EDB for tumor treatment and diagnosis. These other antitumor drugs are composed of mAbs and cytokines (such as IL-12, IL-15 and IL-21), TNF-α, IFN-γ and tissue factor to deliver cytokines directly to the TME to activate the immune response (117).

The L19-interleukin-2 fusion protein. L19-IL2, a novel fusion protein consisting of the single-chain variable fragment of the humanized monoclonal antibody L19 targeting FN-EDB and IL-2 represents a promising advancement in cancer therapeutics (118). This targeted approach enhances the delivery of IL-2 directly to the TME, thereby mitigating the systemic side effects associated with conventional IL-2 therapy.

The L19 antibody, which selectively binds to FN-EDB overexpressed in various malignancies, has been effectively fused with IL-2 to create darleukin. This fusion protein not only exhibits high tumor specificity but also activates cytotoxic T lymphocytes and natural killer (NK) cells, bolstering the immune response against cancer cells (119). Moreover, L19-IL2 may reshape the immunosuppressive TME, facilitating the activation and proliferation of tumor-specific T cells and the maturation and antigen presentation of dendritic cells.

Preclinical studies have demonstrated the efficacy of L19-IL2 in suppressing the growth of FN-EDB-positive tumors and extending survival in animal models, including nude and SCID mice (120). The transition of L19-IL2 into clinical trials marks a significant step, with ongoing research aimed at assessing its safety, tolerability and efficacy in human subjects. Initial clinical investigations are focused on determining the maximum tolerated dose, recommended phase II dose and characterizing the pharmacokinetic and pharmacodynamic profiles of L19-IL2 (121).

The potential for L19-IL2 to be combined with other therapeutic modalities, such as immune checkpoint inhibitors, chemotherapy and radiotherapy, is currently being explored in clinical trials. For example, in a randomized phase II clinical trial, one group of patients received dacarbazine (DTIC; 1,000 mg/m^2 on day 1 of a 21-day cycle) as a single agent, while in the other two groups, L19IL2 was increased (22.5 million international units of IL 2 equivalents) depending on two different dosing regimens. Analysis of efficacy results showed that patients receiving L19IL2 combined with DTIC had statistically significant results for overall response rate and median progression-free survival compared with DTIC monotherapy (122). The synergy between L19-IL2 and stereotactic ablation body radiotherapy (SABR) is of particular interest, as evidenced by a phase II clinical trial investigating their combined use in patients with oligometastatic non-small cell lung cancer (NSCLC) (123). This multicenter, randomized controlled trial, involving 126 patients, is designed to evaluate the 1.5-year progression-free survival as the primary endpoint, highlighting the potential of integrating L19-IL2 with SABR to enhance local tumor control and systemic immune responses (124).

The L19-interleukin-12 fusion protein. IL-12 was covalently linked with a monoclonal antibody to produce an IL-12 antibody drug conjugate (ADC) to deliver IL-12 to tumor cells enriched at the target antigen level. This IL-12 delivery targeting a specific antigen minimizes IL-12 exposure in normal tissues, allowing it to show lower toxicity and an improved therapeutic index (125). The development of ADC therapy underwent several stages. The first generation ADC has limited therapeutic efficacy due to problems such as linker instability and insufficient payload toxicity. The second generation ADC improved efficacy by improving the stability of the linker and using more payloads. The third generation of ADC further optimized the selectivity of antibodies, the design of linker and the toxicity and bystander effects of payload, so that ADC therapy has achieved significant therapeutic effects in some hematological tumors and solid tumors (126,127).

AS1409 is formed by covalently linking the humanized anti-FN-EDB antibody BC-1 with IL-12, aiming to deliver IL-12 to the tumor-associated vasculature (128). Ongaro *et al* (129) developed a tandem-dimer form of a full-human fusion protein, L19-L19-IL12, created by fusing human IL-12 as a tandem dimer with the L19 antibody using protein engineering techniques. This variant, equipped with an optimized linker, demonstrated enhanced tumor targeting, prolonged retention at the tumor site and rapid clearance from

Table II. Tumor immunotherapy strategies targeting FN-EDB.

First author/s, year	Treatment	Antibody	Conjugate	Name	Combination	Tumor therapy	Research progress	Study identifier	(Refs.)
Danielli *et al*, 2015	Antibody therapy	L19	IL2	L19-IL-2 + L19-TNF-α	L19-TNF	GMs	Clinical Phase I/II	NCT02076633	(137)
Saif *et al*, 2022		L19	IL2	L19IL2 + L19TNF	L19-TNF	Cutaneous melanoma	Clinical Phase IIIB/IIIC	NCT03567889	(141)
Spitaleri *et al*, 2013		L19		L19+TNF	TNF	Colorectal cancer	Clinical Phase I/II	NCT01253837	(139)
Yu *et al*, 2022		Anticotinine antibody	VEGF	HyPEP (EDB-VEGF) + PD-1	PD-1	Metastatic melanoma pancreatic cancer	Animal experiment	-	(147)
Johannsen *et al*, 2010		L19	IL2	L19-IL2		Renal cell carcinoma	Clinical phase I/II	NCT01058538	(118)
Kim *et al*, 2019		Abcot	APTEDB	HC (cot-APTEDB-Stereotactic ablative body radiotherapy/Abcot)	Stereotactic ablative body radiotherapy/ Abcot	MGs	Animal experiment	-	(149)
Van Limbergen *et al*, 2021		L19	IL2	L19-IL2 + stereotactic body radiation therapy	Stereotactic body radiation therapy	NSCLC	Clinical Phase I/II	NCT02086721	(123)
Schliemann *et al*, 2009		L19	IL2	L19-IL2 + rituximab	Rituximab	B cell NHL	Clinical phase II	NCT02957019	(121)
Weide *et al*, 2019		L19	IL2	L19-IL2 + dacarbazine	Dacarbazine	Stage IV melanoma	Clinical phase II	NCT02076646	(122)
Ongaro *et al*, 2022		L19-L19	IL2	L19-L19-IL2+PD-1	PD-1	solid tumor	Animal experiment	-	(129)
Orecchia *et al*, 2019		L19	IL2	L19-IL2 + 46F2SIP	Anti-SDC1 46F2SIP (small immuno protein) antibody	EOC	Animal experiment	-	(120)
Papadia *et al*, 2013		L19	TNF	L19-TNF + melphalan	Melphalan	extremity melanoma	Clinical Phase II	NCT01213732.	(138)
Lieverse *et al*, 2020		L19	IL2	L19-IL2 + SABR	SABR	NSCLC	phase IV	NCT03705403	(124)
Trachsel *et al*, 2007		L19	IL-10	L19-IL-10	IL-10	RA	Animal experiment	-	(132)
Kaspar *et al*, 2007		L19	IL-15/ GM-CSF	L19-IL-15 L19-GM-CSF	IL-15/GM-CSF	Teratocarcinoma and colon carcinoma	Animal experiment	-	(133)
Niu *et al*, 2023		L12	IFN-γ + PD-1	IL-12-IFN-γ + PD-1	PD-1	Solid tumor	Clinical phase I/II	NCT04370587	(136)
Spaeth *et al*, 2006	Radiation therapy	L19-SIP	^{131}I,^{124}I	^{131}I-L19-SIP	RIT	Colorectal tumour	Animal experiment	-	(140)

Table II. Continued.

First author/s, year	Treatment	Antibody	Conjugate	Name	Combination	Tumor therapy	Research progress	Study identifier	(Refs.)
Tijink *et al*, 2009		L19-SIP	^{131}I	^{131}I-L19-SIP	RIT squamous cell carcinoma	Head and neck	Animal experiment	-	(180)
Tang *et al*, 2023	Cell Therapy	CAR-T	APT0/ CGS2	APT0 CAR-T CGS2 CAR-T		Solid tumor cells	Cell experiment	-	(163)
Zhang *et al*, 2022		CAR-T	rTCR	rTCR-CAR-T		Solid tumor cells	Xenograft models	-	(167)
Xiong *et al*, 2024		CAR-T	CD70	CD70 CAR-T		Renal cell carcinoma	Xenograft model and *in vivo* tests	-	(170)
Zhu *et al*, 2022		CAR-T	CD70	CD70 CAR-T+ oncolytic herpes simplex virus-1	Oncolytic herpes simplex virus-1	GBM	Animal experiment	-	(171)
Li *et al*, 2023		CAR-T	PD-L1	CAR-T +PD-L1		Hematological malignancies	Cell experiment	-	(173)
Li *et al*, 2023		CAR-T	Rituximab	CAR-T + rituximab		Relapsed/refractory B cell acute lymphoblastic leukemia	Retrospective study	-	(174)
Noh *et al*, 2021	Nanoparticle-drug		cyNP	APTEDB-cyNP@ CpG	CpG adjuvant	Colon tumor	Animal experiment	-	(104)
Saw *et al*, 2021				APT EDB-DSPE-DTX	DSPE-DTX	MGs	Gene expression profiles and survival analysis	-	(154)
Saw *et al*, 2018			NPs	APT-EDB NPs-experiments)		GBM	*In vitro* and *in vivo*	-	(152)

FN, fibronectin; EDB, extra domain B;NSCLC, non-small cell lung cancer; HyPEP, hybrid peptide; GBM ,glioblastoma multiforme; MG, malignant Glioma; NP, Nanoparticles; HC, hepatocellular Carcinoma; NHL, non-Hodgkin's Lymphoma; 46F2SIP, Anti-Syndecan-1 46F2 Small Immuno Protein; EOC, epithelial Ovarian Cancer; SABR, stereotactic Ablative Body Radiotherapy; GM-CSF, granulocyte-Macrophage Colony Stimulating Factor; RIT, Radioimmunotherapy; RA, rheumatoid Arthritis; CAR-T, Chimeric Antigen Receptor T-Cell Therapy; rTCR, Recombinant T Cell Receptor; CyNP @ CpG,a nanoparticle containing CpG oligonucleotides; DSPE-DTX, 1,2-distearyl-sn-glycerol-3-phosphorylethanolamine-docetaxel.

blood and normal organs, suggesting its potential as a promising alternative to L19-IL12 and warranting further clinical investigation.

Clinical trials are currently underway to evaluate ADCs targeting FN-EDB across different types of tumor for safety, pharmacokinetics and antitumor effects. PYX-201, an ADC comprising a monoclonal antibody against FN-EDB, a cleavable linker and an auristatin payload, is being assessed in Phase I clinical trials for various solid tumors, including squamous cell carcinoma of the head and neck, non-small cell lung cancer, ovarian cancer, soft tissue sarcoma and pancreatic ductal adenocarcinoma (130). The ADC's internalization and endosomal-lysosomal pathway release the payload, achieving precise cytotoxicity against tumor cells.

EDB-ADCs have demonstrated antitumor activity and enhanced immune cell infiltration in multiple cancer models, with improved efficacy when combined with immune checkpoint blockade and have shown good safety profiles in non-human primates (131). These findings underscore the potential of FN-EDB-targeting ADCs as a valuable tool in the oncology therapeutic arsenal, providing a rationale for their further development and clinical application.

L19 with other immune cytokines. L19 can also be coupled to other cytokines, including IL-10 (132), IL-15 (133) and granulocyte-macrophage colony-stimulating factor (granulocyte-macrophage colony stimulating factor; GM-CSF) (133), IFN-γ (134-136), and TNF-α (137-140), Most of these cytokines are produced by immune cells and act on the tumor.

Trachsel *et al* (132) demonstrated that L19-IL-10 fusion protein, when delivered to rheumatoid arthritis models, exhibits superior efficacy in alleviating arthritis symptoms and inhibiting disease progression compared with non-targeted IL-10. This targeted approach suggests a potential therapeutic benefit in chronic inflammatory conditions. Kaspar *et al* (133) investigated the antitumor properties of L19-IL-15 and L19-GM-CSF, revealing potent antitumor activities in both subcutaneous and metastatic cancer models. These immunocytokines, by selectively binding to tumor vasculature, effectively inhibited tumor growth and metastasis, underscoring the potential of L19-based targeted therapy in oncology (133). IFNγ, a critical immunomodulatory cytokine, has been explored in the context of L19 fusion proteins to enhance tumor immunogenicity and promote immune cell infiltration. Ruan *et al* (134) developed an L19-IFNγ variant fusion protein with reduced affinity for its cognate receptor, thereby minimizing off-target organ capture and enhancing tumor-specific localization. Di Nitto *et al* (135) designed an L19-IFNγ KRG fusion protein that demonstrated improved tumor homing and pharmacokinetic properties in preclinical models. Niu *et al* (136) show that the combination of L19-IFNγ KRG with immune checkpoint inhibitors, such as anti-PD-1 antibodies, induce tumor growth delay and increase intra-tumoral concentrations of T cells and NK cells, highlighting the synergistic potential of this combination therapy.

TNF-α is known for its potent antitumor effects. L19-TNF is an immune cytokine fusion protein targeting tumor vasculature resulting from the fusion of the human monoclonal antibody L19 to TNF. L19 antibody can specifically bind FN-EDB on the surface of tumor vascular endothelial cells and accurately deliver TNF to tumor tissue, which has also been studied in the form of L19-mTNF fusion protein (140). Spitaleri *et al* (139) reported on the efficacy and safety of L19-TNF monotherapy in patients with advanced solid tumors in a phase I/II clinical trial (139). A total of 34 patients were enrolled into six dose groups, with L19-TNF doses increasing from 1.3-13 μg/kg, every 3 weeks, administered on days 1, 3 and 5. The aforementioned study showed that in terms of safety, the patients mainly experienced mild chills, nausea and vomiting but no hematology or unexpected serious toxicity. In terms of efficacy, no objective tumor response was observed, but out of 31 evaluable patients, 19 had transient stable disease-states (137). Therefore, its potential for combination chemotherapy to play a greater role in the treatment of advanced solid tumors can be further explored. In an additional exploratory clinical trial, the investigators evaluated the safety and clinical activity of L19-TNF in combination with melphalam-isolated limb perfusion (ILP) containing melphalam in patients with locally advanced limb melanoma (138). A total of 17 patients were included, of which 7 received 325 μg L19-TNF and 10 received 650 μg L19-TNF. The results showed that the non-hematologic toxicity of L19-TNF ILP was low, but four patients developed severe myelosuppression. Although the TNF equivalent dose of L19-TNF was only 3.13 and 6.25% of the approved TNF (Beromun®) dose of 4 mg, the clinical efficacy was marked. In the 650 μg dose group, 89% of patients had objective responses, five achieved complete response (CR) and four patients had CR status until 12 months; no CR was observed in the 325 μg group. This suggests that L19-TNF ILP has promising clinical activity and potential application in the treatment of locally advanced limb melanoma (138). Danielli *et al* (137) fused two immune cytokines, IL-2 and TNF-α, to a combination of monoclonal L19 antibody that binds to FN-EDB and this selectively targeting L19 antibody could allow the drug to accumulate in the injected lesion, potentially producing a more durable immunoreactive. Melanoma is an aggressive skin malignancy (141), especially in the local advanced stage (stage IIIB/C). Traditional surgical resection may not achieve ideal results, and patients face a high risk of recurrence and metastasis. New treatments are needed. The Neo-DREAM trial is a phase III study to evaluate the efficacy of neoadjuvant intratumoral injection of Darleukin/Fibromun (L19IL2 + L19TNF) in patients with clinical stage IIIB/C melanoma. The study randomizes patients into two groups: Darleukin/Fibromun, followed by surgery and adjuvant therapy and surgery and adjuvant therapy alone (142). Darleukin/Fibromun precisely targets FN-EDB in the tumor extracellular matrix (ECM) by using immune cytokines IL-2 and TNF-α with the monoclonal antibody L19 to stimulate the local immune response. According to the June 2022 issue of Ann Surg Oncol, the study observed significant patient objective response rates (ORR) and excellent complete response rates (CR) and partial response rates (PR) (143). Notably, the incidence of distant effect (abscopal effect) in non-injected lesions was ≤53.8% (7/13), indicating that Darleukin/Fibromun can not only effectively control tumors at the injection site, but also produce immune killing in distant uninjected lesions. In terms of safety, the side effects recorded in the study were relatively few and controllable and Darleukin/Fibromun showed improved safety compared with traditional treatments.

These studies collectively illustrate the versatility and potential of L19-based immunocytokines in modulating

the TME and enhancing antitumor immune responses. The ongoing clinical development of L19-IL2 and other L19-based fusion proteins signifies a promising direction in cancer immunotherapy, with the potential to improve patient outcomes through targeted delivery of therapeutic agents to the tumor site. The synergistic effects observed with combination therapies further emphasize the importance of exploring multimodal treatment strategies to maximize clinical benefits.

Tumor-specific peptide-drug conjugate (PDC). Tumor-specific PDCs) represent a burgeoning class of therapeutics in cancer immunotherapy, harnessing the targeting capabilities of tumor-specific peptides to deliver cytotoxic agents directly to cancer cells (144). This strategy aims to enhance therapeutic efficacy while minimizing damage to healthy tissues, offering a promising approach to improve cancer treatment.

The design of PDCs necessitates careful selection of a linker that ensures drug stability and is cleavable within the TME to release the active drug. Equally critical is the choice of toxin, which must possess potent antitumor activity and retain its pharmacological properties post-conjugation. Researchers have developed a bispecific peptide, HyPEP (EDB-VEGF), capable of binding to cancer cells overexpressing EDB and VEGF (145). In animal models, this conjugate has demonstrated the ability to inhibit tumor growth, with enhanced effects when combined with anti-PD-1 antibodies, suggesting its potential in immunotherapy (146). The PL1 peptide, identified for its ability to bind to FN-EDB and Tenascin C subtypes in the tumor ECM, triggers cellular uptake primarily through electrostatic interactions.

This finding provides a novel perspective for developing targeted cancer therapeutics. By conjugating carboplatin (CBT) with a cyclic cell-penetrating peptide, a new drug delivery system has been developed. This conjugate selectively binds to cancer cells overexpressing integrins and EDB-FN and releases CBT in the acidic tumor environment, exhibiting high selectivity and low toxicity for cancer cells (147). In the treatment of prostate cancer, PDCs targeting EDB-FN have shown therapeutic effects in animal models by providing selective cytotoxicity (148). To overcome the short circulation half-life of PDCs, a strategy involving the formation of hybrid complexes with anti-hapten antibodies has been developed, significantly extending the PDC's circulation half-life, enhancing tumor accumulation and penetration, thereby inhibiting tumor growth (149). The expression of VEGF, closely associated with the development of various tumors, has been targeted by cetuximab (Erbitux), a monoclonal antibody that inhibits tumor angiogenesis by blocking the binding of VEGF to its receptors, thus exerting antitumor effects (150).

Current research on PDCs in cancer therapy has made significant progress. As clinical trials continue to assess the safety and efficacy of PDCs, their potential to revolutionize cancer treatment strategies continues to grow.

Nanoparticle-drug conjugates. In the medical research domain, innovative nanosystems have been developed to enhance drug targeting and bioavailability while minimizing systemic side effects (104,151,152). Gold nanoparticles (NPs) with distinct surface modifications have demonstrated the ability to selectively bind various structural domains of fibronectin (FN), with interaction strengths influenced by the NPs' physicochemical properties. Particularly, cationic NPs (NP-NH3+) interact with acidic domain-rich regions of FN, potentially impairing its function (153).

For instance, a system encapsulating bevacizumab within immunoglobulin-1 functionalized PLGA nanoparticles has been investigated for the treatment of atherosclerosis, showing improved drug targeting and bioavailability with reduced systemic adverse effects (151). Noh *et al* (104) developed APTEDB-cyNP @ CpG nanomedicine, which coupled APTEDB to PEG and DSPE to build cyanine nanoparticles coated with CpG adjuvant for photothermal therapy and immunotherapy of cancer. In addition, APTEDB-DSPE-docetaxel (DTX) nanoparticles formed by combining APTEDB peptide and DTX show excellent targeting and anti-tumor effects in glioma treatment (154). These nanodrugs have exhibited significant therapeutic effects in tumor models, inducing immunogenic cell death and enhancing T-cell antitumor activity.

To enhance the therapeutic efficiency for malignant glioblastoma multiforme (152), aptamer-like peptide-modified liposomal nanoplatforms for systemic small interfering RNA (siRNA) delivery have been developed. These nanoplatforms specifically target tumor cells and suppress tumor growth by silencing the expression of key genes, such as cyclophilin A. Furthermore, FN-EDB expression in atherosclerotic plaques has been explored, leading to the design of FN-EDB-specific nanoparticles for enhanced plaque detection and localized drug delivery, demonstrating favorable targeting and drug delivery outcomes in animal models (155).

These studies collectively highlight the potential of nanotechnology in improving drug delivery efficiency and tumor therapy, offering new directions for future clinical applications.

Small molecule inhibitors. Small molecule inhibitors targeting FN-EDB disrupt FN-EDB interactions with integrin receptors to impede tumor cell adhesion, migration and invasion. These inhibitors mitigate tumor growth and metastasis by attenuating interactions between cancer cells and the ECM, modulating signaling pathways within the TME that influence angiogenesis and immune cell infiltration (156).

BRAF/MEK inhibitors exemplify cell growth suppressants that induce immunogenic cell death, activating antitumor T cell responses, particularly in melanomas harboring BRAF (V600E) mutations (157). VEGF-VEGFR inhibitors, such as bevacizumab, disrupt the VEGF pathway, curtailing recruitment of immunosuppressive cells and enhancing T cell tumor infiltration, demonstrating significant efficacy in combination with atezolizumab for non-small cell lung cancer (158).

CSF1R inhibitors, by modulating immune cell activity in the TME, reduce the number of TAMs, bolstering the antitumor immune response (159). Metabolic pathway inhibitors, including glutaminase and arginase inhibitors, are under investigation to regulate the metabolism of both tumor and immune cells, further potentiating immune responses (160).

The advent of small molecule immune checkpoint inhibitors offers a novel avenue for directly targeting the PD-1/PD-L1 axis, promising similar therapeutic effects to antibody drugs with improved pharmacokinetics and oral bioavailability. Emerging small molecule immunotherapeutic candidates, such as ABBV-CLS-484, target both tumor and immune

cells, enhancing immune cell activity and sensitizing tumors to immune attack, showing significant antitumor effects in animal models (161).

These advances highlight the key role of small molecule inhibitors in activating and augmenting the immune system against cancer, offering new therapeutic prospects for precision oncology.

Chimeric antigen receptor T-cell (CAR-T) therapy. CAR-T cell therapy has revolutionized cancer treatment by genetically engineering the T cells of patients to recognize and attack specific tumor cells. This approach has demonstrated marked efficacy in hematological malignancies, leading to the approval of several CAR-T products. The evolution of CAR-T therapy, from the first generation with a single-chain variable fragment to the fourth generation incorporating multiple signaling pathways, has been driven by the pursuit of enhanced efficacy and safety.

Despite the success in blood cancers, CAR-T cell therapy faces challenges in solid tumors due to the suppressive TME and the lack of specific tumor-associated antigens (162). To address this, researchers have turned to FN-EDB, a cancer-fetal antigen highly expressed in the tumor vasculature and cancer cells, making it a potential target for CAR-T cell therapy in solid tumors (163).

Investigators have developed recombinant T cells expressing T cell receptors (rTCRs) fused with CARs (rTCR-CAR T cells) that target FN-EDB (164). These rTCR-CAR T cells have shown the ability to bypass TME suppression by combining the single-chain variable fragment with CD3ε. Preclinical studies have shown that rTCR-CAR T cells exert antitumor effects on various solid tumors, including thyroid cancer and breast cancer, by inhibiting the effects of FN-EDB in cell adhesion, migration and angiogenesis (165,166). These rTCR-CAR T cells exhibited cytotoxicity against EDB-positive tumor cells *in vitro* and effectively inhibited tumor growth and reduced tumor vascular density *in vivo* without significant toxicity.

Further research has constructed a BBz CAR targeting EDB-FN, using lentiviral transduction to express CAR molecules on T cells (167). *In vitro* and *in vivo* experiments confirm the ability of CAR T cells to activate and lyse EDB-positive cells, inhibiting tumor growth and vascular density in U-87 MG xenograft models.

The innovative rTCR-CAR T cell therapy is currently being evaluated in multiple clinical trials for its safety and efficacy in cancer treatment, particularly in targeting FN-EDB within the TME (168,169). Combination therapy strategies under exploration include the use of CD70 CAR-T cells with PARP inhibitors, aiming to enhance efficacy by optimizing CAR-T cell structures and constructing bispecific CAR-T cells (170).

Additionally, the combination of CD70 CAR-T cells with oncolytic viruses (OVs) exploits the selective replication and destructive capacity of OVs within tumor cells, promoting an inflammatory TME to improve treatment outcomes for solid tumors (171). Epigenetic modulators, such as decitabine and givinostat, have shown potential in enhancing the efficacy of CD70 CAR-T cells by increasing CD70 expression levels (172).

The combination of CAR-T cells with immune checkpoint inhibitors, such as anti-PD-1 and anti-PD-L1 antibodies, restores the function of tumor-infiltrating lymphocytes, enhancing their ability to attack cancer cells and improving the persistence and efficacy of CAR-T cells within the TME (173). Furthermore, the co-expression of cytokines such as IL-12, IL-15 and IL-18 with CAR-T cells has demonstrated advantages in boosting their activity and durability (174).

Moreover, the combination of CAR-T cells with small molecule drugs, such as the BTK inhibitor ibrutinib, has potential to improve patient responses in clinical trials (175). The scope of combination therapies for CAR-T cells extends beyond the aforementioned strategies to include radiotherapy, chemotherapy, hematopoietic stem cell transplantation and targeted therapies, all aiming to enhance treatment efficacy.

In summary, CAR-T cell therapy targeting FN-EDB holds significant potential in the treatment of solid tumors, with ongoing clinical trials exploring its effectiveness. The future of cancer treatment may benefit from more effective and personalized options offered by CAR-T cell therapies. As research progresses, optimizing CAR-T cell design, enhancing treatment efficacy, minimizing side effects and exploring combination applications with other therapeutic modalities will remain a focal point in this field.

6. Radiotherapy for targeting the FN-EDB

FN-EDB, a tumor-associated antigen, is highly expressed in the TME and serves as an ideal target for radiotherapy, particularly in radioimmunotherapy (RIT). RIT combines the precision of mAbs with the cytotoxic effects of radioactive isotopes, offering a targeted approach to cancer treatment (176).

The L19 mAb, which selectively binds to FN-EDB, has been instrumental in RIT. Variants such as L19-SIP, L19 (ScFv)2 and AP39, have demonstrated significant success in imaging and treating various types of cancer (177). These antibodies have been conjugated with radioisotopes like ^{131}I, ^{124}I, ^{125}I, ^{90}Y and ^{111}In, showing promising results in preclinical models by significantly inhibiting tumor growth (178).

Radiotherapy strategies targeting FN-EDB have potential in several types of tumor. The EDBp probes developed by Li *et al* (179), including Cy 5-PEG 4-EDBp, (^{18}F)-NOTA-PEG 4-EDBp and (^{177}Lu)-DOTA-PEG 4-EDBp, respectively, for surgical navigation, radionuclide imaging and treatment. These probes showed high tumor uptake and good therapeutic effects in thyroid cancer models, providing new tools for the diagnosis and treatment of various types of tumors. In a study by Spaeth *et al* (140), ^{131}I-labeled L19 (SIP) was evaluated in a C6 glioma rat model, revealing a significant extension in median survival time compared with the control group, underscoring the potential of ^{131}I-SIP (L19)-RIT in glioma treatment. Tijink *et al* (180) prepared high-purity ^{124}I-L19-SIP for immuno-PET imaging of tumor angiogenesis, supporting its use as a preclinical tool to predict the biodistribution of ^{131}I-L19-SIP in RIT.

Moosmayer *et al* (181) compared the therapeutic potential of directly radioiodinated L19-SIP with a pretargeting approach using ^{111}In-labeled HSG peptide and AP39xm679 bispecific mAb. The pretargeting strategy showed superior tumor accumulation and dose delivery, suggesting that using ^{90}Y-labeled half-antigen peptides could enhance therapeutic outcomes.

A phase I study by Del Conte *et al* (182) evaluated the safety and tumor targeting of ^{131}I-L19SIP in 51 patients with soft tissue sarcoma (SC) and hematological malignancies. The

treatment exhibited acceptable toxicity with thrombocytopenia as the main side effect. Partial responses and disease stabilization were observed in Hodgkin's lymphoma patients, confirming the potential of EDBF as a target for angiogenesis-based antibody therapy.

Research on FN-EDB expression in thymic epithelial tumors (TET) and its application in R-RIT has been conducted through prospective clinical trials, demonstrating the feasibility of using ED-B FN-specific human recombinant antibody radretumab for the treatment of recurrent TET patients (183).

In summary, RIT targeting FN-EDB has shown considerable potential in various types of cancer, with ongoing clinical trials aimed at optimizing treatment strategies. Future research should focus on refining RIT approaches, enhancing treatment efficacy, reducing side effects and exploring combination therapies with other modalities such as chemotherapy, immunotherapy and targeted agents to improve patient outcomes.

7. Discussion

Summary and outlook. The present study focused on the involvement of FN-EDB and FN-EDB and its diagnostic role as a biomarker in tumors and FN-EDB inhibition as a strategy for immunotherapy. FN-EDB has emerged as a pivotal player in tumorigenesis, with its elevated expression levels in malignant tissues correlating with aggressive tumor behavior, including invasion, lymph node metastasis and poor prognosis. This association positions FN-EDB as a potential biomarker for cancer diagnosis and a therapeutic target. The utility of FN-EDB as a biomarker has been harnessed in the development of various imaging techniques, such as MRI contrast agents using FN-EDB-specific peptides like ZD2, enhancing the specificity and sensitivity of tumor detection. Significant advances have been made in targeting FN-EDB for cancer immunotherapy, with approaches including ADCs, CAR-T cell therapy and immune checkpoint blockade. Clinical trials for several therapeutic and diagnostic modalities targeting FN-EDB are underway, with some, like the phase III trial of daromun for neoadjuvant treatment, showing promising results (184,185).

Despite these developments, challenges remain in translating FN-EDB-targeted therapies into clinical practice. The precise mechanisms by which FN-EDB contributes to the TME are not fully understood and its complex interactions with cancer cell signaling pathways require further investigation. The differential expression of FN-EDB across tumor types hints at diverse roles in oncogenesis, necessitating a deeper exploration of the underlying biological mechanisms. While preclinical studies have shown promise for FN-EDB-targeted conjugate therapies, the transition from animal models to clinical settings demands rigorous clinical trials to validate safety and efficacy in humans. Future research should focus on elucidating the role of FN-EDB in tumor biology, dissecting its signaling pathways in oncogenesis and understanding the basis for its variable expression across different tumors.

A thorough understanding of FN-EDB's function will pave the way for the development of more effective and safer immunotherapies targeting FN-EDB. Concurrently, the pursuit of safer and more effective conjugate drugs, along with the development of non-invasive and precise diagnostic strategies for tumor grading, will be crucial for enhancing cancer treatment and diagnosis outcomes. Although challenges persist, ongoing research into FN-EDB holds the promise of yielding more potent immunotherapeutic agents and clinical strategies, offering renewed hope for patients with cancer.

8. Conclusion

FN-EDB, a key player in tumor aggression, angiogenesis and immune evasion, presents a promising therapeutic target. Strategies targeting FN-EDB, including peptide-drug conjugates, antibody-drug conjugates, nanoparticles and immunotherapies, have demonstrated significant antitumor effects in various cancer models. Future research should focus on optimizing targeting ligands, exploring combination therapies, accelerating clinical translation and understanding resistance mechanisms to enhance the precision and efficacy of cancer treatments.

Despite the growing interest in tumor-associated FN-EDB as a potential therapeutic target, there is a lack of articles available for a comprehensive review of the field. Thus, the present study provided a broad body of knowledge carefully compiled for the current research, highlighting the role of FN-EDB in tumor progression and its implications for clinical oncology. By elucidating the underlying mechanisms, including tumor angiogenesis, internal and external cell signaling and summarizing the latest targeted therapy and diagnostic strategies in the field, it provided necessary guidance for researchers, clinicians and pharmaceutical developers and to promote informed decision-making. In conclusion, the present study could provide important information for future research directions and treatment strategies that could guide the development of new diagnostic and treatment modalities in cancer treatment in the future.

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Authors' contributions

YZ wrote the first draft and reviewed and edited the article. JL helped to conceive the article, guided the writing direction and ideas, designed the research content, and drafted the article. YP and TC performed the literature review, were responsible for collecting and organizing the relevant literature, and performed a preliminary analysis of the literature. In addition they participated in the initial collation and analysis of the data, providing valuable insights into the conception and design of this study. YZ, TC, YP and JL contributed to revising the manuscript, provided important intellectual content. All authors agree to be accountable for all aspects of the work,

ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved and have read and approved the final manuscript. Data authentication is not applicable.

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.

References

1. Tiwari A, Trivedi R and Lin SY: Tumor microenvironment: Barrier or opportunity towards effective cancer therapy. J Biomed Sci 29: 83, 2022.
2. Kolesnikoff N, Chen CH and Samuel MS: Interrelationships between the extracellular matrix and the immune microenvironment that govern epithelial tumour progression. Clin Sci (Lond) 136: 361-377, 2022.
3. Efthymiou G, Saint A, Ruff M, Rekad Z, Ciais D and Van Obberghen-Schilling E: Sha up the tumor microenvironment with cellular fibronectin. Front Oncol 10: 641, 2020.
4. Kim SE, Yun S and Doh J: Effects of extracellular adhesion molecules on immune cell mediated solid tumor cell killing. Front Immunol 13: 1004171, 2022.
5. Parmar D and Apte M: Angiopoietin inhibitors: A review on targeting tumor angiogenesis. Eur J Pharmacol 899: 174021-174021, 2021.
6. Peng Z, Lv X and Huang S: Recent progress on the role of fibronectin in tumor stromal immunity and immunotherapy. Curr Top Med Chem 22: 2494-2505, 2022.
7. Neri D and Sondel PM: Immunocytokines for cancer treatment: Past, present and future. Curr Opin Immunol 40: 96-102, 2016.
8. Wang J, Li R, Li M and Wang C: Fibronectin and colorectal cancer: Signaling pathways and clinical implications. J Recept Signal Transduct Res 41: 313-320, 2021.
9. Wagner J, Wickman E, Shaw TI, Anido AA, Langfitt D, Zhang J, Porter SN, Pruett-Miller SM, Tillman H, Krenciute G and Gottschalk S: Antitumor effects of CAR T cells redirected to the EDB Splice variant of fibronectin. Cancer Immunol Res 9: 279-290, 2021.
10. Zhou F, Sun J, Ye L, Jiang T, Li W, Su C, Ren S, Wu F, Zhou C and Gao G: Fibronectin promotes tumor angiogenesis and progression of Non-small-cell lung cancer by elevating WISP3 expression via FAK/MAPK/HIF-1α axis and activating wnt signaling pathway. Exp Hematol Oncol 12: 61, 2023.
11. Pankov R and Yamada KM: Fibronectin at a glance. J Cell Sci 115: 3861-3863, 2002.
12. Kumra H and Reinhardt DP: Fibronectin-targeted drug delivery in cancer. Adv Drug Deliv Rev 97: 101-110, 2016.
13. Wang K, Seo BR, Fischbach C and Gourdon D: Fibronectin mechanobiology regulates tumorigenesis. Cell Mol Bioeng 9: 1-11, 2016.
14. Kraft S, Klemis V, Sens C, Lenhard T, Jacobi C, Samstag Y, Wabnitz G, Kirschfink M, Wallich R, Hänsch GM and Nakchbandi IA: Identification and characterization of a unique role for EDB fibronectin in phagocytosis. J Mol Med (Berl) 94: 567-581, 2016.
15. Dalton CJ and Lemmon CA: Fibronectin: Molecular structure, fibrillar structure and mechanochemical signaling. Cells 10: 2443, 2021.
16. Singh P, Carraher C and Schwarzbauer JE: Assembly of fibronectin extracellular matrix. Annu Rev Cell Dev Biol 26: 397-419, 2010.
17. Ventura E, Sassi F, Parodi A, Balza E, Borsi L, Castellani P, Carnemolla B and Zardi L: Alternative splicing of the angiogenesis associated Extra-domain B of fibronectin regulates the accessibility of the B-C loop of the type III repeat 8. PLoS One 5: e9145, 2010.
18. Barrow-McGee R, Kishi N, Joffre C, Ménard L, Hervieu A, Bakhouche BA, Noval AJ, Mai A, Guzmán C, Robbez-Masson L, *et al*: Beta 1-integrin-c-Met cooperation reveals an inside-in survival signalling on autophagy-related endomembranes. Nat Commun 7: 12392, 2016.
19. Dinesh NEH, Campeau PM and Reinhardt DP: Fibronectin isoforms in skeletal development and associated disorders. Am J Physiol Cell Physiol 323: C536-C549, 2022.
20. Guerrero-Barberà G, Burday N and Costell M: Shaping oncogenic microenvironments: Contribution of fibronectin. Front Cell Dev Biol 12: 1363004, 2024.
21. Rick JW, Chandra A, Dalle Ore C, Nguyen AT, Yagnik G and Aghi MK: Fibronectin in malignancy: Cancer-specific alterations, protumoral effects and therapeutic implications. Semin Oncol 46: 284-290, 2019.
22. Park JH, Ryu JM and Han HJ: Involvement of caveolin-1 in fibronectin-induced mouse embryonic stem cell proliferation: Role of FAK, RhoA, PI3K/Akt and ERK 1/2 pathways. J Cell Physiol 226: 267-275, 2011.
23. Fei D, Meng X, Yu W, Yang S, Song N, Cao Y, Jin S, Dong L, Pan S and Zhao M: Fibronectin (FN) cooperated with TLR2/TLR4 receptor to promote innate immune responses of macrophages via binding to integrin β1. Virulence 9: 1588-1600, 2018.
24. Menrad A and Menssen HD: ED-B fibronectin as a target for antibody-based cancer treatments. Expert Opin Ther Targets 9: 491-500, 2005.
25. Hall RC, Vaidya AM, Schiemann WP, Pan Q and Lu ZR: RNA-Seq analysis of extradomain a and extradomain B fibronectin as extracellular matrix markers for cancer. Cells 12: 685, 2023.
26. Faisal SM, Comba A, Varela ML, Argento AE, Brumley E, Abel C II, Castro MG and Lowenstein PR: The complex interactions between the cellular and non-cellular components of the brain tumor microenvironmental landscape and their therapeutic implications. Front Oncol 12: 1005069, 2022.
27. Chen CW, Yang CH, Lin YH, Hou YC, Cheng TJ, Chang ST, Huang YH, Chung ST, Chio CC, Shan YS, *et al*: The fibronectin expression determines the distinct progressions of malignant gliomas via transforming growth Factor-beta pathway. Int J Mol Sci 22: 3782-3782, 2021.
28. Hall RC, Ayat NR, Qiao PL, Vaidya AM, Ma D, Aminoshariae A, Stojanov I and Lu ZR: Preclinical assessment of the effectiveness of magnetic resonance molecular imaging of Extradomain-B fibronectin for detection and characterization of oral cancer. Mol Imaging Biol 22: 1532-1542, 2020.
29. Vaidya A, Wang H, Qian V, Gilmore H and Lu ZR: Overexpression of Extradomain-B fibronectin is associated with invasion of breast cancer cells. Cells 9: 1826, 2020.
30. Nail HM, Chiu CC, Leung CH, Ahmed MMM and Wang HD: Exosomal miRNA-mediated intercellular communications and immunomodulatory effects in tumor microenvironments. J Biomed Sci 30: 69, 2023.
31. Yuan Z, Li Y, Zhang S, Wang X, Dou H, Yu X, Zhang Z, Yang S and Xiao M: Extracellular matrix remodeling in tumor progression and immune escape: From mechanisms to treatments. Molecular Cancer 22: 48, 2023.
32. Burrows L, Clark K, Mould AP and Humphries MJ: Fine mapping of inhibitory anti-α5 monoclonal antibody epitopes that differentially affect integrin-ligand binding. Biochem J 344: 527-527, 1999.
33. Farndale RW and Jarvis GE: Integrins in GtoPdb v.2023.1. IUPHAR/BPS guide to pharmacology CITE. Apr26, 2023. doi:10.2218/gtopdb/f760/2023.1.
34. Takagi J: Structural basis for ligand recognition by RGD (Arg-Gly-Asp)-dependent integrins. Biochem Soc Trans 32: 403-406, 2004.
35. Kolasangiani R, Bidone TC and Schwartz MA: Integrin conformational dynamics and mechanotransduction. Cells 11: 3584, 2022.
36. Moro L, Venturino M, Bozzo C, Silengo L, Altruda F, Beguinot L, Tarone G and Defilippi P: Integrins induce activation of EGF receptor: Role in MAP kinase induction and adhesion-dependent cell survival. EMBO J 17: 6622-6632, 1998.
37. Su Y, Xia W, Li J, Walz T, Humphries MJ, Vestweber D, Cabañas C, Lu C and Springer TA: Relating conformation to function in integrin α 5 β 1. Proc Natl Acad Sci USA 113: E3872-E3881, 2016.
38. Widmaier M, Rognoni E, Radovanac K, Azimifar SB and Fässler R: Integrin-linked kinase at a glance. J Cell Sci 125: 1839-1843, 2012.

39. Matsuura S, Thompson CR, Kah NS, Karagianni A, Torres CW, Mazzeo CS, Leiva O, Malara A, Balduini A and Ravid K: Integrin-mediated adhesion to extracellular matrix protein fibronectin drives megakaryocytosis in $JAK2^{V617F+}$ primary myelofibrosis. Blood 134 (Suppl 1): S4205, 2019.
40. Andreucci E, Bugatti K, Peppicelli S, Ruzzolini J, Lulli M, Calorini L, Battistini L, Zanardi F, Sartori A and Bianchini F: Nintedanib-αVβ6 integrin ligand conjugates reduce TGFβ-Induced EMT in human Non-small cell lung cancer. Int J Mol Sci 24: 1475, 2023.
41. Haarmann A, Nowak E, Deiß A, van der Pol S, Monoranu CM, Kooij G, Müller N, van der Valk P, Stoll G, de Vries HE, *et al*: Soluble VCAM-1 impairs human brain endothelial barrier integrity via integrin α-4-transduced outside-in signalling. Acta Neuropathologica 129: 639-652, 2015.
42. Su Y, Iacob RE, Li J, Engen JR and Springer TA: Dynamics of integrin α5β1, fibronectin and their complex reveal sites of interaction and conformational change. J Biol Chem 298: 102323, 2022.
43. Durrant TN, van den Bosch MT and Hers I: Integrin αIIbβ3 outside-in signaling. Blood 130: 1607-1619, 2017.
44. Pang X, He X, Qiu Z, Zhang H, Xie R, Liu Z, Gu Y, Zhao N, Xiang Q and Cui Y: Targeting integrin pathways: Mechanisms and advances in therapy. Signal 8: 1, 2023.
45. Shams H and Mofrad MRK: Molecular mechanisms underlying the inside-out signaling through focal adhesions. Biophysical J 106: 574a, 2014.
46. Huang C, Rajfur Z, Yousefi N, Chen Z, Jacobson K and Ginsberg MH: Talin phosphorylation by Cdk5 regulates Smurf1-mediated talin head ubiquitylation and cell migration. Nat Cell Biol 11: 624-630, 2009.
47. Han SB, Lee G, Kim D, Kim JK, Kim IS, Kim HW and Kim DH: Selective suppression of Integrin-ligand binding by single molecular tension probes mediates directional cell migration. Adv Sci (Weinh) 11: e2306497, 2024.
48. Kim HJ, You SJ, Yang DH, Chun HJ and Kim MS: Preparation of novel RGD-conjugated thermosensitive mPEG-PCL composite hydrogels and in vitro investigation of their impacts on Adhesion-dependent cellular behavior. J Industrial Engineering Chemistry 84: 226-235, 2020.
49. Legate KR, Wickström SA and Fässler R: Genetic and cell biological analysis of integrin outside-in signaling. Genes Dev 23: 397-418, 2009.
50. Betriu N, Andreeva A, Alonso A and Semino CE: Increased stiffness downregulates focal adhesion kinase expression in pancreatic cancer cells cultured in 3D Self-assembling peptide scaffolds. Biomedicines 10: 1835-1835, 2022.
51. Martinez OE and Sudhamsu J: Abstract A025: Investigating the molecular mechanisms of regulation of the RAS guanine nucleotide exchange factor, SOS1 by Grb2 and 14-3-3. Mol Cancer Res 21 (5_Suppl): A025, 2023.
52. Lagarrigue F and Gingras AR: Src-mediated phosphorylation of RIAM promotes integrin activation. Structure 29: 305-307, 2021.
53. Kang BW and Chau I: Molecular target: Pan-AKT in gastric cancer. ESMO Open 5: e000728, 2020.
54. Wu W, Xue X, Chen Y, Zheng N and Wang J: Targeting prolyl isomerase Pin1 as a promising strategy to overcome resistance to cancer therapies. Pharmacol Res 184: 106456, 2022.
55. Alanko J and Ivaska J: Endosomes: Emerging platforms for Integrin-mediated FAK signalling. Trends Cell Biol 26: 391-398, 2016.
56. Wan J: The regulatory role of the mitotic checkpoint component Mad1 in interphase and tumor progression. University of Wisconsin-Madison, 2019.
57. Liu J, Lu F, Ithychanda SS, Apostol M, Das M, Deshpande G, Plow EF and Qin J: A mechanism of platelet integrin αIIbβ3 outside-in signaling through a novel integrin αIIb subunit-filamin-actin linkage. Blood 141: 2629-2641, 2023.
58. Shams H and Mofrad MRK: α-Actinin induces a kink in the transmembrane domain of β3-Integrin and impairs activation via talin. Biophys J 113: 948-956, 2017.
59. Fraser J, Simpson J, Fontana R, Kishi-Itakura C, Ktistakis NT and Gammoh N: Targeting of early endosomes by autophagy facilitates EGFR recycling and signalling. EMBO Rep 20: e47734, 2019.
60. Miao M, Collins J, Moreira Bahnson ES, Chubinskaya S and Loeser RF: Reactive oxygen species regulate fibronectin fragments-induced map kinase signaling and metalloproteinase 13 release in human chondrocytes through nadph oxidase 2 and endocytosis of chondrocyte integrins. Osteoarthritis 28 (Suppl): S45-S46, 2020.
61. Teran OY, Zanotelli MR, Joy Lin MC, Cerione RA and Wilson KF: Dock7 regulates AKT and mTOR/S6K activity required for the transformed phenotypes and survival of cancer cells. bioRxiv, Jan 3, 2023. doi: 10.1101/2023.01.03.522657.
62. Gagné D, Benoit YD, Groulx JF, Vachon PH and Beaulieu JF: ILK supports RhoA/ROCK-mediated contractility of human intestinal epithelial crypt cells by inducing the fibrillogenesis of endogenous soluble fibronectin during the spreading process. BMC Mol Cell Biol 21: 14, 2020.
63. Nadel G, Maik-Rachline G and Seger R: JNK Cascade-induced Apoptosis-A unique role in GqPCR signaling. Int J Mol Sci 24: 13527-13527, 2023.
64. Karunakaran D, Nguyen MA, Geoffrion M, Vreeken D, Lister Z, Cheng HS, Otte N, Essebier P, Wyatt H, Kandiah JW, *et al*: RIPK1 expression associates with inflammation in early atherosclerosis in humans and can be therapeutically silenced to reduce NF-κB activation and atherogenesis in mice. Circulation 143: 163-177, 2021.
65. Zhang H, Meng F, Wu S, Kreike B, Sethi S, Chen W, Miller FR and Wu G: Engagement of I-branching {beta}-1, 6-N-acetylglucosaminyltransferase 2 in breast cancer metastasis and TGF-{beta} signaling. Cancer Res 71: 4846-4856, 2011.
66. Yang S, Liu Y, Li MY, Ng CSH, Yang SL, Wang S, Zou C, Dong Y, Du J, Long X, *et al*: FOXP3 promotes tumor growth and metastasis by activating Wnt/β-catenin signaling pathway and EMT in non-small cell lung cancer. Mol Cancer 16: 124, 2017.
67. Weller M, Silginer M, Goodman SL, Hasenbach K, Thies S, Schraml P, Tabatabai G, Moch H, Tritschler I and Roth P: Effect of the integrin inhibitor cilengitide on TGF-beta signaling. J Clin Oncol 30 (15_suppl): S2055, 2012.
68. Valdembri D and Serini G: Angiogenesis: The importance of RHOJ-mediated trafficking of active integrins. Current Biology 30: R652-R654, 2020.
69. Nilsson M and Heymach JV: Vascular endothelial growth factor (VEGF) pathway. J Thorac Oncol 1: 768-770, 2006.
70. Liu Z and Dong Z: A cross talk between HIF and NF-κB in AKI. Am J Physiol Renal Physiol 321: F255-F256, 2021.
71. Zhao Y, Wang J, Qin W, Hu Q, Li J, Qin R, Ma N, Zheng F, Tian W, Jiang J, *et al*: Dehydroepiandrosterone promotes ovarian angiogenesis and improves ovarian function in a rat model of premature ovarian insufficiency by up-regulating HIF-1α/VEGF signalling. Reprod Biomed Online 49: 103914, 2024.
72. Chatterjee S and Naik UP: Pericyte-endothelial cell interaction. Cell Adh Migr 6: 157-159, 2012.
73. Surazynski A, Donald SP, Cooper SK, Whiteside MA, Salnikow K, Liu Y and Phang JM: Extracellular matrix and HIF-1 signaling: The role of prolidase. Int J Cancer 122: 1435-1440, 2007.
74. Caporali A, Martello A, Miscianinov V, Maselli D, Vono R and Spinetti G: Contribution of pericyte paracrine regulation of the endothelium to angiogenesis. Pharmacol Ther 171: 56-64, 2017.
75. Harrell CR, Simovic Markovic B, Fellabaum C, Arsenijevic A, Djonov V and Volarevic V: Molecular mechanisms underlying therapeutic potential of pericytes. J Biomed Sci 25: 12, 2028.
76. Simonavicius N, Ashenden M, van Weverwijk A, Lax S, Huso DL, Buckley CD, Huijbers IJ, Yarwood H and Isacke CM: Pericytes promote selective vessel regression to regulate vascular patterning. Blood 120: 1516-1527, 2012.
77. Smart N: Understanding the recruitment process. Arteriosclerosis Thrombosis Vascular Biol 40: 2564-2565, 2020.
78. Senger DR and Davis GE: Angiogenesis. Cold Spring Harb Perspect Biol 3: a005090, 2011.
79. Huang Y, Snuderl M and Jain RK: Polarization of Tumor-associated macrophages: A novel strategy for vascular normalization and antitumor immunity. Cancer Cell 19: 1-2, 2011.
80. Poto R, Cristinziano L, Modestino L, de Paulis A, Marone G, Loffredo S, Galdiero MR and Varricchi G: Neutrophil extracellular traps, angiogenesis and cancer. Biomedicines 10: 431-431, 2022.
81. Van Hinsbergh VW and Koolwijk P: Endothelial sprouting and angiogenesis: Matrix metalloproteinases in the lead. Cardiovasc Res 78: 203-212, 2008.
82. Zhou X, Zhang J, Lv W, Zhao C, Xia Y, Wu Y and Zhang Q: The pleiotropic roles of adipocyte secretome in remodeling breast cancer. J Exp Clin Cancer Res 41: 203, 2022.
83. Tang KH, Ma S, Lee TK, Chan YP, Kwan PS, Tong CM, Ng IO, Man K, To KF, Lai PB, *et al*: CD133(+) liver tumor-initiating cells promote tumor angiogenesis, growth, and self-renewal through neurotensin/interleukin-8/CXCL1 signaling. Hepatology 55: 807-820, 2012.

84. Engelmann D, Mayoli-Nüssle D, Mayrhofer C, Fürst K, Alla V, Stoll A, Spitschak A, Abshagen K, Vollmar B, Ran S and Pützer BM: E2F1 promotes angiogenesis through the VEGF-C/VEGFR-3 axis in a feedback loop for cooperative induction of PDGF-B. J Mol Cell Biol 5: 391-403, 2013.
85. Azizi M, Dianat-Moghadam H, Salehi R, Farshbaf M, Iyengar D, Sau S, Iyer AK, Valizadeh H, Mehrmohammadi M and Hamblin MR: Interactions between tumor biology and targeted nanoplatforms for imaging applications. Adv Funct Mater 30: 1910402, 2020.
86. Vaidya A, Ayat N, Buford M, Wang H, Shankardass A, Zhao Y, Gilmore H, Wang Z and Lu ZR: Noninvasive assessment and therapeutic monitoring of drug-resistant colorectal cancer by MR molecular imaging of extradomain-B fibronectin. Theranostics 10: 11127, 2020.
87. Lewandowski S, Diao L, Quigley A, Crochiere M and Pinkas J: EDB+ FN is an attractive therapeutic target in oncology: Insights from protein expression analysis of solid tumors. Cancer Res 84 (6_Suppl): S2908, 2004.
88. Qiao P, Ayat NR, Vaidya A, Gao S, Sun W, Chou S, Han Z, Gilmore H, Winter JM and Lu ZR: Magnetic resonance molecular imaging of extradomain B fibronectin improves imaging of pancreatic cancer tumor xenografts. Front Oncol 10: 586727, 2020.
89. Feng Y, Hao Y, Wang Y, Song W, Zhang S, Ni D, Yan F and Sun L: Ultrasound molecular imaging of bladder cancer via extradomain B Fibronectin-targeted biosynthetic GVs. Int J Nanomedicine 18: 4871-4884, 2023.
90. Zhang Y, Zheng X, Huang Y, Li S, Li X and Zhu L: EDB-FN-targeted probes for near infrared fluorescent imaging and positron emission tomography imaging of breast cancer in mice. Sci Rep 14: 22056, 2024.
91. Mohammadgholi M, Sadeghzadeh N, Erfani M, Abediankenari S, Abedi SM, Emrarian I, Jafari N and Behzadi R: Human fibronectin Extra-domain B (EDB)-Specific aptide (APTEDB) radiolabelling with technetium-99m as a potent targeted Tumour-imaging agent. Anticancer Agents Med Chem 18: 277-285, 2018.
92. Qiao PL, Gargesha M, Liu Y, Laney VEA, Hall RC, Vaidya AM, Gilmore H, Gawelek K, Scott BB, Roy D, *et al*: Magnetic resonance molecular imaging of extradomain B fibronectin enables detection of pancreatic ductal adenocarcinoma metastasis. Magn Reson Imaging 86: 37-45, 2022.
93. Sergeeva O, Zhang Y, Gao S, Chan ER, Sergeev M, Iyer R, Sexton S, Avril N, Lu ZR and Lee Z: PET imaging of hepatocellular carcinoma using ZD2-(Ga-NOTA). J Hepatocell Carcinoma 10: 291-301, 2023.
94. Han Z, Sergeeva O, Roelle S, Cheng H, Gao S, Li Y, Lee Z and Lu ZR: Preparation and evaluation of ZD2 peptide Cu-DOTA conjugate as a positron emission tomography probe for detection and characterization of prostate cancer. ACS Omega 4: 1185-1190, 2019.
95. Lu ZR, Laney V and Li Y: Targeted contrast agents for magnetic resonance molecular imaging of cancer. Acc Chem Res 55: 2833-2847, 2022.
96. Han Z, Sergeeva O, Roelle S, Cheng H, Gao S, Li Y, Lee Z and Lu ZR: Preparation and evaluation of ZD2 Peptide ^{64}Cu-DOTA conjugate as a positron emission tomography probe for detection and characterization of prostate cancer. ACS Omega 4: 1185-1190, 2019.
97. Han Z, Li Y, Roelle S, Zhou Z, Liu Y, Sabatelle R, DeSanto A, Yu X, Zhu H, Magi-Galluzzi C and Lu ZR: Targeted contrast agent specific to an oncoprotein in tumor microenvironment with the potential for detection and risk stratification of prostate cancer with MRI. Bioconjug Chem 28: 1031-1040, 2017.
98. Ye XX, Zhao YY, Wang Q, Xiao W, Zhao J, Peng YJ, Cao DH, Lin WJ, Si-Tu MY, Li MZ, *et al*: EDB Fibronectin-Specific SPECT Probe 99mTc-HYNIC-ZD2 for breast cancer detection. ACS Omega 2: 2459-2468, 2017.
99. Zhang W, Liang X, Zhang X, Tong W, Shi G, Guo H, Jin Z, Tian J, Du Y and Xue H: Magnetic-optical dual-modality imaging monitoring chemotherapy efficacy of pancreatic ductal adenocarcinoma with a low-dose fibronectin-targeting Gd-based contrast agent. Eur J Nucl Med Mol Imaging 51: 1841-1855, 2024.
100. Yang N, Huang Y, Wang X, Wang D, Yao D and Ren GL: Fibronectin-targeting Dual-Modal MR/NIRF imaging contrast agents for diagnosis of gastric cancer and peritoneal metastasis. Bioconjug Chem 35: 843-854, 2024.
101. Wang MD, Lv GT, An HW, Zhang NY and Wang H: In Situ Self-assembly of bispecific peptide for cancer immunotherapy. angewandte chemie international edition. Angew Chem Int Ed Engl 61: e202113649, 2022.
102. Ranjbar L, Maleki F, Sadeghzadeh N, Abediankenari S, Mardanshahi A and Masteri Farahani A: In vitro/in vivo assessment of the targeting ability of [99mTc] Tc-labeled an aptide specific to the extra domain B of fibronectin (APTEDB) for colorectal cancer. Ann Nucl Med 34: 460-466, 2020.
103. Ghaffari H, Atashzar MR and Abdollahi H: Molecular imaging in tracking cancer stem cells: A review. Med J Islam Repub Iran 34: 90, 2020.
104. Noh I, Son Y, Jung W, Kim M, Kim D, Shin H, Kim YC and Jon S: Targeting the tumor microenvironment with amphiphilic near-infrared cyanine nanoparticles for potentiated photothermal immunotherapy. Biomaterials 275: 120926, 2021.
105. Henze J: Immunotherapy of solid tumors: Multimodal imaging strategies for chimeric antigen receptor T cell tracking in the tumor microenvironment. Dissertation, Göttingen, Georg-August Universität, 2021.
106. Sun Y, Kim HS, Park J, Li M, Tian L, Choi Y, Choi BI, Jon S and Moon WK: MRI of breast tumor initiating cells using the extra domain-B of fibronectin targeting nanoparticles. Theranostics 4: 845-857, 2014.
107. Sun Y, Kim HS, Kang S, Piao YJ, Jon S and Moon WK: Magnetic resonance Imaging-guided drug delivery to breast cancer Stem-like cells. Adv Healthc Mater 7: e1800266, 2018.
108. Park J, Kim S, Saw PE, Lee IH, Yu MK, Kim M, Lee K, Kim YC, Jeong YY and Jon S: Fibronectin extra domain B-specific aptide conjugated nanoparticles for targeted cancer imaging. J Control Release 163: 111-118, 2012.
109. Jailkhani N, Ingram JR, Rashidian M, Rickelt S, Tian C, Mak H, Jiang Z, Ploegh HL and Hynes RO: Noninvasive imaging of tumor progression, metastasis and fibrosis using a nanobody targeting the extracellular matrix. Proc Natl Acad Sci USA 116: 18745, 2019.
110. Reeves KM: Applying PET imaging to cancer immunotherapy to improve clinical outcomes. The University of Alabama at Birmingham, 2022.
111. Rossin R, Berndorff D, Friebe M, Dinkelborg LM and Welch MJ: Small-animal PET of tumor angiogenesis using a (76)Br-labeled human recombinant antibody fragment to the ED-B domain of fibronectin. J Nucl Med 48: 1172-1179, 2007.
112. Berndorff D, Borkowski S, Moosmayer D, Viti F, Müller-Tiemann B, Sieger S, Friebe M, Hilger CS, Zardi L, Neri D and Dinkelborg LM: Imaging of tumor angiogenesis using 99mTc-labeled human recombinant anti-ED-B fibronectin antibody fragments. J Nucl Med 47: 1707-1716, 2006.
113. Zhao Q, Zong H, Zhu P, Su C, Tang W, Chen Z and Jin S: Crosstalk between colorectal CSCs and immune cells in tumorigenesis and strategies for targeting colorectal CSCs. Exp Hematol Oncol 13: 6, 2024.
114. Morgos DT, Stefani C, Miricescu D, Greabu M, Stanciu S, Nica S, Stanescu-Spinu II, Balan DG, Balcangiu-Stroescu AE, Coculescu EC, *et al*: Targeting PI3K/AKT/mTOR and MAPK signaling pathways in gastric cancer. Int J Mol Sci 25: 1848, 2024.
115. Yu J, Li S, Chen D, Liu D, Guo H, Yang C, Zhang W, Zhang L, Zhao G, Tu X, *et al*: SIRPα-Fc fusion protein IMM01 exhibits dual anti-tumor activities by targeting CD47/SIRPα signal pathway via blocking the 'don't eat me' signal and activating the 'eat me' signal. J Hematol Oncol 15: 167, 2022.
116. Li R, Wang Q, She K, Lu F and Yang Y: CRISPR/Cas systems usher in a new era of disease treatment and diagnosis. Mol Biomed 3: 31, 2022.
117. Dabas P and Danda A: Revolutionizing cancer treatment: A comprehensive review of CAR-T cell therapy. Med Oncol 40: 275, 2023.
118. Johannsen M, Spitaleri G, Curigliano G, Roigas J, Weikert S, Kempkensteffen C, Roemer A, Kloeters C, Rogalla P, Pecher G, *et al*: The tumour-targeting human L19-IL2 immunocytokine: Preclinical safety studies, phase I clinical trial in patients with solid tumours and expansion into patients with advanced renal cell carcinoma. Eur J Cancer 46: 2926-2935, 2010.
119. Schliemann C, Börschel N, Schwöppe C, Liersch R, Kessler T, Dreyling M, Klapper W, Menssen HD, Neri D, Berdel WE and Mesters RM: Targeting Interleukin-2 to the neovasculature potentiates Rituximab's activity against mantle cell lymphoma in mice. Blood 120: 3716-3716, 2012.
120. Orecchia P, Balza E, Pietra G, Conte R, Bizzarri N, Ferrero S, Mingari MC and Carnemolla B: L19-IL2 immunocytokine in combination with the anti-syndecan-1 46F2SIP antibody format: A new targeted treatment approach in an ovarian carcinoma model. Cancers (Basel) 11: 1232, 2019.

121. Schliemann C, Palumbo A, Zuberbühler K, Villa A, Kaspar M, Trachsel E, Klapper W, Menssen HD and Neri D: Complete eradication of human B-cell lymphoma xenografts using rituximab in combination with the immunocytokine L19-IL2. Blood 113: 2275-2283, 2009.
122. Weide B, Eigentler T, Catania C, Ascierto PA, Cascinu S, Becker JC, Hauschild A, Romanini A, Danielli R, Dummer R, *et al*: A phase II study of the L19IL2 immunocytokine in combination with dacarbazine in advanced metastatic melanoma patients. Cancer Immunol Immunother 68: 1547-1559, 2019.
123. Van Limbergen EJ, Hoeben A, Lieverse RIY, Houben R, Overhof C, Postma A, Zindler J, Verhelst F, Dubois LJ, De Ruysscher D, *et al*: Toxicity of L19-Interleukin 2 Combined with Stereotactic Body Radiation Therapy: A Phase 1 Study. Int J Radiat Oncol Biol Phys 109: 1421-1430. 2021.
124. Lieverse RIY, Van Limbergen EJ, Oberije CJG, Troost EGC, Hadrup SR, Dingemans AC, Hendriks LEL, Eckert F, Hiley C, Dooms C, *et al*: Stereotactic ablative body radiotherapy (SABR) combined with immunotherapy (L19-IL2) versus standard of care in stage IV NSCLC patients, ImmunoSABR: A multicentre, randomised controlled open-label phase II trial. BMC Cancer 20: 557, 2020.
125. Zhang S, Mills D, Kim JY, Knudsen N, Nelson J, Buechler Y and Skidmore L: 41P Evaluation of ARX517: A next-generation anti-PSMA antibody drug conjugate for prostate cancer treatment, in preclinical enzalutamide-resistant and enzalutamide-sensitive pharmacology models and in toxicology models. Ann Oncol 34 (Suppl 2): S199, 2023.
126. Nakada T, Sugihara K, Jikoh T, Abe Y and Agatsuma T: The latest research and development into the antibody-drug conjugate, (fam-)Trastuzumab Deruxtecan (DS-8201a), for HER2 cancer therapy. Chem Pharm Bull (Tokyo) 67: 173-185, 2019.
127. Ceci C, Lacal PM and Graziani G: Antibody-drug conjugates: Resurgent anticancer agents with multi-targeted therapeutic potential. Pharmacol Ther 236: 108106, 2022.
128. Rudman SM, Jameson MB, McKeage MJ, Savage P, Jodrell DI, Harries M, Acton G, Erlandsson F and Spicer JF: A phase 1 study of AS1409, a novel Antibody-cytokine fusion protein, in patients with malignant melanoma or renal cell carcinoma. Clin Cancer Res 17: 1998-2005, 2011.
129. Ongaro T, Gouyou B, Stringhini M, Corbellari R, Neri D and Villa A: A novel format for recombinant antibody-interleukin-2 fusion proteins exhibits superior tumor-targeting properties in vivo. Oncotarget 11: 3698-3711, 2020.
130. Wilks S, Carneiro BA, Coté GM, Henry J, Sen S, Spira AL, Tsai F YC, Wang JS, Crochiere M, He S, *et al*: 762 A first-in-human phase 1 clinical study evaluating safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of the EDB+FN targeting ADC PYX-201 in participants with advanced solid tumors. J Immunother Cancer 11 (Suppl 1): A1-A1731, 2023.
131. Hooper AT, Marquette K, Chang CB, Golas J, Jain S, Lam MH, Guffroy M, Leal M, Falahatpisheh H, Mathur D, *et al*: Anti-extra Domain B splice variant of fibronectin antibody-drug conjugate eliminates tumors with enhanced efficacy when combined with checkpoint blockade. Mol Cancer Ther 21: 1462-1472, 2022.
132. Trachsel E, Bootz F, Silacci M, Kaspar M, Kosmehl H and Neri D: Antibody-mediated delivery of IL-10 inhibits the progression of established collagen-induced arthritis. Arthritis Res Ther 9: R9, 2007.
133. Kaspar M, Trachsel E and Neri D: The antibody-mediated targeted delivery of interleukin-15 and GM-CSF to the tumor neovasculature inhibits tumor growth and metastasis. Cancer Res 67: 4940-4948, 2007.
134. Ruan JI: Blocking leptin-STAT3 axis-induced fatty acid oxidation: A novel approach to activate $CD8^+$ T effector cells in breast cancer. Thoracic Cancer 11: 3422-3424, 2020.
135. Di Nitto C, Gilardoni E, Mock J, Nadal L, Weiss T, Weller M, Seehusen F, Libbra C, Puca E, Neri D and De Luca R: An engineered IFNγ-antibody fusion protein with improved tumor-homing properties. Pharmaceutics 15: 377, 2023.
136. Niu J, Kaufman HL, Kichenadasse G, Haydon AM, Barve MA, Ganju V, Iannotti Buchbinder E, Spira AI, Pang W, Fu W, *et al*: Updated results from an ongoing phase 1/2a study of T3011, an oncolytic HSV expressing IL-12 and PD-1 antibody, administered via IT injection as monotherapy or combined with pembrolizumab in advanced solid tumors. J Clin Oncol 41 (16_suppl): 9535, 2023.
137. Danielli R, Patuzzo R, Di Giacomo AM, Gallino G, Maurichi A, Di Florio A, Cutaia O, Lazzeri A, Fazio C, Miracco C, *et al*: Intralesional administration of L19-IL2/L19-TNF in stage III or stage IVM1a melanoma patients: Results of a phase II study. Cancer Immunol Immunother 64: 999-1009, 2015.
138. Papadia F, Basso V, Patuzzo R, Maurichi A, Di Florio A, Zardi L, Ventura E, González-Iglesias R, Lovato V, Giovannoni L, *et al*: Isolated limb perfusion with the tumor-targeting human monoclonal antibody-cytokine fusion protein L19-TNF plus melphalan and mild hyperthermia in patients with locally advanced extremity melanoma. J Surg Oncol 107: 173-179, 2013.
139. Spitaleri G, Berardi R, Pierantoni C, De Pas T, Noberasco C, Libbra C, González-Iglesias R, Giovannoni L, Tasciotti A, Neri D, *et al*: Phase I/II study of the tumour-targeting human monoclonal antibody-cytokine fusion protein L19-TNF in patients with advanced solid tumours. J Cancer Res Clin Oncol 139: 447-455, 2013.
140. Spaeth N, Wyss MT, Pahnke J, Biollaz G, Trachsel E, Drandarov K, Treyer V, Weber B, Neri D and Buck A: Radioimmunotherapy targeting the extra domain B of fibronectin in C6 rat gliomas: A preliminary study about the therapeutic efficacy of iodine-131-labeled SIP (L19). Nucl Med Biol 33: 661-666, 2006.
141. Saif A, Rossi AJ, Sarnaik A, Hernandez JM and Zager JS: Efficacy of neoadjuvant intratumoral Darleukin/Fibromun (L19IL2 + L19TNF) in patients with clinical stage IIIB/C melanoma (Neo-DREAM). Ann Surg Oncol 29: 3377-3378, 2022.
142. Hauschild A, Hassel JC, Ziemer M, Rutkowski P, Meier FE, Flatz L, Gaudy-Marqueste C, Santinami M, Russano F, von Wasielewski I, *et al*: Phase 3 study (PIVOTAL) of neoadjuvant intralesional daromun vs. immediate surgery in fully resectable melanoma with regional skin and/or nodal metastases. J Clin Oncol 42 (17_suppl): LBA9501, 2024.
143. Borga G, Sucre S, Sucre O, Vivas L, Salazar H and Sucre CE: Use of tumor mutational burden as a predictive marker of response to immuno-oncology agents: Initial experience at an academic center in Venezuela. J Clin Oncol 41 (16_suppl): e14613, 2023.
144. Park SE: Design and Sythesis of conjugages of amphiphilic cell-penetrating peptides containing anticancer drug and ligand for extra cellular matrix biomarker to provide efficient tumor-targeting. Irvine, CA, Chapman University, 2021.
145. Yu B, Hwang D, Jeon H, Kim H, Lee Y, Keum H, Kim J, Lee DY, Kim Y, Chung J and Jon S: A hybrid platform based on a bispecific peptide-antibody complex for targeted cancer therapy. Angew Chem Int Ed Engl 58: 2005-2010, 2019.
146. Park SE, El-Sayed NS, Shamloo K, Lohan S, Kumar S, Sajid MI and Tiwari RK: Targeted delivery of cabazitaxel using cyclic cell-penetrating peptide and biomarkers of extracellular matrix for prostate and breast cancer therapy. Bioconjug Chem 32: 1898-1914, 2021.
147. Yu B, Yoo D, Kim KH, Kim TW, Park S, Kim Y, Son Y, Kim J, Noh I, Whang CH, *et al*: Effective combination immunotherapy through vessel normalization using a cancer-targeting antiangiogenic peptide-antibody hybrid. Advanced Therapeutics: 5: 2100151, 2022.
148. Park SE, Shamloo K, Kristedja TA, Darwish S, Bisoffi M, Parang K and Tiwari RK: EDB-FN targeted peptide-drug conjugates for use against prostate cancer. Int J Mol Sci 20: 3291-3291, 2019.
149. Kim H, Hwang D, Choi M, Lee S, Kang S, Lee Y, Kim S, Chung J and Jon S: Antibody-assisted delivery of a peptide-drug conjugate for targeted cancer therapy. Mol Pharm 16: 165-172, 2018.
150. Kim YJ, Bae J, Shin TH, Kang SH, Jeong M, Han Y, Park JH, Kim SK and Kim YS: Immunoglobulin Fc-fused, neuropilin-1-specific peptide shows efficient tumor tissue penetration and inhibits tumor growth via anti-angiogenesis. J Control Release 216: 56-68, 2015.
151. Hong Y, Gao D, Zhao B, Ma J, Yang Z and Guo H: Thermal immuno-nanomedicine: A new strategy for cancer treatment. Clin Transl Med 23: e1256, 2023.
152. Saw PE, Zhang A, Nie Y, Zhang L, Xu Y and Xu X: Tumor-associated fibronectin targeted liposomal nanoplatform for cyclophilin A siRNA delivery and targeted malignant glioblastoma therapy. Front Pharmacol 9: 1194, 2018.
153. Javid H, Oryani MA, Rezagholinejad N, Hashemzadeh A and Karimi-Shahri M: Unlocking the potential of RGD-conjugated gold nanoparticles: A new frontier in targeted cancer therapy, imaging, and metastasis inhibition. J Mater Chem B 12: 10786-10817, 2024.

154. Saw PE, Xu X, Kang BR, Lee J, Lee YS, Kim C, Kim H, Kang SH, Na YJ, Moon HJ, *et al*: Extra-domain B of fibronectin as an alternative target for drug delivery and a cancer diagnostic and prognostic biomarker for malignant glioma. Theranostics 11: 941-957, 2021.
155. Yu M, Leitao R, Xu X, Saw PE, Ortega CA, Si K, Ahn S, Liu J, Lotfi A, Lee I-H, *et al*: Fibronectin Extradomain B (FN-EDB) expression is specific to the atherosclerotic lesion types III, IV, and V, and the FN-EDB targeting nanomedicine enhances atherosclerotic plaque detection and local delivery of model drug cargo. Arteriosclerosis Thrombosis Vascular Biol 37 (suppl_1): A467, 2017.
156. Zhou Y, Qian M, Li J, Ruan L, Wang Y, Cai C, Gu S and Zhao X: The role of tumor-associated macrophages in lung cancer: From mechanism to small molecule therapy. Biomed Pharmacother 170: 116014-116014, 2024.
157. Lee JH, Carlino MS and Rizos H: Dosing of BRAF and MEK inhibitors in melanoma: No point in taking a break. Cancer Cell 38: 779-781, 2020.
158. Kapoor AR and Mittal V: Immunoregulatory role of club cell secretory proteins in non-small cell lung cancer. Cancer Res 83 (7_Suppl): 4424, 2023.
159. Ji P, Gong Y, Jin M, Hu X, Di G and Shao Z: 23P in vivo multi-dimensional CRISPR screens identify LGALS2 as an immunotherapy target in triple-negative breast cancer. Ann Oncol 33: S133, 2022.
160. Canè S, Barouni RM, Fabbi M, Cuozzo J, Fracasso G, Adamo A, Ugel S, Trovato R, De Sanctis F, Giacca M, *et al*: Neutralization of NET-associated human ARG1 enhances cancer immunotherapy. Sci Transl Med 15: eabq6221, 2023.
161. Baumgartner CK, Ebrahimi-Nik H, Iracheta-Vellve A, Hamel KM, Olander KE, Davis TGR, McGuire KA, Halvorsen GT, Avila OI, Patel CH, *et al*: The PTPN2/PTPN1 inhibitor ABBV-CLS-484 unleashes potent anti-tumour immunity. Nature 622: 850-862, 2023.
162. Jogalekar MP, Rajendran RL, Khan F, Dmello C, Gangadaran P and Ahn BC: CAR T-cell-based gene therapy for cancers: New perspectives, challenges, and clinical developments. Front Immunol 13: 925985, 2022.
163. Tang J, Liu N, Zhu Y, Li Y and Zhao X: CAR-T therapy targets extra domain b of fibronectin positive solid tumor cells. Immunol Invest 52: 985-996, 2023.
164. Zhang Z, Liu C, Wang M, Sun R, Yang Z, Hua Z, Wu Y, Wu M, Wang H, Qiu W, *et al*: Treating solid tumors with TCR-based chimeric antigen receptor targeting extra domain B-containing fibronectin. J Immunother Cancer 11: e007199, 2023.
165. Gentile D, Orlandi P, Banchi M and Bocci G: Preclinical and clinical combination therapies in the treatment of anaplastic thyroid cancer. Med Oncol 37: 19, 2020.
166. Zhang S, Hao R, Wang H, Yi QY, Yantao Y, Zhong Y and Sun M: Peri cruiser CAR-T: An innovative platform to reduce on-target off-tumor toxicity of CAR-T therapy. J Clin Oncol 41 (16_suppl): 2539, 2023.
167. Zhang Z, Liu C, Yang Z and Yin H: CAR-T-cell therapy for solid tumors positive for fibronectin extra Domain B. Cells 11: 2863, 2022.
168. Martínez Bedoya D, Gustave R, Corlazzoli F, Dutoit V and Migliorini D: 53P A multispecific non-integrating RNA CAR T platform to overcome the clinical challenge of glioblastoma heterogeneity. Ann Oncol 32: S1395, 2021.
169. Parikh RH and Lonial S: Chimeric antigen receptor T-cell therapy in multiple myeloma: A comprehensive review of current data and implications for clinical practice. CA Cancer J Clin 73: 275-285, 2023.
170. Xiong Q, Wang H, Shen Q, Wang Y, Yuan X, Lin G and Jiang P: The development of chimeric antigen receptor T-cells against CD70 for renal cell carcinoma treatment. J Transl Med 22: 368, 2024.
171. Zhu G, Zhang J, Zhang Q, Jin G, Su X, Liu S and Liu F: Enhancement of CD70-specific CAR T treatment by IFN-γ released from oHSV-1-infected glioblastoma. Cancer Immunol Immunother 71: 2433-2448, 2022.
172. Wu G, Guo S, Luo Q, Wang X, Deng W, Ouyang G, Pu JJ, Lei W and Qian W: Preclinical evaluation of CD70-specific CAR T cells targeting acute myeloid leukemia. Front Immunol 14: 1093750, 2023.
173. Li X, Zhu T, Wang R, Chen J, Tang L, Huo W, Huang X and Cao Q: Genetically programmable vesicles for enhancing CAR-T therapy against solid tumors. Adv Mater 35: e2211138, 2023.
174. Li Y, Liu Y, Cui Q, Liu L, Li Z, Cui W, Li M, Zhu X, Kang L, Yu L, *et al*: Rituximab improves clinical outcomes of CAR-T therapy for r/r B-ALL via sensitizing leukemia cells to CAR-T-mediated cytotoxicity and reducing CAR-T exhaustion. Blood 142 (Suppl 1): 6803, 2023.
175. Simmons ME, McIntosh J, Zhang T, Li Y, Yan F, Yao Y, Nie L, Lee HH, Wang W, Jiang VC, *et al*: The reversible BTK inhibitor nemtabrutinib demonstrates favorable antitumor efficacy and enhances the function of CAR T cells in mantle cell lymphoma. Blood 142 (Suppl 1): 5789, 2023.
176. Gholamrezanezhad A, Shooli H, Jokar N, Nemati R and Assadi M: Radioimmunotherapy (RIT) in brain tumors. Nucl Med Mol Imaging 53: 374-381, 2019.
177. Leung K: I-Human recombinant anti-ED-B fibronectin antibody small immunoprotein. In: Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD), National Center for Biotechnology Information (US), April 17, 2007.
178. Borsi L, Balza E, Bestagno M, Castellani P, Carnemolla B, Biro A, Leprini A, Sepulveda J, Burrone O, Neri D and Zardi L: Selective targeting of tumoral vasculature: Comparison of different formats of an antibody (L19) to the ED-B domain of fibronectin. Int J Cancer 102: 75-85, 2002.
179. Li R, He H, Li X, Zheng X, Li Z, Zhang H, Ye J, Zhang W, Yu C, Feng G and Fan W: EDB-FN targeted probes for the surgical navigation, radionuclide imaging and therapy of thyroid cancer. Eur J Nucl Med Mol Imaging 50: 2100-2113, 2023.
180. Tijink BM, Perk LR, Budde M, Stigter-van Walsum M, Visser GW, Kloet RW, Dinkelborg LM, Leemans CR, Neri D and van Dongen GA: (124)I-L19-SIP for immuno-PET imaging of tumour vasculature and guidance of (131)I-L19-SIP radioimmunotherapy. Eur J Nucl Med Mol Imaging 36: 1235-1244, 2009.
181. Moosmayer D, Berndorff D, Chang CH, Sharkey RM, Rother A, Borkowski S, Rossi EA, McBride WJ, Cardillo TM, Goldenberg DM and Dinkelborg LM: Bispecific antibody pretargeting of tumor neovasculature for improved systemic radiotherapy of solid tumors. Clin Cancer Res 12: 5587-5595, 2006.
182. Del Conte G, Erba PA, Fasolo A, Chiesa C, Grana C, Menssen H, Neri D, Mariani G, Bombardieri E and Gianni L: Radioimmunotherapy (RIT) with ^{131}I-L19SIP in solid cancers (SC) and lymphoproliferative diseases: Final results of the first human trial. J Clin Oncol 28 (15_suppl): 2523, 2010.
183. Petrini I, Sollini M, Bartoli F, Barachini S, Montali M, Pardini E, Burzi IS and Erba PA: ED-B-containing isoform of fibronectin in tumor microenvironment of thymomas: A target for a theragnostic approach. Cancers (Basel) 14: 2592, 2022.
184. Miura JT and Zager JS: Neo-DREAM study investigating Daromun for the treatment of clinical stage IIIB/C melanoma. Future Oncol 15: 3665-3674, 2019.
185. Vaidya AM, Wang H, Qian V and Lu ZR: Extradomain-B Fibronectin is a molecular marker of invasive breast cancer cells. bioRxiv Aug 22, 2019. doi: 10.1101/743500.