REVIEW

Integrins as attractive targets for cancer therapeutics

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Abstract

Integrins are transmembrane receptors that have been implicated in the biology of various human physiological and pathological processes. These molecules facilitate cell–extracellular matrix and cell–cell interactions, and they have been implicated in fibrosis, inflammation, thrombosis, and tumor metastasis. The role of integrins in tumor progression makes them promising targets for cancer treatment, and certain integrin antagonists, such as antibodies and synthetic peptides, have been effectively utilized in the clinic for cancer therapy. Here, we discuss the evidence and knowledge on the contribution of integrins to cancer biology. Furthermore, we summarize the clinical attempts targeting this family in anti-cancer therapy development.

KEY WORDS

Integrins; Extracellular matrix; Tumor progression; Targeted drug; Antagonists; Clinical trial

Abbreviations: ADAMs, adisintegrin and metalloproteases; AJ, adherens junctions; CAFs, cancer-associated fibroblasts; CAR, chimeric antigen receptor; CSC, cancer stem cell; CRC, colorectal cancer; ECM, extracellular matrix; EGF, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; ERK, extracellular regulated kinase; FDA, U.S. Food and Drug Administration; HIF-1\textalpha, hypoxia-inducible factor-1\textalpha; HUVECs, human umbilical vein endothelial cells; ICAMs, intercellular adhesion molecules; IGF, insulin-like growth factor receptor; IMD, integrin-mediated death; JNK, c-Jun N-terminal kinase; JNK, c-Jun N-terminal kinase 16; mAb, monoclonal antibodies; MAPK, mitogen-activated protein kinase; MMP2, matrix metalloprotease 2; NF-\kappaB, nuclear factor-\kappaB; NSCLC, non-small cell lung cancer; PDGF, platelet-derived growth factor receptor; PE3K, phosphatidylinositol 3-kinase; RGD, Arg-Gly-Asp; RTKs, receptor tyrosine kinases; SAPKs, stress-activated MAP kinases; sdCAR-T, switchable dual-receptor CAR-engineered T; SDF-1, stromal cell-derived factor-1; SH2, Src homology 2; siRNA, small interfering RNA; STAT3, signal transducer and activator of transcription 3; TCGA, The Cancer Genome Atlas; TICs, tumor initiating cells; TNF, tumor necrosis factor; uPA, urokinase-type plasminogen activator; VCAMs, vascular cell adhesion molecules; VEGFR, vascular endothelial growth factor receptor.

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1. Introduction

Integrins are a family of ubiquitous cell membrane adhesion receptors that make up the extracellular matrix (ECM). These include 24 heterodimers that span the plasma membrane and are consist of 18 α and 8 β integrin subunits. The two main functions of integrins include: (1) mechanical attachment to the ECM; and (2) activation of signal transduction pathways that control various cellular functions that are essential to solid tumor initiation, progression, and metastasis.

Integrins play a crucial role in several physiological processes by maintaining cell viability via its attachment to the ECM. Moreover, these can directly control the migration and invasion of cancer cells by binding to ECM components and establishing traction for cellular motility and invasion. ECM remodeling is also regulated by integrins that control ECM protease localization and activity. Furthermore, integrins also influence the proliferation, survival, and metastasis of cancer cells. Integrins have the ability to detect a wide range of extracellular ligands, such as transmembrane receptors on the surface of other cells and ECM proteins involved in cell–cell junctions; integrins bind to different receptors, including adisintegrin and metalloproteases (ADAMs) or molecules expressed by endothelial cells and leukocytes, such as intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs). Moreover, various extracellular ligands are involved in cell–ECM interactions, including collagen, laminin, and fibronectin. However, integrins can also recognize various other physiological ligands and act as receptors for viruses, snake venoms, and other pathogens. Some integrins can only bind to particular ECM ligands, such as α5β1 integrin binds to fibronectin, whereas others show a wider ligand-binding repertoire, such as αvβ3 integrin binds to vitronectin, fibronectin, thrombospondin, and fibrinogen. Almost all integrins can bind to the ECM using the RGD (Arg-Gly-Asp) peptide motifs. The EILDV and REDV sequences have also been shown to mediate integrin–ECM adhesion.

This review presents the crucial and often contradictory function of integrins in controlling tumor cell survival, other than their ligation-dependent effects, as well as recent developments in integrin treatment schemes, particularly in cancer.

2. Integrins in cancer

Integrins occurring on cancer cells and other cell types in the tumor microenvironment have essential roles in controlling intracellular activity as well as intercellular communication. These have a paradoxical role in cancer, ligated integrins can enhance cell survival and promote cell proliferation; in contrast, whereas unligated integrins present in the surroundings of tumor cells can initiate apoptotic cascade. However, cancer cell–ECM interactions are essential to maintaining homeostasis between the two states, including “inside-out” and “outside-in” signaling, which are almost altered during cancer progression.

Integrins are proteins that span the plasma membrane and are capable of bidirectional signaling (Fig. 1). Various cytoplasmic interactions control integrin activation, altering its affinity to extracellular ligands that transmit “inside-out” signals. Here, the recruitment of the adaptor protein talin to the tail region of the integrin protein induces a conformational change in the extracellular domain of the integrin, resulting in its activation and increase in affinity for ECM ligands. Integrins are capable of regulating intracellular pathways in response to the ECM or other extracellular matrix that binds to “outside-in” signals. Integrin–ECM interactions, in turn, result in the recruitment of adaptors and signaling proteins to the cytoplasmic domain of integrins, and promote macromolecular complex assembly, which is also known as focal adhesion.

The major function of integrins expressed on the cell surface is to adhere to the ECM, ligation provides traction that is essential tumor cell survival and invasion. Cell migration and invasion are also controlled by integrins by influencing the activity and localization of matrix-degrading proteases, such as urokinase-type plasminogen activator (uPA) and matrix metalloprotease 2 (MMP2). In addition, deregulation in integrin recycling by the crab GTPas or mediated by integrins themselves also results in enhanced growth factor signaling and cell migration. Integrin-controlled cell migration is largely mediated by signaling pathways involving members of the focal adhesion kinase (FAK)-SRC family kinase. However, it is highly dependent on an integrin-specific mechanism. Integrin–ligand adhesion triggers an increase in FAK tyrosine (Tyr) 397 phosphorylation, which creates a binding site for the SRC kinases domains SRC homology 2 (SH2) and SH3. Then, SRC phosphorylates other tyrosines that contribute to the full activation of FAK. This activated FAK/SRC complex facilitates various key signaling cascades that regulate cell motility. The other well-established role of integrins involves cancer cell proliferation and survival. Integrin adhesion activates the cyclin-dependent kinase inhibitor family pathway and cyclin D1, thereby regulating the entry of cells into the S phase of the cell cycle while deregulating cell proliferation and anchorage-independent growth. In addition, integrins contribute to cell survival through several complex- and context-dependent pathways. In addition to PS3 activation, integrin ligation also triggers the upregulation of BCL-2 and FLIP pro-survival molecules, the activation of mitogen-activated protein kinase (MAPK)/extracellular regulated kinase (ERK) pathway, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway, c-Jun N-terminal kinase (JNK)16, and stress-activated MAP kinases (SAPKs) or nuclear factor-κB (NF-κB) signaling.

However, integrins in the unligated form could in contrast negatively influence tumor survival. Integrins in adherent cells are predominantly unligated and result in the cleavage of caspase 8 that in turn triggers tumor cell apoptosis via a mechanism known as integrin-mediated death (IMD). In contrast to IMD, which is induced by cell adhesion, anokisis-induced apoptosis is induced by the complete detachment of the cell from the ECM. Recent studies have revealed that tumor cells are resistant to IMD, which is attributable to the complete loss of caspase 8. In this situation, integrins promote cell survival and metastasis. In summary, more studies are required to better understand the effects of...
ligated and unligated integrins, which is a determining factor in the clinical evaluation of integrin antagonists.

2.1. The composition and function of integrin family in cancer

Numerous integrins have been investigated for their presence and contribution to tumor progression. Some integrins detected in tumors are retained from the normal epithelial cells before tumorigenesis, i.e., α2β1, α3β1, αvβ5, α6β1, and α6β4, mainly mediate cell adhesion as well as cell migration, proliferation and survival. More interestingly, some integrins have been proven to be upregulated in tumor cells while expressed at low or undetectable levels in normal tissues, notably, integrins αvβ3, α5β1 and αvβ6 and with less frequency αvβ5, α6β4, and α4β1 (Table 1). The expression of these integrins has been correlated with pathological outcomes including disease stage, tumor metastasis, treatment resistance, and patient survival.

2.1.1. αvβ6
The integrin subunit β6 is selectively expressed during wound healing and embryonic development and is associated with tissue remodeling. In cancer, αvβ6 integrin is upregulated in various carcinomas and strongly correlated to cell migration, invasion, and survival, in particular in breast cancer, and αvβ6 acts via the activation of TGF-β, which is a key initiator of matrix remodeling and fibrosis. Then, subsequent studies have found that colorectal cancer (CRC) cells expressing integrin αvβ6 secrete inactive TGF-β, which is then activated by integrin αvβ6 that subsequently activates fibroblasts that promote CRC cell invasion. Furthermore, it has been shown that αvβ6 expression is correlated to poor prognosis in individuals with triple-negative breast cancer. The crosstalk between αvβ6 integrin and epidermal growth factor receptor (EGFR) controls bidirectional force transmission and regulates breast cancer invasion; it influences matrix stiffness, transcriptional reprogramming, force transmission to the nucleus, as well as microenvironment rigidity. αvβ6 interactions trigger EGFR and MAPK signaling, whereas αvβ6–EGFR crosstalk controls mutual receptor trafficking mechanisms. Blocking αvβ6 alone or together with trastuzumab administration has been reported for the treatment of high-risk and trastuzumab-resistant breast cancer patients.

2.1.2. α5β1
Integrin α5β1 has been described as the central regulator of angiogenesis. High α5β1 integrin expression levels have been observed in endothelial cells, and they are correlated with reduced cellular survival. In addition, vascular remodeling defects were observed in mice with α5β1 integrin abnormalities, leading to adhesion and migration alterations. It has been proven that α5β1 integrin supports survival of cells with fibronectin attachment via upregulation of BCL-2 expression, whereas αvβ1 integrin (binds the fibronectin onto the same RGD such as α5β1) does not suppress cell apoptosis.

In colon cancer, HT29 cells expressing α5β1 have been shown to be resistant to serum starvation-induced apoptosis, and a correlation between acquisition of α5β1 integrin and ADAM-15 downregulation and poor prognosis has been reported. In line with this, a previous study described that hypoxia upregulates the α5β1 integrin subunit, which in turn promotes colon cancer progression. In addition, fibronectin generated by peritoneal tissues activates α5β1 integrin on ovarian cancer cells, stimulating their invasive process by increasing MMP-9 activity. Several human ovarian cancer cell lines express α5β1 integrin, and its binding is disrupted specifically by anti-α5β1 integrin antibodies or a ligand of α5β1 integrin, namely, endostatin.

**Figure 1**  Composition and signal transduction of the integrin family in cancer progression. Depending on the ligation status of integrins expressed by the cancer cells or cells present in TME, they can trigger pro-survival or paradoxically a pro-apoptotic signal. In the most common situations, integrins are ligated and initiate the pro-survival signal by overexpression of the pro-survival molecules BCL-2 and FLIP factor-xB, activation of NF-κB or PI3K-AKT, and downregulation of P53. In an unligated status, the integrins initiate a process known as integrin-mediated death (IMD) via the cleavage of caspase 8.
ERBB2, an oncogene strongly associated with metastasis and poor prognosis in breast cancer, triggers the upregulation of α5β1 integrin in mammary adenocarcinomas, thereby promoting tumor cell survival. A recent study has indicated that hypoxia selectively enhances the expression of integrin α5β1 receptor in breast cancer to promote metastasis as well. The expression of α5β1 integrin has also been reported in lung cancer, melanoma, and glioma and it has respectively been associated with the activation of the PI3K/AKT pathway, Rho-GTPases, and the IL-6-STAT3 pathway.

2.1.3. αvβ3

Beside its role in various physiologic processes such as wound healing and angiogenesis, αvβ3 integrin is highly involved in tumor pathogenesis. It has been associated with the growth, survival, invasion, and metastasis of various cancer cells. It has also been correlated with tumor progression and lower patient survival rates in breast cancer, melanoma, and colon carcinoma, in addition to increasing migration and invasion of tumor cells. Integron αvβ3 binds to a spectrum of ECM molecules using the RGD triple-peptide motif, which includes von Willebrand factor, fibronectin, fibrinogen, proteolyzed forms of collagen and laminin, and vitronectin, whereas other integrins, including α5β1, can only selectively bind to fibronectin. In prostate cancer, the upregulation of αvβ3 has been associated with resistance to radiotherapy via the regulation of survivin expression levels. The application of αvβ3 integrin antagonist or survivin inhibition with siRNA enhances IR-induced inhibition of anchorage-independent cell-growth.

2.1.4. β1/β3 balance

A strong correlation has been reported between β1 and β3 integrin expression in cancer cells. The association between the two integrins remains complicated and paradoxical, but it has been utilized as an indicator of cancer prognosis and in clinical treatment. The downregulation of β1 integrin has been shown to be compensated by an upregulation of β3 integrin to maintain the metastasis of breast cancer cells, and tumor proliferation was only affected when β1 and β3 integrins were simultaneously downregulated. This effect has not been observed in normal cells. Furthermore, the roles of β1 integrin include promoting proliferation and inhibiting metastasis, and β1 integrin inhibition affects TGF-β function that in turn attenuates the expression of E-cadherin, decreasing the activity of cell–cell junctions while enhancing motility and migration. In contrast, β3 integrin induces epithelial–mesenchymal transition (EMT) and activates a non-canonical FAKs-independent signaling pathway that in turn prevents cancer cells from undergoing IMD and promoting cancer cell metastasis.

### 2.2. Integrins and angiogenesis

Angiogenesis plays a central role in wound healing and embryonic development, various diseases, such as cancer, psoriasis, rheumatoid arthritis, and diabetic retinopathy, are orchestrated, in part, by a pathological angiogenic response. Angiogenesis also plays a major role in tumorigenesis, and the formation of new blood vessels is important to provide tumor cells with oxygen and nutrients for the maintenance of growth of solid tumors. In addition, neovascularized tumor cells could be shed into the circulation, leading to cancer metastases. Harold Dvorak has stated “tumors make bad blood vessels” and tumor-associated blood vessels are structurally and biologically different from quiescent vessels in that their tortuous and leaky characteristics influence blood flow, disrupt drug delivery, promote fibrosis, and allow tumor cell intravasation. Angiogenesis is dependent on endothelial cell adhesion to ECM proteins, such as vitronectin, through integrins, particularly, integrin β1, β3, and αvβ3. Lugano et al. demonstrated that β1 integrin activation is critical for the organization of fibronectin fibrillogenesis during tumor vascularization. They claimed that CD93 controls β1 integrin signaling, phosphorylation of focal adhesion kinase (FAK), as well as fibronectin fibrillogenesis in endothelial cells. In turn, tumor vessels in gliomas orthotopically implanted in CD93-deficient mice exhibited diminished activation of β1 integrin, with fibronectin showing disorganized fibrillar structures. In addition, studies have revealed β3 upregulation on newly developed blood vessels in tumors, whereas it is not generally observed on blood vessels of normal tissues, and it acts through the ligation onto proteolyzed collagen at its RGD site to induce tumor neovascularization. In fact, various animal models lacking β3 integrin expression or directly blocking β3 integrin have been shown to have decreased angiogenesis and induction of tumor regression. Moreover, αvβ3 integrin has been investigated for its indirect effect via VEGF2 promoting a VEGF-induced angiogenesis. In endothelial cells, an interaction between VEGFR2 and integrin αvβ3 is an essential process during vascularization, and studies have shown that the disruption of the αvβ3/VEGFR2 crosstalk and its downstream pathways slows down tumor angiogenesis.
2.3. Integrins and cancer stem cells

Cancer stem cell (CSC)-tumor initiating cells (TICs) are the most aggressive and dangerous cells in tumors as these have the capacity to self-renew and differentiate. Accumulated evidence suggests that CSCs are drivers of tumor progression, disease relapse, and drug resistance. Integrins have been proved to have a pivotal part in both cancer initiation and progression, as well as cell differentiation, which may serve as evidence for their contribution to CSC biology. However, several of these integrins that are enriched in normal adult stem cells and progenitor cells are also indicators of CSCs, including \( \alpha_1, \alpha_2, \beta_3, \) and \( \beta_4 \) integrin subunits. Interestingly, a complete inhibition of tumorigenesis was detected in mice lacking \( \beta_1 \)-integrin function, and the mammary gland-specific loss of function of \( \beta_1 \) integrin can essentially block the generation or the amplification of CD24\( ^{\text{hi}} \)CD29\( ^{\text{lo}} \)CD61\( ^{\text{hi}} \) cancer cells, thereby resulting in tumorigenesis. Barnawi et al. analyzed the expression profiles of 530 patients from the TCGA (The Cancer Genome Atlas) database and reported a statistically significant correlation between \( \beta_1 \) integrin and fascin expression; fascin-mediated regulation of \( \beta_1 \) integrin plays a critical role for various breast cancer cell functions, such as adhesion to different ECMs, self-renewability, and chemoresistance. Furthermore, a significant relationship among coexpression of fascin and \( \beta_1 \) integrin, short disease-free intervals, and overall survival was reported in chemo-treated breast cancer. The expression of a dominant-negative \( \beta_4 \) integrin mutant has been shown to delay the progression and to inhibit metastasis in a breast-cancer mouse model that was induced by an activated HER2 (ERBB2) receptor. The proposed mechanism was that \( \beta_4 \) integrin directly interacts with the ERBB2 receptor, and activates the JUN and STAT3 pathways, which have been associated with tumor proliferation, survival, and resistance to immunotherapy. Other signaling pathways have also shown that \( \beta_4 \) integrin is essential to cell survival and resistance to apoptosis. The P63 pathway, which is normally and particularly expressed in basal cells (either normal or cancerous mammary basal epithelial cells or stem cells) can activate \( \beta_4 \)-integrin expression that mediates resistance to anoikis (an apoptotic mechanism that is caused by loss of cell anchorage) via a STAT3-dependent mechanism. The second pathway is through NF-\( \kappa \)B signaling mechanism that promotes \( \beta_4 \)-integrin-mediated resistance to apoptosis. Hoogland et al. assessed the immunohistochemical expression of stem cell markers in 481 patients with prostate cancer, and \( \alpha_6 \) expression was observed in 28.4% of these patients and was described as a predictive biochemical marker for local recurrence of prostate cancer and disease-specific death. Furthermore, integrin expression was correlated to high aggressiveness of squamous cell carcinomas, and serial limit dilution transplantation assays revealed that \( \alpha_6^\text{lo} \beta_1^\text{lo} \) populations can initiate secondary tumors, but \( \alpha_6^\text{hi} \beta_1^\text{lo} \) populations cannot, regardless of whether the cells were CD34\( ^{\text{hi}} \) or CD34\( ^{\text{lo}} \). Taken together, these findings strongly argue that targeting integrins may potentially be utilized as a relevant strategy for cancer treatment to restrict cancer-stem cell survival and aggressiveness.

2.4. Integrins and cancer-associated fibroblasts (CAFs)

CAFs are the predominant stromal cell type in the tumor microenvironment that can contribute to cancer progression through interactions with tumor cells. Numerous experimental studies support that integrins play bidirectional regulatory roles between cancer cells and CAFs. CAFs that express IL-32 contain an RGD cell attachment sequence that binds to integrin \( \beta_3 \)-positive cancer cells to promote breast cancer cell invasion and metastasis. CAF-derived extracellular vesicles that express annexin A6 plays a pivotal role in gastric cancer drug resistance via activation of \( \beta_1 \) integrin-FAK-YAP signaling. CRC cells express integrin avb6-activated CAFs through TGF-\( \beta \), which subsequently secrete stromal cell-derived factor-1 (SDF-1) and promote CRC cell metastasis. These research studies reveal that integrins act as receptors that regulate the interactions between CAFs and cancer cells in tumor progression and drug resistance.

2.5. Integrins and cancer immunity

Myeloid cells and lymphocytes rely on cell adhesion receptors (including integrins) for trafficking inflamed tissues and tumors, which are involved in both innate immune and adaptive immune responses. Previous studies have revealed that integrin avb1 regulates myeloid cell trafficking into tumors during tumor progression. Integrin avM2 inhibits immune suppression by restraining immunosuppressive macrophage polarization. Moreover, the relative expression level and activation state of integrin avb5 and avb2 on T cells mediates movement within the tumor microenvironment through direct interactions with ligands on tumor cells, stromal cells, and other immune cells. Integrin \( \beta_3 \) signaling regulates the balance between protumor and anti-tumor immune cells via STAT6/STAT1 signaling, which partly explains the varied clinical results of integrin antagonists. Thus, integrins may serve as critical components of the tumor immune microenvironment and can be an effective immunotherapeutic target.

3. Crosstalk between integrins and other signaling pathways in cancer

Integrins signaling in cancer involves not only activation of certain pathways downstream of specific receptors, but also crosstalk with growth factors, growth factor receptors, cytokines, oncogenes, and enzymes. Integrins can associate with numerous receptor tyrosine kinases (RTKs) and trigger their cross-phosphorylation, such as EGFR, vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor receptor (IGF), platelet-derived growth factor receptor (PDGFR), and c-Met. This cooperative signaling differentially activates RAF, which in turn enhances cell survival. Signaling via integrin avb3 and the fibroblast growth factor receptor induces the phosphorylation of RAF Ser338 and Ser339, thereby protecting cells from the intrinsic apoptosis pathway. The ligation of integrin avb5 and VEGFR2 phosphorylates RAF Tyr340 and Tyr341, preventing apoptosis through the extrinsic pathway. The engagement of integrin avb1 with fibronectin controls the activity of RhoA by inducing Src-mediated P190 RhoGAP tyrosine phosphorylation.

In addition to the crosstalk, integrin-mediated adhesion to ECM can also enhance growth factor signaling on its receptor, and in some cases, interactions with ECM may aid in the effective presentation of growth factors to their receptors. Integrins crosstalk with EGF and/or its receptor EGFR has been extensively investigated. It has been revealed that cooperation between integrins and members of the EGFR family (EGFR and ERBB2) may affect tumor initiation, proliferation, migration,
and invasion. Examples include α6β4, αvβ6, and αvβ5 integrins. α6β4-ERBB2 induces the activation of signal transducer and activator of transcription 3 (STAT3) and Jun, resulting in the loss of cell polarity and hyperproliferation, respectively. A previous study has also revealed the consequence of αvβ6—EGFR crosstalk in the bidirectional transmission of mechanical signals between the nucleus and ECM. The crosstalk between αvβ6 integrin and EGFR involves a complex regulatory mechanism that impacts matrix stiffness and force transmission to the nucleus. The adhesion of αvβ6 to ECM induces EGFR and MAPK signaling, and αvβ6—EGFR crosstalk controls mutual receptor trafficking mechanisms that in turn regulate tumor cell invasion. Furthermore, the activation of αvβ5 integrin in breast cancer is EGFR-dependent and occurs via SRC phosphorylation of the p130Cas substrate domain, followed by the activation of the GTPase RAP1A, which is a known mediator of integrin activation. Similar as that in breast cancer, it has been published that integrin ligation itself regulates EGF signaling, inducing an EGFR-independent EGFR phosphorylation and crucially influencing tumor cell susceptibility to treatment, resulting in increased MAPK activation, tumor cell proliferation, survival, and resistance for anticancer therapy.

Integrins and VEGF/VEGFR interaction is another example of integrin crosstalk occurring on endothelial cells not tumor cells themselves and promoting tumor angiogenesis. The VEGFR2—integrin αvβ5 pair induces SRC-dependent phosphorylation of RAF (Tyr340 and Tyr341) and resistance to extrinsic vascular endothelial cellular apoptosis that is induced by inflammatory mediators, including tumor necrosis factor (TNF)102. Furthermore, VEGF influences integrin αvβ3 signaling by controlling the affinity state or activating integrins. αvβ3 integrin activation can in turn enhance tumor cell secretion of VEGF, thereby providing a feedback loop that increases tumor growth. In addition, the recruitment of SRC to VEGFR2 promotes the SRC-dependent tyrosine phosphorylation of the integrin αvβ3 cytoplasmic domain, thereby enhancing angiogenesis.

Integrins and MMPs also exhibit strong cross-reactivity. MMP-2 and MMP-9 are major factors influencing cancer cell migration and invasion; these degrade ECM proteins and facilitate tumor invasion. It has been reported that αvβ3 is essential to MMP-2 activation, its blockade inhibits MMP-2 activation by collagen 1, consequently affecting cell migration and invasion. αvβ5 ligation participates in the activation of MMP-9 and an upregulation of VEGF, inducing an increase in cell migration in melanoma.

E-cadherin is a Ca2+-dependent cell surface glycoprotein that influences cell—cell adhesion. It acts as a single-pass transmembrane protein that controls homophilic cell—cell interactions. During tumorigenesis E-cadherin loses its function and transforms to a more motile and invasive phenotype in coordination with integrin-mediated adhesions to the surrounding ECM. An ultimate crosstalk exists between integrin and E-cadherin in controlling cell motility and invasiveness. Integrin engagement activates several signaling cascades that are controlled by FAK and SRC and Rho GTPases. This signaling network downstream of integrins alters actin dynamics, which regulate adherent junctions (AJ) principally formed by E-cadherin. In addition, activation of these pathways results in changes in the transcriptional and post-transcriptional regulation of AJ components, the regulation of E-cadherin endocytosis, and the enhancement of the cell motility and migration.

In addition to the direct phosphorylation of AJ components, AJs may also be regulated by SRC and FAK by altering the expression and stability of E-cadherin protein expression. The transcriptional control of the E-cadherin promoter (HIF-1α) and FAK and SRC can also control the endocytosis of E-cadherin and thus its membrane localization and control of the strength of AJs, Canel et al.114 reported that small interference RNA (siRNA)-mediated depletion of β1 integrin or FAK inhibits E-cadherin endocytosis and is correlated to strengthening of cell—cell adhesion and decrease in collective invasion.

4. Integrin targeted therapy

The importance of integrins as indicated by earlier studies has prompted the development of integrin—antagonist molecules that disrupt tumor growth of both tumor cells and tumor-associated cells, notably endothelial cells. Current integrin antagonists that are now being investigated in clinical trials include RGD peptide mimetics and monoclonal antibodies (mAb).

4.1. Antibodies

4.1.1. Bevacizumab

Bevacizumab (Avastin, LM609, Genentech) is a mouse anti-human integrin αvβ3 and anti-VEGF mAb. Its anti-angiogenic mechanism is attributed to the inhibition of bFGF and TNF-α induced angiogenesis. Avastin shows good efficacy and tolerance in all of the clinical trials and thus has been approved by the U.S. Food and Drug Administration (FDA) as a first- or second-line treatment for metastatic breast cancer or as part of a combination chemotherapy scheme for metastatic colorectal cancer. Avastin is also used in glioblastoma, metastatic renal carcinoma, and NSCLC. Several humanized versions of Avastin, such as Vatixin I (MEDI-523) and Abeegrin (Vatixin II, MEDI-522) have been developed, but these failed to show antitumor efficacy in clinical trials. Recently, it has been revealed that Avastin treatment may increase the number of CSCs in breast cancer, suggesting a possible explanation for why this molecule does not lead to longer survival. Conley et al. attributed this effect to enhanced intra-tumoral hypoxia that in turn activates hypoxia-inducible factor 1α (HIF-1α). Furthermore, several reports have shown the critical role of hypoxia and HIF in the proliferation, self-renewal, and maintenance of cancer stem cells.

Becherirat et al. observed a significant increase in proangiogenic factors when therapy was stopped in mice with colorectal cancer. Upon withdrawal of bevacizumab, and after a drug-break period, a notable increase in CSCs was observed, indicating that Avastin treatment needs to be maintained because discontinuous administration triggers tumor regrowth and increases tumor resistance and CSC heterogeneity.

4.1.2. Intetumumab

Intetumumab (CNTO 95, Centocor) is a fully humanized antibody with multiple integrin inhibition property, it recognizes and binds with high affinity multiple αv integrins. In a phase I clinical trial, CNTO 95 has been shown to be safe and well tolerated, and a prolonged response was observed in patients with tumor cells expressing αvβ3 integrin, whereas a partial response was detected in a patient whose tumor expressed αvβ1 integrin. The development of this drug was discontinued during its phase II clinical trial for treatment of melanoma and prostate cancer.
4.1.3. Abciximab
Abciximab (c7E3) is a Fab fragment of the 7E3 chimeric human-murine monoclonal antibody. It has been developed as a platelet aggregation inhibitor mainly by targeting αIIbβ3 receptors on platelets. However, it can also effectively bind to αvβ3 integrin, and, more importantly, it can redistribute between the two receptors in vitro. The FDA has approved c7E3 Fab as adjunct therapy against cardiac ischemic complications in individuals undergoing percutaneous coronary intervention. An in vitro angiogenesis assay has shown that c7E3 Fab inhibits the migration of αvβ3-mediated human umbilical vein endothelial cells (HUVECs) and cell adhesion, migration, and invasion in melanoma, in addition to bFGF stimulating the proliferation of HUVECs. An animal study has shown that c7E3 Fab partially suppresses human melanoma tumor growth in nude mice and completely interferes with the formation and growth of human melanoma tumor in nude rats. Furthermore, it has been reported that abciximab could induce a proapoptotic effect in MCF-7 breast cancer cells through the activities of proline oxidase, ERK1/2, NF-κB, HIF-A1, VEGF, and collagen biosynthesis.

4.1.4. LM609-derived antibody
Vitaxin (MEDI-532) is the developmental precursor of Abegrin (Etaracizumab, MEDI-522). These are two humanized versions of the LM609 monoclonal antibody that have been shown to specifically recognize αvβ3 integrin and target angiogenic blood vessels, thereby suppressing tumor growth in various animal models. It has been revealed that Vitaxin has no effect on the rates of wound closure or integrity (indicating a disadvantage to anti-angiogenic therapy) in two different animal species, implying that this molecule is safe. In a pilot trial involving patients with metastatic cancer who failed standard therapy, no significant toxicity and no immune response to Vitaxin were noted. In addition, three patients who received two cycles of therapy had stable disease on Day 85 when taken off study.

Phase I clinical trial revealed that Vitaxin treatment provides clinical benefit to patients with tumors without causing significant side effects. The two antibodies are currently being assessed in phase II clinical trials as a therapeutic regimen for melanoma and prostate cancer. Combination therapy with paclitaxel generated stable disease on Day 85 when taken off study. In phase II clinical trials, cilengitide showed a high efficacy and safety in an individual with glioblastoma. Unfortunately, in a randomized, controlled, multicenter UEORTC phase III clinical trial, treatment with cilengitide did not improve the overall survival of patients with newly diagnosed glioblastoma, which may be due to various reasons such as dose dependency, absence of reliable biomarker for the assessment of tumor activity and/or the selection of cancer type. Future investigations need to take account into the unique pharmacokinetics of the drug.

4.2. Synthetic peptides
Synthetic peptides mimic the organization of the natural ligands of integrins, these are mostly RGD mimetics that effectively bind to integrins and block their effect. There is no currently FDA-approved integrin-blocking peptide for cancer.

4.2.1. Cilengitide
Cilengitide (EMD 12) is a cyclic RGD pentapeptide [Arg-Gly-Asp-(D-Phe-(NMeVal))] that acts as a potent αvβ3 and αvβ5 integrin inhibitor of integrin-mediated adhesion and migration. This αv inhibitor apparently acts by inhibiting the FAK/SRC/AKT pathway and triggering apoptosis in endothelial cells. Preclinical studies have revealed positive antiangiogenic effects in vitro and antitumor effects in vivo against melanoma, and also in head and neck cancer, breast cancer, and brain tumor. Cilengitide has been used in trials for the treatment of sarcoma, glioma, lymphoma, leukemia, and lung cancer.

Phase I clinical trials involving patients with recurrent malignant glioma has shown that its well-tolerated doses are 2400 mg/m², with stable disease observed in four patients. In phase II clinical trials, cilengitide showed a high efficacy and safety in an individual with glioblastoma. In a randomized, controlled, multicenter UEORTC phase III clinical trial, treatment with cilengitide did not improve the overall survival of patients with newly diagnosed glioblastoma, which may be due to various reasons such as dose dependency, absence of reliable biomarker for the assessment of tumor activity and/or the selection of cancer type. Future investigations need to take account into the unique pharmacokinetics of the drug.

4.2.2. ATN-161
ATN-161 is a non-RGD-based pentapeptide (PHSRN) derived from the fibronectin synergy region. This noncompetitive inhibitor of fibronectin binds exclusively to the integrin β subunit, and it inhibits the function of several integrins implicated in tumor angiogenesis. In vivo experiments revealed that treatment with ATN-161 alone or combined with 5-fluorouracil (5-FU) could significantly reduce tumor cell proliferation and enhance overall survival in mice with metastatic colon cancer, and promising effects were detected in breast cancer treatment, in which ATN-161 caused a significant dose-dependent reduction in tumors and blocked the incidence and frequency of metastases.

A phase I clinical trial revealed that the drug is safe and potentially active, and it does not induce dose-limiting toxicity, with prolonged stable disease occurring in some of the treated patients.

4.2.3. HM-3 and PEG-HM-3
HM-3 is 18-amino-acids RGD-containing peptide that specifically targets integrin αvβ3. Its long-lasting form PEG-HM-3 was developed to prolong its half-life and change its mode of administration from IV injection to subcutaneous administration. It has been proven that this molecule, not only targets the endothelial cells, but also some integrin-expressing tumor cells. Preclinical tests revealed that the drug exhibits a strong antiangiogenic and antitumor bioactivity, and the PEGylated peptide achieved an impressive tumor inhibitory rate in human NSCLC.
gastric, breast, and colon cancer xenograft models with minimal cytotoxicity. A mechanistic study revealed that PEG-HM-3 targets integrin αvβ3, thus disrupting the downstream ERK and AKT pathways, resulting in the downregulation of VEGF, AKT, p-AKT1, MEK1, p-MEK1, ERK1/2, and p-ERK1/2 and the expression of integrins αv and β3 decreased after HUVECs were incubated in the presence of mPEG-SC20k-HM-3 for 24 h. Recently, Zhao et al. reported that an enhanced therapeutic effect of HM-3 on lung cancer in vivo by administering a combination of Salmonella VNP20009 carrying a SOX2 shRNA construct and targeting cancer stem cells and the SOX2 gene.

4.2.4. AP25
AP25 is a 25-amino acid RGD-modified polypeptide that targets αvβ3 and α5β1 integrins that are secreted by endothelial and tumor cells. AP25 has an extraordinary antitumor effect on different types of cancer, including gastric carcinoma, melanoma, hepatic carcinoma, and breast cancer. Recently, Li et al. presented a novel strategy involving fusion of the AP25 peptide and GnRH Fc fragment that not only retains the bifunctional biological activity of angiogenesis inhibition as well as GnRH receptor blocking, but it also prolongs its half-life, which provides a reliable approach for future pre-clinical research.

4.3. New emerging integrin-targeted therapy
Whilding et al. developed αvβ3-specific CAR-T cells and assessed their antitumor function in preclinical models both in vitro and in vivo. αvβ3-CAR-T cells rapidly and specifically destroyed αvβ3-positive tumor cells, secreted IFN-γ and IL-2 (CD4+ > CD8+), and underwent productive proliferation in vitro. In a murine xenograft model for metastatic A-375 melanoma, the molecule triggered complete elimination of melanoma lesions, resulting in long-term tumor-free survival.

Integrins have also been employed to generate specific, controllable, and improved cytotoxicity of CAR-T therapy in a novel approach that was developed by our team with novel switchable dual-receptor CAR-engineered T (sdCAR-T) cells as well as a new switch FITC-HM-3 molecule, as described earlier (above, bifunctional molecule, FHBM). Furthermore, to improve the specificity of CAR-T cells, human mesothelin-expressing sdCAR-T cells against cognate tumor cells and integrin αvβ3 were also generated. The results of the study revealed that in the presence of FHBM, these designed sdCAR-T cells were highly active, which include activation and proliferation and exhibited specific cytotoxicity following a time- and dose-dependent manner in vitro. In nude mice treated with a combination of FHBM, sdCAR-T cells disrupted the growth of MSLN K562 cells and exhibited downregulation of cytokines, including interleukin-2, interleukin-6, interferon γ, and TNF-α relative to conventional CAR-T cells. These findings imply that targeting integrins can effectively control the timing and dose of injected CAR-T cells. Furthermore, sdCAR-T cells exert significant antitumor activity as these releasing lower amounts of cytokines for MSLN- and integrin αvβ3-expressing cognate tumor cells.

5. Conclusions
In the past few years, pre-clinical assays have revealed that integrin targeted therapy, including mAbs and synthetic molecules, imparts strong antitumor effects. However, clinical assays have failed to translate this effect on tumor progression inhibition. The disappointing results in relation to patient survival time, disease stabilization, and occurrence of metastasis may be due to the complexity of integrin mechanisms, the development of resistance to anoikis, and their ability to compensate each other and induce a poor phenotype. Using a more complex mechanism, a vessel co-option was reported as a pathway that allows tumor cells to receive nutrients from blood via pre-existing vasculature without developing new vessels as a resistance response against anti-angiogenic therapy.

In addition, studies have also shown that improper doses of integrin inhibitors may break the balance and produce the opposite effect. For example, Xu et al. found that RGD modified peptide HM-3 has significant anti-tumor activity at the effective dose, while HM-3 promotes tumor growth and metastasis when exceeded the effective dose. Therefore, to improve the efficacy of integrin targeted therapy, the mechanism of integrins should be comprehensively investigated. Furthermore, exploring the appropriate drug dosage is important to rationally design clinical dosing regimens. Recently, the endocytosis of integrins and their transfer through exosomes were described as key factors in integrin-therapy failure by influencing integrin recycling. Integrin inhibition in the tumor microenvironment remains challenging because in complex organisms, factors systemically interfere with each other, making drug development even more difficult.

Together, integrin inhibition may be potentially utilized as a target for drug development when combined with other targeted therapies (tyrosine kinase inhibitors, anti-growth factors antibodies, or CAR-T therapy) for anticancer treatment, but it needs to be extensively assessed in the pre-clinical phase, possibly considering all of the plausible escape mechanisms by which tumor cells can develop.

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Meng Li and Hannei Xu were responsible for the conception and design of the review. Meng Li, Sara Settemrahmane and Mengwei Li analyzed the reports, summarized the results, and wrote the manuscript. Ying Wang and Xuezhen Wu revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest
The authors declare no conflicts of interest.

References
1. Moreno-Layseca P, Icha J, Hamidi H, Ivaska J. Integrin trafficking in cells and tissues. Nat Cell Biol 2019;21:122–32.
2. Humphries JD, Byron A, Humphries MJ. Integrin ligands at a glance. J Cell Sci 2006;119:3901–3.
3. Guo W, Giancotti FG. Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 2004;5:816–26.

4. Han S, Khuri FR, Roman J. Fibronectin stimulates non-small cell lung carcinoma cell growth through activation of Akt/mammalian target of rapamycin/S6 kinase and inactivation of LKB1/AMP-activated protein kinase signal pathways. Cancer Res 2006;66:315–23.

5. Vellon L, Menendez JA, Lupu R. AlphaVbeta3 integrin regulates heregulin (HRG)-induced cell proliferation and survival in breast cancer. Oncogene 2005;24:3759–73.

6. Hynes RO. Integrins: bidirectional, allosteric signalling machines. Cell 2002;110:673–87.

7. Hynes RO, Zhao Q. The evolution of cell adhesion. J Cell Biol 2000;150:89–96.

8. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol 2009;10:21–33.

9. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 2010;10:9–22.

10. Longmate W, DiPersio CM. Beyond adhesion: emerging roles for integrins in control of the tumor microenvironment. F1000Res 2017;6:1612.

11. Cooper J, Giancotti FG. Integrin signaling in cancer: mechano-transduction, stemness, epithelial plasticity, and therapeutic resistance. Cancer Cell 2019;35:347–67.

12. Schwartz MA, Ginsberg MH. Networks and crosstalk: integrin signalling spreading. Nat Cell Biol 2002;4:E65.

13. Askari JA, Buckley PA, Mould AP, Humphries MJ. Linking integrin conformation to function. J Cell Sci 2009;122:165–70.

14. Legate KR, Wickström SA, Fässler R. Genetic and cell biological analysis of integrin outside-in signalling. Genes Dev 2009;23:397–418.

15. Hamidi H, Piettiá M, Ivaska J. The complexity of integrins in cancer and new scopes for therapeutic targeting. Br J Cancer 2016;115:1017–23.

16. Hamidi H, Ivaska J. Every step of the way: integrins in cancer progression and metastasis. Nat Rev Cancer 2018;18:533–48.

17. Yue J, Zhang K, Chen J. Role of integrins in regulating proteases to mediate extracellular matrix remodeling. Cancer Microenviron 2012;5:275–83.

18. Playford MP, Schaller MD. The interplay between Src and integrins in normal and tumor biology. Oncogene 2004;23:7928–46.

19. Brunton VG, Frame MC. Src and focal adhesion kinase as therapeutic targets in cancer. Curr Opin Pharmacol 2008;8:427–32.

20. Caswell PT, Chan M, Lindsay AJ, McCaffrey MW, Boettiger D, Norman JC. Rab-coupling protein coordinates recycling of integrin and EGFR1 to promote cell migration in 3D microenvironment. J Cell Biol 2008;183:143–55.

21. Tuguzbaeva G, Yue E, Chen X, He L, Li X, Ju J, et al. PEP06 polypeptide 30 is a novel cluster-dissociating agent inhibiting av integrin/FAK/Src signaling in oral squamous cell carcinoma cells. Acta Pharm Sin B 2019;9:1163–73.

22. Fournier AK, Campbell LE, Castagnino P, Liu WF, Chung BM, Weaver VM, et al. Rac-dependent cyclin D1 gene expression regulated by cadherin- and integrin-mediated adhesion. J Cell Sci 2008;121:226–33.

23. Carrano AC, Pagano M. Role of the F-box protein Skp2 in adhesion-dependent cell cycle progression. J Cell Biol 2001;153:1381–90.

24. Matter ML, Ruoslahti E. A signaling pathway from the α5β1 and αvβ3 integrins that elevates bcl-2 transcription. J Biol Chem 2001;276:27757–63.

25. Uh ml JH, Dooley NP, Kyritsis AP, Rao JS, Gladson CL. Vimenticon, a glioma-derived extracellular matrix protein, protects tumor cells from apoptotic death. Clin Cancer Res 1999;5:1587–94.

26. Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli CM. NF-κB mediates αvβ3 integrin-induced endothelial cell survival. J Cell Biol 1998;141:1083–93.

27. Courtier DL, Lomas L, Scatena M, Giachelli CM. Src kinase activity is required for integrin αvβ3-mediated activation of nuclear factor-κB. J Biol Chem 2005;280:12145–51.

28. Bao W, Strömblad S. Integrin αvβ3-mediated inactivation of p53 controls a MEK1-dependent melanoma cell survival pathway in threedimensional collagen. J Cell Biol 2004;167:745–56.

29. Stupack DG, Puente XS, Boutsaboulay S, Storgard CM, Cheresh DA. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. J Cell Biol 2001;155:459–70.

30. Zhao H, Ross FP, Teitelbaum SL. Unoccupied αvβ3 integrin regulates osteoclast apoptosis by transmitting a positive death signal. Mol Endocrinol 2005;19:771–80.

31. Vlahakis A, Debnath J. The interconnections between autophagy and integrin-mediated cell adhesion. J Mol Biol 2016;429:515–30.

32. Frisch SM, Scroaten RA. Anoikis mechanisms. Curr Opin Cell Biol 2001;13:555–62.

33. Stupack DG, Teitz T, Potter MD, Mikolson D, Houghton PL, Kidd V, et al. Potentiation of neuroblastoma metastasis by loss of caspase-8. Nature 2006;439:95–9.

34. Cooper CR, Chay CH, Pienta KJ. The role of αvβ3 in prostate cancer progression. Neoplasia 2002;4:191–4.

35. Zhang Z, Vuori K, Reed JC, Ruoslahti E. The α5β1 integrin supports survival of cells on fibronectin and up-regulates Bcl-2 expression. Proc Natl Acad Sci U S A 1995;92:6161–5.

36. Schaffner F, Ray AM, Doniwell M. Integrin α5β1, the fibronectin receptor, as a pertinent therapeutic target in solid tumors. Cancers 2013;5:27–47.

37. O’Brien V, Frisch SM, Juliano RL. Expression of the integrin α5 subunit in HT29 colon cancer cells suppresses apoptosis triggered by serum deprivation. Exp Cell Res 1996;224:208–13.

38. Toquet C, Colson A, Jarry A, Bezieau S, Volteau C, Boissoe P, et al. ADAM15 to α5β1 integrin switch in colon cancer cells: a late event in cancer progression associated with tumor dedifferentiation and poor prognosis. Int J Cancer 2011;130:278–87.

39. Koike T, Kimura N, Miyazaki K, Yabuta T, Kumatoko K, Takehoitita S, et al. Hypoxia induces adhesion molecules on cancer cells: a missing link between Warburg effect and induction of selectin-ligand carbohydrates. Proc Natl Acad Sci U S A 2004;101:8132–7.

40. Shibata K, Kikkawa F, Nawa A, Suganuma N, Hamaguchi M. Fibronectin secretion from human peritoneal tissue induces Mr 92,000 type IV collagenase expression and invasion in ovarian cancer cells. Cancer Res 1997;57:5416–20.

41. Yokoyama Y, Ramakrishnan S. Binding of endostatin to human ovarian cancer cells inhibits cell attachment. Int J Cancer 2007;121:2402–9.

42. Yokoyama Y, Sedgewick G, Ramakrishnan S. Endostatin binding to ovarian cancer cells inhibits peritoneal attachment and dissemination. Cancer Res 2007;67:10813–22.

43. Ignatowski KM, Maehama T, Markwart SM, Dixon JE, Livant DL, Ethier SP. ERBB-2 overexpression confers PI 3 kinase-dependent invasion capacity on human mammary epithelial cells. Br J Cancer 2000;82:666–74.

44. Spangenberg C, Lausch EU, Trost TM, Prawitt D, May A, Keppler R, et al. ERBB2-mediated transcriptional up-regulation of the α5β1 integrin fibronectin receptor promotes tumor cell survival under adverse conditions. Cancer Res 2006;66:3715–25.

45. Ju JA, Gedot I, Ye IC, Ryu J, Jayati11a K, Lee SJ, et al. Hypoxia selectively enhances integrin α5β1 receptor expression in breast cancer to promote metastasis. Mol Cancer Res 2017;15:723–34.

46. Dingemans AM, van den Boogaart V, van Suylen RJ, Griffioen AW, Thijssen VL. Integrin expression profiling identifies integrin α5 and β1 as prognostic factors in early stage non-small cell lung cancer. Mol Cancer 2010;9:152.

47. Arpaia E, Blaser H, Quintela-Fandino M, Duncan G, Leong HS, Ablack A, et al. The interaction between caveolin-1 and Rho-
Integrins for cancer therapeutics

48. Dudovský Staněković N, Bicker F, Keller S, Jones DT, Harter PN, Kienzl A, et al. EGFl7 enhances surface expression of integrin α9β1 to promote angiogenesis in malignant brain tumors. *EMBO Mol Med* 2018;10:e8420.

49. Janousková H, Maglott A, Leger DY, Bossert C, Noulet F, Guerin E, et al. Integrin αvβ3 plays a critical role in resistance to temozolomide by interfering with the p53 pathway in high-grade glioma. *Cancer Res* 2012;72:3463–70.

50. Liu Z, Wang F, Chen X. Integrin αvβ3-targeted cancer therapy. *Drug Dev Res* 2008;69:329–39.

51. Pecheur I, Peyruchaud O, Serre CM, Guglielmi J, Voland C, Bourre F, et al. Integrin αvβ3 expression confers on tumor cells a greater propensity to metastasize to bone. *FASEB J* 2002;16:1266–8.

52. Hood JD, Cheshir DA. Role of integrins in cell invasion and migration. *Nat Rev Cancer* 2002;2:91–100.

53. Song Y, Guo XF, Fu JJ, He B, Wang XQ, Dai WB, et al. Dual-targeting nanovesicles enhance specificity to dynamic tumor cells in vitro and in vivo via manipulation of αvβ3-ligand binding. *Acta Pharm Sin B* 2020;10:1823–97.

54. Van der Flier A, Sonnenberg A. Function and interactions of integrins. *Cell Tissue Res* 2001;305:285–98.

55. Kuonen F, Surbeck I, Sarin KY, Dontenwill M, Ruegg C, Gilliet M, et al. EGFL7 enhances surface expression of integrin αβ3 in breast cancer. *Cell Mol Med* 2004;8:225–34.

56. Vasudev NS, Reynolds AR. Anti-angiogenic therapy for cancer: current status, unresolved questions and future directions. *Angiogenesis* 2014;17:471–94.

57. Lugano R, Venmi K, Yu D, Bergqvist M, Smits A, Eissand M, et al. CD93 promotes β1 integrin activation and fibronectin fibrillogenesis during tumor angiogenesis. *J Clin Investig* 2018;128:3280–97.

58. Hill EE, Kim JK, Jung Y, Neeley CK, Pienta KJ, Taichman RS, et al. Integrin αvβ3 targeting by a conjugated therapeutic reduces fibroblast-mediated prostate tumor progression and metastasis. *J Cell Biochem* 2018;119:8074–83.

59. Wu YP, Wang Q, Liu YC, Xie Y. Molecular basis for the targeted binding of RGD-containing peptide to integrin αvβ3. *Biomaterials* 2014;35:1667–75.

60. Somanath PR, Malinin NL, Byszova TV. Cooperation between integrin αvβ3 and VEGFR2 in angiogenesis. *Angiogenesis* 2009;12:177–85.

61. Shlamovitch T, Aharon L, Kosalwsky D, Einav Y, Popo N. Targeting the Tie2-αvβ3 integrin axis with bi-specific reagents for the inhibition of angiogenesis. *BMC Biol* 2018;16:92.

62. Moore KM, Thomas GJ, Duffy SW, Warwick J, Gabe R, Chou P, et al. Therapeutic targeting of integrin αvβ6 in breast cancer. *J Natl Cancer Inst* 2014;106:du169.

63. Clarke MF. Clinical and therapeutic implications of cancer stem cells. *Nat Engl J Med* 2019;380:2237–45.

64. Medema JP. Cancer stem cells: the challenges ahead. *Nat Cell Biol* 2013;15:338–44.

65. Martin TA, Jiang WG. Evaluation of the expression of stem cell markers in human breast cancer reveals a correlation with clinical progression and metastatic disease in ductal carcinoma. *Oncol Rep* 2014;31:262–72.

66. Zheng Y, de la Cruz CC, Sayles LC, Alleyne-Chin C, Vaka D, Knaak TD, et al. A rare population of CD24+ITGB4+Notch6 cells drives tumor propagation in NSCLC and requires Notch3 for self-renewal. *Cancer Cell* 2013;24:59–74.

67. Lahlo H, Sanguin-Gendreau V, Zuo D, Cardiff RD, McLean GW, Frame MC, et al. Mammary epithelial-self-disruption of the focal adhesion kinase blocks mammary tumor progression. *Proc Natl Acad Sci U S A* 2007;104:20302–7.

68. White DE, Kurpios NA, Zuo D, Hassell JA, Blaess S, Mueller U, et al. Targeted disruption of β3-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. *Cancer Cell* 2004;6:159–70.

69. Barnawi R, Al-Khalili S, Majed Sleiman G, Sarkar A, Al-Dhifyan A, Al-Mohanna F, et al. Fascin is critical for the maintenance of breast cancer stem cell pool predominantly via the activation of the notch self-renewal pathway. *Stem Cell* 2016;34:2799–813.

70. Jeong BY, Cho KH, Jeong KJ, Park YY, Kim JM, Rha SY, et al. Rab3B augments cancer cell invasiveness through a β3 integrin/EGFR/VEGFR-A/Snail signaling axis and expression of fascin. *Exp Mol Med* 2018;50:e435.

71. Guo W, Pilyayeva Y, Pepe A, Yoshioka T, Muller WJ, Ingham G, et al. β4 integrin amplifies ErbB2 signaling to promote mammary tumorigenesis. *Cell* 2006;126:489–502.

72. Urinci-Siegel J, Schade B, Cardiff RD, Muller WJ. Insights from transgenic mouse models of ERBB2-induced breast cancer. *Nat Rev Cancer* 2007;7:389–97.

73. Carroll DK, Carroll JS, Leong CO, Cheng F, Brown M, Mills AA, et al. p63 regulates an adhesion programme and cell survival in epithelial cells. *Nat Cell Biol* 2006;8:551–61.

74. Weaver VM, Petersen OW, Cheng F, Brown M, Mills AA, et al. p63 regulates an adhesion programme and cell survival in epithelial cells. *Nat Cell Biol* 2006;8:551–61.

75. De Witt NC, van den Boogaard LMA, Kruitwagen RP, de la Cruz I, Nauta MJ, et al. Targeted disruption of β3-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. *Cancer Cell* 2004;6:159–70.

76. Schade B, Cardiff RD, Muller WJ. Insights from transgenic mouse models of ERBB2-induced breast cancer. *Nat Rev Cancer* 2007;7:389–97.

77. Carroll DK, Carroll JS, Leong CO, Cheng F, Brown M, Mills AA, et al. p63 regulates an adhesion programme and cell survival in epithelial cells. *Nat Cell Biol* 2006;8:551–61.

78. Weaver VM, Petersen OW, Cheng F, Brown M, Mills AA, et al. p63 regulates an adhesion programme and cell survival in epithelial cells. *Nat Cell Biol* 2006;8:551–61.
104. De S, Razorenova O, McCabe NP, O’Toole T, Qin J, Byzova TV. VEGF-integrin interplay controls tumor growth and vascularization. *Proc Natl Acad Sci U S A* 2005;102:7589–94.

105. Borrioukan W, Lafleur MA, Mercuri FA, Blick T, Price JT, Fridman R, et al. The type I collagen induction of MT1-MMP-mediated MMP-2 activation is repressed by avb3 integrin in human breast cancer cells. *Matrix Biol* 2007;26:291–305.

106. Desch A, Strozyk EA, Bauer AT, Hück V, Niemeyer V, Wieland T, et al. Highly invasive melanoma cells activate the vascular endothelium via an MMP-2/integrin avb5-induced secretion of VEGF-A. *Am J Pathol* 2012;181:693–705.

107. Chechaj A, Ivaska J, Roca-Cusachs P. Integrins as biomechanical sensors of the microenvironment. *Nat Rev Mol Cell Biol* 2019;20:457–73.

108. Arthur WT, Petch LA, Burridge K. Integrin engagement suppresses RhoA activity via a c-Src-dependent mechanism. *Curr Biol* 2000;10:719–22.

109. Menke A, Gehlk K. Regulation of adherens junctions by Rho GTPases and p120-catenin. *Arch Biochem Biophys* 2012;524:48–55.

110. Canel M, Serrels A, Frame MC, Brunton VG. E-cadherin–integrin cross-talk in cancer invasion and metastasis. *J Cell Sci* 2013;126:393–401.

111. Cicchini C, Laudadio I, Corazzari M, Steindler C, Conigliaro A, et al. TGFb3-induced EMT requires focal adhesion kinase (FAK) signaling. *Exp Cell Res* 2008;344:143–52.

112. Coluccia AM, Benati D, Delkhi H, De Filippo A, Lan C, Gamba-corti-Passerini C. SKI-606 decreases growth and motility of colorectal cancer cells by preventing pp60 (c-Src)-dependent tyrosine phosphorylation of b-catenin and its nuclear signaling. *Cancer Res* 2006;66:2279–86.

113. Canel M, Serrels A, Miller D, Timpson P, Serrells B, Frame MC, et al. Quantitative in vivo imaging of the effects of inhibiting integrin signalling via Src and FAK on cancer cell movement; effects on E-cadherin dynamics. *Cancer Res* 2010;70:9413–22.

114. Cheresh DA. Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. *Proc Natl Acad Sci U S A* 1987;84:6471–5.

115. Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. Definition of two angiogenic pathways by distinct avb integrins. *Science* 1995;270:1500–2.

116. Wang Z, Zheng Y, Fang Z. The clinical efficacy and safety of BSR20180243. *Cancers* 2020;12:343.

117. Menke A, Shattil SJ, et al. Cross-activating c-Met/VEGF-integrin receptor tyrosine kinases and TLR/IL1Rs unex-

118. Uchihara T, Miyake K, Yonemura A, Komohara Y, Itoyama R, Ellies LG, et al. Receptor tyrosine kinases promote tumor inflammation and progression. *Cancer Cell* 2011;19:715–27.

119. Peng C, Zou X, Xia W, Gao H, Li Z, Liu N, et al. Integrin avbβ6 plays a bi-directional regulation role between colon cancer cells and cancer-associated fibroblasts containing annexin A6 induces FAK-YAP activation by stabilizing avb3 integrin, enhancing drug resistance. *Cancer Res* 2020;80:3222–35.

120. Wang SE, Xiang B, Zent R, Quaranta V, Pozzi A, Arteaga CL. A novel mechanism for integrin-mediated Ras activation in breast carcinoma cells: the phorylation of specific EGF receptor tyrosines. *Cancer Res* 2002;62:344–52.

121. Jahangiri A, Nguyen A, Chandra A, Sidorov MK, Yagnik G, Rick J, et al. Cross-activating c-Met/integrin complex drives metastasis and invasive resistance in cancer. *Proc Natl Acad Sci U S A* 2017;114:E8685–94.

122. Allen MD, Thomas GJ, Clark S, Dawoud MM, Vallath S, Payne SJ, et al. Altered microenvironment promotes progression of preinvasive breast cancer: myoepithelial expression of avb6 integrin in DCIS identifies high-risk patients and predicts recurrence. *Clin Cancer Res* 2014;20:344–57.

123. Yoon SO, Shin S, Lipscomb EA. A novel mechanism for integrin-mediated Ras activation in breast carcinoma cells: the avb5 integrin regulates ErbB2 translation and transactivates epidermal growth factor receptor/ErbB2 signaling. *Cancer Res* 2006;66:2732–9.

124. Baart VM, van Duijn C, van Egmond SL, Dijkstra RA, Jansen JC, Vahrmeijer AL, et al. EGFR and avb6 as promising targets for molecular imaging of cutaneous and mucosal squamous cell carcinoma of the head and neck region. *Cancers* 2020;12:1474.

125. Schlaepfer DD, et al. Specific cross-talk between epidermal growth factor receptor and integrin avb5 promotes carcinoma cell invasion and metastasis. *Cancer Res* 2009;69:1383–91.

126. Moro L, Dolce L, Cabodi S, Bergatto E, Boeri Erba E, Smeriglio M, et al. Integrin-induced epidermal growth factor (EGF) receptor activation requires c-Src and p130Cas and leads to phosphorylation of specific EGF receptor tyrosines. *J Biol Chem* 2002;277:9405–14.

127. Wu H, Beuerlein G, Nie Y, Smith H, Lee BA, Hensler M, et al. Stepwise in vitro affinity maturation of Vitaxin, an avb3-specific humanized mAb. *Proc Natl Acad Sci U S A* 1998;95:6037–42.

128. Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Pardoll DM, et al. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. *Proc Natl Acad Sci U S A* 2012;109:2784–9.

129. Becherirat S, Valamanesh F, Karimi M, Faussat AM, Launay JM, Pimpie C, et al. Discontinuous schedule of bevacizumab in colorectal cancer induces accelerated tumor growth and phenotypic changes. *Transl Oncol* 2018;11:406–15.

130. Mullamitha SA, Ton NC, Parker NJ, Jackson A, Julyan PJ, Roberts C, et al. Phase I evaluation of a fully human anti-av integrin monoclonal antibody (CNTO 95) in patients with advanced solid tumors. *Clin Cancer Res* 2007;13:2128–35.

131. Tam SH, Sassoli PM, Jordan RE, Nakada MT. Abciximab (ReoPro, cimaPlex 7E3 Fab) demonstrates equivalent affinity and functional blockade of glycoprotein IIb/IIIa and avb3 integrins. *Circulation* 1998;98:1085–91.

132. Cohen SA, Trikha M, Maselli MA. Potential future clinical applications for the GPIIb/IIIa antagonist, abciximab in thrombosis, vascular and oncological indications. *Pathol Oncol Res* 2000;6:163–74.
Integrins for cancer therapeutics

124. Trikha M, Zhou Z, Nemeth JA, Chen Q, Sharp C, Emmell E, et al. CNTO 95, a fully human monoclonal antibody that inhibits αv integrins, has antitumor and antiangiogenic activity in vivo. Int J Cancer 2004;110:326−35.

125. Vamer JA, Nakada MT, Jordan RE, Coller BS. Inhibition of angiogenesis and tumor growth by murine 7E3, the parent antibody of c7E3 Fab (abciximab; ReoPro). Angiogenesis 1999;3:53−60.

126. Alghisi GC, Rüegg C. Vascular integrins in tumor angiogenesis: mediators and therapeutic targets. Endothelium 2006;13:113−35.

127. Trikha M, Zhou Z, Timar J, Raso E, Kennel M, Emmell E, et al. Multiple roles for platelet GPIIb/IIIa and αvβ3 integrins in tumor growth, angiogenesis, and metastasis. Cancer Res 2002;62:2824−33.

128. Kononczuk J, Surazynski A, Czyzewska U, Prokop I, Tomczyk M, Palka J, et al. αIIbβ3−integrin ligands: abciximab and epifibatide as proangiogenic factors in MCF-7 human breast cancer cells. Curr Drug Targets 2015;16:1429−37.

129. Guthrie JC, Campbell TN, Pierce PR, Watkins JD, Huse WD, et al. The first anti-integrin αv integrin antagonist, in patients with solid tumours. Br J Cancer 2006;94:1621−6.

130. Posey JA, Khazaelli MB, DelGrosso A, Saleh MN, Lin CY, Huse W, et al. A pilot trial of vitaxin, a humanized anti-vitronectin receptor (anti αvβ3) antibody in patients with metastatic cancer. Cancer Biother Radiopharm 2001;16:125−32.

131. Carter P, Smith L, Ryan M. Identification and validation of cell surface antigens for antibody targeting in oncology. Endocr Relat Cancer 2004;11:859−87.

132. Wallstabe L, Mades A, Frenz S, Einsele H, Rader C, Hudecek M. CAR T cells targeting αβ, integrin effect is effective against advanced cancer in preclinical models. Adv Cell Gene Ther 2018;1:e11.

133. Almkadem S, Belani CP. Volociximab in cancer. Expet Opin Biol Ther 2012;12:251−7.

134. Besse B, Tsao LC, Chao DT, Fang Y, Soria JC, Almkadem S, et al. Phase Ib safety and pharmacokinetic study of volociximab, an anti-α5β1 integrin antibody, in combination with carboplatin and paclitaxel in advanced non-small-cell lung cancer. Ann Oncol 2010;21:90−6.

135. Mas-Moruno C, Rechenmacher F, Cilengitide Kessler H. The first anti-angiogenic small molecule drug candidate design, synthesis and clinical evaluation. Anticancer Agents Med Chem 2010;10:753−68.

136. Nisato RE, Tille JC, Jonczyk A, Goodman SL, Pepper MS. αvβ3 and αvβ5 integrin antagonists inhibit angiogenesis in vitro. Angiogenesis 2003;6:105−19.

137. Burke PA, DeNardo SJ, Miers LA, Lamborn KR, Matzku S, DeNardo GL. Cilengitide targeting of αvβ3 integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts. Cancer Res 2002;62:4263.

138. Raguse JD, Gath HJ, Bier J, Riess H, Oettle H. Cilengitide (EMD 137. Burke PA, DeNardo SJ, Miers LA, Lamborn KR, Matzku S, DeNardo GL. Cilengitide targeting of αvβ3 integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts. Cancer Res 2002;62:4263.

139. Smith JW. Cilengitide merck. Curr Opin Invest Drugs 2003;4:741−5.

140. Eisele G, Wick A, Eisele AC, Clément PM, Tomn J, Tabatabai G, et al. Cilengitide treatment of newly diagnosed glioblastoma patients does not alter patterns of progression. J Neuro Oncol 2014;117:141−5.

141. Kocher AS, Madhavan M, Manjila S, Scoco A, Belle VK, Geertman RT. Contemporary updates on clinical trials of antiangiogenic agents in the treatment of glioblastoma multiforme. Asian J Neurosurg 2018;13:546−54.

142. Doñate F, Parry GC, Shaked Y, Hensley H, Guan X, Beck I, et al. Pharmacology of the novel antiangiogenic peptide ATN-161 (Ac-

PHSCN-NH2): observation of a U-shaped dose-response curve in several preclinical models of angiogenesis and tumor growth. Clin Cancer Res 2008;14:2137−44.

143. Sökeland G, Schumacher U. The functional role of integrins during intra- and extravasation within the metastatic cascade. Mol Cancer 2019;18:12.

144. Stoeltzing O, Liu W, Reinmuth N, Fan F, Parry GC, Parikh AA, et al. Inhibition of integrin α5β1 function with a small peptide (ATN-161) plus continuous 5-FU infusion reduces colorectal liver metastases and improves survival in mice. Int J Cancer 2010;104:496−503.

145. Khalili P, Arakelian A, Chen G, Plunkett ML, Beck I, Parry GC, et al. A non-RGD-based integrin binding peptide (ATN-161) blocks breast cancer growth and metastasis in vivo. Mol Cancer Therapeutics 2006;5:2271−80.

146. Cianfrocca ME, Kimmel KA, Gallo J, Cardoso T, Brown MM, Hudes G, et al. Phase 1 trial of the antiangiogenic peptide ATN-161 (Ac-PhSCN-NH2), a δ integrin antagonist, in patients with solid tumours. Br J Cancer 2006;94:1621−6.

147. Xu H, Pan L, Ren Y, Yang Y, Huang X, Liu Z. RGD-modified angiogenesis inhibitor HM-3 dose: dual function during cancer treatment. Bioconjug Chem 2011;22:1386−93.

148. Zhou K, Zheng X, Xu HM, Zhang J, Chen Y, Xi T, et al. Studies of poly(ethylene glycol) modification of HM-3 polypeptides. Bioconjug Chem 2009;20:932−6.

149. Setterrarramane S, Yu J, Hao J, Zheng H, Xu H. Novel production method of innovative antiangiogenic and antitumor small peptides in Escherichia coli. Drug Des Dev Ther 2017;11:3207−20.

150. Yassin S, Hu J, Xu H, Li C, Setterrarramane S. In vitro and in vivo activities of an antitumor peptide HM-3: a special dose-efficacy relationship on an HCT116 xenograft model in nude mice. Oncol Rep 2016;36:2951−9.

151. Liu Z, Ren Y, Pan L, Xu HM. In vivo anti-tumor activity of polypeptide HM-3 modified by different polyethylene glycols (PEG). Int J Mol Sci 2011;12:2650−63.

152. Hu L, Wang J, Wang Y, Xu H. An integrin αvβ3 antagonistic modified peptide inhibits tumor growth through inhibition of the ERK and AKT signaling pathways. Oncol Rep 2016;36:1953−62.

153. Zhao C, He J, Cheng H, Zhi Z, Xu H. Enhanced therapeutic effect of an antiangiogenesis peptide on lung cancer in vivo combined with salmonine VNP2009 carrying a Sox2 shRNA construct. J Exp Clin Cancer Res 2016;35:107.

154. Hu J, Cheng T, Zheng L, Sun B, Deng L, Xu H. Anti-tumor peptide AP25 decreases cyclin D1 expression and inhibits MGC-803 proliferation via phospho-extracellular signal-regulated kinase-, Src-, c-Jun N-terminal kinase- and phosphoinositide 3-kinase-associated pathways. Mol Med Rep 2015;12:4396−402.

155. Yin R, Zheng H, Xi T, Xu HM. Effect of RGD-4C position is more important than disulfide bonds on antiangiogenic activity of RGD-4C modified endostatin derived synthetic polypeptide. Bioconjug Chem 2010;21:1142−7.

156. Li M, Xu H, Wang J. Optimized functional and structural design of dual-target LMRAP, a bifunctional fusion protein with a 25-amino-acid antitumor peptide and GnrRH Fc fragment. Acta Pharm Sin B 2020;10:262−75.

157. Whilding LM, Halim L, Draper B, Parente-Pereira AC, Zabinski T, Davies DM, et al. CAR T-cells targeting the integrin αvβ6 and co-expressing the chemokine receptor CXCR2 demonstrate enhanced homing and efficacy against several solid malignancies. Cancers 2019;11:674.

158. Zhang E, Gu J, Xue J, Lin C, Liu C, Li M, et al. Accurate control of dual-receptor-engineered T cell activity through a bifunctional antiangiogenic peptide. J Hematol Oncol 2018;11:44.