Integrins in cancer: biological implications and therapeutic opportunities

Jay S. Desgrosellier and David A. Cheresh

Abstract | The integrin family of cell adhesion receptors regulates a diverse array of cellular functions crucial to the initiation, progression and metastasis of solid tumours. The importance of integrins in several cell types that affect tumour progression has made them an appealing target for cancer therapy. Integrin antagonists, including the αvβ3 and αvβ5 inhibitor cilengitide, have shown encouraging activity in Phase II clinical trials and cilengitide is currently being tested in a Phase III trial in patients with glioblastoma. These exciting clinical developments emphasize the need to identify how integrin antagonists influence the tumour and its microenvironment.

Desmoplasia
The growth of fibrous or connective tissue.

Cilengitide
An RGD-containing cyclic pentapeptide that inhibits ligand binding to αv integrins.

Glioblastoma
The most common human brain tumour, it originates from glial cells and has no known cure.

Department of Pathology, Moores University of California at San Diego Cancer Center, 3855 Health Sciences Drive, Room 2344, La Jolla, CA 92093-0803, United States. Correspondence to D.A.C.
e-mail:
dcheresh@ucsd.edu
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Much of the classic literature regarding cancer and integrins has implicated this family of adhesion receptors in tumour cell proliferation, migration and survival (BOX 1). The role of integrins in cell migration and invasion is one of their most studied functions in tumour biology and has recently been reviewed elsewhere[1,2]. Integrins directly bind components of the extracellular matrix (ECM) and provide the traction necessary for cell motility and invasion. ECM remodelling is also controlled by integrins, which regulate the localization and activity of proteases. In addition to their well-established role in migration and invasion, integrins can regulate proliferation[3]. Although adhesion-dependent control of proliferation is deregulated in tumour cells, integrins continue to regulate cell growth in some tumours[4,5]. Recent studies have shed new light on the crucial, and often contradictory, role integrins have in regulating tumour cell survival. In addition to their ligation-dependent effects, it is now becoming clear that in some cases unligated integrins can positively or negatively influence tumour cell survival, thereby affecting tumour growth and metastasis. How integrins affect tumour cell survival both in the ligated and unligated states could be a crucial determinant of the efficacy of integrin antagonists in cancer.

In addition to their role in tumour cells, integrins on the surface of tumour-associated host cells can profoundly influence the malignant potential of a tumour. The tumour microenvironment is comprised of many host cell types, including endothelial cells, perivascular cells, fibroblasts and inflammatory cells, which contribute to tumour progression by mediating angiogenesis, lymphangiogenesis, desmoplasia and inflammation. The involvement of integrins in angiogenesis is well described, and recent studies have demonstrated that they also influence many other host cell responses to cancer. Therefore, integrin antagonists targeting the tumour microenvironment might significantly curtail tumour progression.

Integrins span the lipid bilayer of cells and promote intracellular signalling, typically in the context of activated cytokine receptors or growth factor receptors. Consequently, tumour growth and invasion probably depend on integrin crosstalk with growth factor receptors or oncogenes in both tumour cells and tumour-associated cells. Recent studies have demonstrated that some growth factors and oncogenes require specific integrins for tumour initiation and progression. These studies highlight the importance of understanding crosstalk mechanisms, as they could influence the tumour response to inhibitors of growth factor or integrin signalling.

In recent years, great progress has been made towards targeting integrins in cancer. Preclinical as well as clinical studies with various integrin antagonists have demonstrated their effectiveness in blocking tumour progression. Phase II clinical trials with cilengitide (developed by Merck KGaA), an αvβ3 and αvβ5 integrin antagonist, have shown clinical activity and few side effects in patients with glioblastoma. These positive clinical findings have led to the initiation of the first Phase III clinical trial with an integrin antagonist. The advance of integrin

At a glance

- Integrin signalling regulates diverse functions in tumour cells, including migration, invasion, proliferation and survival. In several tumour types, the expression of particular integrins correlates with increased disease progression and decreased patient survival.
- In addition to tumour cells, integrins are also found on tumour-associated host cells, such as the vascular endothelium, perivascular cells, fibroblasts, bone marrow-derived cells and platelets. Integrin signalling crucially regulates the contribution of these cell types to cancer progression. Therefore, integrin antagonists may inhibit tumour progression by blocking crucial signalling events in both the tumour microenvironment and the tumour cells themselves.
- Integrins have a profound influence on tumour cells, both in the ligated and unligated states, in which they regulate tumour cell survival and malignancy.
- Although integrins alone are not oncogenic, recent data have found that some oncogenes may require integrin signalling for their ability to initiate tumour growth and invasion. These effects may be due to the important contribution of integrin signalling in maintaining the cancer stem cell population in a given tumour.
- Crosstalk between integrins and growth factor or cytokine receptors on both tumour and host cell types is vital for many aspects of tumour progression. Mechanisms of crosstalk include both direct and indirect association of integrins with growth factor or cytokine receptors, which affects the expression, ligand affinity and signalling of the receptors.
- The important contribution of integrins to the biology of both tumour cells and tumour-associated cell types has made them appealing targets for the design of specific therapeutics. Of particular interest, the integrin αv inhibitor cilengitide is now in a Phase III clinical trial in glioblastoma, and because this is the first integrin antagonist to achieve this milestone it places anti-integrin therapy on the doorstep of clinical availability.
- In addition to their use as therapeutic targets, integrins can be imaging biomarkers for assessing the efficacy of anti-angiogenic and anti-tumour agents. Integrin-targeted nanoparticles with a diverse array of anti-tumour payloads also represent a particularly promising area of research that may decrease the toxicities associated with systemic delivery of radiation or chemotherapy.

antagonists into the clinic highlights the importance of continued research to determine the role integrins have in tumour progression and to identify the factors that influence the effectiveness of these inhibitors.

Integrin biology

The integrin family of cell adhesion receptors. Integrins are heterodimeric cell surface receptors that mediate adhesion to the ECM and immunoglobulin superfamily molecules. At least 24 distinct integrin heterodimers are formed by the combination of 18 α-subunits and 8 β-subunits. Specific integrin heterodimers preferentially bind to distinct ECM proteins. The repertoire of integrins present on a given cell dictates the extent to which that cell will adhere to and migrate on different matrices. αv integrins and integrin α5β1 recognize the RGD sequence on their respective ligands. In fact, these integrins were first identified on the basis of their ability to recognize the RGD sequence[6]. Other adhesive sequences in ECM proteins have also been observed, including the EILDV and REDV sequences that are recognized by integrin α4β1 in an alternatively spliced form of fibronectin. On ligation to the ECM, integrins cluster in the plane of the membrane and recruit various signalling and adaptor proteins to form structures known as focal adhesions. The composition of these adhesions differs depending on whether the contacts occur in two-dimensional or three-dimensional conditions[7]. Although integrins lack kinase activity, by clustering they recruit and activate kinases, such as focal adhesion kinases (FAKs) and Src family kinases (SFKs), in addition to scaffolding molecules, such as p130 CRK-associated substrate (p130CAS; also known as BCAR1). Integrins also couple the ECM to the actin cytoskeleton by recruiting proteins, including talin, paxillin, α-actinin, tensin and vinculin. Additionally, a ternary complex consisting of an integrin-linked kinase, PINCH (also known as LIMS1), and parvin regulates many scaffolding and signalling functions required for integrin-mediated effects on cell migration and survival[8]. Furthermore, integrin recruitment to membrane microdomains by tetraspanins might crucially regulate integrin function in tumour cells[9]. Regulation of the recruitment and activation of these and other focal adhesion proteins influences cell adhesion and migration on the ECM. In fact, many of these molecules are themselves being investigated as possible targets for cancer therapy. In some cases, the function of an integrin is related to its ligand affinity. Increased affinity or activation can be induced by either ligand-mediated integrin clustering on the cell surface or increased intracellular signalling through molecules, such as the GTPase RAP1A[10]. Therefore, signalling that is induced by oncogenes or growth factor receptors may outwardly influence integrin affinity and function.

Integrin expression in cancer. A wide variety of integrins contribute to tumour progression. As many solid tumours originate from epithelial cells, the integrins expressed by epithelial cells (including α6β4, α6β1, αvβ5, α2β1 and α3β1) are generally retained in the tumour, though expression levels may be altered. These integrins typically mediate epithelial cell adhesion to the basement membrane, but might contribute to migration, proliferation and survival in tumour cells. However, integrin expression can also vary considerably between normal and tumour tissue. Most notably, integrins αvβ3, α5β1 and αvβ6, are usually expressed at low or undetectable levels in most adult epithelia but can be highly upregulated in some tumours. Expression levels of some integrins, such as α2β1, decrease in tumour cells, potentially increasing tumour cell dissemination[11]. In fact, re-expression of α2β1 in breast cancer cells reversed some of the malignant properties of those cells, suggesting that α2β1 could function as a tumour suppressor[12]. Studies correlating integrin expression levels in human tumours with pathological outcomes, such as patient survival and metastasis, have identified several integrins that might have an important role in cancer progression. Tumour cell expression of the integrins αvβ3, αvβ5, α5β1, α6β4, α4β1 and αvβ6 is correlated with disease progression in various tumour types (TABLE 1), therefore, these are the most studied integrins in cancer. However, this is by no means a complete list and other integrins certainly contribute to cancer progression, particularly on some of the host cell types in the primary tumour.

Focal adhesions
Dynamic, macromolecular protein complexes that link the ECM to the actin cytoskeleton through integrins.

Intrinsic apoptosis
Cell death initiated by cell stress or DNA damage and induced by mitochondrial release of cytochrome *c* and activation of pro-apoptotic caspases.

Extrinsic apoptosis
Cell death induced by ligand binding to transmembrane death receptors and the activation of caspases, including caspase 8.

Integrin-mediated death
(IMD). Apoptotic cell death resulting from the recruitment and activation of caspase 8 by unligated integrins on otherwise adherent cells.

Dasatinib
A dual ABL1 and SFK inhibitor manufactured by Bristol-Meyers Squibb and approved for treatment of patients with chronic myelogenous leukaemia.

Integrin regulation of cell survival and apoptosis

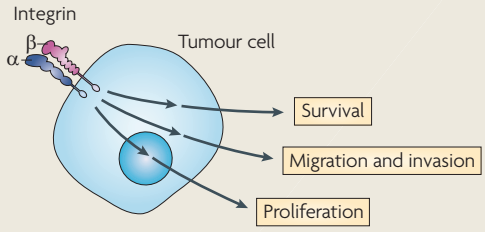
Depending on environmental cues, integrins have the ability to either enhance cell survival or initiate apoptosis (FIG. 1). Integrins constantly interrogate their environment through their capacity to interact with the ECM. Ligated integrins relay survival signals, and unligated integrins can promote pro-apoptotic cascades. The balance of these signals results in cell survival or apoptosis based on the ability of the cell to interact with the surrounding ECM. In this way, integrins maintain the integrity of different organs and tissues by preventing cells from surviving in an improper environment.

Integrin ligation enhances cell survival through several mechanisms, including increased expression of BCL-2 (REFS 13,14) or FLIP (also known as CFLAR)[15], activation of the PI3K–AKT pathway[16] or nuclear factor-κB (NF-κB) signalling[17,18], and/or p53 inactivation[19]. These cell survival pathways are differentially regulated by specific integrin–growth factor receptor pairs. For example, in endothelial cells integrin αvβ3 crosstalk with fibroblast growth factor receptor (FGFR) prevents apoptosis through the intrinsic apoptosis pathway, and αvβ5 and the vascular endothelial growth factor receptor 2 (VEGFR2) function together to inhibit extrinsic apoptosis[20,21]. Although integrin antagonists directed to αvβ3 and αvβ5 promoted endothelial cell death, which led to decreased angiogenesis, genetic deletion of *Itgb3* (which encodes integrin β3) or deletion of both *Itgb3* and *Itgb5* in mice did not inhibit angiogenesis. However, mice deficient in these integrins showed increased VEGF-mediated angiogenesis[22], reflecting a compensatory increase in VEGFR2 in these mice[23]. Interestingly, *Itgb3*$^{-/-}$ mice did show abnormal cardiac endothelial cell morphology associated with increased VEGF signalling[24]. These results highlight crucial differences between studies involving genetic deletion of an integrin during early development and studies in which integrin antagonists were used to suppress integrin function in adult animals. They also illustrate the important role compensation could have in the interpretation of such knockout studies in mice.

Recent studies have highlighted the role integrins have in modulating apoptosis. Early work hinted at a possible dual role for integrins in both promoting cell survival and inducing cell death. Studies have shown that although some integrins, such as αvβ3 and α6β4, enhance tumour progression[25], paradoxically, others such as α5β1 inhibit oncogene-induced transformation[26,27]. Further experiments showed that the pro-tumorigenic integrin αvβ3 could inhibit tumour progression in some mouse models of glioblastoma[28] and melanoma[29]. This apparent discrepancy might be explained by the discovery that unligated integrins can induce apoptosis[30,31]. In a process termed integrin-mediated death (IMD), unligated integrins on adherent cells recruit and activate caspase 8, resulting in apoptotic cell death[30]. IMD is distinct from anoikis, which is apoptosis that occurs in response to cellular detachment from the ECM[32]. Further studies demonstrated that the loss of caspase 8 is one mechanism by which tumour cells can avoid IMD, allowing increased metastatic dissemination[33]. It is still unclear what part IMD plays in the therapeutic effects of integrin antagonists. However, it is thought that by inhibiting adhesion to the ECM, integrin antagonists can induce IMD and therefore have a greater effect in IMD-sensitive tumours.

In recent work, we have shown that in IMD-resistant tumour cells the unligated integrin αvβ3 substantially increases anchorage-independent tumour cell survival *in vitro* and metastasis *in vivo*[34] (FIG. 2). These effects specifically required integrin αvβ3 recruitment and the activation of the non-receptor tyrosine kinase SRC, which leads to a FAK-independent survival pathway. This anchorage-independent integrin αvβ3–SRC signalling module might explain the association between integrin αvβ3 and tumour progression, as observed in various clinical studies[35–41], and could have important clinical ramifications. First, it suggests that αvβ3 antagonists that function by blocking ligand binding to tumour cells might be ineffective in treating some αvβ3-positive tumours. However, it remains possible that such antagonists could still function as anti-angiogenic agents. Second, our study shows that integrin αvβ3-expressing tumours that recruit and activate SRC in this manner may be particularly sensitive to SFK inhibitors such as dasatinib.

Box 1 | **Integrins in tumour cells**



Integrins expressed in tumour cells contribute to tumour progression and metastasis by increasing tumour cell migration, invasion, proliferation and survival (see the figure). Integrin adhesion to the extracellular matrix (ECM) provides the traction required for tumour cell invasion. Integrins also contribute to tumour cell invasion by regulating the localization and activity of matrix-degrading proteases, such as matrix metalloprotease 2 (MMP2) and urokinase-type plasminogen activator (uPA). Integrin-mediated migration generally requires focal adhesion kinase (FAK)–Src family kinase (SFK) signalling. However, integrin-specific mechanisms do exist in the signalling pathways that result in cell motility. For example, in neuroblastoma cells although the integrin α5β1 uses the expected FAK-mediated activation of SRC, integrin α4β1 activates SRC through a FAK-independent mechanism[168]. Some integrins inhibit tumour cell motility, as integrin β1 (*Itgb1*) deletion increased tumour cell dissemination in a mouse model of spontaneous pancreatic islet cancer[11]. Regulation of integrin recycling is also crucial for tumour cell invasion. Rab GTPases direct integrins to the leading edge of invading tumour cells[79] and coordinately regulate integrin and growth factor receptor recycling, resulting in enhanced growth factor signalling[79]. Differences in the recycling pathways mediated by particular integrins also influence random rather than persistent cell migration[169]. In addition to their well-established role in migration and invasion, integrins also regulate proliferation. Integrin ligation controls the expression of key cell cycle proteins, including cyclin D1 (REF. 170) and the cyclin-dependent kinase inhibitor family, which regulate entry into the S phase of the cell cycle[171]. Adhesion-dependent control of cell proliferation is deregulated in tumour cells, as anchorage-independent growth is a hallmark of malignant transformation. However, integrins also have an important role in tumour growth[4,5]. Further research is needed to determine the mechanisms by which integrins continue to regulate proliferation in tumour cells. Integrins also control tumour cell survival. Ligated integrins prevent pro-apoptotic signalling cascades initiated by anoikis or integrin-mediated death and increase survival signalling. However, recent evidence also points to a role for unligated integrins in regulating tumour cell survival and malignancy.

Table 1 | **Integrins in cancer progression**

Tumour type	Integrins expressed	Associated phenotypes
Melanoma	αvβ3 and α5β1	Vertical growth phase [35,172–174] and lymph node metastasis[173,175]
Breast	α6β4 and αvβ3	Increased tumour size and grade[176], and decreased survival[177] (α6β4). Increased bone metastasis[36–38,64] (αvβ3)
Prostate	αvβ3	Increased bone metastasis[39]
Pancreatic	αvβ3	Lymph node metastasis[40]
Ovarian	α4β1 and αvβ3	Increased peritoneal metastasis[178] (α4β1) and tumour proliferation[179] (αvβ3)
Cervical	αvβ3 and αvβ6	Decreased patient survival[41,180]
Glioblastoma	αvβ3 and αvβ5	Both are expressed at the tumour–normal tissue margin and have a possible role in invasion[181]
Non-small-cell lung carcinoma	α5β1	Decreased survival in patients with lymph node-negative tumours[182]
Colon	αvβ6	Reduced patient survival[109]

Integrin regulation of cancer stem cells

Cancer stem cells represent a highly tumorigenic subset of cells in the primary tumour. Recent evidence has implicated integrins as markers of both normal progenitor and stem cell populations and cancer stem cells. In particular, the integrin αvβ3 represents a marker of luminal progenitor cells in the mammary ductal epithelium[42]. In a mouse model of spontaneous mammary tumorigenesis, expression of the proto-oncogene *WNT1* caused the expansion of the luminal progenitor cell population, among which the luminal marker integrin β3 (also known as CD61) represented a highly tumorigenic cancer stem cell population[43]. Integrin β3 expression identified cancer stem cells in around 50% of *Trp53*$^{+/-}$ tumours, but interestingly an integrin β3-positive cancer stem cell population was not found in the more homogeneous ERBB2 (also known as Neu)-positive tumours[43]. This finding may explain the lack of any observed effect when *ERBB2* was used to drive tumour initiation in *Itgb3*$^{-/-}$ mice[44]. Integrin signalling seems to maintain the cancer stem cell population in tumours, as ablation of *Ptk2* (which encodes FAK) decreased the pool of cancer stem cells in spontaneously forming mouse mammary tumours[45]. Additionally, integrins may regulate the expression of cancer stem cell markers, such as CD44 (REF. 46). As cancer stem cells are thought to represent the most tumorigenic and aggressive subset of a particular tumour, it is tempting to speculate that the expression of specific integrins could enhance cancer stem cell properties through cooperation with tumour-initiating oncogenes or growth factor receptors.

The host cellular response to cancer

In addition to their role in tumour cells, integrins are also important for the host cellular response to cancer. Endothelial cells, fibroblasts, pericytes, bone marrow-derived cells, inflammatory cells and platelets all use integrins for various functions, including angiogenesis, desmoplasia and the immune response (FIG. 3). In addition to integrins expressed on tumour cells, integrins present on many of these cell types might be potential therapeutic targets.

Angiogenesis. The contribution of angiogenesis to tumour progression is well established and the role of integrins has recently been reviewed[47]. Tumour-associated blood vessels are structurally and biologically distinct from quiescent vessels, and according to Harold Dvorak "tumours make bad blood vessels" (REF. 48). Their tortuous and leaky characteristics compromise blood flow, impair drug delivery, promote fibrosis and facilitate tumour cell intravasation leading to haematogenous or lymphatic metastasis. We have established that, unlike quiescent endothelium, tumour-associated vessels express integrin αvβ3 (REF. 49). It is possible that increased expression of integrins αvβ3 and αvβ5 allow angiogenic endothelial cells to bind provisional matrix proteins such as vitronectin, fibrinogen, von Willebrand factor, osteopontin and fibronectin that are deposited in the tumour microenvironment. In addition, proteolyzed, but not native, collagen is a ligand for integrin αvβ3 owing to the exposure of RGD sites made available by proteolysis[50]. These adhesive interactions could provide survival cues and/or traction for invading endothelial cells.

Through genetic deletion, or treatment with integrin antagonists, several additional integrins have been identified as crucial for angiogenesis, including α1β1, α2β1, α4β1, α5β1, α6β1, α9β1 and α6β4 (REF. 47). Integrin cooperation with particular growth factor receptors seems to confer responsiveness to specific angiogenic growth factors that are highly expressed in tumours. For example, αvβ3 and FGFR interaction induces angiogenesis downstream of FGF binding, and αvβ5 and VEGFR2 promote VEGF-induced angiogenesis[51]. The development of cilengitide as an anti-tumour and anti-angiogenic agent directed to both integrins αvβ3 and αvβ5 was partly based on these findings. These distinct pathways of angiogenesis highlight the fact that integrins can integrate cues from the ECM and growth factors to drive specific intracellular signalling events.

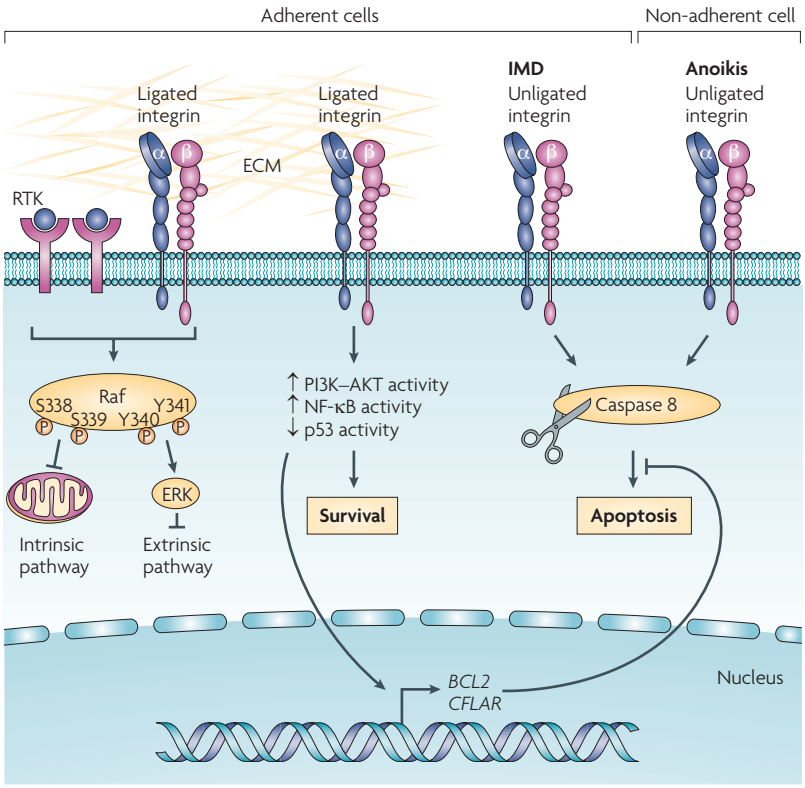


Figure 1 | **Integrin-mediated survival versus apoptotic pathways.** Integrins can paradoxically initiate pro-survival as well as pro-apoptotic signals. Which pathway is more active depends on the ligation status of the surface integrins expressed by a given cell. In a cell in which most of the integrins are ligated, a pro-survival pathway is initiated through increased nuclear factor-κB (NF-κB) or PI3K–AKT activity, decreased p53 activation and increased expression of the pro-survival molecules BCL-2 and FLIP (also known as CFLAR). Cooperative signalling between growth factor receptors and integrins also differentially activates Raf leading to distinct mechanisms of cell survival. Signalling through integrin αvβ3 and the fibroblast growth factor receptor promotes phosphorylation of Ser338 and Ser339 of Raf, protecting cells from the intrinsic pathway of apoptosis, and integrin αvβ5 and vascular endothelial growth factor receptor 2 phosphorylate Tyr340 and Tyr341 of Raf, preventing apoptosis through the extrinsic pathway. In adherent cells in which many of the integrins are unligated, the unligated integrins initiate cleavage of caspase 8, triggering apoptosis through integrin-mediated death (IMD). On complete loss of adhesion, cell death is initiated through a process termed anoikis. Apoptosis induced by anoikis may proceed through either the intrinsic or extrinsic pathways. ECM, extracellular matrix; RTK, receptor tyrosine kinase.

Perivascular cells. Angiogenesis not only depends on the invading endothelium but also requires perivascular cells, such as pericytes and vascular smooth muscle cells, which associate with the developing endothelium and promote blood vessel maturation. Tumours typically express immature blood vessels with reduced perivascular coverage[52]. This leads to tortuous and leaky vessels that account for much of the hypoxia and poor perfusion typically observed in tumours. Integrins regulate the interaction between endothelial cells and the vascular basement membrane, and recent studies suggest that the endothelial cell integrin α4β1 is necessary for an interaction with vascular cell adhesion molecule 1 (VCAM1) on pericytes, resulting in endothelial cell–pericyte interaction and vessel stabilization[53]. Recent studies have described an important role for blood vessel recruitment of pericytes in regulating blood vessel branching and patency in tumours through vascular normalization[54], the manipulation of which may improve the delivery of chemotherapeutics. According to Jain and colleagues[54], normalizing the tumour vasculature with agents such as bevacizumab or other VEGF pathway inhibitors should make it possible to increase drug delivery to the tumour and gain an improved therapeutic index for a wide range of anti-tumour agents. In fact, crosstalk between growth factor receptors, such as VEGFR2 and platelet-derived growth factor receptor (PDGFR), regulates pericyte recruitment to tumour-associated blood vessels[55]. Integrin cooperation with these growth factor receptors may be vital for regulating blood vessel normalization in tumours.

Desmoplasia. Abundant collagen deposition is a hallmark of the desmoplastic reaction in both primary tumours and their metastases. Through integrin signalling, the deposited collagen increases tumour cell proliferation, survival and chemoresistance, possibly contributing to the establishment and progression of metastatic lesions[56]. Integrins that are expressed on stromal fibroblasts also contribute to enhanced tumour growth. Integrin α11 is commonly overexpressed in stromal fibroblasts that are associated with non-small-cell lung carcinoma (NSCLC). Expression of α11β1 on fibroblasts increased tumour growth by stimulating the release of insulin-like growth factor 2 (IGF2)[57]. This study highlights the importance of the regulation of growth factor signalling by integrins for the tumour-promoting effects of the host stroma. Targeting the tumour stroma with integrin antagonists could represent a new avenue for tumour therapy.

Bone marrow-derived cells. Circulating bone marrow-derived cells are recruited to solid tumours, in which they can suppress tumour growth and also secrete pro-angiogenic growth factors and cytokines that contribute to tumour progression. Immune cells, including macrophages and natural killer cells, are crucial for tumour suppression. For example, macrophage tumour infiltration is decreased in *Itgb3*$^{-/-}$ mice and contributes to increased tumour burden, demonstrating that the expression of integrin αvβ3 on macrophages is important for their tumour suppressive function[58]. Alternatively, the tumour-homing of bone marrow-derived cells can result in increased tumour progression by increasing angiogenesis. Bone marrow cells expressing a functionally inactive integrin β3 mutant failed to be recruited to sites of neovascularization, resulting in decreased pathological angiogenesis[59]. Homing of endothelial and monocyte precursors to tumours also requires integrin α4β1 (REF. 60). Expression of integrin α4β1 on bone marrow-derived cells promotes adhesion to the tumour-associated endothelium, and blockade of integrin α4β1 reduced blood vessel density[60,61]. It is not clear whether blocking tumour homing of bone marrow-derived cells represents a viable therapeutic strategy as their tumour-suppressive effects might outweigh their pro-angiogenic potential.

Vascular normalization
The process of restoring the integrity and function of the vasculature through 'pruning' immature vessels and increasing pericyte and basement membrane coverage of the remaining vessels.

Tumour stroma
The fibroblasts, immune cells, pericytes, endothelial cells and inflammatory cells that surround a tumour and have a major role in tumour growth and progression.

Polyoma middle T
Derived from the polyomavirus, the middle T antigen is commonly used to induce spontaneous tumorigenesis in mouse mammary epithelial cells as a model of breast cancer.

Platelets. Multiple studies have linked tumour cell–platelet interactions with increased tumour metastasis. The ECM protein fibrinogen functions as a bridge between integrins αIIbβ3 on platelets and αvβ3 on tumour cells. This interaction facilitates tumour cell arrest in the vasculature, leading to metastasis to various sites, including the bone marrow[62–64]. Combined blockade of both tumour integrin αvβ3 and platelet integrin αIIbβ3 increased the anti-angiogenic and anti-tumour effects compared with blocking tumour integrin αvβ3 alone[65], suggesting that antagonists that target both integrins on platelets and endothelial cells could have greater clinical efficacy in patients.

Integrin cooperation with oncogenes

Although integrins lack the ability to transform cells, and therefore do not function as oncogenes, several integrins cooperate with oncogenes or receptor tyrosine kinases to enhance tumorigenesis. In spontaneous mouse models of tumorigenesis, integrins such as α6β4 cooperate with ERBB2 to increase breast tumour onset and invasion[66]. Integrin β1 mediates breast cancer that is driven by the polyoma middle T oncoprotein[67], and integrin α1 is required for KRAS-G12D-induced tumours in the lung[68]. There seems to be some specificity to the ability of integrins to crosstalk with particular oncogenes, as integrin signalling through FAK is required for oncogenesis through Ras and PI3K[69,70], whereas tumorigenesis induced by ERBB2 required integrin α6β4 (REF. 70). In another example, *in vitro* and *in vivo* experiments showed that integrin αvβ3 synergizes with the *SRC* oncogene to increase tumorigenic potential[71]. Interestingly, this effect enhanced only the oncogenic effects of SRC, and not morphological transformation[72]. These studies suggest that some oncogenes are dependent on integrin signalling, a property that could potentially be exploited therapeutically.

Integrin crosstalk with growth factor cytokines

Growing evidence supports a central role for cooperative signalling between integrins, growth factor receptors and cytokine receptors in many aspects of tumour progression (FIG. 4). Integrin crosstalk not only regulates tumour cell adhesion, migration, invasion and survival, but also affects many aspects of the host response to cancer, particularly in the angiogenic endothelium. However, not all crosstalk is pro-tumorigenic, as some integrins can inhibit tumorigenesis that is induced by certain oncogenes[73]. From the numerous examples of crosstalk that have been described, several themes have emerged regarding the underlying mechanisms involved. In some instances cooperative signalling, possibly mediated by the formation of an integrin–growth factor receptor complex[74–77], potentiates activation of downstream kinases such as MAPK[78] or AKT[79] and therefore enhances cell migration and survival. In other examples, integrins and growth factor or cytokine receptors reciprocally regulate the surface expression of one another[80–89], or the release of their respective ligands[90,91]. In addition to these general mechanisms, recent studies have elucidated other models of crosstalk (described below) that could have important implications for tumour metastasis and the acquisition of drug resistance.

EGF and its receptors. Members of the epidermal growth factor (EGF) receptor family, including EGFR and ERBB2, contribute to tumour formation and metastasis in many tumour types, including breast and pancreatic cancer. Increased expression and hyperactivation of EGF receptors occurs in many cancers, and overexpression of ERBB2 is oncogenic. In tumour cells, cooperation between integrins and members of the EGF receptor family affect many aspects of tumour progression, including tumour initiation, proliferation, migration and invasion. The integrin α6β4 may be particularly vital to tumour formation in the subset of patients with breast cancers that express high levels of ERBB2 as it cooperates with integrin α6β4 to induce spontaneous mammary tumour formation and tumour cell invasion[66]. This cooperative effect could be due to the formation of an integrin α6β4–ERBB2 complex that enhances the activation of signal transducer and activator of transcription 3 (STAT3) and JUN, leading to the loss of cell polarity and hyperproliferation, respectively[66]. Furthermore, these studies found that the deletion of *Itgb4* increased the efficacy of targeted ERBB2-specific therapy[66], highlighting the potential importance of combination therapy using antagonists targeting integrin and EGF receptor family members.

In pancreatic cancer, the EGF pathway is often hyperactivated, which potentiates the tumour cell migration and metastasis of this highly aggressive disease. EGF stimulates pancreatic tumour cell migration on

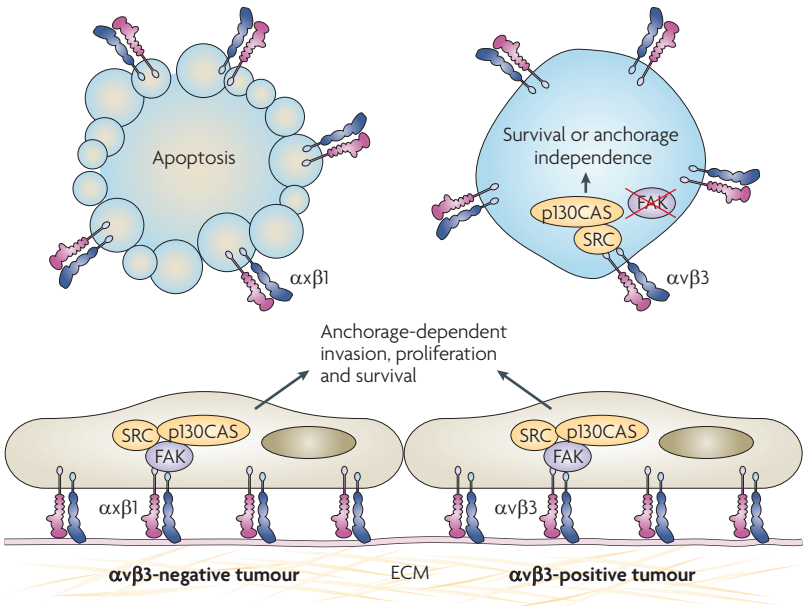


Figure 2 | **An integrin αvβ3–SRC oncogenic unit promotes anchorage independence.** In tumour cells, both β1 integrins (that is, αxβ1) and integrin αvβ3 induce adhesion-dependent activation of focal adhesion kinase (FAK) and SRC, in addition to phosphorylation of the adaptor protein p130 CRK-associated substrate (p130CAS). These signalling events result in invasion, proliferation and survival of tumour cells bound to the extracellular matrix (ECM). In suspended tumour cells unligated integrin αvβ3 signals directly through SRC and p130CAS to increase cell survival independently of FAK. This effect occurs in tumour cells that are already resistant to integrin-mediated death.

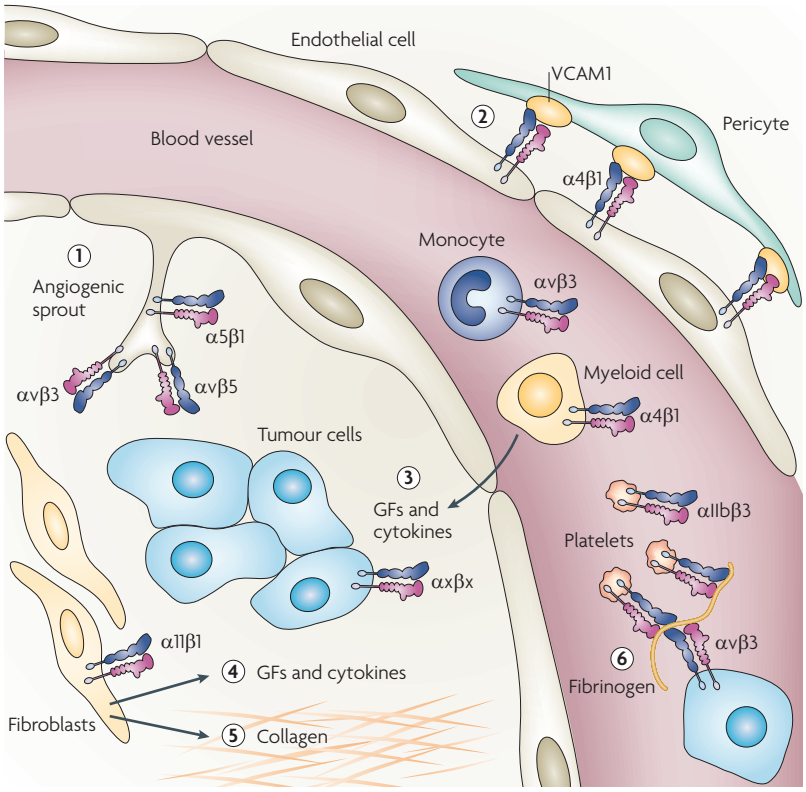


Figure 3 | **Integrins in the host response to cancer.** Integrins expressed in many tumour-associated cell types have crucial roles in increasing tumour progression and metastasis. In endothelial cells, integrins regulate the migration, proliferation and survival necessary for angiogenesis (step 1). The interaction between pericytes and endothelial cells is crucial for the stabilization of newly formed vessels during angiogenesis. Binding of integrin α4β1 on endothelial cells to vascular cell adhesion molecule 1 (VCAM1) on pericytes plays an important part in pericyte recruitment to the neovasculature (step 2). Myeloid cells and monocytes in primary tumours contribute to disease progression by secreting cytokines and growth factors (GFs) that initiate angiogenesis and tumour cell migration (step 3). Several studies have shown that integrins have an essential role in the homing of myeloid cells and monocytes to tumours. Fibroblast infiltration into the primary tumour, known as desmoplasia, also contributes to tumour progression through increased growth factor secretion (step 4). In addition, the invading fibroblasts deposit large amounts of collagen that might result in resistance to therapy in some tumours (step 5). A recent study showed that integrins, such as α11β1, are crucial regulators of growth factor secretion by these fibroblasts. Platelet expression of αIIbβ3 may be important for interacting with tumour cells through a fibrinogen bridge, possibly aiding in metastatic dissemination (step 6).

vitronectin *in vitro*[92–94] and metastasis *in vivo*[93,94], and these effects require integrin αvβ5 (REF. 94). Interestingly, before activation in non-stimulated cells integrin αvβ5 is unable to cluster and form focal adhesions on its own[95], which may be a prerequisite for integrin-mediated cell migration. Instead, αvβ5 requires EGF-dependent activation of SRC for its ability to mediate cell migration[94]. Further studies revealed a requirement for SRC phosphorylation of the p130CAS substrate domain and subsequent activation of the GTPase RAP1A (REF. 94), a known mediator of integrin activation. EGF–integrin crosstalk is not limited to pancreatic cancer, and it also increases the migration of colon cancer cells through integrins α3β1 and α6β4 (REF. 96), and hepatocellular carcinoma through integrins α1β1 and α2β1 (REF. 97). Therefore, EGF signalling in tumour cells may increase the ability of particular integrins to mediate cell migration and survival, resulting in increased metastatic potential.

Other studies have demonstrated that integrin ligation itself regulates EGF signalling, crucially influencing tumour cell susceptibility to treatment. In fact, integrin ligation can induce EGFR phosphorylation independently of EGF, resulting in increased MAPK activation, tumour cell proliferation and survival[98] through a SRC–p130CAS pathway[99]. The ability of integrins to increase EGFR signalling may be particularly important in breast cancers expressing high levels of ERBB2. A recent study found that integrin signalling increased EGF secretion and ERBB2 clustering in breast cancer cells, resulting in resistance to the ERBB2 inhibitor trastuzumab[90]. Inhibition of integrin signalling reversed trastuzumab resistance, suggesting that this combined approach may prove therapeutically efficacious in ERBB2-expressing breast cancers.

HGF and receptors. The hepatocyte growth factor (HGF) receptor MET is implicated in tumour initiation and the metastasis of various cancers, and integrin cooperation with MET results in enhanced tumour progression. In particular, integrin β4 synergizes with MET to enhance the transformation of fibroblasts and increase tumorigenic potential[100]. In breast cancer cells, HGF binding to MET increases anchorage-independent growth by inducing phosphorylation of integrin β4, resulting in the recruitment of proteins such as SHP2 (also known as PTPN11) and the subsequent activation of SRC and ERK[101]. Complex formation between MET and integrin α6β4 enhances HGF-induced signals, including tumour cell invasion[77]. This effect may be due to the potentiation of HGF-induced Ras and PI3K signalling by α6β4-mediated recruitment of proteins such as SHC1 and PI3K[77]. However, integrin β4 is not required for HGF-induced tumour cell invasion[102]. Additionally, MET cooperates with other integrins, as integrin αvβ5 contributes to MET signalling by controlling the expression of HGF-induced genes required for cell migration[103]. Integrin–MET crosstalk is also indirectly regulated by other molecules. For example, the tetraspanin KAI1 (also known as CD82) suppresses integrin-mediated activation of MET resulting in reduced tumour cell invasion[104]. Therefore, the role of MET in cancer seems to depend on crosstalk with tumour cell-associated integrins.

TGFβ and receptors. Although generally known for its anti-proliferative effects, transforming growth factor-β (TGFβ) is a well-characterized inducer of epithelial–mesenchymal transformation (EMT) in tumour cells, resulting in enhanced cell migration and invasion. Integrins are instrumental in the activation of TGFβ signalling. TGFβ ligands are secreted as inactive complexes with a latency-associated peptide (LAP). The TGFβ1 LAP was first identified as a ligand for integrin αvβ6, and expression of integrin αvβ6 regulates TGFβ1 activation[105]. It is now known that multiple αv integrins can bind the RGD motif in the LAP of TGFβ1, but only

Trastuzumab
A humanized monoclonal antibody that binds ERBB2 on tumour cells and prevents uncontrolled proliferation caused by aberrant ERBB2 signalling.

binding of integrin αvβ6 and αvβ8 results in TGFβ1 activation[106]. In cancers such as basal cell carcinoma, increased integrin αvβ6 expression correlates with aggressive disease, possibly owing to increased TGFβ1 activation forming a dense tumour stroma[107]. Further studies have shown that integrin αvβ6 activates TGFβ1 *in vivo*, contributing to tumour growth[108], and that upregulation of integrin αvβ6 in tumour cells is associated with EMT, TGFβ1 activation and increased migration[109].

Integrin signalling can also directly modulate TGFβ responses. Integrin αvβ3 and SRC cooperate with TGFβ to induce EMT of mammary epithelial cells[110], and this requires SRC-dependent phosphorylation of TGFβ receptor type 2 (TGFBR2)[111]. Additionally, TGFβ stimulation induces phosphorylation of the cytoplasmic domain of integrin β1, resulting in integrin activation and tumour cell invasion[112]. As TGFβ is predominantly secreted by tumour stromal cells, crosstalk between integrins and TGFβ may have an important role in the contribution of the tumour stroma to cancer progression.

VEGF, FGF and their receptors. Integrin–growth factor crosstalk not only occurs on tumour cells, but also has a role in various host cell types, including endothelial cells, in which it contributes to tumour angiogenesis. During angiogenesis, both integrins and growth factors are vital to endothelial cell migration, proliferation and survival. Therefore, considerable effort has gone into identifying specific functional interactions between individual integrins and growth factor receptors. In fact, distinct integrin–growth factor pairs have been described that contribute to angiogenesis through different signalling pathways.

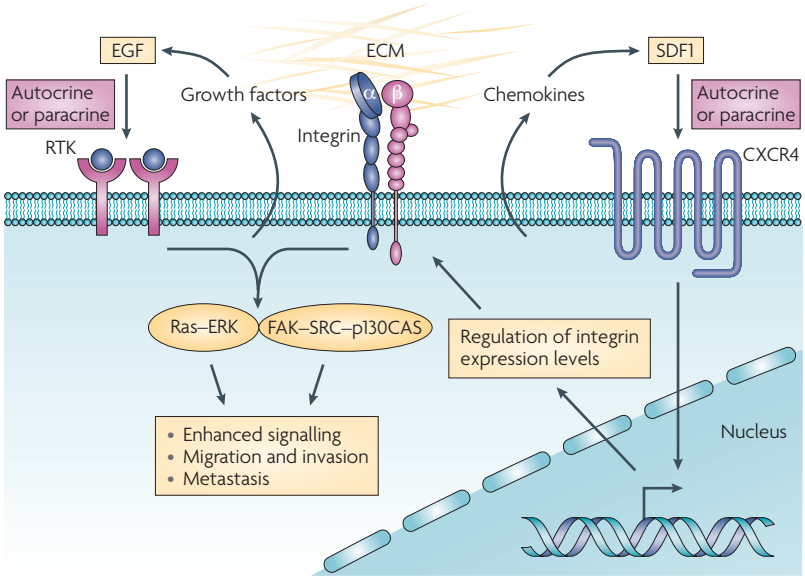


Figure 4 | **Integrin–growth factor and integrin–cytokine receptor crosstalk.** Cooperation between integrin and growth factor signalling or integrin and cytokine signalling is crucial to tumour progression. Several crosstalk mechanisms have so far been elucidated. Integrin ligation may lead directly to the increased secretion of growth factors and/or cytokines, which can then bind to their receptors in an autocrine or paracrine manner to further induce signalling. In addition, signalling induced by either integrin ligation or growth factor binding may activate common downstream pathways resulting in enhanced signalling overall compared with the activation of either receptor alone. This signalling seems to most commonly converge on kinases such as Src family kinases (SFKs), scaffolding proteins such as p130 CRK-associated substrate (p130CAS), and GTPases, such as the Ras family. Alternatively, both chemokine and growth factor signalling may regulate integrin function by directly controlling integrin expression levels. ECM, extracellular matrix; EGF, epidermal growth factor; FAK, focal adhesion kinase; SDF1, stromal cell-derived factor 1; RTK, receptor tyrosine kinase.

During tumour angiogenesis endothelial cells in the tumour microenvironment must resist cell death that is induced by stresses such as hypoxia and nutrient deprivation (intrinsic apoptosis) or inflammatory mediators (extrinsic apoptosis). We have previously described distinct pathways of angiogenesis that are mediated by specific integrin–growth factor receptor pairs[49]. These pathways signal to protect endothelial cells from distinct apoptotic stimuli through differential activation of Raf[19,20] (FIG. 2). FGFR cooperates with integrin αvβ3 to increase the phosphorylation of Raf Ser338 and Ser339 through PAK[19,20], resulting in Raf–ASK1 (also known as MAP3K5) complex formation in the mitochondria, inhibiting the intrinsic pathway of apoptosis[113]. By contrast, VEGFR2 cooperates with integrin αvβ5, leading to SRC-dependent phosphorylation of Raf Tyr340 and Tyr341 and resistance to extrinsic apoptosis that is induced by inflammatory mediators such as tumour necrosis factor (TNF)[19,20]. This underscores the importance of Raf during tumour angiogenesis and documents how distinct integrin–growth factor pairs can differentially influence downstream endothelial survival pathways. A central role for Raf in angiogenesis was established by targeting mutationally inactive Raf to the tumour vasculature, which potently inhibited angiogenesis and tumour growth in mice[114].

Additional examples of crosstalk between VEGFR2 and αv integrins have also been described in vascular endothelial cells. For example, SRC recruitment to VEGFR2 promotes crosstalk with integrin αvβ3 and SRC-dependent tyrosine phosphorylation of the cytoplasmic domain of integrin β3 (REF. 113). This may be related to the increased levels of VEGFR2 in *Itgb3*$^{-/-}$ mice leading to a compensatory increase in angiogenesis[22]. Another mechanism by which VEGF influences integrin αvβ3 signalling is through regulating the affinity state, or activation of the integrin[115]. Activation of integrin αvβ3 can in turn increase tumour cell secretion of VEGF, providing a feedback loop resulting in increased tumour growth[91]. Specific integrin–growth factor pairs have also been identified in FGF-mediated angiogenesis. Integrin β4 contributes to FGF-mediated angiogenesis, as a targeted deletion of the signalling portion of the cytoplasmic domain of integrin β4 resulted in decreased FGF-induced angiogenesis and reduced tumour size[116]. Therefore, signalling through distinct integrin–growth factor receptor pairs has crucial roles in tumour angiogenesis.

CXCR4. Although best characterized for its role in the recruitment of haematopoietic cells to sites of injury or infection, the chemokine receptor CXCR4 is also expressed on tumour cells and various tumour-associated cell types. Binding of CXCR4 to its cognate ligand stromal cell-derived factor 1 (SDF1; also

known as CXCL12) induces tumour cell migration and contributes to metastasis. SDF1 stimulation of CXCR4 on tumour cells increases the expression of integrins, such as α5β1 and αvβ3, increasing cell adhesion and invasion *in vitro*[87,88] and experimental metastasis *in vivo*[117]. In addition to increasing integrin surface expression, SDF1 controls adhesion by augmenting integrin activation[118]. In another example, integrin ligation reciprocally regulates CXCR4 expression levels[89]. Further studies are needed to determine the relevance of integrin–CXCR4 crosstalk to tumour growth and spontaneous metastasis.

Integrins as targets for cancer therapy

The expression of integrins in various cell types that are involved in tumour progression and their ability to crosstalk with growth factor receptors has made them appealing therapeutic targets. Preclinical studies showed that integrin antagonists inhibit tumour growth by affecting both tumour cells and tumour-associated host cells, most notably the angiogenic endothelium. Integrin antagonists currently in clinical trials include monoclonal antibodies and RGD peptide mimetics (see Avraamides *et al.*[47] for a complete review). Years of preclinical studies and early clinical trials have now culminated in the initiation of a Phase III clinical trial in glioblastoma with the RGD peptide mimetic cilengitide. In this section, we discuss the current status of integrin antagonists in cancer therapy, including the studies supporting the design of the first Phase III clinical trial of an integrin inhibitor in cancer.

Targeting αvβ3 and αvβ5. Integrin αvβ3 is upregulated in both tumour cells and angiogenic endothelial cells, making it an attractive therapeutic target. Function-blocking monoclonal antibodies, such as LM609, were among the first integrin antagonists developed, and showed considerable anti-angiogenic activity in preclinical models[119]. As a result of these studies, etaracizumab (MEDI-522), a humanized version of LM609, was developed. In addition to its anti-angiogenic effects, etaracizumab inhibited tumour growth by directly affecting tumour cells[120], and impaired bone resorption by inhibiting osteoclast attachment, suggesting possible efficacy in reducing bone metastasis[121]. As a result of its efficacy in preclinical studies, etaracizumab was one of the first integrin antagonists introduced into clinical trials. Phase I trials with vitaxin, the precursor of etaracizumab, showed anti-angiogenic activity[122], low toxicity and disease stabilization in some patients with advanced solid tumours[123] and renal cell cancer[124]. A Phase II study showed some efficacy in metastatic melanoma[125]. The human αv integrin-specific monoclonal antibody CNTO 95, which targets both αvβ3 and αvβ5 integrins, also had anti-tumour and anti-angiogenic effects in xenograft tumour models[126,127]. In a Phase I trial, CNTO 95 was non-toxic[128], localized to tumours and showed signs of anti-tumour activity[129]. Both CNTO 95 and etaracizumab are being further evaluated in additional clinical trials.

Cilengitide is an inhibitor of both αvβ3 and αvβ5 integrins, and it was selected in our laboratory by screening a library of cyclic RGD peptides in a cell-free receptor assay for their capacity to inhibit integrins αvβ3 and αvβ5 but not αIIbβ3 (REF. 130). Cilengitide is currently being tested in Phase II trials in patients with lung and prostate cancer[131], and Phase II and Phase III trials are currently underway in glioblastoma. So far, cilengitide has shown significant promise in patients with late-stage glioblastoma by extending patient survival with minimal side effects (discussed below)[132–135]. Nevertheless, in mouse studies, Reynolds *et al.*[22] found that the continuous infusion of very low concentrations of RGD peptides paradoxically stimulates tumour growth and angiogenesis by promoting VEGF-induced endothelial cell migration[22]. These results are consistent with published accounts from other groups showing that low concentrations of soluble integrin antagonists can function as integrin agonists in some cases[136–138]. Such studies might be relevant to recent studies in which inhibitors of VEGF increased tumour perfusion resulting in enhanced tumour progression[139,140]. However, the increased tumour perfusion associated with anti-angiogenic therapy might be exploited to increase the delivery of chemotherapeutic agents, potentially explaining why anti-angiogenic agents such as cilengitide are most effective when used in combination with chemotherapy. It is also important to consider that cilengitide and other anti-angiogenic therapies might target multiple cell types in the tumour microenvironment, including the tumour cells themselves, and therefore their anti-tumour effects may not be entirely due to anti-angiogenic activity.

Glioblastomas are aggressive, highly vascularized brain tumours for which patient survival is only marginally increased by current therapies. Once diagnosed, patients typically have a short life expectancy of only a few months. Consequently, the development of therapeutic options that control this disease is crucial. These highly vascularized tumours express integrin αvβ3 on angiogenic blood vessels, as well as the tumour cells themselves, suggesting that antagonists to this integrin might be therapeutically beneficial in patients with glioblastoma. In preclinical studies, cilengitide effectively inhibited angiogenesis and the growth of orthotopic glioblastoma[141,142]. Importantly, the brain microenvironment was a crucial determinant of the susceptibility of these tumours to cilengitide, as tumours that formed in the flank of these same mice were unaffected by treatment with this drug[141]. In addition, high-grade glioblastomas abundantly express the ECM protein vitronectin, an integrin αvβ3 ligand, and this interaction affects tumour cell survival[14] and invasion[143]. Therefore, the relatively large quantity of vitronectin present in the brain microenvironment surrounding glioblastomas might explain why these tumours are susceptible to cilengitide treatment.

These promising preclinical studies were the basis for clinical trials in patients with glioblastoma. Phase I studies with cilengitide in patients with recurrent glioblastoma showed that it was well tolerated and produced durable responses that seemed to be related to changes in

relative cerebral blood flow[132]. Another Phase I study in children with refractory brain tumours determined that it was well tolerated and produced stable disease, with a full response in some patients[133]. A Phase II trial with cilengitide showed anti-tumour efficacy and minimal toxicity in patients with recurrent glioblastoma[134]. A different Phase I/II trial examined cilengitide in patients with newly diagnosed glioblastoma and met its primary end point with 69% of patients progression-free after 6 months[135]. Importantly, this trial made the observation that patients with lowered expression of *O*-6-methylguanine-DNA methyltransferase (MGMT), owing to promoter methylation, exhibited a higher rate (91%) of progression-free survival at 6 months. The *MGMT* promoter is a prognostic marker in patients with glioblastoma: tumours with unmethylated *MGMT* promoters indicate a lower probability of patient survival, as MGMT is thought to increase resistance to drugs such as temozolomide, which is a current standard therapy.

The favourable results obtained from these early clinical trials provided the impetus for a Phase III trial with cilengitide that began in October 2008. The CENTRIC trial will enroll approximately 500 patients and measure the effect of cilengitide on the survival of patients with *MGMT* promoter methylation in combination with temozolomide and radiotherapy. This is the first Phase III oncology trial carried out with any integrin antagonist. As a companion to the CENTRIC trial, the Phase II CORE trial will assess the efficacy of cilengitide in a large number of patients whose tumours have unmethylated *MGMT* promoters.

Targeting β1 integrins. Strategies that target β1 integrins, particularly α5β1, have also shown efficacy in reducing tumour burden in preclinical models. An integrin β1 inhibitory antibody significantly affected *in vitro* and *in vivo* growth of human breast cancer tumour cells[144]. Volociximab, a function-blocking monoclonal antibody against integrin α5β1, inhibits angiogenesis and impedes tumour growth[145,146]. A Phase I trial in patients with advanced solid malignancies showed that volociximab was well tolerated and may have clinical efficacy[147]. Volociximab is currently in Phase II clinical trials for solid tumours[148]. ATN-161 is a non-RGD-based peptide inhibitor of integrin α5β1 that blocks breast cancer growth and metastasis *in vivo*[149]. In mouse models of colon cancer metastasis to the liver, combination therapy with ATN-161 and fluorouracil significantly reduced tumour burden and liver metastases compared with either treatment alone[150]. ATN-161 was tested in patients with advanced solid tumours and was well tolerated and prolonged stable disease in one-third of the patients[151].

Additional integrin antagonists. Several additional integrin antagonists have shown efficacy in preclinical studies, but have not yet made it to the clinic. In xenograft tumour models, the integrin αvβ3 small-molecule antagonist S247 inhibited breast cancer bone metastases[152], decreased colon cancer metastasis and angiogenesis, and increased survival[153]. A non-peptide antagonist of integrin αvβ3, PSK1404, inhibited breast and ovarian cancer bone metastases without affecting osteoclast activity[154]. Administration of the RGD peptidomimetics S137 and S247 produced anti-metastatic effects[155]. Preclinical studies showed that antisense to either *ITGAV* or *ITGB3* suppressed the growth of subcutaneously injected human hepatocellular carcinoma cells[156]. A monoclonal antibody against integrin αvβ6, 6.3G9, blocked the growth of human pharyngeal carcinoma cells both *in vitro* and *in vivo*[108]. Interestingly, treatment of cells with this antibody also inhibited TGFβ signalling, suggesting that at least some of its efficacy might require crosstalk with the TGFβ receptors. It will be interesting to observe the performance of these new agents in clinical trials, and study how their efficacy may be optimized in combination with additional therapeutic strategies.

Targets for cancer imaging and drug delivery

Imaging. Currently, there are no validated biomarkers for clinically assessing the efficacy of anti-angiogenic therapies, including cilengitide. Although candidate markers are being investigated, including serum levels of VEGF, FGF and placental growth factor, as well as the abundance of circulating endothelial cells and their precursors, these markers have not yet consistently predicted tumour response. As a result, better vascular imaging techniques are being developed to monitor responsiveness to treatment. In particular, considerable effort has been expended on characterizing integrin antagonists for their ability to specifically deliver diagnostic agents to tumour cells and associated blood vessels. Coupling of the integrin αvβ3 antibody LM609 or other antagonists to a paramagnetic contrast agent[157] or radionuclides[158] has allowed the detection of angiogenic vessels in rabbit and mouse tumour models. An integrin αvβ3-targeted magnetic resonance imaging nanoparticle has also been used to detect the neovasculature of minute solid tumours in a xenograft tumour model[159]. Additionally, angiogenic vessels can be detected by contrast enhanced ultrasound with microbubbles targeting αv integrins[160]. RGD peptides labelled with ^{64}Cu, ^{18}F and ultrasmall superparamagnetic iron oxide particles have also been used to detect αv integrins in xenograft models of breast[161], brain[162] and lung cancer[163], respectively. Some of these imaging agents have recently undergone evaluation in cancer patients. Scintigraphic imaging using a radiolabelled integrin αvβ3-targeted peptide (^{99m}Tc-NC100692) detected a high proportion of malignancies in patients with breast cancer[164,165]. In another study, delivery of ^{18}F-galacto-RGD in combination with positron emission tomography (PET) provided non-invasive quantitative assessment of integrin αvβ3 expression in human tumours[166]. These studies suggest that labelled integrin antagonists could provide important diagnostic tools for assessing the efficacy of anti-angiogenic and anti-tumour therapies.

Table 2 | **Integrin targeting methods**

Therapeutic agent	Targeting moiety	Tumour model(s)	Results	Refs
Mutant *RAF1*	Organic αvβ3 ligand	Subcutaneous human melanoma cells	Regression of established primary and metastatic tumours and apoptosis of the tumour-associated vasculature	113
Nanoparticle loaded with doxorubicin	cRGD	Orthotopic mouse pancreatic tumour cells and human melanoma and renal tumour cells	Suppressed spontaneous metastases by disrupting the associated vasculature at very low doses	167
Nanoparticle loaded with fumagillin	cRGD	Vx-2 rabbit adenocarcinoma	Suppressed angiogenesis and tumour development at low doses	183
Oncolytic measles virus	cRGD	Subcutaneous human myeloma cells	Targeted delivery of virus to tumour neovessels	184
TRAIL	RGD	Subcutaneous human colon cancer cells	Inhibited primary tumour burden to a greater extent than TRAIL alone	185
p53	RGDK-lipopeptide	Orthotopic mouse melanoma cells	Targeted tumour vasculature and inhibited tumour volume	186
Radionucleotide	cRGD	Subcutaneous human ovarian cancer cells	Increased survival compared with untreated mice	187, 188
Radionucleotide	Etaracizumab	Orthotopic human glioblastoma cells and subcutaneous human colon cancer cells	Decreased angiogenesis and reduced tumour volume better than delivery of the antibody alone	189
Cytotoxic immunoconjugates	CNTO 95	Subcutaneous human colon and lung cancer cells	Reduced primary tumour burden above the level observed with antibody alone	190

cRGD, cyclic arginine-glycine-aspartic acid; RGDK, arginine-glycine-aspartic acid-lysine; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.

Targeted delivery of therapy. Integrin-targeted therapeutics have recently proved beneficial in delivering chemotherapeutics, oncolytic viruses, pro-apoptotic peptides (such as TNF and TNF-related apoptosis-inducing ligand (TRAIL)) and radionucleotides to both tumour cells and the supporting vasculature (TABLE 2). The use of integrin targeting to deliver therapeutics to the tumour vasculature was first shown by Hood *et al.*[114], who used an integrin αvβ3-targeted nanoparticle to selectively deliver a mutant *RAF1* gene to the tumour vasculature, resulting in apoptosis of endothelial cells and tumour regression. More recent studies showed that delivery of targeted nanoparticles loaded with doxorubicin to integrin αvβ3-positive tumour vasculature inhibited the growth of metastases while eliminating the toxicity and weight loss associated with systemic administration of this drug[167]. This delivery method resulted in a 15-fold improvement in tumour and anti-metastatic activity when compared with administration of the free drug. The preferential activity of these nanoparticles on metastases suggests that growing metastatic tumours may have a greater dependence on angiogenic vessels and so could be more susceptible to integrin αvβ3-targeted therapy.

Conclusions

Integrins expressed by tumour and tumour-associated host cells mediate a diverse array of cellular effects resulting in tumour progression and metastasis. Important among these is the role integrins have in determining tumour cell survival. In the past few years studies have revealed new roles for unligated integrins in this process. Under some circumstances unligated integrins can induce tumour cell apoptosis through IMD by recruiting and activating caspase cleavage. Tumour cells that are resistant to IMD gain the ability to metastasize. In this case unligated integrins may promote cell survival, resulting in increased anchorage-independence and metastasis. These effects of unligated integrins may be clinically relevant and probably represent important factors in determining tumour cell sensitivity to integrin antagonists.

Crosstalk with growth factor receptors is required for many of the cancer-promoting effects of integrins. Recent studies have shown that certain growth factor receptors and oncogenes require specific integrins for their effects on tumorigenesis and metastasis. This suggests that it may be plausible to tailor the use of integrin antagonists in individual patients whose tumours are responsive to particular growth factors or oncogenes. Alternatively, the ECM composition of the tumour microenvironment may have a vital role in determining the sensitivity of a tumour to integrin antagonists. This may partly explain the clinical responsiveness of patients with glioblastoma treated with cilengitide. Future studies will have to elucidate the factors responsible for tumour susceptibility to these inhibitors and these will ultimately influence how effective these agents are as cancer therapeutics.

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Competing interests statement
The authors declare no competing financial interests.

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