

## Review Article

# Integrin Signaling as a Cancer Drug Target

**Erik H. J. Danen**

*Division of Toxicology, LACDR, Leiden University, 2333 CC Leiden, The Netherlands*

Correspondence should be addressed to Erik H. J. Danen; [e.danen@lacdr.leidenuniv.nl](mailto:e.danen@lacdr.leidenuniv.nl)

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Integrins are transmembrane receptors that mediate cell adhesion to neighboring cells and to the extracellular matrix. Here, the various modes in which integrin-mediated adhesion regulates intracellular signaling pathways impinging on cell survival, proliferation, and differentiation are considered. Subsequently, evidence that integrins also control crucial signaling cascades in cancer cells is discussed. Lastly, the important role of integrin signaling in tumor cells as well as in stromal cells that support cancer growth, metastasis, and therapy resistance indicates that integrin signaling may be an attractive target for (combined) cancer therapy strategies. Current approaches to target integrins in this context are reviewed.

## 1. Integrin-Mediated Cell Adhesion

**1.1. Cell Adhesion.** Cells within multicellular organisms are typically attached to each other and to the extracellular matrix (ECM). ECM is a meshwork of various glycoproteins that exists in many forms, including laminin-rich basement membranes that align tissues, pliable matrices made from fibrillar networks of collagens, rigid collagen-based bone matrices, and provisional fibronectin-containing matrices associated with active processes such as wound healing and angiogenesis [1]. Various adhesion molecules, such as those belonging to the cadherin family, mediate cell-cell contacts. Likewise, interactions with the ECM also occur through a variety of receptors, including syndecans, dystroglycans, and integrins.

**1.2. Integrins.** Integrin cell adhesion receptors participate in cell-cell and cell-ECM interactions [2]. This large family of heterodimeric transmembrane receptors recognizes a plethora of extracellular ligands, including transmembrane receptors on other cells and ECM proteins. The common integrin-binding motif, Arg-Gly-Asp (RGD), is shared by several ECM proteins, including fibronectin, vitronectin, and fibrinogen. Integrin binding to laminins and collagens occurs at other recognition motifs. Integrins participating in cell-cell adhesion bind counter receptors such as a disintegrin and metalloproteases (ADAMs), or immunoglobulin-type receptors such as intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs) that are expressed on leukocytes and endothelial cells.

**1.3. Integrin Evolution.** Clearly, integrins are essential receptors in mammalian development, and adult life. Studies in mice have attributed roles to certain integrins already at very early stages of development while others play specific roles later in the adult. In fact, integrins are found throughout metazoan evolution [3]. In the nematode *Caenorhabditis elegans*, one  $\beta$  subunit (termed  $\beta$ pat3) and two  $\alpha$  subunits (termed  $\alpha$ inal and  $\alpha$ pat2) form two integrins. In the fruit fly *Drosophila melanogaster*, five integrins are formed through combination of one  $\beta$  subunit (termed  $\beta$ PS) with five  $\alpha$  subunits (termed  $\alpha$ PS1 through 5). The integrin family has expanded in vertebrates, and as many as 18  $\alpha$  and 8  $\beta$  subunits have been identified in humans, from which 24 different functional integrins are formed.

**1.4. Integrins: Transmembrane Linkers of Extra- and Intracellular Milieu.** A unique feature of integrins that explains their critical role in cell adhesion is their ability to physically couple the extracellular ECM network to the intracellular cytoskeletal network [4]. This involves connections at the very short integrin cytoplasmic tails with a complex of cytoskeletal adapter proteins. Importantly, this coupling is tightly regulated both at the level of integrin-mediated ligand binding as well as at the level of linkage to the cytoskeleton [5, 6]. This allows, for instance, muscle cells to anchor firmly to the tendon but platelets to start coagulation only when needed and leukocytes to temporarily attach to endothelial cells only when stimulated to extravasate.

## 2. Integrin Signaling

*2.1. Bidirectional Signaling Controls Ligand-Cytoskeleton Coupling and Formation of Adhesion Plaques.* Integrins act as bidirectional signaling molecules [2]. Inside-out signaling refers to intracellular signaling pathways that regulate protein interactions at the cytoplasmic tails, which control integrin conformation and thereby affinity. Talins and kindlins are the major proteins that play critical roles in this process [5, 7, 8]. They bind to the integrin tails with their FERM domains, thereby separating the tails, which triggers a conformation change leading to integrin activation. Talin-integrin association can be regulated by disruption of intramolecular interactions within the talin molecule in response to intracellular signaling. For kindlins, modes of regulation are not understood, and the concerted action of these two regulators of integrin affinity also remains obscure. In addition, lateral diffusion and clustering of integrins can further adapt cell adhesion strength.

Vice versa, outside-in signaling refers to integrin-ligand binding and clustering affecting inter- and intramolecular interactions within the cluster of proteins associated at the integrin cytoplasmic tails and connecting to the cytoskeleton. Thus, following initial ligand binding of integrins, a growing adhesion plaque is formed through recruitment of more and more adaptor proteins and linkage to the cytoskeleton is strengthened [6]. In the case of ECM adhesion, this plaque is called a “cell-matrix adhesion” [9]. Such outside-in signaling does not happen when soluble ligands are bound but requires binding to immobilized ligands, for instance, ECM meshworks or receptors on opposing cells (although soluble ligands that cause clustering may suffice to trigger a limited signal).

*2.2. Integrins Are Bidirectional Force Transmitters.* Integrins and several associated cell-matrix adhesion proteins act as force sensors [10, 11]. A cascade of conformational changes in integrins and in associated proteins known to reside in cell-matrix adhesions, including focal adhesion kinase (FAK), p130Cas, vinculin, and others, is activated in response to force in either direction. Extracellular forces, through integrins, exert forces on integrin-associated cytoplasmic proteins, thereby exposing binding sites for new intramolecular interactions that drive a cytoskeletal stiffening response. Vice versa, cytoskeleton-generated forces, probably through similar conformational changes in the integrin-associated protein complex, allow integrins to pull and stretch ECM proteins such as fibronectin. This can cause ECM stiffening: exposure of binding sites driving, for instance, higher order interactions between fibronectin molecules during fibril formation or remodeling of collagen networks [12, 13]. Thus, integrins allow cells to maintain a balance between the intracellular cytoskeletal contractile machinery and ECM stiffness.

*2.3. Activation of Biochemical Signaling Cascades.* Outside-in signaling also involves the activation of kinases in response to integrin-mediated cell adhesion. For instance, the combination of lateral aggregation of integrins in the membrane

and integrin ligand binding leads to recruitment and activation of FAK [14]. This process is not entirely understood, but a conformational change leading to FERM domain displacement in FAK is involved [15, 16]. This change is associated with increased phosphorylation of tyrosine (Tyr)397, which along with an exposed PxxP motif forms a binding site for the Src homology 2 (SH2) and SH3 domains, respectively, of the Src kinase [17]. Src then phosphorylates other tyrosines contributing to full activation of FAK. This active FAK/Src complex mediates a number of important signaling cascades downstream of integrins. Key examples include the control of the ERK MAP kinase pathway, the phosphatidylinositol-3 kinase (PI3K)/AKT pathway, and Rho GTPase activities.

Src can phosphorylate FAK at Tyr925, creating a binding site for the SH2 domain of Grb2 that links FAK to Sos, the guanine nucleotide exchange factor (GEF) for Ras [18]. This is but one of the connections by which integrins can control activity of the Ras-Raf-MEK-ERK pathway. Src can also phosphorylate the scaffolding protein p130Cas that is associated with FAK via its SH3 domain, thereby creating a binding site for the adaptor protein Crk [19]. The interaction with Crk, through association with Sos or through association with C3G, the GEF for Rap-1 that subsequently activates B-Raf, can result in ERK activation [20]. Integrin-mediated adhesion also stimulates the association of the adaptor protein Nck with p130Cas, creating yet another potential link from p130Cas to ERK activation.

PI3K can also associate via the SH2 domain in its 85 kDa subunit with phosphorylated Tyr397 in FAK [21]. Local activity of PI3K can contribute to various signaling pathways via its production of phosphatidylinositol-3,4,5-trisphosphate (PtdInsP3), including the membrane localization of Sos leading to ERK activation. Moreover, this pool of PtdInsP3 stimulates the recruitment of PKB/Akt to the membrane through its PH domain. This allows PDK1, which is also recruited to PtdInsP3 via its PH domain to activate PKB/Akt together with the elusive PDK2 through phosphorylation at Thr-308 and Ser-473, respectively [22].

Integrin-mediated activation of the FAK/Src complex also provides control of the activity of Rho family members [23]. Rho small GTPases are critical regulators of cytoskeletal dynamics. The active FAK/Src complex recruits and phosphorylates p130Cas. Phosphorylated p130Cas recruits Dock180 and ELMO through its association with the adaptor protein Crk. The Dock180/ELMO complex acts as a guanine exchange factor (GEF) for Rac [24–26]. The Rac GTPase is important for Arp2/3-mediated branched F-actin growth that drives membrane protrusions in the form of lamellipodia. Alternatively, the FAK/Src complex phosphorylates another cell-matrix adhesion protein, paxillin, which recruits paxillin kinase linker (PKL) and Pak-interacting exchange factors (PIX), two GEFs for Rac and Cdc42 (another Rho GTPase that drives extension of filopodia, another type of membrane protrusions) [27, 28]. The FAK/Src complex also phosphorylates p190RhoGAP in cells adhering to fibronectin. This GTPase activating protein (GAP) suppresses RhoA GTP levels, thereby suppressing RhoA-mediated actin-myosin contractility and thus facilitating cell spreading upon adhesion to ECM [29, 30]. However, when external forces are applied

to integrins, FAK cooperates with another Src family kinase, Fyn, to activate two GEFs for RhoA, LARG and GEFH1, to enhance cytoskeletal contractility, resulting in cellular stiffening [31]. By coordinating the activities of Rho GTPases as described here, and at the same time recruiting components of the actin polymerization machinery such as Arp2/3, integrin-mediated adhesion complexes are signaling hotspots for local regulation of cytoskeletal dynamics.

**2.4. Crosstalk with Other Receptors in Outside-in Signaling.** The integrin-regulated signaling pathways described previously do not stand on their own but act in concert with signaling by other receptor classes. For instance, in cells adhering to fibronectin, activity of RhoA is controlled by engagement of integrin  $\alpha 5 \beta 1$ , which stimulates Src-mediated p190RhoGAP tyrosine phosphorylation, while syndecan-4 engagement stimulates PKC $\alpha$ -dependent translocation of p190RhoGAP to the cell membrane [32]. In fact, a major part of integrin signaling may involve activation of pathways downstream of other receptors. One of the earliest examples for this notion came from studies investigating adhesion control of the Rac small GTPase. It turned out that epidermal growth factor receptor (EGFR) signaling was required to activate Rac in response to cell adhesion [33]. In the case of growth factor receptors, the ability of integrins to cluster key enzymes and substrates may augment growth factor signaling through these same enzymes, including kinases and GTPases. However, it is now clear that integrin-mediated adhesion may lower the threshold for receptor tyrosine kinase (RTK) activation more directly. Integrins can associate with and/or trigger cross-phosphorylation of a large number of RTKs including EGFR, insulin-like growth factor (IGF)R, vascular endothelial growth factor (VEGF)R, platelet-derived growth factor (PDGF)R, c-Met, and macrophage stimulating 1 receptor (MST1R; Ron). Several studies have shown that Src family kinases mediate such integrin-RTK crosstalk [34–36].

Integrin-mediated adhesion to ECM can enhance growth factor signaling in yet another manner. Many growth factors bind to heparin and heparan sulfate found in ECM proteoglycans [1]. Proteolytic cleavage of ECM proteins may liberate such growth factors (or ECM-derived peptides with signaling potential) for receptor binding and in some cases interactions with ECM are known to aid effective presentation of growth factors to their receptors. ECM proteins also contain growth factor-like motifs such as EGF themselves that may mimic soluble growth factor action [37]. TGF $\beta$  is one of the growth factors associated with ECM proteins. In its inactive form, it is bound to and masked by the latency-associated peptide (LAP). Several  $\alpha v$  integrins can bind to the RGD motif within LAP and cause exposure of active TGF $\beta$ , which can subsequently bind and activate TGF $\beta$ R. Interestingly, this can occur through distinct protease-dependent or protease-independent mechanisms, the latter involving traction forces exerted through the integrin on the TGF $\beta$ -LAP complex by the actin cytoskeleton [38, 39].

**2.5. Control of Signaling by Anchoring and Regulating the Cytoskeleton.** Lastly, the ability of integrins to transmit forces, physically connect to the cytoskeleton, and act as cytoskeletal

anchoring points as well as hotspots for dynamic regulation of the cytoskeleton can strongly impact on the cellular response to growth factors. Cytoskeletal tension allows cells to respond to mechanical forces with changes in gene transcription [40]. This response can be mediated by altered concentrations of second messengers such as calcium and cyclic AMP and crosstalk with growth factor receptor signaling pathways as described earlier. More directly, the cytoskeleton is connected to integrins at the plasma membrane as well as to the nuclear envelope through linker of nucleoskeleton and cytoskeleton (LINC) complexes. Here, nesprin proteins in the outer membrane connect to microtubules, actin fibers, and intermediate filaments, while SUN proteins in the inner membrane bind the nuclear lamina [41]. Thus, extracellular forces, through integrins, are mechanically linked to changes in nuclear orientation and shape [42]. Since chromatin-binding proteins and DNA are attached to the nuclear lamina, extracellular mechanical stress may be propagated into the chromatin and affect gene expression through conformational regulation of DNA and associated proteins [41]. At present, direct evidence for such purely mechanical coupling between ECM and gene expression is lacking.

### 3. Control of Cell Fate Decisions

The various modes of action discussed previously that allow integrins to regulate signaling pathways underlie adhesion control of cell survival, proliferation, and differentiation.

**3.1. Cell Survival.** Most cell types depend on integrin-mediated cell adhesion to ECM for survival and proliferation. Endothelial and epithelial cells rapidly undergo apoptosis when adhesion is disturbed. This process has been termed “anoikis.” The fact that integrin-mediated adhesion supports PI3K-mediated PKB/AKT activity is important in this respect [43, 44]. PKB/AKT activity is a key regulator of cell survival pathways. It phosphorylates/inhibits proapoptotic proteins such as Bad and procaspase-9 [45]. PKB/AKT also suppresses proapoptotic transcriptional responses through Forkhead factors and p53 [46]. Interestingly, integrins that are not ligand bound have been reported to stimulate apoptosis even in adherent cells by recruitment and activation of caspase-8 [47, 48]. This implies that the integrin expression profile must match the ECM environment to prevent cells from entering apoptosis.

FAK, besides contributing to PKB/AKT activity as described previously, can stimulate cell survival through enhanced expression of the antiapoptotic transcription factor NF- $\kappa$ B [49]. Moreover, FAK protects against apoptosis by entering the nucleus, binding p53, and preventing p53-mediated transcription of proapoptotic genes [50]. Notably, the link between integrin-mediated activation of FAK and this mechanism is unclear: FAK nuclear translocation is triggered by disruption of cell adhesion and is independent of its kinase activation. Perhaps, this represents a stress response allowing cells to cope with detachment, but the FAK:p53 interaction may not be important under adherent conditions.

**3.2. Cell Proliferation.** A major checkpoint in the cell cycle is the transition from G1 to S phase. Here, environmental cues drive cyclin-cdk activities that culminate in phosphorylation of the Rb tumor suppressor, which acts as a restriction point for entry in the S phase by sequestering the E2F transcription factor. Progression through the G1 phase depends on growth factor signaling but is also controlled by cell adhesion [51]. Integrin-mediated attachment to ECM supports the sustained MAP kinase signaling that is required for transcription of cyclin D. The cyclin D-cdk4/6 activity leads to partial phosphorylation of the Rb-E2F complex resulting in E2F-mediated transcription of cyclin E. The cyclin E/Cdk2 activity leads to further phosphorylation of the Rb-E2F complex and E2F-mediated transcription of cyclin A and entry in S. Each of these events requires integrin-mediated attachment [52]. Besides the transcriptional regulation of cyclin D through MAP kinase activity, adhesion-dependent activation of Rho GTPases also regulates levels and distribution of cdk inhibitors and cyclin D protein levels [53, 54]. FAK also plays an important role in adhesion control of cell proliferation: the ability of integrin-mediated adhesion to support cell cycle progression requires FAK activation [55].

**3.3. Pluripotency and Differentiation.** Integrin-mediated adhesion regulates the expression of genes associated with differentiation in several cell types. Differentiation of luminal epithelial cells in the mammary gland is impaired when  $\beta 1$  integrins are deleted [56, 57].  $\beta 1$  integrin-mediated attachment to the basement membrane supports signaling through the prolactin receptor and Stat5 to transcribe milk genes [58]. In the developing mammary gland,  $\beta 1$  integrin-mediated adhesion also controls basal-apical cell polarity, which is essential for lumen formation [59]. In myoblasts, integrin-mediated attachment to ECM is also important for expression of desmin and meromyosin and for cell fusion to form contracting myotubes [60]. It has also been shown that integrins can play important roles in restricting differentiation of skin keratinocytes to the suprabasal layers while promoting proliferation in the basal layer [61]. Keratinocytes stop cycling and undergo rapid differentiation characterized by involucrin expression when detached in vitro. In vivo ablation of the various candidate integrins in keratinocytes indicates that general cell adhesion rather than signaling by a specific integrin is required to suppress differentiation in the basal layer of the skin [62].

Stem cells communicate with their microenvironment, “the niche” [63]. The ECM is an intricate part of the niche, and integrin-ECM interactions allow locally embedded factors to support stemness [64].  $\beta 1$  integrin expression has been used as a marker for epithelial stem cell populations [65]. In the gut,  $\beta 1$  integrins are required to compartmentalize intestinal epithelial stem cell proliferation and differentiation through effects on hedgehog signaling [66]. In neuronal progenitors,  $\beta 1$  integrins coordinate Notch signaling, which is involved in cell fate decisions [67]. Mammary gland stem cell self-renewal is supported by  $\beta 1$  integrins [68]. Moreover, several studies show that integrins, in concert with cadherins, regulate centrosome positioning and spindle angle to control

the balance between symmetrical and asymmetrical divisions, which is a key determinant of stem cell properties [69].

**3.4. Force Transmission in Survival, Proliferation, and Differentiation Signaling.** The important role for integrins in sensing of and responding to mechanical aspects of the environment may also explain their role in survival and proliferation signaling. Experiments using micropatterned substrates have shown that cell spreading and cytoskeletal tension rather than the number of integrin-ligand bonds control cell survival [70]. Likewise, MAP kinase activity, cyclin D expression, and cdk inhibitor levels were not properly regulated when cells adhered to soft, rather than stiff collagen matrices leading these cells into quiescence [71]. Again, the ability to generate cytoskeletal tension appeared crucial. On rigid but not soft ECM substrates, FAK is activated causing Rac-mediated cyclin D1 gene induction and cyclin D1-dependent Rb phosphorylation [72]. ECM stiffness also controls angiogenesis in vitro and in vivo by Rho-dependent regulation of the balance between two mutually antagonistic transcription factors that influence expression of the VEGFR [73].

It has been demonstrated that physical properties of the ECM are decisive for lineage specification of mesenchymal stem cells. Soft substrates promote neuronal, and stiff substrates promote osteoblast lineages [74]. There is some debate over what stem cells precisely “feel.” In experiments using hydrogels, stiffness also correlates with ECM protein anchorage point density. The increased resistance to integrin pulling as a result of this in stiffer matrices has been proposed to control stem cell lineage decisions [75]. In either case, integrins will be crucial sensors of physical ECM properties in control of stem cell fate.

## 4. Integrin Expression and Function in Cancer

As discussed previously, integrins allow cells to sense chemical and physical information in their environment and can modulate cell signaling pathways and gene expression profiles in response to that. In doing so, integrins control cell survival, proliferation, and differentiation, which are disrupted from normal environmental control in cancer [76]. One of the hallmarks of cancer cells is their ability to grow in an anchorage-independent fashion. They grow in vitro in semisolid media that do not support growth and lead to anoikis of nontransformed cells [77]. Nevertheless, interactions with the surrounding ECM and neighboring cells also control cancer cell behavior. Numerous studies have shown that integrin expression profiles are subject to change during cancer growth and progression and that such changes contribute to the aggressive behavior of cancer cells [78–81]. Notably, besides tumor cell-autonomous functions, integrins also play important roles in processes in the tumor microenvironment that contribute significantly to tumor progression.

**4.1. Roles for Tumor Cell Integrins.** Transformation of rodent fibroblasts and kidney cells by Src or Ras oncogenes is associated with a decreased synthesis of fibronectin or a loss of

the fibronectin receptor, integrin  $\alpha 5\beta 1$  [82, 83]. This plays a causal role in the oncogenic transformation: ectopic expression of  $\alpha 5\beta 1$  can restore fibronectin matrix assembly and suppress tumor formation in mice of transformed Chinese hamster ovary cells [84]. This appears to reflect a relevant mechanism for human cancer as loss of the tumor suppressor p16INK4a prevents anoikis through downregulation of  $\alpha 5\beta 1$  expression levels [85]. On the other hand, point mutants of the p53 tumor suppressor have been shown to stimulate invasion of cancer cells through enhanced trafficking of integrin  $\alpha 5\beta 1$  and EGFR [86]. Thus, the role of  $\alpha 5\beta 1$  in cancer is context dependent, with distinct and even opposite effects on cancer growth or progression depending on the tumor suppressor or oncogene profile.

The role of  $\alpha \nu\beta 6$  in TGF $\beta$  activation may be important for epithelial-to-mesenchymal transitions (EMT) in cancer. Enhanced expression of this integrin is associated with EMT and poor prognosis in colon carcinoma [87]. Crosstalk with TGF $\beta$  has also been reported for  $\alpha 6\beta 4$ : transgenic suprabasal expression of  $\alpha 6\beta 4$  leads to inhibition of TGF $\beta$  signaling, which relieves TGF $\beta$ -mediated suppression of epithelial proliferation causing tumor growth [88]. Indeed, expression of  $\alpha 6\beta 4$  is frequently enhanced in squamous cell carcinomas and correlates with poor prognosis in patients [89]. Increased expression of  $\alpha 6\beta 4$  in breast cancer cells and secretion of its ECM ligand, laminin-5, support NF $\kappa$ B-mediated survival [90]. In gastric carcinoma cells overexpressing the Met receptor,  $\alpha 6\beta 4$  can associate with and activate Met, which stimulates invasion and metastasis [91].  $\alpha 6\beta 4$  appears to play a dual role in growth and progression of squamous cell carcinomas: initial loss of  $\alpha 6\beta 4$  is associated with tumor growth in squamous cell carcinoma [92]. During progression of skin cancer,  $\alpha 5\beta 1$  is unregulated [93]. This switch from laminin-binding to fibronectin-binding integrins may facilitate detachment from the basement membrane and entry into the stromal compartment. A related switch occurs in melanoma progression when the radial growth phase converts to a vertical growth phase. This is associated with the induction of  $\alpha 5\beta 1$  and  $\alpha \nu\beta 3$  expression, and forced expression of  $\alpha \nu\beta 3$  is sufficient to trigger a conversion from radial to vertical growth [94, 95]. Enhanced matrix degradation through matrix metalloproteases may explain the effect of these integrins on the invasive growth of melanoma [96]. Interestingly, individuals homozygous for an activating polymorphism in the  $\beta 3$  subunit have an increased risk to develop breast cancer, ovarian cancer, or melanoma, indicating that  $\alpha \nu\beta 3$  may involve those cancers as well [97].  $\alpha \nu\beta 3$  expression has also been implicated in bone metastasis in prostate and breast cancer patients [98–101]. One remarkably specific function of  $\alpha \nu\beta 3$  is relevant in the context of the Src oncogene: the  $\beta 3$  subunit cytoplasmic tail binds c-Src and supports its activation, which drives anchorage-independent growth and tumor formation (without effects on morphological Src-mediated transformation) in pancreatic and other cancers [102–104].

The ability of integrins to mediate tensional homeostasis may also be important in cancer. Increased cytoskeletal contractility through Rho GTPases in cancer cells can exert forces on ECM through integrins, resulting in environmental stiffening. A stiffer ECM in turn exerts forces on

the cytoskeleton through integrins. This feed forward loop leading to increased tension may act as a driving force in cancer progression [105].

In melanoma, the expression of  $\alpha 4\beta 1$  correlates with tumor progression [106]. This integrin may be important for tumor cell interactions with VCAM receptors on the endothelial cells allowing arrest and extravasation [107, 108]. Another integrin that has been associated specifically with metastasis is  $\alpha 2\beta 1$ ; forced expression of this integrin in rhabdomyosarcoma cells stimulated metastasis but did not affect primary tumor growth [109].

*4.2. Lessons from Disruption of Integrin Signaling in Transgenic Mouse Models for Cancer.* The role of the  $\beta 1$  family of integrins in cancer initiation, growth, and metastasis has been analyzed in transgenic mouse models. Studies using (conditional) genetic models point to critical roles for  $\beta 1$  integrins in initiation, growth, or progression of a variety of cancers (Table 1). In the context of the Polyoma middle T (PyMT) oncogene, deletion of  $\beta 1$  integrins essentially blocks breast cancer initiation, indicating that transformed cells require  $\beta 1$  integrins to survive. However, in the context of the ErbB2 oncogene, initiation is only delayed. Still, the role for integrin signaling is apparent, as these tumors are smaller, showing less angiogenesis and more apoptotic cells. As a consequence, metastasis to the lungs is drastically impaired. Interestingly, FAK/Src signaling and EGFR phosphorylation is impaired in these tumors. Likewise, in the RIPTag model for pancreatic cancer, deletion of  $\beta 1$  integrins leads to impaired tumor growth and metastasis, and deletion of the  $\alpha 2\beta 1$  integrin also impairs the outgrowth of squamous cell carcinomas. However, in other cancer models,  $\beta 1$  integrins appear to play a tumor suppressor-like role. Deletion of  $\beta 1$  integrins in the TRAMP prostate adenocarcinoma model led to more dramatic expansion of the tumor cell population, enhanced the rate of prostate tumor progression, and decreased overall animal survival [110]. Also, the initiation and outgrowth of ErbB2/Neu-induced mammary tumors were not affected by  $\alpha 2\beta 1$  deletion, but intravasation and metastasis were increased [111]. Taken together,  $\beta 1$  integrins can play remarkably distinct roles in tumor growth, progression, and metastasis depending on the model system. It will be important to understand the apparent metastasis suppressor activity that is conferred by integrins in certain contexts.

The major integrin-associated signal transducer that has been investigated in mouse models for cancer is FAK (Table 2). Four different studies have investigated the role of FAK in PyMT-induced breast cancer, and all four provide evidence that FAK plays an important role in primary tumor growth and progression. Tumor initiation, survival, and proliferation of tumor cells, maintenance of a cancer stem/progenitor cell population, and progression to carcinoma and metastasis are all supported by FAK. Likewise, FAK promotes the formation and growth of chemically induced skin cancer as well as its progression from papilloma to carcinoma.

*4.3. Effects of Integrins in the Microenvironment.* Importantly, integrin signaling not only regulates cancer growth and progression in a tumor cell autonomous fashion. Integrins

TABLE 1: Transgenic mouse models  $\beta 1$  and  $\alpha v$  integrins.

Model	Cancer type	Effect on tumor growth	Effect on metastasis	Reference
$\beta 1^{fl/fl}$ ; MMTV-Cre; PyMT	Breast cancer	Impaired initiation; reduced growth	Not studied	[112]
$\beta 1^{fl/fl}$ ; MMTV-Cre; ErbB2	Breast cancer	Delayed initiation; reduced growth; increased apoptosis; reduced angiogenesis	Reduced lung metastasis	[113]
$\alpha 2^{-/-}$ ; K14-HPV16	Human papilloma virus-induced squamous carcinoma	Reduced growth; role for tumor cell integrin confirmed after orthotopic transplantation	Decreased progression from papilloma to dysplasia but no effect on subsequent progression to carcinoma	[114]
$\beta 1^{fl/fl}$ ; A RR2PBi-Cre; TRAMP <sup>tg/+</sup>	Prostate cancer	Increased proliferation; decreased differentiation	Enhanced progression to poorly differentiated carcinoma	[110]
$\alpha 2^{-/-}$ ; MMTV-c-ErbB2/Neu	Breast cancer	Delayed onset; no effect on tumor growth; no effect on apoptosis; no effect on proliferation	Increased intravasation; increased metastasis	[111]
$\beta 1^{fl/fl}$ ; RCre; RIP1Tag2	Pancreatic $\beta$ cell carcinoma (insulinomas)	Increased senescence; decreased proliferation	Blocked tumor formation and metastasis after transplantation	[115]
Rag2 <sup>KO</sup> ; $\beta 3^{KO}$ ; $\beta 5^{KO}$ ; xenografted WT tumor cells	Melanoma and lung carcinoma xenografts	Increased tumor angiogenesis; increased tumor growth	Not studied	[116]
Rag2 <sup>KO</sup> ; $\beta 3^{KO}$ ; $\beta 5^{KO}$ ; wild type or KO bone marrow transplantations; xenografted WT tumor cells	Melanoma and colon carcinoma xenografts	Increased tumor angiogenesis; increased tumor growth; reduced macrophage infiltration	Not studied	[77]

TABLE 2: Transgenic mouse models: FAK.

Model	Cancer type	Effect on tumor growth	Effect on metastasis	Reference
FAK <sup>fl/fl</sup> ; MMTV-Cre; PyMT	Breast cancer	Delayed initiation; decreased growth	Decreased lung metastasis	[117]
FAK <sup>fl/fl</sup> ; MMTV-Cre; PyMT	Breast cancer	Reduced tumor cell proliferation	Blocked progression from hyperplasia to carcinoma; blocked metastasis	[118]
FAK <sup>fl/fl</sup> ; MMTV-Cre; PyMT	Breast cancer	Reduced initiation; reduced proliferation; reduced cancer stem/progenitor cell population	Not studied	[119]
FAK <sup>fl/fl</sup> ; MMTV-Cre; PyMT	Breast cancer	Reduced initiation; reduced proliferation; increased apoptosis	Decreased progression from hyperplasia to carcinoma	[120]
FAK <sup>fl/fl</sup> ; K14-CreERT2; two-stage chemical carcinogenesis protocol	Skin cancer	Reduced initiation; reduced growth; increased apoptosis	Reduced progression from papilloma to squamous cell carcinoma	[121]

on various cell types in the tumor microenvironment may be equally relevant. For instance, tumor angiogenesis is important for tumor growth and may be instrumental in the metastatic cascade for intravasation [76]. As discussed previously, integrin-mediated adhesion provides endothelial cells with critical survival cues. Expression of  $\alpha v\beta 3$  and  $\alpha v\beta 5$  is induced on endothelial cells during angiogenesis. Based on studies using blocking antibodies, these integrins support the activities of distinct proangiogenic soluble factors [122]. However,  $\alpha v$  null embryos develop normally to E9.5 with extensive vasculogenesis and angiogenesis [123], and mice lacking  $\alpha v\beta 3$ ,  $\alpha v\beta 5$ , or both show no defects in angiogenesis [116]. In the context of cancer, tumor angiogenesis and tumor growth are in fact increased when wild type tumor cells are xenografted in  $\alpha v\beta 3$  knockout or  $\alpha v\beta 3/\alpha v\beta 5$  double knockout mice,

indicating that the blocking antibodies targeting these integrins may have acted as agonists [77, 116]. Interestingly, fewer tumor-infiltrating (but not circulating) macrophages are observed in the knockout mice, and bone marrow transplantation experiments indicate that the  $\alpha v$  integrins are required for tumor suppression by macrophages [77].

Integrin  $\alpha 5\beta 1$  is also induced on angiogenic blood vessels, and its interaction with fibronectin supports angiogenesis [124]. This same integrin may also play an important role on cancer-associated fibroblasts during invasion of squamous cell carcinomas. Fibroblasts remodel ECM to lay tracks along which carcinoma cells can invade the stroma and  $\alpha 5\beta 1$ - and  $\alpha 3\beta 1$ -mediated adhesion is important for Rho GTPase activity, contractility, and ECM remodeling [125]. Besides regulating tumor invasion, cancer-associated fibroblasts also

play important roles in tumor growth. In nonsmall cell lung cancer (NSCLC),  $\alpha 11\beta 1$  on these cells is needed to promote IGF2 expression, which in turn supports tumor growth [126].

The ability of  $\alpha v\beta 6$  and other RGD-binding integrins to control activation of TGF $\beta$  and TGF $\beta$ R signaling may also impact tumor cells as well as cancer-associated fibroblasts.  $\alpha v\beta 6$  is upregulated at the tumor-stroma interface of various squamous cell carcinomas. Local  $\alpha v\beta 6$ -mediated activation of TGF $\beta$  would inhibit proliferation of epithelial cells but also affect the complex interplay between carcinoma cells and stromal cells that control cancer progression [106]. Another recent example of the important role of integrins in other cell types is seen in the remodeling that takes place in lymph nodes to accommodate homing and outgrowth of cancer cells. Tumor cells may create this “metastatic niche” by secreting VEGF, which reaches lymph nodes and locally stimulates lymphangiogenesis and PI3 K-mediated activation of integrin  $\alpha 4\beta 1$ . Thus, an adhesive surface is created where disseminating tumor cells can home through their VCAM receptors and form metastatic colonies [127].

## 5. Current Clinical Use of Integrins as Targets for Anticancer Therapy

**5.1.  $\beta 1$  and  $\alpha v$  Integrins as Therapeutic Targets in Cancer.** From what is mentioned earlier, it is evident that integrins and integrin signaling pathways may represent candidate targets to interfere with cancer growth and progression. Disrupting integrin-ligand interactions has the potential to interfere with key survival and proliferation signals that support cancer growth. Importantly, as discussed, integrin inhibition may simultaneously target key aspects of tumor cell behavior as well as crucial features of the tumor microenvironment such as angiogenesis or functions of cancer-associated fibroblasts that support cancer growth or invasion. Early studies showed that blocking a large subset of integrins, including  $\alpha 5\beta 1$ ,  $\alpha v\beta 3$ ,  $\alpha v\beta 5$ , by using RGD peptides can interfere with tumor cell invasion in vitro and metastasis in mouse models [128]. Subsequently, various synthetic peptides containing the RGD sequence or other integrin binding sequences, nonpeptide RGD mimetics, and disintegrins (integrin-binding proteins isolated from viper snake venoms) have been demonstrated to be able to block experimental tumor cell metastasis in animal model systems [129]. Interestingly, similar approaches could at the same time inhibit tumor angiogenesis [130, 131]. Based on these initial promising findings, a variety of RGD-related peptides, peptides covering alternative integrin recognition motifs, nonpeptide mimetics, and humanized integrin-directed antibodies have been developed. These are in various stages of (pre) clinical testing or already on the market for a variety of diseases. In the context of cancer treatment, at present strategies for targeting  $\beta 1$  integrins or  $\alpha v$  integrins have entered phase I, phase II, and even phase III clinical trials [132–134] (Table 3).

A humanized version of the LM609 anti- $\alpha v\beta 3$  antibody, *vitaxin*, later developed into *etaracizumab*, was among the first to enter clinical testing. In Phase I and Phase II studies toxicity was low. Some signs of efficacy have been observed in

melanoma and other solid tumors, but based on a randomized Phase II study where efficacy was compared to standard chemotherapy in metastatic melanoma, further clinical development appears to have been stopped [135–138]. *CTNO 95*, an  $\alpha v$  antibody targeting  $\alpha v\beta 3$  as well as  $\alpha v\beta 5$ , has also been tested in Phase I and showed little toxicity and some antitumor activity [139]. The cyclic RGD peptide, *cilengitide*, selectively blocks  $\alpha v\beta 3$  and  $\alpha v\beta 5$ . It has already gone through Phase I and Phase II trials for lung cancer, prostate cancer, and glioblastoma and is currently tested in Phase III for glioblastoma treatment [134, 140–143]. As discussed, such approaches targeting  $\alpha v$  integrins may target tumor cells as well as the tumor microenvironment, for example, angiogenic vessels. Because of these potentially versatile effects, efficacy could be high, and further testing, for instance, with higher doses, seems worthwhile. On the other hand, this also means that the mode of action is poorly understood, and the apparent opposite results, for instance, of pharmaceutical inactivation and gene deletion in the case of  $\alpha v\beta 3$  and  $\alpha v\beta 5$  in the context of tumor angiogenesis remain puzzling [116, 122, 130]. Importantly, treatment with low-dose RGD peptides can actually cause enhanced VEGF-mediated angiogenesis and tumor growth [144], clearly indicating that the effects in patients can be unanticipated.

Another integrin that has emerged as a potential target for anticancer therapy is  $\alpha 5\beta 1$ . Again, as discussed previously, this integrin plays important roles on tumor cells, cancer-associated fibroblasts, and angiogenic vessels. Based on the latter, a humanized antibody, *volociximab*, was developed as an antiangiogenic agent. In a Phase I trial, *volociximab* showed little toxicity in patients with solid tumors, and in two cases disease stabilization in response to treatment was observed [145]. An interesting alternative means of targeting  $\alpha 5\beta 1$  makes use of the fact that this integrin requires a second recognition motif, in addition to RGD, for its interaction with fibronectin [146]. *ATN-161* was derived from this so-called “synergy sequence,” PHSRN and inhibits growth and metastasis in animal models [147, 148]. *ATN-161* has been tested in a Phase I clinical trial where no dose-limiting toxicities occurred in patients with advanced solid tumors [149]. Strikingly, one in three patients manifested prolonged stable disease in this study, clearly warranting further testing in Phase II trials.

**5.2. Inhibition of FAK.** Several FAK inhibitors have been developed and have entered cancer trials (Table 3). The FAK homologue, Pyk2, can compensate for lack or inhibition of FAK activity. Therefore, design of FAK inhibitors preferably leads to dual specificity compounds blocking both FAK and Pyk2. The early FAK-specific inhibitors, PF-573228 and NVP-TAC544 from Pfizer and Novartis, respectively, served as backbones for derivatives showing such dual specificity that are in early stages of clinical development. PF-562271 is an ATP-competitive, reversible inhibitor of catalytic activity of FAK and Pyk2. PF-562271 treatment led to dose- and drug exposure-dependent tumor regression in several human-mouse xenograft models without weight loss, morbidity, or mortality [150]. This compound is in Phase I testing for

TABLE 3: Integrin (signaling) inhibitors in anticancer therapy.

Compound	Sponsor	Target	Cancer type	Stage
Vitaxin/etaracizumab	MedImmune Inc., NCI	$\alpha v\beta 3$	Solid tumors, melanoma, colorectal cancer, small intestine cancer, lymphoma, prostate cancer, and renal cell cancer	Phase I, II
CTNO 95	Centocor Inc.	$\alpha v$ integrins	Solid tumors, melanoma, and prostate cancer	Phase I, II
Cilengitide (cyclic RGD)	EMD Pharmaceuticals, Merck	$\alpha v\beta 3, \alpha v\beta 5$	Lung cancer, prostate cancer, melanoma, glioblastoma, leukemia, brain and CNS tumors, breast cancer, and squamous cell cancer	Phase I, II
Volociximab	Protein Design Labs	$\alpha 5\beta 1$	Solid tumors, melanoma, ovarian cancer, renal cancer, pancreatic cancer, and lung cancer	Phase I, II
ATN-161 PHSRN mimetic	Attenuon	$\alpha 5\beta 1$ and other fibronectin-binding integrins	Solid tumors, renal cell carcinoma, and brain and CNS tumors	Phase I
PF-00562271	Verastem Inc.	FAK, Pyk2	Head and neck cancer, prostate cancer, and pancreatic cancer	Phase I
GSK2256098	GlaxoSmithKline	FAK, Pyk2	Solid tumors	Phase I
PF-04554878	Verastem Inc.	FAK, Pyk2	Metastatic, nonhematologic tumors	Phase I
VS-4718	Verastem Inc.	FAK, Pyk2	Metastatic, nonhematologic tumors	Phase I
VS-6063	Verastem Inc.	FAK, Pyk2	Ovarian cancer	Phase I

various solid tumors, and initial results with respect to safety and sporadic cases of improved tumor-related symptoms appear promising [151]. Another inhibitor is TAE-226 (Novartis) that was developed as a FAK inhibitor but also effectively inhibits IGF1R and is not in clinical development currently [152, 153]. Several other FAK/Pyk2 inhibitors are in Phase I testing. Like strategies targeting integrins, antitumor activity with FAK inhibitors may be due to effects on tumor cells or effects on other supporting cell types, such as endothelial cells. Moreover, although FAK is an important player in integrin signaling, effects of these inhibitors may be unrelated to the role of FAK in integrin adhesion complexes. Rather, FAK interacting physically and functionally with transcriptional regulators (e.g., p53) or RTKs (e.g., EGFR) may be the relevant target.

**5.3. Combinatorial Treatment: Sensitization to Radio-, Chemo-, or Targeted Therapy.** Ongoing clinical trials that further evaluate the potential of integrin-blocking strategies described previously usually do so in the context of chemo- or existing targeted therapy. This likely is the most successful application of peptides and antibodies targeting integrins: disrupting prosurvival and proliferation signals in tumor cells and other cell types in the tumor microenvironment that depend on integrin-mediated adhesion and thereby (i) weaken tumor cells directly (e.g., blocking tumor cell interactions with their environment) to render them more sensitive to other therapies, (ii) weaken tumor cells indirectly by corrupting essential input from the environment (e.g., loss of oxygenation by killing endothelial cells; loss of paracrine stimuli from stromal cells), and (iii) preventing therapy-induced responses in the microenvironment that protect

tumor cells (e.g., blocking the enhanced  $\alpha v\beta 3$ -mediated endothelial cell survival triggered by irradiation that would protect cancer cells through enhanced angiogenesis [154]).

Resistance to radio-, chemo-, or targeted therapies represents a major hurdle in cancer therapy. Disrupting adhesion signals that allow cancer cells to evade therapy may significantly improve therapy. Attachment in vitro of small cell lung cancer (SCLC) cells to two-dimensional ECM substrates containing proteins that typically surround SCLC tumors confers protection against apoptosis induced by doxorubicin, cyclophosphamide, and etoposide [155]. This chemoprotective signal can be disrupted using  $\beta 1$  antibodies. ECM attachment also suppresses breast cancer cell apoptosis induced by paclitaxel and vincristine, and  $\beta 1$  antibodies sensitize to these microtubule disruptors that are commonly used in breast cancer therapy [156]. Likewise, attachment of DU145 prostate cancer cells to fibronectin through  $\beta 1$  integrins protects against ceramide or docetaxel [157]. The role of  $\beta 1$  integrins in determining chemosensitivity in 2D cultures appears context dependent: in Src-transformed cells expression of  $\beta 1$  integrins promotes sensitivity to cisplatin and other genotoxicants [158].  $\beta 1$  integrin-mediated ECM attachment also protects against radiation-induced genotoxic injury [159], and interfering with  $\beta 1$  integrins or specifically with  $\alpha 5\beta 1$  integrin-mediated adhesion can also enhance sensitivity to radiotherapy of different human cancer cell types grown in three-dimensional cultures or as xenografts in mice [160–162]. Also, ovarian cancer ascites has been shown to confer protection against TRAIL-induced apoptosis through integrin  $\alpha v\beta 5$  [163]. Activities of FAK and PKB/AKT have been implicated in several of these integrin-mediated protective signaling pathways. Lastly, integrins have also been identified as potential targets for improved efficacy of targeted

therapies: disruption of integrin-mediated laminin adhesion complexes that signal through FAK-sensitized ErbB2 positive breast cancer cells to trastuzumab and lapatinib, antibodies targeting the extracellular and kinase domains of ErbB2 [164].

## 6. Concluding Remarks

Integrins allow cells to interact with their local environment and translate external chemical and physical cues into a concerted intracellular response. A number of distinct connections have been described from integrin adhesion complexes to regulation of gene expression, including local concentration of enzymes and substrates to trigger intracellular signaling, harnessing signal transduction cascades downstream from other receptors, such as RTKs, and physical connections through cytoskeletal elements from integrins to the nucleus that may provide mechanical control of gene transcription. Together, such mechanisms govern cell survival, proliferation, and differentiation. Integrin signaling, like most signal transduction cascades, is typically rewired in cancer cells, but many studies have shown that integrins still regulate tumor growth, progression, and metastasis. Interfering with integrin-mediated attachment or preventing integrin signaling has the potential to disrupt key survival or proliferation cues both in tumor and tumor-associated cells including, for instance, endothelial cells. Altogether, this can lead to tumor shrinkage and increased sensitivity to existing radio-, chemo-, or targeted therapies. Studies using genetically engineered mice support this idea but also show that in some contexts integrins mediate tumor (metastasis) suppressive effects, indicating that use of integrin antagonists may trigger unwanted outcomes. Clearly, much more mechanistic insight is required to determine which integrin-blocking strategies may be applied to which types and stages of cancer. Nevertheless, initial promising results with integrin inhibitors in clinical trials warrant continued translation of findings obtained in cell culture systems to in vivo cancer models and testing in clinical trials. Ultimately, strategies will hopefully be designed where disruption of integrin signaling synergizes with genotoxic and/or other targeted antitumor strategies to effectively eradicate tumors.

## Conflict of Interests

The author declares no direct financial relation with any commercial entities mentioned in the paper that might lead to a conflict of interests.

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