INTEGRIN SIGNALLING DURING TUMOUR PROGRESSION

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Abstract | During progression from tumour growth to metastasis, specific integrin signals enable cancer cells to detach from neighbouring cells, re-orientate their polarity during migration, and survive and proliferate in foreign microenvironments. There is increasing evidence that certain integrins associate with receptor tyrosine kinases (RTKs) to activate signalling pathways that are necessary for tumour invasion and metastasis. The effect of these integrins might be especially important in cancer cells that have activating mutations, or amplifications, of the genes that encode these RTKs.

INTERSTITIAL MATRIX
The extracellular matrix that resides in connective tissues.

TYPE-I TRANSMEMBRANE PROTEIN
A protein that contains a single membrane-spanning domain, with the carboxyl terminus orientated towards the cytoplasm and the amino terminus orientated towards the lumen of membrane compartments or in an extracellular direction.

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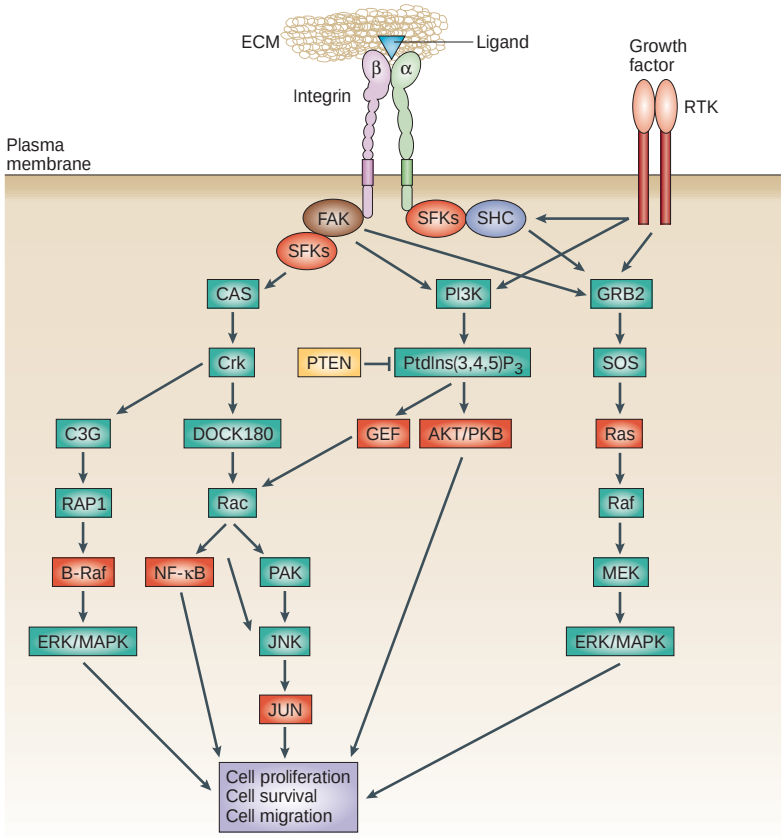
Throughout their lives, cells secrete, assemble and remodel an insoluble network of proteins — the extracellular matrix (ECM). As well as providing a pliable, but resistant, scaffold for the organization of cells in tissues, the ECM exerts an extraordinary control on the behaviour of cells. It is able to dictate whether they will proliferate or undergo growth arrest, migrate or remain stationary, and thrive or undergo apoptotic death. There are two main forms of ECM — the basement membrane and the INTERSTITIAL MATRIX. Each varies in its composition and properties depending on the identity/location of the cells/tissues that it surrounds and the developmental stage of the organism. The effects of the ECM on cells are mainly mediated by the integrins, a large family of cell-surface receptors that bind — and therefore mediate adhesion to — ECM components, organize the cytoskeleton, and activate intracellular signalling pathways. Each integrin consists of two TYPE-I TRANSMEMBRANE subunits: α and β. In mammals, 18 α and 8 β subunits associate in various combinations to form 24 integrins that can bind to distinct, although partially overlapping, subsets of ECM ligand[1,2].

The integrins transmit both mechanical and chemical signals. As well as imparting polarity to the cell and organizing and remodelling its cytoskeleton during adhesion and migration, these signals exert a stringent control on cell survival and cell proliferation. Most integrins activate focal adhesion kinase (FAK) and thereby also SRC-FAMILY KINASES (SFKs), which causes the phosphorylation of, and therefore signalling from, p130CAS and paxillin. A subset of integrins, including $\alpha_1\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$, also activates a pathway that is mediated through the adaptor protein SHC through a PALMITOYLATED SFK, such as Fyn or Yes. Further complexity arises from the existence of integrin-specific mechanisms of signalling, such as those that are exemplified by $\alpha_6\beta_4$ and $\alpha_2\beta_1$ (REFS 3–5; BOX 1). Despite this complexity and specificity, the essence of integrin signalling — that is, what integrins do — is simple: they promote cell survival and impart positional control to the action of RTKs, and therefore determine whether cells proliferate and migrate in response to soluble growth factors and cytokines.

Dominant mutations in oncogenes and recessive mutations in tumour-suppressor genes disrupt the regulatory circuits that control cell fate, conferring on neoplastic cells the ability to survive and proliferate even if appropriate extracellular cues are not available[6]. As a consequence, cells that have undergone neoplastic transformation are much less dependent on ECM adhesion for their survival and proliferation[1]. But despite their relative anchorage independence, cancer cells still benefit from integrin signals, both during tumour initiation and tumour progression[7,8]. It is increasingly clear that neoplastic cells enhance the expression of integrins that favour their proliferation, survival and migration, whereas they tend to lose

Box 1 | Integrin signalling



Autonomous integrin signalling

Integrins signal predominantly through the recruitment and activation of Src-family kinases (SFKs). Most integrins recruit focal adhesion kinase (FAK) through their β subunits. As well as activating signalling from phosphatidylinositol 3-kinase (PI3K) to AKT/protein kinase B (PKB) through phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3), FAK functions as a phosphorylation-regulated scaffold to recruit Src to focal adhesions. Here, Src phosphorylates p130CAS and paxillin, which recruits the Crk–DOCK180 complex, and thereby results in the activation of Rac. Rac then leads to the activation of p21-activated kinase (PAK), Jun amino-terminal kinase (JNK), and nuclear factor κB (NF-κB)[121–123]. FAK also activates extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) by recruiting the RAP1 guanine nucleotide-exchange factor (GEF) C3G through Crk. RAP1 then activates ERK/MAPK through B-Raf. Alternatively, FAK can activate ERK/MAPK by recruiting the growth-factor-receptor-bound-2 (GRB2) and son-of-sevenless (SOS) complex. Certain integrins, including $\alpha_5\beta_1$, $\alpha_1\beta_1$ and $\alpha_v\beta_3$, are coupled to palmitoylated SFKs, such as Fyn and Yes, through their α subunits. In this pathway, caveolin-1 functions as a transmembrane adaptor to facilitate the recruitment of Fyn and Yes. The palmitoylated SFKs recruit and phosphorylate the adaptor SHC, which combines with GRB2–SOS to activate ERK/MAPK signalling from Ras[91,124]. Some integrins can also directly interact with SFKs through the cytoplasmic domain of their β subunits[125]. One integrin, $\alpha_6\beta_4$, is palmitoylated, and it combines with SFKs that are similarly palmitoylated in LIPID RAFTS[126]. The SFKs phosphorylate several tyrosine residues in the cytoplasmic domain of β_4, which causes the recruitment of SHC and activation of Ras–ERK/MAPK and PI3K signalling[81,83,127,128]. The pathways that integrins activate through SFKs are sufficient to induce cell migration and to confer some protection from apoptosis on cells.

Joint integrin–RTK signalling.

The integrins impart positional control to the action of receptor tyrosine kinases (RTKs). Several mechanisms, which function at the membrane-proximal level as well as further downstream, ensure proper integration of integrin- and RTK-dependent signals[3,4]. Examples of these mechanisms are discussed in the main text. Joint integrin–RTK signalling is required for cell proliferation and for optimal cell survival and cell migration/invasion. MEK, MAPK and ERK kinase.

expression of the integrins that exert the opposite effect (BOX 2). Although integrin signalling contributes to primary tumour growth, this review will focus on the molecular basis of integrin function during tumour invasion and metastasis. We propose that dysregulated integrin–RTK signalling is crucial to tumour invasion and metastasis.

The metastatic cascade

Cancer cells spread throughout the body by metastasis. Both genetic and epigenetic changes contribute to the emergence of cells with metastatic capability within a primary tumour. Recent gene-expression analyses indicate that metastatic subclones probably arise from primary tumours that have already progressed to the invasive stage[9]. Several sequential, obligatory steps must then be completed for metastasis to occur (FIG. 1). First, cancer cells need to detach from neighbouring cells, degrade the basement membrane and penetrate into the interstitial stroma. This is an important transition, as tumours that are removed before they complete this process do not generally recur[10]. Second, tumour cells penetrate into blood vessels and lymphatic vessels in a process that is known as intravasation, which thereby gains them access to the circulatory system. To enter into blood vessels, tumour cells must traverse the endothelial basement membrane and disrupt the cell–cell junctions that seal their lumina. Blood vessels in tumours are characterized by a series of abnormalities, including discontinuities of the basement membrane and 'frayed' cell–cell junctions, which facilitate the intravasation of cancer cells[11]. Lymphatic vessels, on the other hand, lack a continuous basement membrane and TIGHT JUNCTIONS, and so provide an accessible route for cancer cells to spread to regional lymph nodes[12]. After reaching the bloodstream, either directly or through the lymphatic system, tumour cells often adhere to PLATELETS and leukocytes, forming emboli that stop in the microcirculation of target organs more easily than isolated tumour cells[13,14]. Finally, metastatic cells exit the bloodstream — by a process that is known as extravasation — and undergo expansive growth within the parenchyma of the target organ. The expansion of metastases follows requirements that are similar to those that have been identified for primary tumours, including the need for a supportive stroma and an adequate blood supply[6].

A set of acquired capabilities contributes to the deadly behaviour of metastatic cells. First and most pre-eminent is the ability to move through, and thereby invade, other tissues. To break away from their tissue of origin, metastatic cells have to loosen their adhesions to neighbouring cells and the basement membrane, acquire a migratory phenotype, and degrade or remodel all the ECMs that impose barriers to their dissemination. As discussed below, changes in adhesion signalling are key to the acquisition of the capacity for tissue invasion. Second, metastatic cells have to induce angiogenesis to escape the limits that passive diffusion of nutrients and oxygen impose on tumour growth. In fact, it is estimated that tumours and their metastases cannot grow beyond a relatively limited size unless they elicit an

Box 2 | Integrin signalling in primary tumour growth

Integrins and receptor tyrosine kinases (RTKs) exert a joint control on survival and mitogenic pathways[3]. Activating mutations in oncogenes and loss-of-function mutations in tumour-suppressor genes cause dysregulated activation of these intracellular-signalling pathways. Such oncogenes include Src-family kinases (SFKs), Ras, various GUANINE NUCLEOTIDE-EXCHANGE FACTORS (GEFs), AKT/protein kinase B (PKB), B-Raf, nuclear factor κB (NF-κB) and c-Jun; and such tumour-suppressor genes include phosphatase and tensin homologue (PTEN), which is a phosphatase of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3). These mutations seem to bypass the requirement for both growth factors and extracellular matrix (ECM) signals, which renders cancer cells self-sufficient for growth and insensitive to apoptotic stimuli (see BOX 1 figure). However, constitutive activation of RTKs enhances mitogenic and survival signalling even in cancer cells that carry oncogenic mutations of cytoplasmic signalling molecules. We hypothesize that the integrins that can cooperate with RTKs enhance mitogenic and survival signalling most effectively in cancer cells that secrete autocrine growth factors or express amplified or activated RTKs. For example, the expression of $\alpha_6\beta_4$ or $\alpha_v\beta_3$ is upregulated in certain cancers[19,21]. The $\alpha_6\beta_4$ integrin cooperates with the receptor for epidermal growth factor (EGF), with ERBB2, and with Met — and so it is likely to promote the growth of carcinomas with activating mutations or amplifications of the genes that encode these RTKs[19,84,87,129]. Similarly, $\alpha_v\beta_3$ might cooperate with the platelet-derived growth factor (PDGF) receptor to enhance the growth of gliomas that secrete large amounts of PDGF[130,131]. Conversely, the expression of other integrins that induce growth-inhibitory signals might need to be downregulated for tumours to grow efficiently. Integrin-mediated inhibition of cell proliferation occurs by direct as well as indirect mechanisms: $\alpha_2\beta_1$ can activate the p38 mitogen-activated protein kinase, which inhibits cell-cycle progression[92,93]; whereas $\alpha_v\beta_6$ and $\alpha_v\beta_8$ bind to latent transforming growth factor-β (TGF-β) and contribute to its activation, thereby exerting a growth-inhibitory effect on carcinoma cells that have not lost sensitivity to the cytostatic action of TGF-β[39,40].

angiogenic response[15]. Furthermore, angiogenesis provides a gateway for tumour cells to enter the circulation and, in the reverse direction, for leukocytes to infiltrate the tumour and provide proteolytic enzymes and chemokines, which facilitate the migration and invasion of tumour cells[16]. Increasing amounts of evidence now imply that integrin signalling has a key role in tumour angiogenesis[17,18]. Third, metastatic cells have to survive in various foreign microenvironments before they colonize their target organ, and they have to survive and proliferate within the stroma of this new organ. Various joint integrin–RTK signals confer these traits on tumour cells during metastasis.

Switches in integrin usage

Pathological studies of human cancer have provided evidence that tumour cells switch their integrins. These changes in integrin expression are complex and depend on the tissue of origin of the tumour, its histological type, and the stage of progression of the disease[19–23]. Although some oncogenes enhance the expression of some integrins and decrease the expression of others by a direct signalling mechanism[24], most switches in integrin expression and usage are likely to be the result of selective pressures that are exerted by the host on genetically unstable cancer cells. As mentioned above, neoplastic cells tend to lose the integrins that secure their adhesion to the basement membrane and help them to remain in a quiescent, differentiated state; and they maintain or overexpress the integrins that foster their survival, migration and proliferation during tumour invasion and metastasis. Although cell-type-dependent changes in integrin signalling make it impossible to rigidly assign each of the integrins to the 'anti-neoplastic' or the 'pro-neoplastic' category, present evidence indicates that $\alpha_2\beta_1$ and $\alpha_3\beta_1$, at least in some cases, suppress tumour progression, whereas $\alpha_v\beta_3$, $\alpha_v\beta_6$ and $\alpha_6\beta_4$ often promote it.

The cellular basis of tissue invasion

Because most human cancers are CARCINOMAS, the transition from ADENOMA to invasive carcinoma has been the subject of intensive investigation. The results indicate that this transition is driven by a discrete series of adhesive changes, which cancer cells implement in a rather stereotypical manner, independent of their tissue of origin. These changes include the loss of cadherin-dependent junctions and, if they were present, the loss of HEMIDESMOSOMES, as well as the acquisition of a motile, invasive phenotype. In some cases, carcinoma cells undergo a real EPITHELIAL–MESENCHYMAL TRANSITION (EMT) and, as well as these adhesive changes, carry out a mesenchymal gene-expression programme. Finally, in contrast to physiological migration, the invasion of cancer cells involves the partial degradation and extensive remodelling of the ECM. Integrins cooperate with various oncogenic signals to promote the many phases of this process.

Disruption of cell–cell adhesion. Classic cadherins, such as E-cadherin, have a key role in epithelial cell–cell adhesion. Whereas their ectodomain mediates homophilic adhesion, the cytoplasmic domain of cadherins interacts with the actin cytoskeleton through the β-catenin–α-catenin complex. Strong evidence indicates that the loss of E-cadherin-mediated adhesion is required for malignant conversion[25,26]. Various mechanisms contribute to the disruption of E-cadherin-dependent junctions in cancer cells. Certain carcinomas acquire loss-of-function mutations in the E-cadherin gene[27]. In other tumours, members of the SNAIL/SLUG family of transcription factors suppress transcription of the E-cadherin gene[28]. Finally, signals from activated RTKs and SFKs can induce the disruption of ADHERENS JUNCTIONS in neoplastic cells that retain expression of E-cadherin and other junctional elements[27].

Increasing evidence indicates that dysregulated joint integrin–RTK signalling contributes to disrupting cell–cell adhesion in cancer cells. Breast carcinoma cells that are embedded in MATRIGEL form disorganized aggregates of non-polarized cells that are devoid of adherens junctions and a defined basement membrane. Antibody blocking of β_1 integrins induces these malignant cells to re-assemble adherens junctions and deposit a basement membrane, which gives rise to ACINI that are characterized by a distinct polarity[29]. By contrast, overexpression of β_1 integrins causes disruption of adherens junctions in normal epithelial cells[30]. Although these results imply that β_1-integrin signalling contributes to the disruption of cell–cell adhesion in cancer cells, the effects of ECM

SRC FAMILY KINASES
(SFKs). Kinases that belong to the Src family of tyrosine kinases, the largest of the non-receptor-tyrosine-kinase families. SFKs include Src, Yes, Fyn, Lck, Lyn, Blk, Hck, Fgr and Yrk.

PALMITOYLATED
The post-translational modification of a protein with palmitic acid.

LIPID RAFTS
Membrane microdomains that are enriched in cholesterol, sphingolipids and lipid-modified proteins such as GPI-linked proteins and palmitoylated proteins. These microdomains often function as platforms for signalling events.

TIGHT JUNCTION
A belt-like region of adhesion between adjacent epithelial or endothelial cells. Tight junctions regulate paracellular flux, and contribute to the maintenance of cell polarity by stopping molecules from diffusing in the plane of the membrane.

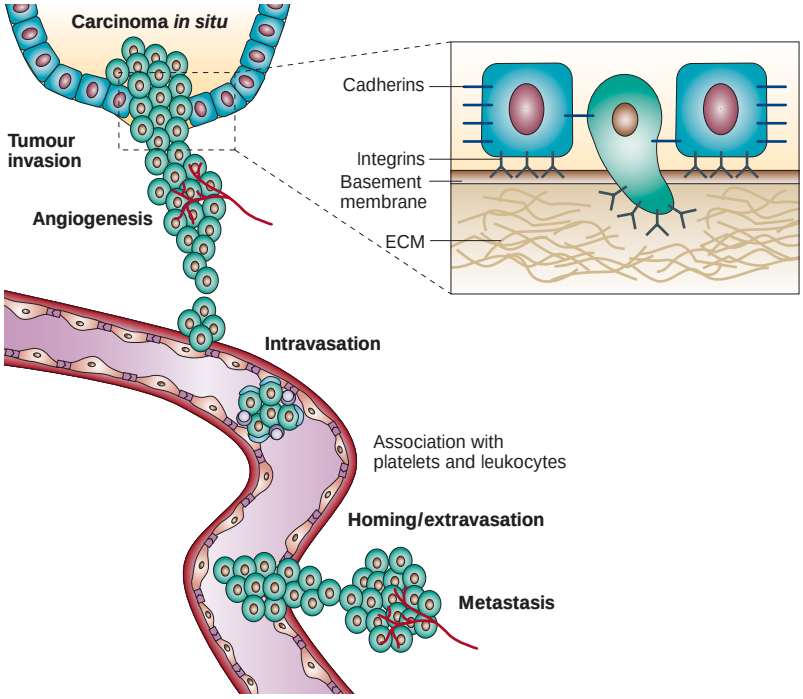


Figure 1 | **The metastatic cascade.** Changes in adhesion are prominent during the metastatic journey. At the onset of this process, cancer cells lose E-cadherin-dependent intercellular adhesions, acquire a migratory phenotype, penetrate the basement membrane, and invade the interstitial matrix. Tumour angiogenesis then allows cancer cells to enter the bloodstream, either directly or through the lymphatic system, by a process called intravasation. In the circulation, tumour cells form small aggregates with platelets and leukocytes. Finally, after stopping in the microcirculation of the target organ, tumour cells exit the bloodstream, by a process called extravasation, and undergo local expansion.

PLATELETS
The smallest blood cells, which are important in haemostasis and blood coagulation.

GUANINE NUCLEOTIDE-EXCHANGE FACTOR
(GEF). A protein that facilitates the exchange of GDP (guanine diphosphate) for GTP (guanine triphosphate) in the nucleotide-binding pocket of a GTP-binding protein.

CARCINOMA
A malignant tumour that originates from epithelial tissue.

ADENOMA
A benign tumour that arises from glandular epithelium.

HEMIDESMOSOMES
Adhesion complexes that connect intracellular keratin filaments with extracellular matrix in the basement membrane, and thereby mediate stable cell–matrix adhesion. These multiprotein complexes are assembled by integrin $\alpha_6\beta_4$, BP180 and plectins.

adhesion on the stability of intercellular adhesions are likely to be integrin specific. In agreement with this hypothesis, activated Rac (a RHO-FAMILY GTPase) promotes or disrupts the assembly of adherens junctions, depending on the composition of the matrix to which the cell adheres[31]. It is possible that the integrins that mediate stable cell–ECM adhesion promote the assembly of cell–cell adhesions and stabilize the epithelial phenotype, whereas the integrins that promote RTK signalling exert the opposite effect.

How does excessive joint integrin–RTK signalling disrupt cell–cell adhesion? Two main mechanisms seem to be involved (FIG. 2). First, activated RTKs and SFKs induce tyrosine phosphorylation of components of the E-cadherin–β-catenin complex. After tyrosine phosphorylation, the E-cadherin–β-catenin complex is recognized by the Cbl-like E3 UBIQUITIN PROTEIN LIGASE Hakai and therefore downregulated by endocytosis[32]. In v-Src-transformed cells, this process requires integrin function and phosphorylation of FAK[33], which implies that v-Src promotes endocytosis of E-cadherin by enhancing integrin signalling (FIG. 2). Second, integrin signalling operates through SNAIL/SLUG to suppress E-cadherin expression and thereby disrupt adherens junctions. In certain epithelial cells, this process seems to be mediated by integrin-linked kinase (ILK)[34,35]. However, studies on the $\alpha_3\beta_1$ integrin have, instead, implicated SFKs in this process[36].

Integrins and transforming growth factor-β in EMT. Transforming growth factor-β (TGF-β) signalling activates a cytostatic programme, which suppresses the proliferation of epithelial cells and antagonizes early carcinoma growth. However, many carcinomas eventually acquire mutations that render them insensitive to the growth-inhibitory action of TGF-β[37]. At this advanced stage of tumour progression, TGF-β signalling induces EMT and cooperates with Ras to promote tumour invasion[38]. Notably, two α_v integrins, $\alpha_v\beta_6$ and $\alpha_v\beta_8$, might promote EMT by contributing to the activation of TGF-β. Both integrins bind to the latent complex that holds TGF-β inactive, but each uses a distinct mechanism to induce the release of active TGF-β[39,40]. The $\alpha_v\beta_6$ integrin, which mediates adhesion to fibronectin and tenascin-C, is expressed at low levels in normal epithelial tissues, but it is upregulated during wound healing and in squamous cell carcinoma[20]. As fibronectin is a component of the interstitial matrix and tenascin-C is upregulated in tumour stroma[41], $\alpha_v\beta_6$ could facilitate the migration of normal and transformed epithelial cells through interstitial matrices. Also, there is evidence that the unique carboxy-terminal extension of the β_6 tail promotes epithelial proliferation[42]. So $\alpha_v\beta_6$ might promote tumorigenesis through a direct effect on the growth of carcinoma cells and a TGF-β-dependent effect on the invasion of carcinoma cells. Although $\alpha_v\beta_8$-mediated activation of TGF-β inhibits the proliferation of certain carcinoma cells[40], it is possible that $\alpha_v\beta_8$ promotes the invasive growth of carcinomas that have become resistant to the anti-proliferative effect of TGF-β.

Migration of tumour cells. To metastasize to a distant organ, neoplastic cells have to traverse several basement membranes and migrate through a variety of interstitial matrices. Cell migration is a complex multi-step process. First, migrating cells extend FILOPODIA in the direction of migration. On adhering to the ECM, these filopodia merge into a lamellipodium. FOCAL ADHESIONS are then nucleated within the LAMELLIPODIUM and become anchored to STRESS FIBRES. Contraction of the stress fibres allows cells to pull forward, as older adhesion complexes at the trailing edge are disassembled[43]. The integrins carry out essential roles during both normal and neoplastic cell migration. As well as providing the anchorage that is necessary for migration, they activate pro-migratory signals that regulate the cytoskeleton as well as gene expression. The mechanisms by which integrin signals orchestrate cell migration are incompletely understood, but at least three main pathways are involved (FIG. 3).

Studies on *FAK*-null fibroblasts have shown that FAK is crucial for cell migration[44]. Notably, many invasive human cancers have elevated levels of FAK[45]. FAK seems to integrate pro-migratory signals from integrins and RTKs, as cell migration that is induced by platelet-derived growth factor (PDGF) or epidermal growth factor (EGF) requires FAK to associate with both RTKs and integrin-containing focal complexes[46]. FAK functions in cell migration as an integrin-regulated scaffold that recruits SFKs to focal adhesions and positions them

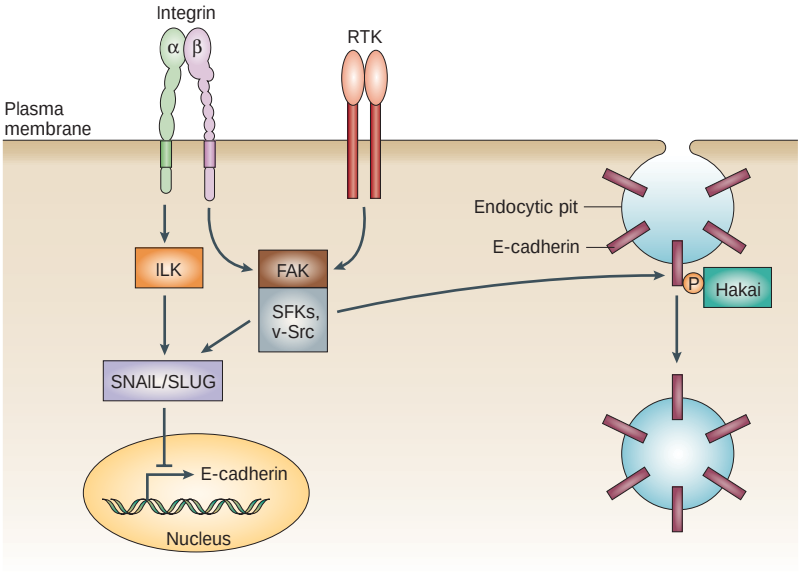


Figure 2 | **Integrin–receptor-tyrosine-kinase signalling disrupts cell–cell adhesion.** v-Src induces endocytosis of E-cadherin, presumably through phosphorylation of the E-cadherin complex and thereby recruitment of the Cbl-like E3 ubiquitin protein ligase Hakai. This process requires integrin signalling to focal adhesion kinase (FAK). Receptor tyrosine kinase (RTK)-induced internalization of E-cadherin might be mediated by a similar mechanism. Also, integrin signalling through integrin-linked kinase (ILK) or Src suppresses transcription of E-cadherin by upregulating the transcriptional repressors SNAIL/SLUG. SFK, Src-family kinase.

close to target-effectors that are crucial for cell migration. FAK–SFK signalling regulates cell migration through several pathways. First, FAK–SFK signalling induces phosphorylation of p130CAS and therefore the recruitment of Crk and the activation of Rac, which is instrumental for lamellipodial extension[47,48]. Second, FAK–SFK signalling causes activation of the tyrosine kinase ETK. Although its mechanism of action is not known, ETK is expressed at high levels in metastatic carcinoma cells, and its suppression blocks the migration of carcinoma cells[49]. Third, FAK–SFK signalling promotes disassembly of focal adhesions[50], and this might be important in the detachment of the trailing edge of cells during migration.

Integrins activate signalling downstream of Ras to extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) through SHC or FAK, and they also activate Jun amino-terminal kinase (JNK) through FAK[1,4]. Interestingly, whereas FAK signalling is necessary for directional cell movement, SHC promotes random cell motility[51]. It is increasingly clear that ERK/MAPK and JNK regulate cell migration by phosphorylating cytoskeletal components as well as by modifying gene expression. ERK/MAPK phosphorylates and activates the myosin light chain (MLC) kinase (MLCK), which induces the contraction of actomyosin fibres through phosphorylation of MLC[52]. JNK induces phosphorylation of paxillin, a component of focal adhesions. Paxillin phosphorylation might regulate cell migration by promoting the turnover of focal adhesions[53]. As paxillin binds to, and induces activation of, ERK/MAPK during the cell morphogenesis that is induced by hepatocyte growth factor (HGF)[54], it might cooperate with FAK to mediate joint integrin–RTK control of cell migration. As well as their cytoplasmic functions, ERK/MAPK and JNK also control cell migration through induction of activator protein-1 (AP-1)-dependent gene expression[55].

The Rho GTPases mediate many of the integrin-dependent modifications of the actin cytoskeleton that are necessary for cell migration[56]. Both Cdc42 and Rac are required for carcinoma migration and invasion[57]. They promote actin polymerization at the leading edge, and thereby the formation of filopodia and lamellipodia. Cdc42 and Rac both activate the ARP2/3 COMPLEX and induce the assembly of actin filaments through Wiskott–Aldrich syndrome protein (WASP)-family proteins[58]. They also activate p21-activated kinase (PAK), which enhances actin polymerization by activating LIM kinase[59]. Finally, Cdc42 contributes to the establishment of cell polarity in migrating astrocytes by associating with, and regulating, a complex of PAR6 and protein kinase Cζ[60]. Similar mechanisms might operate to polarize cancer cells during their migration. Whereas Cdc42 and Rac promote actin polymerization at the leading edge, Rho induces the assembly and contraction of the actomyosin fibres, which contributes to pulling the trailing edge forwards during migration. Two Rho effectors, Rho kinase (ROCK) and mammalian diaphanous (mDIA), function cooperatively to induce the assembly of actomyosin fibres[61]. ROCK inhibits MLC phosphatase (MLCP) and so promotes the phosphorylation of MLC and contraction of actomyosin fibres[62]. Notably, Rho–ROCK signalling might regulate various aspects of carcinoma dissemination — in particular, it is required for cancer cells to invade three-dimensional matrices by amoeboid movement[63] and to penetrate a mesothelial barrier[64]. Furthermore, gene-expression profiling has shown that RhoC is upregulated in metastatic variants of melanoma. As RhoC overexpression enhances colonization to the lung following intravenous injection, RhoC signalling might regulate extravasation[65].

Matrix remodelling. Efficient tumour invasion requires partial degradation of the ECM at the invasion front. In particular, neoplastic cells have to induce local degradation of basement membranes, because these structures are not permeable to cells under physiological conditions. But cancer cells have to control the extent to which they induce degradation of the interstitial matrix, so that they can adhere to it and generate the traction that is required for migration. The matrix metalloproteinases (MMPs) are the main proteases that are involved in remodelling the ECM[66]. They are synthesized as inactive pro-enzymes and converted to active enzymes through the proteolytic removal of an autoinhibitory domain. The MMPs are negatively regulated by the tissue inhibitors of MMPs (TIMPs).

The $\alpha_v\beta_3$ integrin, which binds to RGD-containing components of the interstitial matrix, such as vitronectin, fibronectin and thrombospondin, is upregulated in glioblastomas and melanomas[21,22]. In melanomas, $\alpha_v\beta_3$

EPITHELIAL–MESENCHYMAL TRANSITION
(EMT). The transformation of an epithelial cell into a mesenchymal cell with migratory and invasive properties.

ADHERENS JUNCTION
A cell–cell adhesion complex that contains cadherins and catenins that are attached to cytoplasmic actin filaments.

MATRIGEL
The extracellular matrix secreted by the Engelbrecht–Holm–Swarm mouse sarcoma cell line. It contains laminin, collagen IV, nidogen/entactin and proteoglycans, and so resembles the basement membrane.

ACINI
Differentiated epithelial structures in which a single layer of polarized epithelial cells encompasses a hollow lumen.

RHO-FAMILY GTPases
Ras-related GTPases that are involved in controlling the dynamics of the actin cytoskeleton.

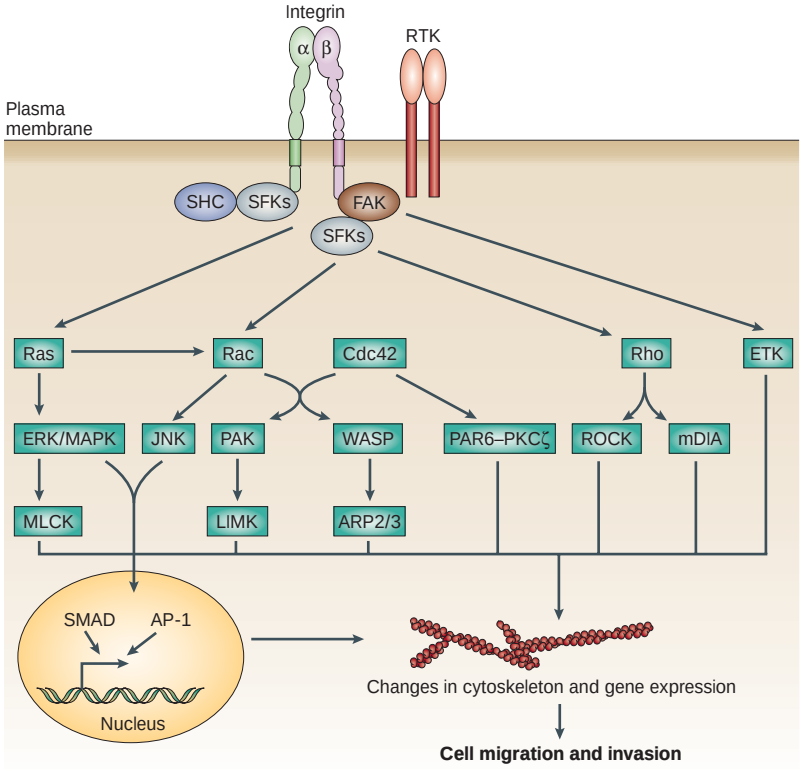


Figure 3 | **Integrin–receptor-tyrosine-kinase signalling induces cell migration and invasion.** Focal adhesion kinase (FAK) and Src-family kinases (SFKs) integrate pro-migratory signals from integrins and receptor tyrosine kinases (RTKs). These signals exert their effect by orchestrating changes in the cytoskeleton and by inducing gene expression. Both Rac and Cdc42 activate Wiskott–Aldrich syndrome protein (WASP)-family proteins and p21-activated kinase (PAK), which then activate the ARP2/3 complex and LIM kinase (LIMK), respectively, to induce actin polymerization. Myosin light chain kinase (MLCK), and the Rho effectors Rho kinase (ROCK) and mammalian diaphanous (mDIA), regulate bundling and contraction of actomyosin fibres. PAR6 and protein kinase C (PKC)ζ function downstream of Cdc42 to control cell polarity during migration. Jun amino-terminal kinase (JNK) and extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK), which can be activated by SHC or FAK, promote cell migration by activating activator protein-1 (AP-1)-dependent gene expression. Signalling through Ras–ERK/MAPK also cooperates with transforming growth factor-β (TGF-β)–SMAD signalling to induce epithelial–mesenchymal transition. Finally, the activation by FAK of ETK tyrosine kinase is also important for cell migration.

facilitates the transition from the radial to the vertical phase of growth, and therefore facilitates penetration of the basement membrane and invasion into the underlying stroma[67]. The $\alpha_v\beta_3$ integrin combines with several RTKs, including the receptors for EGF, PDGF, insulin and vascular endothelial growth factor (VEGF), and cooperates with them to promote cell migration[3,4]. However, its special role in tumour invasion and metastasis seems to arise from its ability to recruit and activate MMP2 and plasmin, which degrade components of the basement membrane and interstitial matrix. The activation of MMP2 involves several steps. When MMP2 binds to its inhibitor TIMP2, the transmembrane metalloproteinase MT1-MMP recruits the complex to the cell surface and it cleaves the pro-domain of MMP2 (REF. 66). The partially active enzyme then interacts with $\alpha_v\beta_3$ at the leading edge of the cell and becomes fully active[68]. An auto-proteolytic fragment of MMP2, PEX, competes with MMP2 for binding to $\alpha_v\beta_3$, which terminates MMP2 activation[69]. The $\alpha_v\beta_3$ integrin is also expressed in angiogenic blood vessels, and PEX suppresses the growth of melanomas, along with angiogenesis, in animal models[69]. These results imply that $\alpha_v\beta_3$ promotes tumour invasion and angiogenesis at least partly by regulating MMP2 (FIG. 4).

Certain integrins, including $\alpha_v\beta_3$, associate with the urokinase plasminogen activator (uPA) receptor, uPAR[70]. This interaction provides a further mechanism to recruit proteolytic activity to the leading edge of migrating cancer cells. The binding of pro-uPA to uPAR is necessary for activating uPA, which then converts plasminogen to plasmin. Plasmin, in turn, degrades ECM components both directly and through the activation of MMPs[71].

As well as recruiting proteases to the cell surface, the integrins regulate matrix degradation through signalling mechanisms. In v-Src-transformed fibroblasts, FAK-mediated activation of JNK enhances the expression of MMP2 and MMP9 and, through this mechanism, enhances tumour invasion and metastasis[72,73]. Conversely, components of the ECM-degradation machinery facilitate integrin signalling. Through uPAR, for example, certain integrins reinforce the activation of RTKs and SFKs and regulate signalling pathways that are important for cell proliferation and migration[74,75].

Increasing evidence indicates that remodelling the ECM exposes new cell-binding sites, which promotes the migration of tumour cells. Neoplastic cells that are situated at the invasive edges of certain carcinomas synthesize laminin-5 (REF. 76). Both MMP2 and MT1-MMP cleave the laminin-5 γ2 chain, which exposes a new pro-migratory site in this matrix protein[77,78]. Similarly, cleavage of collagen IV by MMPs exposes a cryptic $\alpha_v\beta_3$-binding site that is important for the migration of endothelial cells and tumour angiogenesis[79]. So by recruiting activated MMP2, $\alpha_v\beta_3$ promotes matrix degradation, but also contributes to the generation of new integrin-binding sites for migration.

The pro-invasive functions of $\alpha_6\beta_4$. The expression pattern of $\alpha_6\beta_4$ implies that this integrin controls the proliferation of epithelial cells. In normal skin, $\alpha_6\beta_4$ is expressed only in the basal-cell compartment, which includes the rapidly dividing TRANSIT-AMPLIFYING CELLS[80]. In accordance with the hypothesis that $\alpha_6\beta_4$ favours proliferation, ligation of $\alpha_6\beta_4$ allows progression through G1 and entry into S phase in keratinocytes that have been treated with EGF, whereas ligation of $\alpha_2\beta_1$ does not exert this effect[81]. Furthermore, $\alpha_6\beta_4$ and its ligand laminin-5 are required for Ras-mediated transformation of keratinocytes[82].

Studies of tumour biology indicate that $\alpha_6\beta_4$ signalling probably promotes carcinoma invasion. Many carcinomas express elevated levels of $\alpha_6\beta_4$ (REF. 19). Introduction of β_4 in β_4-negative breast carcinoma cells activates signalling from phosphatidylinositol 3-kinase (PI3K) to Rac and increases the invasive ability of these cells *in vitro*[83]. Furthermore, the β_4 tail functions as an

E3 UBIQUITIN PROTEIN LIGASE
The third enzyme in a series — the first two are designated E1 and E2 — that are responsible for ubiquitylation of target proteins. E3 enzymes provide platforms for binding E2 enzymes and specific substrates, thereby coordinating ubiquitylation of the selected substrates.

FILOPODIA
Long, thin protrusions at the periphery of cells and growth cones. They are composed of F-actin bundles.

adaptor and amplifier of pro-invasive signals that are elicited by the HGF receptor Met in cells that are undergoing Met-induced oncogenesis[84]. Finally, introduction of a DOMINANT-NEGATIVE form of β_4 impairs the survival of breast carcinoma cells, and this effect has been linked to the ability of mutant β_4 to interfere with the establishment of a partially polarized phenotype and with the activation of nuclear factor κB (NF-κB) in these cells[85,86]. Collectively, these results indicate that $\alpha_6\beta_4$ promotes cell migration and invasion and confers resistance to apoptosis on carcinoma cells.

What is the relationship between the signalling roles of $\alpha_6\beta_4$ and its essential pro-adhesive function at hemidesmosomes? The $\alpha_6\beta_4$ integrin associates with RTKs, which are often mutated or amplified during tumour progression. And, as well as Met and Ron (which is the receptor for macrophage-stimulating protein), $\alpha_6\beta_4$ combines with the EGF receptor and ERBB2/NEU[84,87–89]. Interestingly, both the EGF receptor and Met induce phosphorylation of β_4 and enhance SHC signalling, which disrupts hemidesmosomes and increases the migration of epithelial cells and carcinoma invasion[84,87]. These results indicate that these RTKs decrease the ability of $\alpha_6\beta_4$ to mediate stable adhesion but increase its function in signalling. The effects of oncogenic or amplified forms of Met in carcinoma cells are independent of adhesion to laminin-5, which implies that constitutive activation of the $\alpha_6\beta_4$-associated RTK might cause ECM-independent joint signalling[84]. Finally, ECM binding to $\alpha_6\beta_4$ seems to increase the activation of ERBB2, which indicates that there might be a reverse pathway of $\alpha_6\beta_4$–RTK crosstalk[88]. Together, these studies indicate that joint $\alpha_6\beta_4$–RTK signalling disrupts hemidesmosomes and promotes the proliferation and migration of epithelial cells, and they imply that dysregulation of this binary signalling system might contribute to carcinoma invasion and growth (FIG. 5).

Contrasting roles of the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins. Tumour progression might be facilitated by the downregulation of integrins that mediate the adhesion of epithelial cells to the basal lamina and that usually help to maintain them in a quiescent state. Expression of the $\alpha_2\beta_1$ integrin, which binds to collagen and laminin, is downregulated in breast carcinoma. Forced expression of $\alpha_2\beta_1$ enables breast carcinoma cells to form gland-like structures in collagen gels and reduces their tumorigenicity *in vivo*[23]. Similarly, overexpression of $\alpha_3\beta_1$, a laminin-5 receptor, decreases the rate of carcinoma formation in the skin[90]. Interestingly, $\alpha_2\beta_1$ and $\alpha_3\beta_1$ cannot recruit SHC or cooperate with RTKs to promote cell-cycle progression[91]. Furthermore, $\alpha_2\beta_1$ activates p38 MAPK[92], which is implicated in cell-growth arrest[93]. However, other reports indicate that $\alpha_2\beta_1$ and $\alpha_3\beta_1$ might facilitate tumour progression. For example, $\alpha_2\beta_1$ enhances the ability of rhabdomyosarcoma cells to metastasize to the lung after intravenous injection in nude mice[94]. Similarly, $\alpha_3\beta_1$ seems to be able to facilitate lung colonization by promoting the adhesion of cancer cells to laminin-5 in exposed areas of the endothelial basement membrane[95]. Perhaps the effects of $\alpha_2\beta_1$ and $\alpha_3\beta_1$ on tumour progression differ depending on the stage of progression that is involved. The effect of other integrins on tumour progression might be similarly dependent on the cellular context and the specific step of tumour progression.

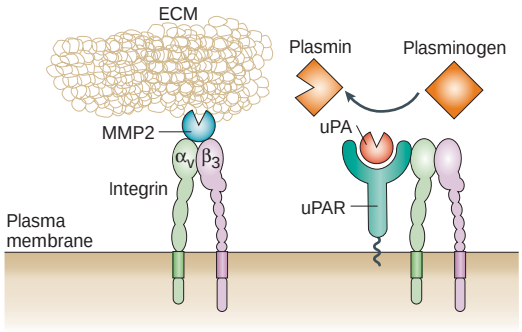


Figure 4 | **Integrins and matrix remodelling.** The $\alpha_v\beta_3$ integrin recruits matrix-metalloproteinase-2 (MMP2) to the cell surface to allow the local degradation of extracellular matrix (ECM) components during the invasion of cancer cells. Several integrins, including $\alpha_v\beta_3$, associate with the urokinase plasminogen activator (uPA) receptor, uPAR. On binding uPAR, uPA is then converted to an active form, which converts plasminogen to plasmin. Plasmin activates pro-MMPs, as well as directly inducing ECM degradation during tumour invasion.

Tumour angiogenesis

Angiogenesis has essential roles during tumour invasion and metastasis. It is estimated that tumours cannot grow beyond a limited size unless they promote angiogenic sprouting and thereby establish a vascular connection with the host[15]. As well as providing oxygen and nutrients to the rapidly proliferating cancer cells, angiogenesis enables tumour cells to gain access to the circulation and leukocytes to infiltrate the tumour stroma and secrete pro-invasive MMPs and chemokines[16].

Tumour angiogenesis occurs by a sprouting mechanism. It commences with de-stabilization and de-differentiation of the host vessels that are closest to the tumour. During the subsequent 'invasive' phase, specific activated endothelial cells migrate and proliferate into the tumour ECM. During the final 'maturation' phase, the endothelial cells become quiescent and organize into functional vessels. In mouse models of cancer, angiogenesis occurs as a discrete step during tumour progression, and often seems to precede tumour invasion of the interstitial matrix. The induction of tumour angiogenesis (the 'angiogenic switch') is thought to result from an increased production of angiogenic factors or a decreased generation of inhibitory factors by cancer cells or stromal cells[15].

Integrins are targets of both angiogenic activators and inhibitors. VEGF and basic fibroblast growth factor (bFGF) enhance the expression, as well as the activation, of several integrins that are involved in angiogenesis[96,97]. Conversely, several endogenous angiogenic inhibitors seem to exert their function by blocking integrins.

FOCAL ADHESION
An integrin-mediated cell–substrate adhesion structure that anchors the ends of actin filaments (stress fibres) and mediates strong attachments to substrates. It also functions as an integrin signalling platform.

LAMELLIPODIA
Flattened, sheet-like structures — which are composed of a crosslinked F-actin meshwork — that project from the surface of a cell. They are often associated with cell migration.

STRESS FIBRE
A bundle of parallel filaments that contain F-actin and other contractile molecules. It often stretches between cell attachments as if under stress.

ARP2/3 PROTEIN COMPLEX
A complex that consists of two actin-related proteins, ARP2 and ARP3, along with five smaller proteins. When activated, the ARP2/3 complex binds to the side of an existing actin filament and nucleates the assembly of a new actin filament. The resulting branch structure is Y-shaped.

RGD SEQUENCE
The primary adhesive motif in many extracellular-matrix molecules, which contains the amino-acid triplet Arg-Gly-Asp.

TRANSIT-AMPLIFYING CELLS
Progenitor cells that are able to divide only 3–5 times before all of their daughters terminally differentiate.

DOMINANT-NEGATIVE
A defective protein that inhibits the function of normal proteins often by competing with normal proteins for interacting molecules through retained interaction capabilities.

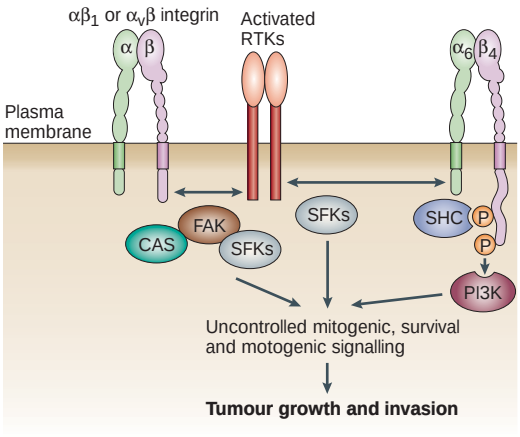


Figure 5 | **Integrins promote oncogenic signals of activated receptor tyrosine kinases.** Receptor tyrosine kinases (RTKs) that are constitutively activated through mutation or overexpression induce phosphorylation of the cytoplasmic domain of β_4, which creates docking sites for signalling molecules, such as SHC and regulators of phosphatidylinositol 3-kinase (PI3K). In this model, the cytoplasmic domain of β_4 functions as a signalling adaptor to amplify the mitogenic, survival and motogenic signals that are elicited by the activated RTK. Other integrins might enhance signalling by activated RTKs through intermediates such as focal adhesion kinase (FAK), Src-family kinases (SFKs) and p130CAS. Through these mechanisms, integrins have important roles in tumour growth and invasion.

For example, endostatin, a fragment of collagen XVIII, interacts with the $\alpha_5\beta_1$ integrin, and tumstatin, a fragment of collagen IV, binds to $\alpha_v\beta_3$ (REFS 98,99). Studies with adhesion-blocking reagents and knockout mice have implicated $\alpha_5\beta_1$ and α_v integrins in angiogenesis[17,18]. However, the mechanisms by which these, and possibly other, integrins function in angiogenesis are not clear[100]. It seems certain that integrins exert essential adhesive functions during angiogenesis, but they might also regulate angiogenic signalling. Furthermore, they might have distinct, and even contrasting, roles, depending on the phase of angiogenesis at which they are functioning. It is possible that the invasive phase of angiogenesis is regulated by integrin–RTK signalling pathways that are similar to those that are involved in the invasion of tumour cells[17]. Because some integrins, such as $\alpha_v\beta_3$, are upregulated in both invasive cancer cells and angiogenic endothelial cells, their targeting might confer a significant therapeutic advantage.

Survival of tumour cells

Loss of ECM adhesion induces normal cells to undergo apoptosis — a process known as ANOIKIS. By contrast, oncogenically transformed cells are relatively resistant to anoikis[101]. As well as facilitating the initial expansion of tumours, resistance to anoikis is key to metastatic dissemination, as tumour cells must survive in several different foreign microenvironments before they can colonize distant organs. FAK promotes the survival of cells by signalling through PI3K to AKT/protein kinase B (PKB)[102,103]. Furthermore, integrin signalling promotes the expression of BCL2 (REF. 104), which is anti-apoptotic, and it suppresses the expression of the pro-apoptotic BCL2-family protein BIM[105]. Extracellular factors such as insulin-like growth factor (IGF)1/2 or interleukin (IL)-3 (REFS 106,107), intracellular signals from mutant Ras or activated Src[103], or deletion of the tumour suppressor phosphatase and tensin homologue (PTEN)[108], all contribute to the anoikis resistance of cancer cells — presumably through activation of PI3K–AKT/PKB signalling. Mutations in p53 are very frequent in tumours, and they confer a selective advantage by allowing cancer cells to avoid apoptosis in response to DNA damage, hypoxia, and aberrant oncogene activation[109]. Notably, loss of p53 allows *FAK*-null fibroblasts to survive in culture, which indicates that FAK might also promote cell survival by inhibiting p53 (REF. 110).

The loss of ECM adhesion can also induce apoptosis by more 'active' mechanisms. Death receptors, such as Fas, and/or death-domain-containing proteins, such as Fas-associated-death-domain protein (FADD), mediate the activation of caspase-8 and, therefore, apoptosis in cells that have lost anchorage to the matrix[111–113]. Lung and colorectal tumours often carry amplifications of the gene that encodes a non-signalling decoy receptor for Fas ligand, and they might therefore be resistant to anoikis that is induced by Fas ligand[114]. Furthermore, there is evidence that unoccupied $\alpha_v\beta_3$ integrins activate apoptosis in cells by recruiting caspase-8 (REF. 115). The mechanisms by which cancer cells overcome this survival control are not well known. Notably, re-expression of the cyclin-dependent kinase (CDK) inhibitor p16 (which is also known as INK4a) restores anoikis sensitivity to a variety of cancer cell lines by enhancing the expression of the $\alpha_5\beta_1$ integrin[116]. This result indicates that the loss of p16 might enhance tumour resistance to apoptosis by reducing the number of integrins that would normally be available to sense the loss of matrix attachment. Similarly, re-introduction of $\alpha_6\beta_4$ into cancer cells that no longer express it activates a p53-dependent death programme, which implies that the loss of $\alpha_6\beta_4$ might enhance the survival of carcinoma cells that possess wild-type p53 and have lost anchorage to a laminin-5-rich basement membrane[117]. By contrast, laminin-5 binding to $\alpha_6\beta_4$ protects cancer cells from several death signals including those that are induced by chemotherapeutic drugs, but this effect requires the formation of a polarized three-dimensional architecture and activation of NF-κB[85,86]. So the same integrin might promote or oppose the survival of cancer cells, depending on whether it is bound to ECM ligand or not. And the ligation of integrins can promote resistance to exogenous apoptotic insults in cancer cells that are already capable of anchorage-independent survival.

Colonization of distant organs

To colonize a distant organ, tumour cells have to survive in the bloodstream, adhere to the endothelium of the capillary bed of the target organ, and extravasate into its parenchyma. Some metastatic lesions, however, expand almost entirely intravascularly. In the circulation,

ANOIKIS
Induction of programmed cell death by detachment of cells from the extracellular matrix.

SELECTINS
A family of adhesion receptors that bind to the carbohydrate groups of heavily glycosylated counter-receptors through their lectin domain. Selectins mediate the adhesion of leukocytes to endothelium.

tumour cells associate with platelets and leukocytes to form small tumour emboli. Through these associations, tumour cells are shielded from shear forces and are provided with adhesive mechanisms that help them to remain in the target organ. SELECTINS and integrins mediate the adhesion of tumour cells to platelets and leukocytes[13,14]. The $\alpha_v\beta_3$ integrin is often expressed in both tumour cells and platelets and, because it binds to fibrinogen and fibrin, it can mediate the co-aggregation of these cells[118]. In accordance with this hypothesis, activation of $\alpha_v\beta_3$ is required for the formation of tumour microemboli and metastasis in a breast carcinoma model[13].

Certain integrins might enable tumour cells to adhere to the vascular endothelium of the target organ and traverse the vessel wall to reach the interstitial matrix. There is evidence that $\alpha_6\beta_4$ binds to CLCA (chloride channel, calcium-activated), a Ca^{2+}-sensitive chloride channel that is expressed by pulmonary endothelial cells. This integrin therefore helps cancer cells to stop in the microvascular bed of the lung, and it promotes their intravascular growth[119]. Also, $\alpha_v\beta_3$ binds to the endothelial cell adhesion molecule L1, and this adhesive interaction might promote migration of melanoma cells across the endothelium[120]. Cancer cells can also attach to the exposed endothelial basement membrane through $\alpha_3\beta_1$–laminin-5 binding[95].

Conclusions and perspectives

Integrins exert a stringent and specific control on the action of RTKs. We propose that switches in integrin usage in conjunction with activating mutations in certain RTKs, such as ERBB2 and Met, cause constitutive joint integrin–RTK signalling, which allows tumour cells to migrate and proliferate independently of positional constraints. Also, many integrins localize at the leading edge of the cell and activate matrix-degrading proteases, such as the MMPs and plasmin. Both types of change are likely to be key to tumour invasion and metastasis. It seems that each tumour type undergoes characteristic and dynamic changes in integrin expression and function during tumour progression. Future studies will uncover the full complexity of these changes, and it might well become apparent that some changes are more important than others. It will then be necessary to test the relevance of these changes in integrin expression and signalling in mouse models of cancer. A genetic validation of the existence of pro-neoplastic integrins should open the way to the development of anti-integrin compounds for tumour therapy. It is likely that certain integrin–RTK signalling pathways will be found to be necessary for both tumour invasion and for angiogenesis. We anticipate that targeting components of these pathways might have increased therapeutic effects.

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