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Recent insights into the role of integrins in cancer metastasis

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Abstract. Integrins have been repeatedly found involved in cancer metastasis. The past two years have seen considerable evolution in our knowledge on the role of these integrins in tumour cells. This includes the elucidation of different signalling pathways by which integrins dictate the anchorage-independent growth, survival and motility of tumour cells. Moreover, integrins may have a more complex role in cancer metastasis as they cooperate with serine proteases and metalloproteases to promote tumour cell invasion and angiogenesis. Finally, integrins favour tumor cell extravasation.

Key words. Integrin; cancer; proliferation; apoptosis; invasion; angiogenesis; extravasation; metastasis.

Introduction

Cancer metastasis is a complex cascade of events that includes tumour growth and invasion, angiogenesis (i.e. the recruitment of new blood vessels, which is an essential feature for the growth of solid tumours), intravasation of tumour cells into the lymphatic or blood circulation, arrest in distant organs via interactions of tumour cells with vascular or lymphatic endothelium, extravasation of the tumour cells from circulation and growth of tumour cells to form secondary tumours in the new organ environment. Considerable research has been directed towards understanding molecular mechanisms involved in these different steps of the metastatic cascade. Here we will discuss one class of molecules, integrins, that have been repeatedly found involved in cancer metastasis. We shall focus on key recent findings implicating integrins in the regulation of tumour cell growth and survival, migration and invasion, angiogenesis and extravasation.

General considerations on the structure and function of integrins

Integrins were originally characterized as a family of cell surface receptors that mediate the attachment of cells to extracellular matrices. Integrins are heterodimeric; they consist of two transmembrane glycoprotein subunits (α and β) that are noncovalently bound (for review see ref. [1]). For adhesion to ligands, the extracellular domain of both integrin subunits is needed, as is the presence of cations. The cytoplasmic domain of integrin α and β subunits anchors the cytoskeleton to the plasma membrane and is required to mediate signalling events. Thus far, the integrin family is composed of 16 α and 8 β subunits that form heterodimers to produce some 22 different $\alpha\beta$ cell surface receptors. Alternative splicing of the α and β subunits adds additional complexity. For example, four β1 isoforms with variant cytoplasmic domains have been described (β1A, β1B, β1C and β1D) (for review, see ref. [2]). Integrins mediate bidirectional transmembrane signalling from the inside to the outside of the cell (a process termed 'inside-out' signalling), and also from the outside to the inside of the cell (a process termed 'outside-in' signalling) [1, 2]. The inside-out signalling regulates the integrin affinity state (or 'activation' state) for a ligand through the propagation of conformational changes from the integrin cytoplasmic domain to the extracellular binding site. In this respect, integrin cytoplasmic domains contain highly conserved motifs which are involved in regulating integrin activation [2]. For

example, point mutations of a highly conserved motif (amino acid sequence KXGFFKR) found in all integrin α subunits converts integrins to a constitutive activated state, whereas point mutations of another highly conserved motif (amino acid sequence NPXY) present in integrin β subunits (with the exception of $\beta 4$) abolish activation of $\beta 1A$ and $\beta 3$ integrins [2]. The switch of integrins from an inactive to an active state could also be regulated by intracellular proteins which bind directly to the cytoplasmic domain of integrins (for reviews, see refs 1, 2). For example, the integrin-linked kinase (ILK) associates with the cytoplasmic domain of integrin β subunits ($\beta 1A$, $\beta 2$ and $\beta 3$) and must be removed during receptor activation, while the calcium-binding protein calreticulin binds only to the highly conserved sequence motif KXGFFKR of α subunits upon integrin activation. The interaction of 'activated' integrins with extracellular matrix proteins induces cell surface integrin clustering at focal sites of the plasma membrane (known as focal adhesion plaques), a process driven from within the cell [3]. These focal adhesion plaques are composed of cytoskeletal proteins (α-actinin, talin, vinculin, paxillin and tensin being a few representative ones) and signalling molecules [calreticulin, focal adhesion kinase (FAK), cadherin-associated substrate (CAS)] [2, 3]. Following inside-out activation, integrin occupation and clustering at focal adhesion plaques elicit outside-in signalling from the extracellular matrix into the cell cytoplasm that leads to cell migration, proliferation, differentiation and survival.

Integrins and tumour cell proliferation

The pattern of integrin expression on a given tumour is intricate, because each tumour type displays a great deal of heterogeneity in integrin expression in situ and integrin expression by cultured cells is not always identical to that by the same cells in tissues. However, there is a general tendency to consider that the down- or upregulation of some integrins in poorly differentiated tumours may represent a hallmark of invasive and metastatic behaviour. For example, decreased expression of integrins $\alpha 2\beta 1$ and $\alpha 5\beta 1$ is consistently observed in poorly differentiated breast carcinomas [4]. Increased expression of integrins $\alpha 6$ and $\alpha 6\beta 4$ also correlates with a poor prognosis in human breast carcinomas and squamous cell carcinomas of the head and neck, respectively [5, 6]. Similarly, increased expression of integrin $\alpha v\beta 3$ is consistently observed in human metastatic melanomas [7]. Changes in the integrin repertoire of solid tumours are therefore thought to play critical roles in the proliferation, migration and invasion of tumour cells. We shall summarize here some recent observations that yield insights into the mechanisms by which integrins regulate tumour cell proliferation. The regulation of tumour cell migration and invasion by integrins will be discussed in another section.

It has been known for a long time that tumour cells, as opposed to normal cells, are independent of anchorage to the extracellular matrix for growth in vitro. This anchorage-independent growth of tumour cells correlates with tumorigenesis in vivo. Because integrins play a central role in mediating cell anchorage to the extracellular matrix, the role of integrins in tumour growth through experimental manipulation of levels of expression of specific integrins has received considerable attention, and a recent excellent review has gone into this subject in detail [8]. Thus, we shall only briefly summarize some of the most salient findings about integrin expression during tumour growth.

A number of studies indicate that the reexpression of $\alpha 2\beta 1$ or $\alpha 5\beta 1$ in tumour cells has a negative effect on anchorage-independent growth of these tumour cells (for review, see ref. [8]). The involvement of integrin $\alpha 6\beta 4$ in the reversion of the malignant phenotype of human breast carcinoma cells in three-dimensional culture has also been recently reported [9]. Interestingly, alternative spliced integrin subunit $\beta 1C$ is constitutively expressed on the surface of growth-arrested endothelial cells (while $\beta 1C$ is absent on growing endothelial cells) [8]. Although the existence of such an alternative splicing of integrin subunit $\beta 1$ has not been documented in tumour cells, downregulation of the expression of $\beta 1C$ could provide an accessory mechanism to promote anchorage-independent cell growth. In contrast, expression of some integrins enhances tumour cell proliferation. Several studies have suggested that constitutive integrin $\alpha v\beta 3$ expression in melanoma cells or overexpression of integrin $\alpha v\beta 6$ in colon carcinoma cells promotes tumourigenicity [8, 10]. The molecular mechanisms by which these integrins negatively or positively regulate tumour cell growth are unclear at present. However, it is possible that constitutive activation of signal transducers downstream from integrins mediates anchorage-independent growth of tumour cells. In normal cells, integrins mediate activation of the Ras/MAP kinase pathway, leading to cell cycle progression [11]. This raises the possibility that some kinases (FAK, Src, ILK being a few representative ones) could serve as upstream regulators of the Ras/MAP kinase pathway. Although integrin-mediated activation of the Ras/MAP kinase pathway is independent of FAK in fibroblasts [12], FAK is consistently overexpressed in invasive human carcinomas [13], and there is recent evidence that the constitutive activation of FAK in epithelial cells (MDCK cell line) confers anchorage-independent (but serum-dependent) growth in soft agar and tumourigenicity in nude mice [14]. Constitutive

activation of the small GTPase Rho in NIH 3T3 cells and, more recently, overexpression of ILK in IEC18 epithelial cells also confer anchorage-independent (but serum-dependent) growth [15, 16]. These findings are appealing, because the anchorage-independence growth property of tumour cells in vitro presumably reflects the tendency of tumour cells to grow in inappropriate locations in vivo. Thus, the constitutive activation or overexpression of integrin signal transducers such as FAK, Rho and ILK could provide a molecular basis to explain the enhanced proliferation of tumour cells.

Integrins and apoptosis

Programmed cell death (or apoptosis) is the process whereby cells are induced to activate their own death. Apoptosis is crucial for maintaining appropriate cell number and tissue organization. The depletion of specific hormones and growth factors induces apoptosis, indicating that they function as survival factors. Like hormones and growth factors, the extracellular matrix also plays a crucial role as a survival factor, since the disruption of integrin-mediated cell-matrix inter-actions induces apoptosis (a phenomenon called anoikis) (for review, see ref. [17]). In this respect, ligation of integrin $\alpha 5\beta 1$ to fibronectin in $\alpha 5$-transfected tumour cells (Chinese hamster ovary, HT29 colon carcinoma and MG-63 osteosarcoma cells) consistently suppresses anoikis. Similarly, ligation of integrin $\alpha v\beta 3$ rescues melanoma cells from anoikis in three-dimensional dermal collagen [18]. The prevention of apoptosis through $\alpha 5$- and αv-transfection presumably results from the upregulation of the protooncogene Bcl-2 (which functions as a death antagonist) and from the downregulation of cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ [17, 19]. This is consistent with the fact that disruption of cell-matrix interactions activates the $p53-p21^{WAF1/CIP1}$ proapoptotic pathway [20]. Moreover, constitutive activation of FAK upon $\beta 1$ and $\beta 3$ clustering also rescues MDCK epithelial cells from anoikis [14]. The importance of FAK in regulating anoikis is further supported by the observation that microinjection of a peptide-mimicking FAK-binding site of the $\beta 1$ cytoplasmic domain inactivates endogenous FAK and induces anoikis [21]. In contrast, ectopic expression of integrin subunit $\beta 4$ in RKO colon carcinoma cells induces expression of $p21^{WAF1/CIP1}$ and subsequent apoptosis [22]. Although these findings indicate that integrins regulate anoikis through multiple signalling pathways, much more work needs to be done to understand integrin-specific signalling involved in the regulation of anoikis. However, several lines of evidence suggest that, at least, $\alpha v\beta 3$-mediated activation of FAK may play a critical role in anoikis resistance and anchorage-independent growth of tumour cells (especially for melanoma cells). Integrin $\alpha v\beta 3$ is consistently overexpressed in metastatic melanomas as it is for FAK in most invasive human tumours [7, 13]; ligation of $\alpha v\beta 3$ mediates activation of FAK in different cell lines including melanoma cells [11, 23], $\alpha v\beta 3$ confers anoikis resistance of melanoma cells [18]; and activation of FAK facilitates anchorage-independent growth [14]. The challenge now is to understand the steps that occur between $\alpha v\beta 3$-mediated activation of FAK and the downstream effects in the nucleus.

Integrins in tumour cell migration and invasion

Cell migration results in a dynamic interaction between the cell, the extracellular matrix and the cytoskeleton. In this respect, integrins serve to connect the extracellular matrix with the cytoskeleton at focal adhesion plaques and provide the traction that is required for cell migration (for reviews, see refs [2, 24]. Correlations between a changing integrin repertoire and alterations in cell motility have been observed both in vitro and in vivo. For example, $\alpha v\beta 1$-expressing Chinese hamster ovary (CHO) tumour cells attach to fibronectin but fail to migrate on fibronectin, although the same cells transfected with $\alpha 5\beta 1$ do attach and migrate on fibronectin [25]. Similarly, overexpression of fibronectin receptors ($\alpha 4\beta 1$ or $\alpha 5\beta 1$) in transfected sarcoma S180 cells enhances their motility on fibronectin in vitro and changes their migratory properties in vivo when grafted into the neuroepithelium of chicken embryos [26]. In addition, there are collaborative interactions among integrins [27]; for example, integrin $\alpha v\beta 3$ regulates $\alpha 5\beta 1$-mediated cell migration towards fibronectin [28]. Cell migration is also dependent on rapid, controlled alterations of the affinity of integrins for their extracellular ligands. Thus, the repeated activation and inactivation of integrins regulates cell migration through coordinated adhesion (at the leading edge of the cell) and de-adhesion events (at trailing edge of the cell). In this respect, the NPXY motif, which regulates the activation of integrin β subunits (with the exception of $\beta 4$), may play a critical role during tumour cell migration because the transfection of CS-1 melanoma cells with a $\beta 3$ complementary DNA (cDNA) containing a mutated NPXY sequence inhibits tumour cell migration in vitro and metastasis formation in vivo [29]. Interestingly, the region of integrins containing the NPXY motif is known to undergo alternative splicing [2]. Transfection experiments have shown that $\beta 1$B (which does not contain the NPXY motif) interferes with $\beta 1$A function when expressed in CHO tumour cells, resulting in a dominant negative effect on cell adhesion and migration [30]. It is therefore tempting to speculate that tumour cells regulate their motility by a splicing mechanism.

Integrin cytoplasmic domain-associated protein-1 (ICAP-1) is a newly described intracellular phosphoprotein which specifically associates with the sequence motif NPXY of integrin β1A [31]. The extent of ICAP-1 phosphorylation is regulated by the guanosine triphosphate (GTP)-binding protein RhoA during cell-matrix interaction [31]. Members of the Rho subfamily are strong candidates implicated in the formation and release of adhesions in migrating cells (for review, see ref. [24]). Interestingly, ICAP-1 is expressed by osteosarcoma cell lines SaOs-2 and UTA-6 [31], suggesting that the Rho-dependent dephosphorylation of ICAP-1 could contribute to tumour cell migration. Apart from RhoA, other members of the small GTP-binding protein family may also be involved in the regulation of integrin inside-out signalling and subsequent cell migration [24]. For example, R-Ras activates integrins [32], whereas H-Ras blocks integrin activation [33]. The molecular basis of this regulation is not known at present. However, this raises the interesting possibility that malignant transformation could produce a sustained activation of H-Ras, leading to integrin inactivation, cell de-adhesion and altered migration. Following inside-out activation, integrin occupation and clustering at focal adhesion plaques elicit transducing signals from the extracellular matrix into the cell cytoplasm. Among these transducing signals, occupation and clustering of β1A and β3 integrins induce autophosphorylation of FAK [2]. Overexpression of FAK increases cell motility [34]. In addition, mesodermal cells cultured from FAK-deficient embryos show decreased spreading and motility [35], suggesting that FAK promotes cell migration by, however, an as yet poorly understood mechanism. Interestingly, haptotaxis (cell migration towards a substratum-bound ligand) of human melanoma cells on vitronectin requires αvβ3-mediated tyrosine phosphorylation of the FAK-associated protein paxillin, while soluble vitronectin stimulates chemotaxis (cell migration towards a soluble ligand) of melanoma cells through a αvβ3-mediated G protein-dependent pathway [23].

Thus, different integrin-dependent cell migratory pathways may be used by tumour cells. Indeed, the convergence of adhesion-mediated and growth factor-mediated signalling pathways may be also required for tumour cell migration. For example, in human pancreatic carcinoma cells, integrin αvβ5 mediates adhesion but not migration on vitronectin, whereas these cells readily migrate on collagen in an α2β1-dependent manner; however, following stimulation with EGF (epidermal growth factor), these carcinoma cells migrate on vitronectin through an αvβ5-mediated protein kinase C (PKC)-dependent pathway without affecting constitutive migration on collagen [36]. Similarly, IGF-I (insulin-like growth factor-I) stimulates motility of human pancreatic and breast carcinoma cells on vitronectin through activation of integrin αvβ5 [37, 38]. The challenge now is to dissect these different pathways to elucidate the precise role of each element in the transmission of signals leading to tumour cell migration.

Cell migration in the surrounding extracellular matrix is also intrinsically linked to a localized cell surface proteolytic activity which favours cell detachment from matrix proteins, thereby promoting cell motility (for reviews, see refs [39, 40]). Key recent findings implicating the coordinated action of integrins with urokinase plasminogen activator (uPA) or metalloproteineases (MMP) in tumour cell migration and invasion have been reported. For example, the induction of cell surface expression of uPA and its receptor (uPAR) by transforming growth factor α (TGFα) is necessary for vitronectin-dependent migration of FG pancreatic carcinoma cells, an event mediated by integrin αvβ5 (these carcinoma cells do not express αvβ3) [41]. FG pancreatic carcinoma cells also promote invasion and in vivo experimental metastasis formation in a manner dependent on both cytokine stimulation and αvβ5 expression [38]. Although the contribution of the uPA/uPAR complex in in vivo FG carcinoma cell dissemination is not known, the relationship between αvβ5 and uPA/uPAR seems to be specific, since FG pancreatic carcinoma cells transfected with integrin subunit β3 express αvβ3 and migrate on vitronectin independently of TGFα and uPA/uPAR expression [41]. As mentioned above, integrin αvβ3 promotes cell motility [23] and is consistently increased in human metastatic melanomas [7]. Recently, it has been demonstrated that MMP-2 (a gelatinase involved in the degradation of the extracellular matrix) colocalizes with integrin αvβ3 in melanoma tumours in vivo and binds directly to αvβ3 in vitro (but not to αvβ5 and α5β1) [42]. The fact that αvβ3 promotes cell motility, while MMP-2 potentiates matrix degradation, suggests that these proteins function in a cooperative manner to favour melanoma cell dissemination. The observation that MMP-2 works in concert with integrin αvβ3 is not unique to tumour cells. MMP-2 and integrin αvβ3 have been specifically colocalized on angiogenic blood vessels, suggesting that αvβ3 also promotes endothelial cell motility [42]. Similarly, an association between integrin α5β1 and the positive regulation of MMP-1 (interstitial collagenase) and MMP-3 (stromelysin-1) expression has been described in fibroblasts [43]. Integrin α2β1 is also a positive regulator of MMP-1 for keratinocyte migration [44]. These findings suggest that the coordinated action of integrins with metalloproteinases during tumour cell invasion is rather a general mechanism occurring during tissue remodelling. The difference may be that cancer cells use uPA or MMPs in conjunction with some integrins at times and places incompatible with normal cellular behaviour.

Integrins and tumour angiogenesis

Angiogenesis is the development of new blood vessels from preexisting ones. In cancer, there is an increase in angiogenesis, which is generally associated with poor prognostic features. Although tumours of about 1 mm^3 can receive all nutriments by diffusion, further growth depends on the development of a blood supply through angiogenesis. The induction of tumour angiogenesis is mediated by the release of angiogenic molecules (VEGF, bFGF, TNFα, TGFα, etc.) from tumour cells and host cells [45]. This stimulates normally quiescent endothelial cells in neighbouring blood vessels to degrade the basement membrane, migrate towards the tumour, proliferate and form tumour vessels which are leaky and abnormal in size and shape. In this respect, integrin $\alpha v\beta 3$ is upregulated on tumour vessels, while it is minimally expressed on quiescent blood vessels [46]. This selective vascular expression of integrin $\alpha v\beta 3$ is related to the fact that the switch of the endothelium from the resting to the angiogenic state is mediated by the *Hox D3* homeobox gene, which in turn increases expression of the integrin $\alpha v\beta 3$ on human endothelial cells [47]. In addition, basic fibroblast growth factor (bFGF) increases expression of *Hox D3* [47], and monoclonal antibody LM609 directed to integrin $\alpha v\beta 3$ blocks angiogenesis on chick chorioallantoic membrane (CAM) induced by bFGF [48], suggesting that bFGF promotes angiogenesis in an $\alpha v\beta 3$-dependent manner through *Hox D3* expression.

Brooks et al. [46] provide direct evidence that integrin $\alpha v\beta 3$ plays a critical role during tumour angiogenesis using a more clinically relevant model of tumour growth and angiogenesis. In this model, $\alpha v\beta 3$-deficient breast carcinoma cells are injected intradermally within the human skin previously transplanted onto severe combined immunodeficient (SCID) mice. Two weeks later, systemic administration of monoclonal antibody LM609 not only disrupts tumour angiogenesis but reduces the growth and invasive properties of human breast carcinoma in the SCID mouse/human chimeric model [46]. Indeed, integrin $\alpha v\beta 3$ antagonists, including cyclic Arg-Gly-Asp (RGD) peptides and antibodies, reduce tumour growth by inducing apoptosis of angiogenic blood vessels [19, 49]. This induction of apoptosis by $\alpha v\beta 3$ antagonists can be explained by the fact that ligation of integrin $\alpha v\beta 3$ promotes a specific adhesion-dependent cell survival signal during angiogenesis, leading to inhibition of p53 activity, decreased expression of $p21^{WAF1/CIP1}$ activity and suppression of the bax cell death pathway [19]. Thus, it is conceivable that angiogenic endothelial cells are protected against apoptosis by the integrin $\alpha v\beta 3$, because $\alpha v\beta 3$ antagonists induce apoptosis. Moreover, the active matrix metalloproteinase MMP-2 and integrin $\alpha v\beta 3$ have been specifically colocalized on angiogenic blood vessels, suggesting that $\alpha v\beta 3$ also promotes endothelial cell motility [42]. Besides $\alpha v\beta 3$ function in angiogenesis, Friedlander et al. [48] have shown that monoclonal antibody P1F6 directed to integrin $\alpha v\beta 5$ inhibits rabbit corneal and chick CAM angiogenesis induced by vascular endothelial cell growth factor (VEGF), but has minimal effect on that induced by bFGF. Moreover, antibody LM 609 directed to integrin $\alpha v\beta 3$ exerts no inhibitory effect on VEGF-induced rabbit corneal and chick CAM angiogenesis [48]. These very interesting findings suggest that there are two cytokine-dependent pathways of angiogenesis: bFGF-induced angiogenesis depends on $\alpha v\beta 3$, whereas angiogenesis initiated by VEGF depends on $\alpha v\beta 5$ [48]. However, it is not known how the endothelial cell dependence on integrin $\alpha v\beta 5$ is mediated at the molecular level. In addition, the ligand that integrin $\alpha v\beta 3$ binds to in mediating tumour angiogenesis is, at present, unidentified. Clarifying the exact role of integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ and their respective ligands in tumour angiogenesis will undoubtedly be an important task for the future.

The critical role of integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ during neovascularization does not preclude contributions by other integrins in tumour angiogenesis. Several members of the $\beta 1$ integrin subfamily are expressed by endothelial cells ($\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 4\beta 1$, $\alpha 5\beta 1$ and $\alpha 6\beta 1$) (for review. see ref. [50]) and $\beta 1$ integrin expression in embryonic stem (ES) cells influences angiogenesis in teratomas [51]. In vitro, some members of the $\beta 1$ integrin subfamily have been involved in collagen-mediated endothelial cell migration ($\alpha 2\beta 1$) [52] and capillary tube formation ($\alpha 2\beta 1$ and $\alpha 6\beta 1$) (for review, see ref. [50]). In addition, integrin $\alpha 5\beta 1$ upregulates the expression of matrix metalloproteinases MMP-1 (interstitial collagenase) and MMP-3 (stromelysin-1) in fibroblasts [43]. Because MMP inhibitors block angiogenesis as assayed in chick and rodent models of neovascularization (for review, see ref. [40]), it is possible that integrin $\alpha 5\beta 1$ (and $\alpha v\beta 3$) expressed by angiogenic endothelial cells may upregulate matrix metalloproteinases, thereby potentiating the angiogenic process.

Integrins and tumour cell extravasation

Blood-borne tumour cells disseminate to distant organs through the microcirculation. As documented by intravital videomicroscopy or positron emission tomography, the arrest of metastatic tumour cells on the vascular endothelium is an essential step for their extravasation from the blood stream in vivo [53–55]. Moreover, intravital videomicroscopy demonstrates that metastatic tumour cells begin to interact with the endothelium by first rolling along the endothelial wall; then they adhere

firmly to endothelial cells and extravasate [53, 54]. In vitro, tumour cell adhesion to cultured endothelial cells has been extensively studied under static conditions, and a plethora of cell surface adhesion molecules encompassing families of integrins, immunoglobulins and selectins have been proposed as mediators of tumour-endothelial cell interactions [56]. However, these findings should be interpreted cautiously, because effects generated by blood flow and the resulting shear forces may critically affect adhesive cell interactions. In this respect, perfusion chambers that mimic physiological blood flow conditions in the vasculature have been recently used to study in vitro tumour-endothelial cell interactions [57]. Under these experimental conditions, E-selectin expressed by interleukin (IL)-1α-activated endothelial cells mediates rolling of tumour cells, while VCAM-1 (vascular cell adhesion molecule-1) mediates tumour cell attachment. E-selectin binds to sialylated oligosaccharides sLe^x and sLe^a, both of which are expressed in abundance on most human colon cancers, and VCAM-1 binds to integrins $\alpha4\beta1$ and $\alpha4\beta7$, which, for example, are expressed by metastatic melanomas and lymphoblastic lymphomas, respectively [56, 58]. Although immunocytochemical analysis demonstrates that $\alpha4\beta1$- and $\alpha4\beta7$-positive metastatic tumour cells colocalize with VCAM-1-positive vascular endothelial cells [58, 59], the contribution of these integrins during tumour cell interactions with the endothelium has not been studied under dynamic flow conditions nor by intravital microscopy. Moreover, overexpression of integrin $\alpha4\beta1$ in B16a melanoma cells (which lack integrin subunit $\beta7$) fails to show any apparent effect on in vivo experimental lung metastasis formation when compared with that observed with the parent cell line [60]. Alternative mechanisms may therefore be involved during tumour cell extravasation. For example, intravital videomicroscopy indicates that a monoclonal antibody directed against integrin $\alpha6\beta1$ inhibits the ability of B16F1 melanoma cells to extravasate during haematogenous metastasis in the liver [61]. After tumour cells have established tight connections with the endothelium, in vivo and in vitro studies demonstrate that tumour cells activate blood platelets [62]. It is generally accepted that platelets may assist haematogenous dissemination of metastatic cells. Perhaps, the most convincing evidence is the inhibition of lung metastasis formation in mice by experimentally induced thrombocytopenia [63, 64]. Upon activation, platelets release 12(S)-HETE (a molecule derived from arachidonic acid metabolism), which leads to enhanced tumour cell adhesion via upregulation of endothelial cell surface expression of integrin $\alpha v\beta3$ [65]. In addition, 12(S)-HETE induces endothelial cell retraction, resulting in the exposure of the subendothelial matrix (for review, see ref. [62]). Tumour cells fail to attach directly to the subendothelial matrix under flow [66, 67]. Indeed, platelets, activated by tumour cells, promote tumour cell adhesion to the subendothelial matrix during blood flow [66, 67]. The association between tumour cells and subendothelium-bound platelets is mediated by platelet integrin $\alpha IIb\beta3$ and by tumour cell integrin $\alpha v\beta3$ [66, 67]. These findings are supported by the fact that platelets from Glanzmann's thrombasthenic patients, which lack integrin $\alpha IIb\beta3$, fail to interact with tumour cells in vitro [68].

Conclusion

Integrins have long been associated with metastasis, and there is no doubt that they are major contributors to the metastatic process. There was a general tendency to consider that changes in the integrin repertoire of solid tumours may play critical roles in the proliferation, migration and invasion of tumour cells in the surrounding extracellular matrix. Recent evidence summarized in this review indicates that the contribution of integrins to the metastatic process occurs through the regulation of different signalling pathways which dictate the motility, survival and anchorage-independent growth of tumour cells. Moreover, integrins may have a more complex role in metastasis as they cooperate with serine proteases and metalloproteases to promote tumour cell invasion and angiogenesis. Finally, integrins mediate cell-cell interactions and favour tumour cell extravasation. An understanding of the molecular mechanisms by which integrins act at each step of this metastatic cascade will be very important in designing new therapeutic approaches.

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