Integrins and metastasis

Kirat Kumar Ganguly, Sekhar Pal, Shuvojit Moulik, and Amitava Chatterjee*
Department of Receptor Biology & Tumor Metastasis; Chittaranjan National Cancer Institute; Kolkata, India

Keywords: integrin, metastasis, MMP, invasion, adhesion

Introduction

Survival of eukaryotic organisms requires balanced interactions between cells and the extracellular matrix (ECM). Homeostasis is maintained by tight regulation of cell survival, proliferation, differentiation, and cell death (e.g., by apoptosis). Cancer cells disobey the normal restraints on cell division and invade to colonize at other sites that are normally reserved for other cells or other tissue components. The neoplastic cells cluster together into a single mass forming a benign tumor, surgical removal of this mass can result in a complete cure. But such treatment is complicated if the tumor becomes malignant, acquiring the ability to invade surrounding tissues, blood, and lymphatic vessels, and forming secondary tumors, called metastases, at distant sites in the body. Both the cell proliferation and metastasis require continuous interaction with the ECM components and modulation of cell signaling. The nature of such interactions with ECM and downstream signaling is often quite different in cancer cells than in normal cells. Extracellular matrix is the tissue components apart from the cells and is mainly filled with intricate network of macromolecules like glycoproteins, fibrous proteins, etc. Some important examples of ECM components are fibronectin (FN), laminin (LM), vitronectin (VN), and collagen-IV (C-IV). The adhesion signal of these matrix components can regulate biological processes such as proliferation, differentiation, survival, wound healing, migration, tumorigenesis, etc., all of which are necessary for execution of successful metastasis. These responses require active participation of several signaling proteins, including Rho GTPases, MAPKs, FAK, JNK, etc., which are found to be recruited at the ECM ligand/integrin binding site.2,3 The ways in which these intracellular mediators regulate gene expression and ultimately the effectors responses can vary among cell types. Thus, selection of the appropriate matrix for experiments using cultured cells is crucial for studying ECM-mediated signaling and its effectors response, including metastasis.

The major difference in behavior between normal and tumor cells lies in the fact that many receptors and signaling components are altered such that they become constitutively active when normally they should be turned on in a particular situation only. Research in the field of metastatic pathways helped to identify some factors that can be targeted to achieve possible therapeutic purpose. The present review covers mainly integrin receptor-mediated signaling pathways leading to metastasis and the present status of some therapeutic approaches targeting these pathways.

Integrins

Integrins are the most predominant and well characterized cell surface receptors of various extracellular matrix (ECM) proteins (e.g., LM, FN, C-IV, VN, etc.). The complete receptor molecule is composed of non-covalent, heterodimeric complexes of an α subunit and a β subunit.1 So far 18 α and 8 β subunits have been found and they combine to form 24 various combinations of different specificities.1 Many members of the integrin family, including α5β1, α8β1, αIIbβ3, αVβ3, αVβ5, αVβ6 and αVβ8 recognize an Arg-Gly-Asp (RGD) motif within their ligands, including fibronectin (FN), fibrinogen, vitronectin, von Willebrand, factor and many other large glycoproteins.4 This ligand receptor recognition can achieve the specificity and high affinity for each ligand receptor pair, by specific residues outside the RGD motif. Such novel non-RGD site, appears to act synergistically with the RGD site to promote cell adhesion, is often designated as “synergy site”. This sequence is highly conserved among fibronectin of diverse species and in human it is localized to a Pro-His-Ser-Arg-Asn (PHSRN) peptide sequence within the ninth type III module of fibronectin structure.3

Sequence studies on integrin genes have shown no detectable homology between α and β subunits, but 30% and 45% of sequence identities among α and β subunits respectively indicates possible evolution of these two gene families by gene duplication.3 In several mammalian α subunits (α1, α2, α10, α11, αL, αM, αX, αD, and αE), an I (insertion or interaction) domain
is present, which contains a metal-ion-dependent adhesive site (MIDAS), and participates in ligand binding. Integrin α3, α4, α5, α6, α7, α8, α9, αV, and αIIb are non-I-domain subunits. Hence, RGD motif is recognized by integrins having non-I-domain α subunits. However, an I-like domain, which contains a structurally similar metal-binding motif, is present in all human integrin β subunits and amino-acid residues from this domain can interact with the RGD peptide of a ligand.5,6

The general structural features of all α and β subunits of integrins appear to be similar. Both the subunits are transmembrane glycoproteins with large globular NH2-terminal extracellular domains that together make up an ellipsoidal head. Each subunit contains a relatively thin transmembrane domain and a relatively small cytoplasmic tail of less than 60 amino acids,1 only known exception is β4 integrin, which has a cytoplasmic domain of over 1000 amino acids.7 As the cytoplasmic tails of integrins are devoid of enzymatic features, integrins transduce signals by associating with adaptor proteins that connect the integrin to the cytoskeleton, cytoplasmic kinases, and transmembrane growth factor receptors.8 Binding of integrins to ECM allows them to cluster in the plane of the cell membrane and associate with a cytoskeletal and signaling complex that promotes the assembly of actin filaments. The reorganized actin filaments cause more integrin clustering, thus enhancing the matrix binding of integrins in a positive feedback system. As a result, adaptor and cytoskeletal signaling proteins assemble into aggregates at the cytoplasmic tails of integrins so that, integrins can serve as integrators of the ECM and cytoskeleton. Such well-developed aggregates are known as focal adhesion sites.8 These sites provide a structural link between the actin cytoskeleton and the extracellular matrix and are regions of signal transduction that relate to growth control9 and regulate several biological processes such as proliferation, differentiation, survival, wound healing, migration, tumorigenesis, etc.1

An interesting feature of integrin-mediated signaling is bidirectional signaling, which is also termed as outside-in and inside-out signaling. Integrin-ligand interaction induces several conformational changes of integrin itself. Interaction with ligands occurs at the extracellular head-domain which induces separation of cytoplasmic domains of integrin subunits. This allows interaction of the cytoplasmic domain with different cytoskeletal and/or signal transducer molecules. This chain of response called outside-in signaling. On the other hand separation of cytoplasmic domain interaction by talin or other proteins, promotes interaction of head-domain and ligands, which is termed inside-out signaling. Another interesting report shows mutation near the tail or at the cytoplasmic domain near the membrane of either integrin subunit shows constitutive activation of this receptor. This again shows the interaction of two subunits as well as inside-out signaling.10,11 A communication has proposed that these two conceptual models may be view as two reflections of same allosteric equilibrium.12 The crystallization of unligated specific domain of integrin αVβ3 have given newer dimension to the study of integrin bidirectional signaling.13,14 An important molecular mediator of integrin bidirectional signaling is Talin, which has been studied by several groups.15,16 A recent report has showed Kindlin-2 as regulator of integrin bidirectional signaling. The authors have shown Kindlin-2 is needed in integrin outside-in signaling to build concrete cell adhesion and spreading. This study indicates need of more molecular studies on this interesting feature of integrin signaling.17 The inside-out signaling is told to be responsible for integrin-ligand interaction which again promotes formation of integrin clusters, focal adhesion sites and subsequent outside-in signaling. The interplay between both directions of integrin signaling is continued in a synchronized fashion which regulates the several physiological behaviors of cell. This synchrony is lost in pathological condition and found to be associated with numerous human disorders including development and metastasis cancer (Fig. 1).

**Metastasis**

**Basics of metastasis.** Metastasis, the series of actions through which cancer cells disseminate from the primary site of tumor, propagate, and form secondary tumors at distant sites, is a serious clinical problem as it is a disseminated disease. Probably this phenomenon makes the difference between cancer and the other diseases. Metastasis is often impossible to eradicate successfully, which results in the death of most cancer patients. Metastasis results from a complex network of sequential molecular events involving many steps. All of those are interlinked through a series of interactions and invasive processes as well as responses to specialized stimuli. Until now, in spite of its clinical significance, it remains incompletely understood. Some of the principle molecular players in the major steps of the metastatic cascade are tumor angiogenesis, disaggregation of cells from the primary tumor mass regulated by group of cadherins and catenins,18,19 invasion and migration of the cells through the basement membrane and extracellular matrix surrounding the tumor epithelium, and subsequent invasion of the BM of the endothelium of local blood vessels. This is mediated through integrins and proteases, including urokinase form of plasminogen activator (uPA), matrix metalloproteinases (MMPs),20,21 cathepsins, and intravasation of the tumor cells into the blood vessels prior to hematogeneous dissemination to distant sites. Another important criterion of this process is adhesion of the circulating tumor cells to the endothelial cell lining at the capillary bed of the target organ site. This occurs through adhesive interactions between cancer cells and endothelial cells involving some cell adhesion proteins like integrins, selectins and members of the immunoglobulin superfamily.22 The next important phase is invasion of the tumor cells through the endothelial cell layer, neighboring basement membrane (this process is called extravasation), and target organ tissue. Then there is step of development of secondary tumor foci at the distant organ. In this section we will discuss some intricate features of each steps of tumor metastasis (Fig. 2).

**Sequential events of metastasis. Invasion from primary tumor site.** Initiation of metastatic journey of cancer cells occurs through separation of some cancer cells from the mass of primary tumor and their protrusion through tumor-associated stroma and thereafter into the adjacent normal tissue parenchyma. The first strong barrier for the cancer cells invasion is the basement
membrane. The basement membrane is a form of extracellular matrix that plays critical role in maintaining architecture of epithelial tissues, partially by separating the epithelial and stromal compartments. The basement membrane is also found to store embedded growth factor molecules that can be liberated by carcinoma-secreted proteases. Moreover, the BM also plays crucial roles in signal transduction events within carcinoma cells via pathways initiated by integrin-mediated cell-matrix adhesions, leading to alterations in cell polarity, proliferation, invasiveness, and survival. Budding evidence indicates that the minute tissue architecture of normal epithelium serves as an intrinsic barrier to invasiveness. This barrier must be overcome by incipient metastatic carcinoma cells before they can develop their invasive and malignant property. In the mammary gland, myoepithelial cells oppose invasion by helping to maintain BM integrity; indeed, co-implantation with myoepithelial cells reversed the invasiveness of breast carcinoma xenografts. Similarly, in ovarian carcinomas, the mesothelial cell layer that lines peritoneal and pleural organs serves as an obstacle to further dissemination that can be overcome by carcinoma cell-exerted, myosin-dependent traction forces that physically displace mesothelial cells. Moreover, modulation of ECM stiffness, achieved by altering collagen crosslinking, affects breast carcinoma progression via altered integrin signaling. At a cell-biological level, most types of carcinomas can invade as cohesive multicellular units through a process known “collective invasion”. Alternatively, individual tumor cells may invade via two distinct programs: the protease-, stress-fiber-, and integrin-dependent “mesenchymal invasion” program or the protease-, stress-fiber-, and integrin-independent, Rho/ROCK-dependent “amoeboid invasion” program. Indeed, differential expression of molecules that enable either mesenchymal or amoeboid invasion can be observed in signatures of local invasiveness derived from mammary carcinoma models. Tumor cells can apparently interconvert between these various invasion strategies in response to changing microenvironmental conditions. This has caused some to propose that robust suppression of single-cell invasion requires concomitant inhibition of the mesenchymal and amoeboid invasion programs. Certain regulators of invasion function as pleiotropically acting factors that simultaneously modulate components of both pathways. For example, microRNA (miRNA) miR-31 inhibits breast cancer invasion via concurrent suppression of key effectors of both the mesenchymal (such as integrin α5) and amoeboid (such as RhoA) invasion programs. The single-cell invasion pathways cited above are clearly incompatible with one critical element of epithelial tissue organization, specifically the E-cadherin-mediated intercellular junctions that knit together epithelial cell sheets and prevent dissociation of individual epithelial cells from their neighbors. In order to overcome this and other obstacles to invasion, carcinoma cells may appoint a cell-biological program known as epithelial mesenchymal transition (EMT), which is critical for multiple aspects of normal embryonic morphogenesis. The EMT program, which involves dissolution of adherens and tight junctions and a loss of cell polarity, dissociates the cells within epithelial cell sheets into individual cells that exhibit multiple mesenchymal attributes, including heightened invasiveness. EMT programs are orchestrated by a set of pleiotropically acting transcription factors, including Slug, Snail, Twist, ZEB1, and ZEB2, which organize entrance into a mesenchymal state by suppressing expression of epithelial markers and inducing...
invading tumor cells, MMP-expressing cells also secrete growth factors that remain there and thereby induce cancer cell proliferation.\textsuperscript{34} Once invading carcinoma cells have dissolved the basement membrane, they pass in the stroma, where they are threatened with several of tumor-associated stromal cells. The composition of these cells is directed by the maturity of tumor. With the progression of primary tumor, the stroma is developed to more responsive and it gets many of the new features of the stroma.\textsuperscript{35} The tumor cells invading into a stromal environment encounter different types of cells like fibroblasts and myofibroblasts, endothelial cells, adipocytes, and various bone marrow-derived cells such as mesenchymal stem cells, as well as macrophages and other immune cells.\textsuperscript{36} These stromal cells take part in further enhancing the aggressive features of carcinoma cells by inducing different types of signaling. Reports have showed that breast cancer invasiveness may be stimulated through the local adipocytes secreted interleukin-6 (IL-6).\textsuperscript{37} Moreover, by stimulating tumor-associated macrophages (TAMs), stromal CD4\textsuperscript{+} T lymphocytes promote mammary carcinoma invasion through activation of epidermal growth factor receptor (EGFR) signaling.\textsuperscript{38} This background knowledge suggests a both side interaction of carcinoma cells and stroma, where the cells induces formation of reactive stroma and the stroma enhances the malignant nature.
of the carcinoma cells. This indicates toward a feedback interaction between these two tissue components. Microarray analysis of stroma from breast cancer tissue have shown characteristics features capable of inducing invasive property of cell. Recent observations are accumulating knowledge showing relevance of stroma in making cancer more vulnerable.

**Intravasation.** Intravasation defines entry of invasive carcinoma cells into the lumen of lymphatic or blood vessels. Spread of carcinoma cells through lymphatic system is a common part of metastasis and used as prognostic marker of advancement of cancer, but spread of carcinoma cells through blood circulation system is the most prevalent mechanism of tumor cell dissemination. Intravasation needs changes of the cells in molecular level that facilitate the ability of carcinoma cells to move through the walls of microvessels made by pericyte and endothelial cell. In colon cancer the transcriptional modulator N-terminal enhancer split are found to inhibit the intravasation of cells by impairing trans-endothelial invasion through Notch-dependent mechanisms. Again report shows cytokine-transforming growth factor-β (TGF-β) induces mammary carcinoma intravasation, by increasing carcinoma cell penetration of microvessel walls or increasing invasiveness. Perivascular TAMs are found to enhance intravasation process through a positive feedback loop. In this loop involve reciprocal secretion of epidermal growth factor (EGF) and colony stimulating factor-1 (CSF-1) by TAMs and cancer cells, respectively. Interestingly, the event of intravasation depends on formation and structural characteristics of surrounding blood vessels. Tumor cells induce formation of new blood vessels in its microenvironment through many vascular endothelial growth factors (VEGF)-regulated mechanisms. These vessels are generally fragile, and immature compared with the vasculatures present in normal tissue. Reports show that intravasation is also facilitated by interaction between adjacent endothelial cells that constitute microvasculatures and absence of pericyte coverage. In this connection this may be noted that cyclooxygenase-2 (COX-2), epiregulin (EREG), MMP-1, and MMP-2, which are found to stimulate formation of new defective vessels surrounding tumor cells, also induce intravasation.

**Self defense of carcinoma cells in circulation.** After entering blood vessels the cells are able to spread in many directions through circulation. Recent techniques have made it possible to track those cells within the blood vessels of the patients. With these techniques the cells circulating between primary and metastatic site can be detected. To make an effective metastasis, circulating cells have to survive and reach an ambient environment. During the flow in circulation tumor cells face different adverse environmental stresses. One of those is absence of integrin-dependent attachment to the extracellular matrix (ECM), which is essential for cell survival. In absence of that the cells will undergo anoikis, a type of apoptosis induced by loss of anchorage. There are some metabolic and/or signaling pathways, like pentose phosphate pathway and regulation of glucose uptake, tyrosine kinase TrkB, which gives the cells protection from this type of death. There are several schools of thought regarding the lifetime of cancer cells in circulation. A report has proposed that breast cancer cells survive in circulation for several hours. Another hurdle to the circulating cells are their size. Typically the blood capillaries are about 8 μm in diameter, whereas diameters of carcinoma cells are generally between 20–30 μm. So, it may be considered that the cells are trapped within the capillaries during their first pass, which happens within minutes after intravasation. This indicates the tumor cells that escape these barriers spend much less time within circulation and their overcome death. Two other threats to circulating tumor cells are dynamic force of blood flow and patients own innate immunity. But tumor cells battle with these problems by forming emboli through interacting with platelets. A class of protein that helps in this interaction is called selectin. Launch at a distant site. It is seen though the circulating tumor cells disseminate to all directions of body, but there is strict specificity where they can settle. This sites are depends on the primary site of the tumor. A specific type of cancer is destined to form secondary tumor at a specific distant site. A very early report by Stephen Padget (1889) explained this theory as the “seed and soil hypothesis”. After several decades the same idea was resumed by other groups. Some proposals suggested that trapping is higher in organs that possess more frequent capillaries than other microvessels. For example, for colorectal cancer, the liver is a site for frequent trapping site, where metastatic cells are drained through portal veins. Another proposal says this site specificity is a predetermined, and mediated by specific combinations of attachment proteins present in the tumor cells and cells of that site.

**Extravasation.** After settling within microvessels of distant site, circulating tumor cells starts to grow which eventually form a microcolony. This microcolony sometimes breaks the hosting walls of vessel and makes contact with surrounding tissue. In another way, cells penetrate endothelial and pericytic cell layer which separate tissue microenvironment and blood vessels. This step is called extravasation. In contrast with supporting factors of intravasation, in extravasation the TAM cells are not expected to be equally available and blood vessels are highly active and non-fragile. So these environmental factors build another barrier to the tumor cells, even after escaping from adverse situation within circulation. To overcome these physical barriers, tumor cells secrete some molecules like matrix metalloproteinases (MMP-1, MMP-2, MMP-3, and MMP-10) angiopoietin-like-4 (Angpt-2 and Angptl4), as well as the pleiotropically acting factors EREG, COX-2, etc., which degrade those physical barriers and increases the permeability of distant microenvironment. These molecules facilitate the process of extravasations along with some specialized mechanisms like CCL-2-dependent recruitment of inflammatory monocyte in pulmonary metastasis. Micrometastases. The microenvironment of this distant site is usually far different from the primary site of the tumor, in terms of types of stromal cells, composition of ECM, available growth factors and cytokines, etc. So at first the cells have to adapt and survive the environment. Some groups have suggested that the tumor cells primarily adopt the situation by forming some premetastatic niche. According to this hypothesis, primary tumor releases signals by secreting lysyl oxidase (LOX), which induces host fibroblasts to secrete fibronectin (FN). A group has proposed that prior arrival of the circulating tumor cells; FN in turn
attracts VEGF receptor-1 positive hematopoetic progenitor cells from the bone marrow to that site, by interacting with integrin α4β1 expressed on those cells. This signaling event induces MMP-9 expression, secretion and activation which along with other sequestered proteins alters the microenvironment and acts as chemotactants for the coming metastatic tumor cells.\textsuperscript{58}

Colonization of tumor cells. It is seen that even after formation of stable microcolonies at distant site, cells cannot form mature metastatic colonies for long-term. Some groups indicate that formation of larger colony is hindered due to inadaptability of microenvironment.\textsuperscript{59} Some groups have indicated this dormancy is caused by inability to form integrin β1, focal adhesion kinase (FAK), Src complex and underlying signaling.\textsuperscript{60,61} It is reported that this process is sometime overcome by induction through molecules like osteopontin (OPN) or stromal cell-derived factor 1 (SDF-1). An alternative way suggests that micrometastases may proliferate continuously.\textsuperscript{59}

In this way the term metastasis includes a series of interdependent physical and signaling events. Ultimately this flow of cellular activities form a mass of transformed cells at an organ other than the initially damaged organ and this makes cancer more devastating.

Integrin-Mediated Signaling and Metastasis

Metastasis is a multistep process which depends on regulation of some cellular properties like cell attachment-detachment, phenotypic changes of cell, e.g., epithelial to mesenchymal transformation, cell survival, cell migration and invasion, etc. These processes are regulated by different molecular events within the cells which are reflected in the regulation of metastasis. From that point of view cell adhesion molecules and growth factor receptors are the important regulators of metastasis. Integrins are the type of cell adhesion molecule that takes crucial part in regulation of metastasis.

Integrins are the important example of bidirectional receptor signaling. Inside-out signals induce conformational changes between the integrin headpiece and the cytoplasmic domains with exposure of neoepitopes, i.e., the ligand-induced binding sites. Attainment of high-affinity states is necessary for binding of outside-in signals which regulates cellular responses to environment. This bidirectional signaling is very important in cancer because it is seen that constitutive activation of integrins from endogenous stimuli mediates stronger binding to the ECM and therefore a more dynamic interaction of these adhesion receptors with their substrates. This is necessary for migration and metastasis studies correlating integrin expression levels in human tumors with pathological outcomes, such as patient survival and metastasis. Studies have identified several integrins that might have an important role in cancer progression. Tumor cell expression of the integrins αvβ3, αvβ5, α5β1, α6β4, α4β1, and αvβ6 is correlated with disease progression in various tumor types. Study on non-small cell lung cancer has showed importance of integrin expression development and progression of lung cancer. The group has suggested integrin α5, β1, and β3 expression as prognostic marker of overall survival in early stage of that type of cancer. They have also shown expression of those integrins as marker for lymph node metastasis and recurrence. Notably, integrins αvβ3, α5β1, and αvβ6, most of the times are found to be expressed at very low even undetectable levels in most adult epithelial cells. Whereas those can be highly overexpressed in some tumors.\textsuperscript{62} Keller and Brown showed frequent association of bone metastasis with advanced prostate cancer is determined by integrin-mediated interaction of metastatic cancer cells and bone microenvironment.\textsuperscript{63} A recent observation showed integrin αvβ3 is responsible for making the choice of attachment to the bone microenvironment and subsequent growth of tumor.\textsuperscript{64} These observations shows integrin as regulator of metastasis.

To colonize distant target organs, cancer cells must cut the connections from the primary tumor, gain access to blood vessels and survive within the vasculature exposed to shear forces which physically oppose cell attachment. Activated αvβ3 may rescue blood circulating cancer cells from shear-induced tumor cell arrest by binding to leukocytes and platelets to survive.\textsuperscript{65} Once localized to the metastatic environment, cancer cells of different tissue origins may utilize distinct integrin-ligand combinations to colonize the same target organ and receive local mitogenic stimuli. In most cancers the αvβ3 integrin is the prime initial receptor to support adhesion and migration to bone matrix. Moreover, overexpression of all three integrins, N-cadherins, and melanoma cell adhesion molecule (MCAM) in a melanoma tissue microarray\textsuperscript{66} was associated with a higher incidence of worse prognosis gastrointestinal metastasis, according to American Joint Committee on Cancer (AJCC) staging metastatic category (M1c), rather than development of more favorable subcutaneous and lymph node metastasis (AJCC M1a, M1b). In their activated state, several integrins recognize ligands, which are usually not bound when inactive. Such aberrant binding may favor colonization of certain microenvironments, such as the bone matrix via sialoprotein or interact with molecules which further enhance malignant behavior, such as osteopontin. Osteopontin is a secreted glycoprotein that is overexpressed in a number of different carcinomas and can promote adhesion, migration and metastasis by binding predominantly to the αvβ3 integrin and the CD44 antigen. This indicates, more aggressive cancers not only have the potency to express more osteopontin but also may be more responsive to this protein.\textsuperscript{65,66}

In addition to their ligation-dependent effects, some cases have shown that unligated integrins can positively or negatively influence tumor cell metastasis. How integrins affect tumor cell survival both in the ligated and unligated states may be a critical determinant of the efficacy of integrin-targeted therapeutic strategies in cancer. A recent report has shown that in integrin-mediated death-resistant tumor cells the unligated integrin αvβ3 significantly upregulates anchorage-independent tumor cell metastasis in vivo.\textsuperscript{69} Growing evidences are showing integrin crosstalk with growth factor cytokines could have important implications for tumor metastasis and the acquisition of drug resistance.\textsuperscript{70,71}

Our lab has shown in cervical cancer cell SiHa, integrin α5β1-fibronectin interaction induces expression, appreciable activation of pro-MMP-9 and moderate change of pro-MMP-2 activity.
involving FAK, ILK, ERK, PI-3K, and NFκB signaling cascade. This ligand-integrin interaction was also found to accelerate cell migration. Another study from our lab showed, in the same cervical cancer cell line, interaction of integrin α5β1 with laminin also induced MMP-9 expression and activation involving FAK, PI-3K and ERK signaling. Interestingly we found induction also induced MMP-9 expression and activation involving FAK, where activation of FAK is induced by its association with α5β1 integrin. In addition to this several other groups have focused on importance of integrin α5β1 in development and/or progression of cancer. For instance, in pancreatic cancer model upregulation of integrin α5β1 was reported to be associated with radiation induced invasion of the cells. A very recent publication on cervical cancer system has reported direct association of integrin α5β1 with an important receptor tyrosine kinase, c-Met. They have shown this association activates c-Met in a hepatocyte growth factor/scatter factor (HGF/ SF) independent pathway, subsequently which plays crucial role in angiogenesis. Studies on a pool of breast cancer tissue sample shows that integrin α5β1 and its downstream signaling cascade is the pathway through which angiopoietin-2 induces breast cancer cell invasion and thereby metastasis. A report by Murillo et al. showed in a colon cancer model, inhibition of integrin α5β1 resulted in decreased cell adhesion and PI3K activation. Another report on colon cancer model reported unusual upregulation of the same integrin found to be linked with metastatic progression. In a hepatocarcinoma cell system inhibition of integrin α5β1 was reported to induce cell invasion involving ERK1/2 and p38 MAPK signaling cascades. In breast cancer cell line MCF-7, fibronectin-α5β1 interaction was found to increase expression and activation of both MMP-2 and MMP-9. Uptregulation of both of these MMPs were found when human melanoma cell A375 was exposed to fibronectin. This interaction also reported to upregulate MMP-9 in human myeloid leukemia cells K562. Our group also showed in a highly invasive breast cancer cell line MDA-MB-231, fibronectin-α5β1 interaction upregulates MMP-9 expression and activity. In cervical cancer cell line SiHa, not only integrin α5β1 but integrin αvβ3 was found to be associated with MMP activity.

Studies have shown expression of integrin αvβ3 expression to be a major determinant of breast cancer cell bone metastasis. Integron αvβ3 is found as modulator of MMP-2 activation lymph node metastasis. Role of β3 and β5 integrins in growth and metastasis of murine mammary carcinomas is established by several groups. An interesting observation shows crucial involvement of platelet and osteoclast β3 integrins in bone metastasis. It was found that in blood flow breast cancer cells attachment is dependent on integrin αvβ3 activation status. Another integrin combination, integrin αvβ5 found to build crosstalk with epidermal growth factor receptor. This link between integrin and growth factor receptor promotes carcinoma cell metastasis. Cell invasion and metastasis was found to be stimulated by epidermal growth factor treatment. This was found to be retarded by blocking integrin αvβ5 suggesting inter-relation of integrin and growth factor receptors.

Therapeutic Aspects

Altered expression of integrins in different types of cancer, in relation to that with tumor progression, tumor cell metastasis, and their ability to crosstalk with growth factor receptors, has made this protein a point of target for therapeutic interventions. Different groups have shown inhibitors of integrin have shown suppressive effect on the metastatic cascade. Integrin inhibitors are now in clinical trials, which include monoclonal antibody and RGD peptide analog. Another section will focus on the strategies that use integrin as a tool to battle metastasis.

Approaches targeting αvβ3 and αvβ5. Integron αvβ3 is upregulated in both tumor cells and angiogenic endothelial cells, which makes it an attractive therapeutic target. The first strategy of integrin deactivation was anti-integrin monoclonal antibody approach. Among those LM609 may be mentioned which showed appreciable anti-angiogenic activity in preclinical model experiments. An altered form of this molecule was developed with name of etaracizumab (MEDI-522) for treatment in humans.

This new molecule along with its anti-angiogenic effect also inhibited tumor growth with direct effect on tumor cells. Due to its high efficiency etaracizumab (MEDI-522) was subjected to clinical trial. At the initial phases of trial, vatinax, the precursor of etaracizumab, showed appreciable suppression of angiogenic activities with low toxicity and disease stability in some patients with renal cancer and solid tumors. In later stages, it showed effectiveness in advanced stages of melanoma.

In xenograft tumor models anti-integrin αv monoclonal antibody (CnTo 95) showed anti-tumor and anti-angiogenic effect. In Phase I trial has shown nontoxic effect and found to be localized to tumors. Cilengitide is an interesting molecule, having ability to inhibit both αvβ3 and αvβ5 integrins. Currently cilengitide is under phase trials in lung cancer, prostate cancer and glioblastoma patients where it is showing extended survival with minimum side effects.

These results were also supported by the observation by other groups showing effects of low concentrations of integrin antagonists in different cases.

Targeting β1 integrins. Among integrin-antagonist strategies another crucial target is integrin-β1, precisely α5β1. This way have demonstrated appreciable efficacy in reducing tumor load in several preclinical studies. Anti-integrin-β1 inhibitory antibody substantially affected in vitro and in vivo growth of human breast cancer tumor cells. Volociximab is an anti-integrin α5β1 monoclonal antibody which blocks the function of integrin α5β1. Volociximab is found to inhibit angiogenesis and hinder tumor growth. Volociximab is found to inhibit angiogenesis and hinder tumor growth. This observation led this to phase II trial for solid tumor. A group has demonstrated a mechanism-based receptor binding model to describe the pharmacokinetic and pharmacodynamic of volociximab in cancer patients.

A different approach has built a non-RGD-based peptide inhibitor, ATn-161. This is an inhibitor of integrin α5β1. In vivo studies have shown its efficacy in blocking breast cancer growth.
and metastasis.\textsuperscript{107} A combination therapy of ATN-161 with fluorouracil showed significant reduction of tumor burden and liver metastasis in a mouse model study of colon cancer metastasis to the liver.\textsuperscript{108} ATN-161 was found to be well tolerated in patients with advanced solid tumor.\textsuperscript{109}

Other integrin targeted inhibitors. There are bunches of integrin-antagonists that have shown appreciable efficiency in preclinical studies, but those have to pass through the clinical trials. S247, an integrin αβ3 antagonist inhibited breast cancer metastasis in a xenograft tumor model.\textsuperscript{110} The compound also decreased colon cancer metastasis, angiogenesis and increased survival.\textsuperscript{111} Another integrin αβ3 antagonist, Pk1404, was found to inhibit breast and ovarian cancer bone metastases without hampering osteoclastic activity.\textsuperscript{112} Anti-metastatic effect was found upon treatment of two RGD-peptide-mimic, α3β3 and αvβ6, on integrin-targeted therapeutic treatment of human hepatocellular carcinoma cell was found to be arrested when either ItGAV or ItGB3 was silenced using antisense approach.\textsuperscript{114} Another group has demonstrated that both in vivo and in vitro growth of human pharyngeal carcinoma cells were arrested by treatment of integrin αβ6 inhibitor, 6.3G9 monoclonal antibody.\textsuperscript{115} In addition this monoclonal antibody was found to inhibit TGFB signaling, indicating some of its efficiency involve crosstalk with TGFB receptor mediated signaling. These compounds draw attention to move for clinical trial with these molecules, standardization of their combinations with other clinical parameters.

Conclusion

Both in physiological and pathological conditions, cell attachment to it surrounding extracellular matrix (ECM), plays a key role in inducing several biological activities like cell migration-invasion, cell survival-apoptosis, and cell proliferation. Integrin is one of the most important cell attachment molecules and it is found to be involved in regulating all of the above mentioned cellular activities. In spite of emphasis given on different targets of cancer research like growth factor related pathways, angiogenic regulators, and cancer immunology. Integrin biology truly demand its place in cancer research, since it is linked with all of these and many more pathways which regulate each and every step of cancer development and progression. As metastasis is a multistep process involving a number of integrin-regulated cellular activities, so this will be a meaningful approach to target different integrin molecules to regulate metastasis. The mode of treatment may be either combinatorial or single-agent treatment, which may be more firmly decided after more studies on integrins and metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Berrier AL, Yamada KM. Cell-matrix adhesion. J Cell Physiol 2007; 213:565-73; PMID:17680633; http://dx.doi.org/10.1002/jcp.21237
2. Miyamoto S, Teramoto H, Gojo OA, Gukindent JS, Burbejo PD, Akiyama SK, et al. Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. J Cell Biol 1995; 131:791-805; PMID:7593197; http://dx.doi.org/10.1083/jcb.131.5.791
3. Zaidel-Bar R, Itskovitz-Sagi Y, Mayer I, Geiger B. Functional atlas of the integrin adhesome. Nat Cell Biol 2007; 9:858-67; PMID:17671451; http://dx.doi.org/10.1038/ncho.8007-838
4. Takagi J, Strokovich K, Springer TA, Walc T. Structure of integrin alpha6beta1 in complex with fibronectin. EMBO J 2003; 22:4607-15; PMID:12970173; http://dx.doi.org/10.1093/emboj/cdg445
5. Takada Y, X. Simon S. The integrins. Genome Biol 2007; 8:215; PMID:17543146; http://dx.doi.org/10.1186/gb-2007-8-5-215
6. Xiong JP, Stehle T, Zhang R, Joachimak A, Frech M, Goodman SL, et al. Crystal structure of the extracellular segment of integrin alphaV beta3 in complex with an Arg-Gly-Asp ligand. Science 2002; 296:339-45; PMID:11548639; http://dx.doi.org/10.1126/science.1064535
7. Arnaout MA, Mahalingam B, Xiong JP. Integrin structure, allosteroy, and bidirectional signaling. Annu Rev Cell Dev Biol 2005; 21:381-410; PMID:16212508; http://dx.doi.org/10.1146/annurev.cellbio.21.090704.151217
8. Calderwood DA, Yan B, de Pereda JM, Alvarez-Borrego F, Fujikata Y, Liddington RC, et al. The phosphotyrosine binding-like domain of talin activates integrins. J Biol Chem 2002; 277:21749-58; PMID:11932255; http://dx.doi.org/10.1074/jbc.M111996200
9. Wegener KL, Partridge AW, Han J, Pickford AR, Liddington RC, Ginsberg MH, et al. Structural basis of integrin activation by talin. Cell 2007; 128:171-82; PMID:17218623; http://dx.doi.org/10.1016/j.cell.2006.10.048
10. Montanez E, Ussar S, Schifferer M, Boil M, Zent R, Moser M, et al. Kindlin-2 controls bidirectional signaling of integrins. J Cell Biol 2007; 9:858-67; PMID:17671451; http://dx.doi.org/10.1038/ncho.8007-838
11. O’Toole TE, Mandelman D, Fororhy J, Shatil SJ, Plow EF, Ginsberg MH. Modulation of the affinity of integrin αIIbβ3 (GPIIb-IIIa) by the cytoplasmic domain of alpha IIb. Science 1991; 254:845-7; PMID:19488065; http://dx.doi.org/10.1126/science.19488065
12. O’Toole TE, Karagyi Y, Faull RJ, Peter K, Tamura R, Quatanta V, et al. Integrin cytoplasmic domains mediate inside-out signal transduction. J Cell Biol 1999; 142:1047-59; PMID:7510712; http://dx.doi.org/10.1083/jcb.142.6.1047
13. Hynes RO. Integrin bidirectional, allosteric signaling molecules. Cell 2002; 110:673-87; PMID:12297042; http://dx.doi.org/10.1086/340092
14. Goodwin SL, et al. Crystal structure of the extracellular segment of integrin alpha Vbeta3. Science 2001; 294:339-45; PMID:11548639; http://dx.doi.org/10.1126/science.1064535
15. Martin MD, Marrissin LM. The other side of MMPs: protective roles in tumor progression. Cancer Metastasis Rev 2007; 26:677-94; PMID:17717634; http://dx.doi.org/10.1007/s10555-006-7886-9
16. Gassmann B, Lang ME, Mees ST, Haier J. In vivo tumor cell adhesion in the pulmonary microvascu- lature is exclusively mediated by tumor cell–endothelial cell interaction. BMC Cancer 2010; 10:177; PMID:20933713; http://dx.doi.org/10.1186/1471-2407-10-177
17. Bisell MJ, Hines WC. Why don’t we get more cancer? A proposed role of the microenvironment in restram- ing cancer progression. Nat Med 2011; 17:240-9; PMID:21385745; http://dx.doi.org/10.1038/nm.2328
18. Hu M, Yao J, Carroll DK, Weremowicz S, Chen H, Carrasco D, et al. Regulation of in situ invasive breast carcinoma transition. Cancer Cell 2008; 13:494-406; PMID:18455123; http://dx.doi.org/10.1016/j.ccr.2008.03.007
19. Iwamiuchi MP, Davidowitz RA, Ng MR, Besser A, Munzen E, Merritt M, et al. Ovarian cancer spheroids use myosin-generated force to clear the mesothelium. Cancer Discov 2011; 1:144-57; PMID:21939516; http://dx.doi.org/10.1158/2159-8274.CD-11-0010

©2013 Landes Bioscience.
expresses and secretes MMP-9 in serum-free culture

PMID:20224727.

PMID:20224727.65

PMID:20224727.97.

PMID:20224727.120.

PMID:20224727.152.

PMID:20224727.184.

PMID:20224727.216.

PMID:20224727.248.

PMID:20224727.280.

PMID:20224727.312.

PMID:20224727.344.

PMID:20224727.376.

PMID:20224727.408.

PMID:20224727.440.

PMID:20224727.472.

PMID:20224727.504.

PMID:20224727.536.

PMID:20224727.568.

PMID:20224727.591.

PMID:20224727.623.

PMID:20224727.655.

PMID:20224727.687.

PMID:20224727.719.

PMID:20224727.751.

PMID:20224727.783.

PMID:20224727.815.

PMID:20224727.847.

PMID:20224727.879.

PMID:20224727.911.

PMID:20224727.943.

PMID:20224727.975.

PMID:20224727.1007.
113. Shannon KE, Keene JL, Sertle SL, Duffin TD, Nichols MA, Westlin M, et al. Anti-metastatic properties of RGD-peptidomimetic agents S137 and S247. Clin Exp Metastasis 2004; 21:129-38; PMID:15168730; http://dx.doi.org/10.1023/B:CLIN.0000024764.93092.5f

114. Li J, Tan H, Dong X, Xu Z, Shi C, Han X, et al. Antisense integrin alphaV and beta3 gene therapy suppresses subcutaneously implanted hepatocellular carcinomas. Dig Liver Dis 2007; 39:557-65; PMID:17374519; http://dx.doi.org/10.1016/j.dld.2007.01.025

115. Van Aarsen LA, Leone DR, Ho S, Dolinski BM, McCoon PE, LePage DJ, et al. Antibody-mediated blockade of integrin alpha v beta 6 inhibits tumor progression in vivo by a transforming growth factor-beta-regulated mechanism. Cancer Res 2008; 68:561-70; PMID:18199553; http://dx.doi.org/10.1158/0008-5472.CAN-07-2307