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Stromal Cells and Integrins: Conforming to the Needs of the Tumor Microenvironment[1]

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Abstract

The microenvironment of a tumor is constituted of a heterogenous population of stromal cells, extracellular matrix components, and secreted factors, all of which make the tumor microenvironment distinct from that of normal tissue. Unlike healthy cells, tumor cells require these unique surroundings to metastasize, spread, and form a secondary tumor at a distant site. In this review, we discuss that stromal cells such as fibroblasts and immune cells including macrophages, their secreted factors, such as vascular endothelial growth factor, transforming growth factor β, and various chemokines, and the integrins that connect the various cell types play a particularly vital role in the survival of a growing tumor mass. Macrophages and fibroblasts are uniquely plastic cells because they are not only able to switch from tumor suppressing to tumor supporting phenotypes but also able to adopt various tumor-supporting functions based on their location within the microenvironment. Integrins serve as the backbone for all of these prometastatic operations because their function as cell-cell and cell-matrix signal transducers are important for the heterogenous components of the microenvironment to communicate.

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Introduction

With more than half a million people dying in the United States from cancer each year, the prevention and treatment of cancer have come to the forefront of biomedical research in the last three decades. Extensive research on neoplasia has led to a profound understanding of how normal cells acquire specific genetic mutations in their progression to either benign or malignant cells. Through these genetic alterations, cancer cells acquire the ability to survive and proliferate with minimal adhesion to the extracellular matrix (ECM) and to evade antigrowth signals and apoptosis. They also are able to multiply without limit and sustain nutrients by angiogenesis. Although these mutations characterize all cancer cells, only malignant cells rather than benign ones are able to invade the surrounding tissue and spread to other parts of the body by metastasis. In order for the progression and metastasis of tumor cells to occur, several obligatory steps must be completed. Cancer cells must first separate from the primary tumor, degrade the physically barring basement membrane and interstitial matrix of the ECM, and invade the walls of the circulatory system in a process known as intravasation. Because cancer cells must penetrate the basement membrane and breach cell-cell adhesions, they often enter the circulatory system through blood vessels or lymph vessels, both of which, in the cancerous condition, have discontinuous basement membranes and irregular cell-cell junctions [1,2]. The process of intravasation is crucial to the metastatic process because tumors that are removed before these initial steps seldom recur [3]. Once they have entered the circulatory system, cancer cells must be transported through the body, a rigorous process wherein normal epithelial cells do not survive. Immune recognition, anoikis (cell death associated with detachment from the ECM), and the physical stress of the circulatory system are all factors that the cancer cell must evade before it can come to rest at a distant organ. Finally, the cancer cell must leave the bloodstream and gain access to the secondary site by inducing angiogenesis and/or by appropriating existing blood vessels [4].

Nevertheless, genetic mutations alone are not sufficient to allow cancer cells to fully undergo the complex metastatic process. The tumor's microenvironments at the primary tumor, during circulation, and at the secondary site are all pivotal to tumor metastasis and progression. According to the Paget "seed and soil" hypothesis, "bad seed" (tumors) will only develop in a "good soil" (environment) that supports tumor

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formation. Previously, it had been thought that cancer cells had the potential to metastasize in any number of locations by releasing factors that modify surrounding normal cells into cancer cells. However, the "seed and soil" hypothesis suggests that it is the mutual interaction between the cancer cell and its specific microenvironment that contributes to tumor formation [4]. Just as cancer cells affect the surrounding normal cells, the heterogeneous mixture of cells in the surrounding environment will, in turn, promote progression of the tumor by stimulating growth, survival, invasion, and metastasis. In addition, tumor growth is dependent on the conditions of the tumor microenvironment, such as low glucose, low pH, and low levels of oxygen, called hypoxia [5]. However, at this point, very little is understood about the mechanisms by which this interaction occurs. This review broadly summarizes recent progress in the understanding of how different cell types participate in the development of a microenvironment that favors tumor cell invasion and metastasis. In this review, we will discuss the important role of macrophages, fibroblasts, and integrins in the tumor microenvironment.

Protumorogenic Activities of Stromal Cells in the Microenvironment

Defining the Microenvironment

The microenvironment consists of an amalgam of secreted soluble factors, noncellular solid material, and stromal cells that directly surround the tumor cells. Secreted soluble factors include chemokines such as CXCR-4 and CXCL-12, matrix-altering enzymes such as matrix metalloproteinases (MMPs), protease inhibitors, and growth factors such as vascular endothelial growth factor (VEGF), all of which are stored in the surrounding ECM and released when required by the tumor cells. The surrounding ECM itself, which is composed of the interstitial matrix and basement membrane, constitutes the noncellular solid material that is critical in the anchorage and migration of malignant cancer cells. However, a central focus of this review will be on stromal cells, particularly fibroblasts, endothelial cells, and macrophages, (Figure 1), which are present in the surrounding environment but are recruited by tumors to promote malignant progression, cell escape and survival, and growth at the secondary site.

Macrophages

Macrophages are released by the bone marrow, after which they navigate through the circulatory system before landing at tissues in the bone, liver, or lung. Once mature, macrophages then act as a cardinal defense mechanism against wounding and/or infection. This includes alerting the immune system to the presence of harmful tumor cells by secreting chemokines that recruit other immune cells to the area and by producing growth factors and angiogenic factors such as VEGF.

Whereas macrophages under normal conditions often exhibit antitumorogenic properties, macrophages under cancerous conditions have also been shown to be key participants in the prometastatic process by enhancing tumor cell migration, invasion, and intravasation [6,7]. Furthermore, they also promote angiogenesis and thereby promote greater tumor size and grade [8]. Macrophages are able to possess both tumor-suppressing and tumor-promoting properties because of their distinct M1 and M2 phenotypes. These two phenotypes not only give macrophages a unique sense of plasticity but also allow the tumor to elicit distinct functions from these macrophages depending on the stage of tumor progression [8]. "Classically activated" M1 macrophages contribute to

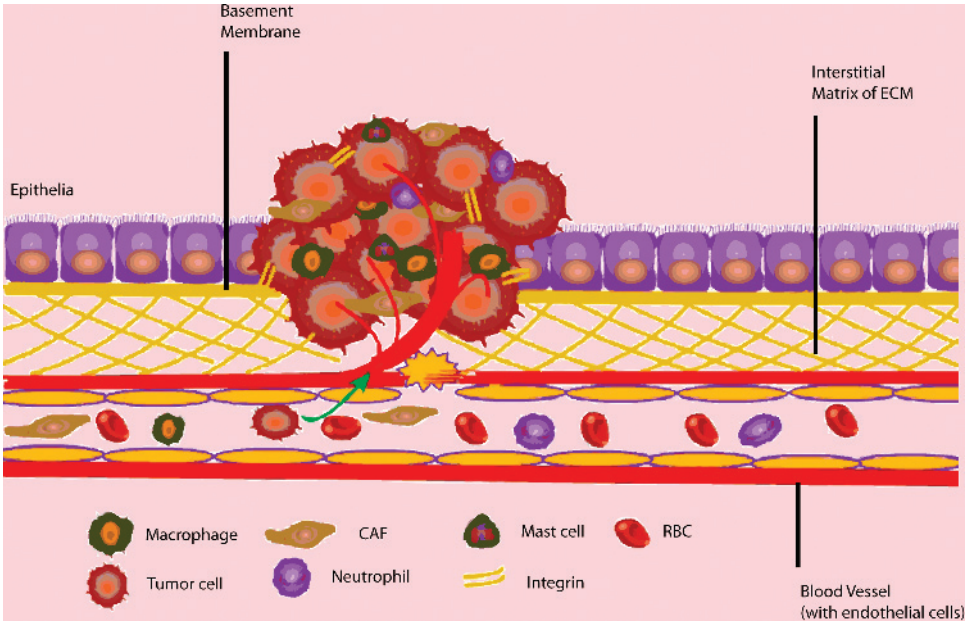

Figure 1. Summary of the microenvironment. The tumor mass microenvironment is composed of a heterogeneous mixture of stromal cells (such as fibroblasts, endothelial cells, and immune cells such as macrophages) and ECM components. The tumor mass uses these various cell types to secrete chemokines such as CXCR4/CXCL12, growth factors such as VEGF and TGF-β, and matrix-degrading proteins (MMPs) to create a prometastatic niche that supports the tumor during invasion, angiogenesis, and extravasation. In addition, integrins and their receptors mediate cellular attachment and communication.

tumor rejection through type 1 cytokine production and antigen presentation [9]. However, during tumor progression, a phenotypical switch occurs: macrophages begin to express the "alternatively activated" M2 phenotype instead of the M1 phenotype. Expression of the M2 phenotype, consequently, leads to enhanced angiogenesis, matrix remodeling, and suppression of the immune system's ability to alert other immune cells to the presence of cancer cells [9]. Thus, the tumor microenvironment causes inflammatory cells such as macrophages to become cancer promoting rather than cancer suppressing.

Tumor-associated macrophages (TAMs), in particular, have been shown to share the M2 phenotype [10]. TAMs are a large component of the tumor microenvironment because they can comprise up to 80% of the tumor mass in breast cancer [11]. Clinical studies have found a correlation between high TAM content of tumors and poor patient prognosis [12]. Joyce and Pollard suggest that the tumor microenvironment contains several subpopulations of TAMs, each with various functions based on the stage of tumor progression and on its geographical location in the microenvironment. For instance, TAMs located in the hypoxic region of the microenvironment support angiogenesis [13], whereas TAMs at the intersection of tumor cells and stromal cells support both invasion and angiogenesis. In studies that depleted the macrophage population through genetic manipulation or pharmacological knockout, substantially fewer macrophages were found in the tumor microenvironment, which correlated to a marked decrease in angiogenesis, tumor growth, and metastasis [14].

Angiogenesis is essential in providing the metastatic tumor with the blood, nutrients, and oxygen necessary for uninhibited proliferation and sustainability. Without this private blood supply, tumors are only be able to grow to a minimal volume of 1 to 2 mm^3, a volume that expands rapidly to 1 to 3 cm^3 when vasularization occurs [15]. Angiogenesis is supported by the large presence of TAMs in areas of low oxygen concentration [13]. Hypoxia activates TAMs, causing them to indirectly upregulate VEGF [16]. VEGF is a principal mediator in angiogenesis and has been observed to be one of the most potent angiogenic factors to have been discovered. In a cascade of events, hypoxic conditions in the microenvironment cause TAMs to increase the gene expression of hypoxia-inducing factor 1 (HIF-1). HIF-1, in turn, upregulates VEGF [17]. Expression of VEGF results in increased vascular permeability and digestion of the ECM so that a new blood vessel can form and furnish the growing tumor with essential nutrients and supplies. In rat carcinoma models, the level of microvessel density positively correlated to the levels of both HIF-1 and VEGF [18] and to the level of TAM density [19], suggesting that the presence of all three factors in the microenvironment are important in tumor development by promoting angiogenesis.

Fibroblasts

Along with macrophages, fibroblasts have recently garnered attention as having a greater influence on the development and progression of tumors than was previously thought. Under normal circumstances, fibroblasts help maintain the structural integrity of connective tissue by synthesizing precursors for the ECM and the stroma for tissues. However, in the cancer microenvironment, fibroblasts have been shown to secrete cell surface proteins and growth factors that prop the microenvironment to be in a tumor-enhancing position. In particular, activated fibroblasts, or myofibroblasts, appear shortly before the invasive stage of tumors and promote degradation of the ECM by secreting serine proteases, MMPs, and urokinase plasminogen activator (uPA) receptor (uPAR). By doing so, fibroblasts not only stimulate their own migration into the tumor but also promote survival, proliferation, and invasion of cancer cells, thereby enhancing metastasis [20].

Fibroblasts are particularly useful to the tumor mass in the hypoxic conditions of the microenvironment. The supply of insufficient oxygen levels to the tumor leads to an anaerobic condition of lactic acid build-up [21]. The accumulation of this lactic acid establishes a microenvironment that is not only hypoxic but also acidic. Only those cells that can survive these highly acidic conditions can go on to join the growing tumor cell mass. Koukourakis et al. [22] demonstrated that in colorectal cancer specimens, fibroblasts are able to remove toxic metabolites and buffer the acidity generated by cancer cells. Thus, the presence of fibroblasts in the microenvironment allows more cells to survive the low pH conditions and join the growing tumor mass, thereby increasing the rate of tumor progression and metastasis. Furthermore, fibroblasts located in tissues closer to the outside environment of the body secrete interferon β (IFN-β) [23]. These interferons are part of the larger family of cytokines, which defend the body against tumor cells by activating natural killer cells, macrophages, and other inflammatory response cells. In fact, IFN-β secreted by some fibroblasts is suggested to have an anticancer effect because mice that have reduced IFN-β activity show increased tumor formation, progression, and invasiveness [24]. Thus, fibroblasts assume different phenotypes, whether it is the protumorigenic phenotye of buffering acidity or the antitumorigenic phenotype of recruiting immune cells based on the stimuli generated by the microenvironment.

Several studies pinpointed activated fibroblasts, namely carcinoma-associated fibroblasts (CAFs), as being indispensable to a microenvironment that promotes tumor growth and angiogenesis. First, CAFs possess a larger concentration of α-smooth muscle actin, which are characteristic of the myofibroblasts found in the stroma of invasive breast cancers, and collagen elasticity, which contributes to an increased rate of ECM permeability [25]. Second, breast cancer cell lines that were coinjected with CAFs resulted in tumors that possessed 7.6-fold greater vasculature and 4.7-fold greater blood microvessel density than those tumors that were coinjected with normal stromal fibroblasts [26]. This finding indicates that CAFs aid not only in tumor angiogenesis but also in proliferation because those tumors that are better vascularized are able to receive more nutrients essential to sustain growth. Thirdly, CAFs retained their protumorigenic properties despite losing contact with the breast carcinoma cells [26], indicating that CAFs may be a critical part of the microenvironment that sustains the tumor as it travels from the primary tumor to its distant secondary site (Figure 2).

The transition of stromal fibroblasts from their normal state to their activated CAF state may be induced by increased levels of MMP-1 and the chemokine CXCR4 [27]. MMP-1 and CXCR4 were particularly chosen for study because they are found at increased levels in breast cancer cells and primary breast tumors but not in normal tissues [28,29]. Eck et al. found that SUM102 breast cancer cells induced normal fibroblasts to attain CAF-like properties by stimulating the expression of MMP-1 and CXCR4. The normal fibroblasts with increased concentrations of MMP-1 and CXCR4 and therefore CAF-like properties demonstrated increased migratory and invasive capabilities.

Thus, the presence of both macrophages and fibroblasts in the tumor microenvironment is pivotal to the growing tumor mass. Without these stromal cells in the microenvironment, tumors would be unable to secrete the factors necessary to succeed in angiogenesis, ECM degradation, invasion, and migration.

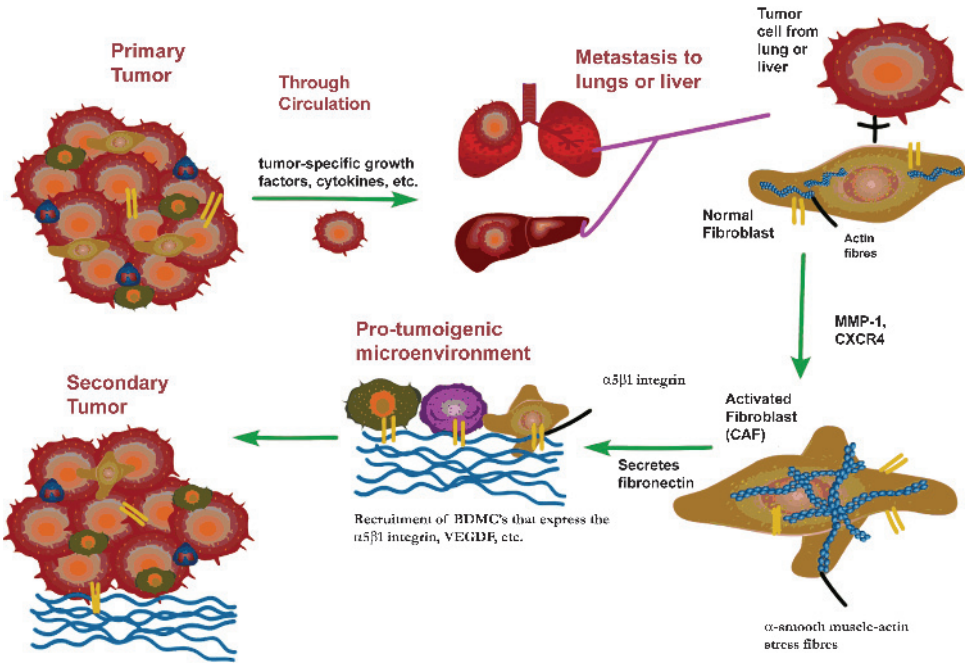

Figure 2. The process of metastasis. The primary tumor mass secretes growth factors, cytokines, and MMPs that allow metastatic cells to invade the circulatory system and travel to distant organs such as lungs and liver. Once they extravasate into the secondary site, the tumor cells induce normal fibroblasts to assume a CAF phenotype. CAFs, in turn, recruit protumorigenic bone marrow–derived cells (BDMCs) to the microenvironment, thereby supporting the growth, proliferation, and angiogenesis of the secondary tumor.

Epigenetic Alterations in Stromal Cells of the Microenvironment

To understand the dual role of protumorigenic and antitumorigenic macrophages and fibroblasts on a molecular level, Ma et al. [30] conducted a comparative analysis of gene expression changes in normal epithelia, normal stroma, tumor epithelia, and tumor-associated stroma using laser capture microdissection and DNA microarrays. Their study found that the genetic changes of the tumor-associated stroma were similar to those of the tumor epithelium. In particular, they found that tumor stroma that had reached the invasive stage expressed an increased level of MMPs, such as MMP-2. These findings support the theory that the tumor mass and its microenvironment have reciprocal relationships with both undergoing genetic expression alterations even in the pre-invasive stage of cancer [31].

Whereas studies have found genetic abnormalities in tumor epithelial cells [32], few studies to date have demonstrated genetic mutations in stromal cells of the tumor microenvironment [33–35]. In an attempt to explain why tumor-associated stromal cells maintain altered genetic expression despite a lack of genetic mutation, Hu et al. [36] suggest that tumor-associated stromal cells maintain their protumorigenic phenotypes because of epigenetic modifications, such as DNA methylation. DNA methylation changes between normal and neoplastic cells have been found in breast cancer [36], lung cancer [37], and prostate cancer [38]. For example, lung and breast cancer cells were found to have lost expression of RRAD, a Rad-related GTPase, whereas the expression of RRAD in normal lung and breast cancer cells remained unaffected. However, the expression of RRAD was restored when either the breast or the lung cancer cells were treated with the demethylating agent 5-Aza-2′-deoxycytidine [37]. Recent discoveries have found more than 50 biomarkers of breast cancer that are based on DNA methylation. These biomarkers have been accurate in detecting DNA hypermethylation in more than 90% of breast cancer cases, suggesting possible therapeutic routes during tumor development [39].

Protumorogenic Activities of Integrins in the Microenvironment

In addition to the agglomeration of macrophages and fibroblasts, the microenvironment of a tumor also includes a large family of cell surface receptors called integrins. Integrins consist of various combinations of α and β subunits, each with its own binding specificity and signaling properties. They have been shown to bind ECM components and to regulate cytoskeleton organization, thereby exerting stringent control on cell survival, proliferation, adhesion, and migration. Integrins are further critical in the microenvironment of a cell because they transfer information between cells and between cells and their surrounding matrix. Regarding the tumor microenvironment, recent analyses have shown that the expression of prometastatic integrins is significantly enhanced, whereas the expression of those integrins that hinder tumor proliferation, survival, and migration is repressed [40]. Therefore, the types of integrins present are dependent on stimuli produced by the microenvironment, rendering integrin expression pertinent to the understanding of tumor formation and progression (Figure 2).

As previously discussed, the local tumor environment is hypoxic, and this hypoxic condition leads to the expression of the proangiogenic factor VEGF. In relation to integrins, VEGF enhances the expression and activation of several integrins that are pivotal to tumor angiogenesis [41,42]. These integrins that play a leading role in new vessel formation include the $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrins. The $\alpha_5\beta_1$-integrin is of particular interest because it acts as the receptor for fibronectin (FN) during

neovascularization [43]. Current research has supported the theory that FN dimers that are bound to the $\alpha_5\beta_1$-integrin stretch and assemble into insoluble fibrils that compose the FN matrix [44]. The fibronectin matrix, which requires the interaction between FN and $\alpha_5\beta_1$ in the microenvironment, has become increasingly important in recruiting fibroblasts and macrophages to the tumor area [14]. Also, keen interest is the finding that $\alpha_v\beta_3$ integrins have been found to participate in the formation of the FN matrix, perhaps suggesting an alternative pathway for matrix assembly [45].

In our previous research, we have found that the protein Nischarin interacts preferentially with the $\alpha_5\beta_1$ integrin [46]. An interplay between Nischarin and $\alpha_5\beta_1$ causes profound inhibition of cell migration on fibronectin by interrupting the p21-activated kinase (PAK) pathway [47]. Inhibiting PAK is critical in reducing cell migration because the overexpression of PAK has been shown to increase the migration potential of epithelial cancer cells by overturning stable focal contacts at the leading edge of the tumor mass [48]. Conversely, decreasing the levels of endogenous Nischarin results in PAK activation and increased cell migration [47]. Interestingly, endogenous Nischarin has been found to be expressed in fibroblast cells. Future study may be conducted on how altered expression of Nischarin by fibroblasts modifies cellular dependency on the $\alpha_5\beta_1$-integrin to migrate on fibronectin.

Although it has been established that $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrins are integral to matrix and therefore vessel formation, recent studies have shown that the inhibition of either $\alpha_v\beta_3$- or $\alpha_5\beta_1$-integrin alone has little effect on slowing capillary tube formation. Rather, some argue that it is the simultaneous inhibition of both the $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrins that reduces capillary tube formation by 78% [49]. Furthermore, studies have shown that the $\alpha_v\beta_3$ integrin requires the ligation of $\alpha_5\beta_1$ to internalize the matrix protein vitronectin [50]. Removing vitronectin is part of tissue remodeling, a process necessary for tumor invasion.

Although many studies are consistent with the important role of $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrins in angiogenesis and migration, further studies have shown that $\alpha_4\beta_1$-integrins and their ligand vascular cell adhesion molecule-1 (VCAM-1) are also crucial to proper vessel development. The knockout of either leads to embryonic lethality in mice [51] because the suppression of $\alpha_4\beta_1$-integrin and VCAM-1 expression weakens the normal association between endothelial and mural cells. Furthermore, increased apoptosis and reduced angiogenesis were all observed in experiments that interfered with the interaction between $\alpha_4\beta_1$ and VCAM-1 [52]. This study of $\alpha_4\beta_1$ and VCAM-1 along with the study of the paired function of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ provides support for the role of integrins in tumor angiogenesis.

In addition to aiding tumor angiogenesis, integrins also promote cell invasion and migration. Tumor cell migration is a key characteristic of metastatic progression, and because migration is perhaps the most threatening element of cancer, understanding the migrational process is key to preventing it. Before the tumor can relocate to a secondary site in the body, it must first break through the ECM components that are the physical barriers for cell migration. Under normal conditions, the basement membrane is a specialized network composed of collagen, laminins, and proteoglycans and acts as an impermeable barrier to malignant tumors [29]. As such, the basement membrane of the ECM must be degraded and reorganized at the invasion front for the metastatic tumor to migrate. However, complete degradation of the ECM is not ideal. The tumor cells must control the extent to which the interstitial matrix is reduced so that they can adhere to the interstitial matrix and generate the traction necessary for migration [40]. Essentially, the tumor must remodel its microenvironment, including both the basement membrane and interstitial matrix of the ECM, to reach the invasive stage of metastasis.

An integral part of ECM degradation is the disruption of cell-cell adhesions in the basement membrane. When the components of the basement membrane are lost or degraded, then tumor progression can speed up without the barrier to migration. Integrins play a fundamental role in maintaining cell-matrix adhesions in the basement membrane because they possess a transmembrane structure and have the ability to bind to many extracellular ligands. Disrupted integrin signaling leads to the irregular cell-cell adhesions that are hallmarks of cancer [40]. Important in this arena are the β_1-integrins, the largest family of integrin subunits. The overexpression of β_1 integrins causes disruption of intercellular adhesions and cell scattering [53]. Conversely, the down-regulation of β_1 integrin expression and function correlates with the reduced degradation of basement membrane-collagen in prostate and breast cancer cell lines [54].

In a more precise focus than the family of β_1-integrins, recent studies have revolved around the effect of $\alpha_v\beta_3$-integrin on tumor intravasation and extravasation. In addition to inhibiting the formation of new vessels in angiogenesis, the $\alpha_v\beta_3$-integrin plays a functional role in the activation and expression of matrix-degrading proteases. The $\alpha_v\beta_3$-integrin has been found to colocalize with MMP-2 at the forefront of the tumor, corresponding to heightened collagen deterioration and subsequent invasion [55]. For instance, blocking the interaction between $\alpha_v\beta_3$ and MMP-2 reduces the tumor progression in ovarian cancer [56]. In addition, recent studies have found that $\alpha_v\beta_3$ also colocalizes with MT1-MMP on migrating endothelial cells, suggesting that an association between the two plays a functional role in tumor cell migration and invasion [57]. Thus, integrins are interdependent on the MMPs in the microenvironment to proteolyze ECM components so that the tumor can move through the ECM and into the circulatory system.

Beyond directly activating MMPs to degrade components of the ECM, the $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrins also work through an alternative mechanism, mainly associating with uPAR, to recruit proteolytic activity to the leading edge of migrating cancer cells [58]. 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 [59]. Thus, the activation and expression of both β_1- and $\alpha_v\beta_3$-integrins in the tumor microenvironment contribute to the breakdown of the surrounding ECM, significantly freeing the pathway for tumor migration and invasion.

As it pertains to the actual process of cell migration, Defilles and Lissitsky [60] suggest that it is not one specific integrin but a complex of $\alpha_v\beta_5/\beta_6$- and $\alpha_2\beta_1$-integrins that modulate cell migration toward type I collagen. In two colon carcinoma cell lines, inhibition of $\alpha_v\beta_5/\beta_6$-integrin function lead to enhanced $\alpha_2\beta_1$-integrin function through focal adhesion rearrangements and matrix-cell signaling, as indicated by increased levels of phosphorylated focal adhesion kinase and mitogen-activated protein kinase (extracellular signal–regulated kinases 1 and 2). Heightened expression of the $\alpha_2\beta_1$ integrin, consequently, led to a more than two-fold increase in integrin-dependent cell migration toward type I collagen in colon carcinoma cells. Conversely, interfering with the $\alpha_2\beta_1$-integrin through the use of disintegrins or anti-α_2 or anti-β_1 monoclonal antibodies led to significantly decreased levels of cell migration [61].

Besides the $\alpha_v\beta_5/\beta_6$- and $\alpha_2\beta_1$-integrin complex suggested by Defilles and Lissitsky [60], the $\alpha_v\beta_3$-integrin seems to play a role in tumor cell migration in addition to angiogenesis and ECM degradation. Integrin $\alpha_v\beta_3$ has been shown to bind to fibroblast growth factor-1 (FGF-1), an interaction essential to tumor cell migration [62]. In fact, FGF-1

specifically binds to $\alpha_v\beta_3$, with only a loose association to the β_1 family of integrins. The pivotal role of $\alpha_v\beta_3$ is supported by the defective migration of mutant FGF-1 cells. The mutant FGF-1 cells were still able to bind to their fibroblast growth factor receptors but unable to bind to the $\alpha_v\beta_3$-integrin. Without the integrin interaction, the FGF-1 mutant cells took significantly longer to migrate than those with active FGF-1/$\alpha_v\beta_3$ associations, suggesting that direct integrin binding to FGF-1 in the microenvironment is critical for cell migration [62].

Paracrine Signaling Pathways between the Microenvironment and Tumor Epithelial Cells

We have discussed the abundance of different stromal cells, integrins, endothelial, and epithelial cells in the microenvironment but not yet the source of their recruitment, which involves a system of paracrine signaling. The cross talk involves a number of soluble factors, such as transforming growth factor (TGF) and various chemokines, which act in a paracrine fashion by binding to their respective receptors. In addition, they can work in conjunction with integrins to coordinate cellular functions and signals.

Under normal conditions, TGF-β acts as a tumor suppressor by inhibiting proliferation, as previous studies have shown in prostate epithelial cell lines [63]. However, TGF-β has been shown to assume a protumorigenic role as cancer progresses because those signals emitted by CAFs modify the response of adjacent epithelial cells to other aspects of the microenvironment [64]. For example, in a study using an orthotopic xenograft model to reconstruct human mammary gland, results indicated that overexpressing TGF-β in mouse fibroblasts could induce the initiation of breast cancer from normal epithelial tissue [65].

Several studies have indicated that TGF-β emits protumorigenic signals to surrounding cells through CXCR4/stromal cell–derived factor-1 (SDF-1) signaling pathways [63,66]. However, the exact mechanism of CXCR4 regulation of TGF-β is not clearly understood. The expression of TGF-β makes epithelial cells specifically sensitive to elevated levels of SDF-1 [66]. SDF-1 is elevated in human breast cancer stroma in which the CXCR4 pathway plays a role in tumor growth [26]. Thus, in a feedback loop, elevated levels of TGF-β expression increase the expression of CXCR4, which in turn causes SDF-1 to activate Akt by phosphorylation. Phosphorylated Akt permits stroma to ignore the growth-inhibitory effects of TGF-β, allowing for uninhibited cell proliferation [66].

Also acting in a paracrine fashion with the chemokine CXCR4 is its ligand CXCL12, which binds to CXCR4 on the membrane of cancer cells [67]. CXCL12 promotes angiogenesis by recruiting endothelial cells to the newly forming blood vessels. In a study that cografted CXCR4 knockout cells with vascular endothelial cells into immunodeficient mice, significantly fewer blood vessels were formed [68]. CXCL12 has also been shown to trigger the invasion of transgenic MMTV-PyMT tumors, but only through the epidermal growth factor/colony-stimulating factor-1 paracrine loop between cancer cells and macrophages [69]. In *in vivo* studies, those mice that were injected with clodronate-containing liposomes, or those that cause macrophage depletion, exhibited significantly less invasion than in the control group despite CXCL12 expression [69]. Therefore, cross talk between tumor epithelial cells and the microenvironment through the growth factor TGF-β or the chemokines CXCR4/CXCL12 seems to have a major influence on tumor progression and metastasis. Paracrine signaling is merely one mechanism by which the tumor and its microenvironment interact to create a prometastatic niche.

Conclusions

The microenvironmental conditions are important to the progression of tumor metastasis. Macrophages and fibroblasts are critical to the recruitment of prometastatic cells and to the secretion of chemokines and growth factors, all of which the tumor mass requires for proliferation, migration, and invasion. Likewise, integrins play a role in the activation and expression of proteases that aid in ECM degradation, in the cell-cell adhesions that must be broken for migration to occur, and in the formation of new blood vessels. Interestingly, the stromal cells and integrins discussed in this article all seem to have both prometastatic and antimetastatic properties and are able to "activate" one phenotype or the other based on the surrounding microenvironmental and tumorigenic signals. Further study on the manipulation of these signals has potential for therapeutic use to prevent or treat metastatic tumors.

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