

Epithelial and mesenchymal phenotypic switchings modulate cell motility in metastasis

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1. ABSTRACT

The most ominous stage of cancer progression is metastasis, or the dissemination of carcinoma cells from the primary site into distant organs. Metastases are often resistant to current extirpative therapies and even the newest biological agents cure only a small subset of patients. Therefore a greater understanding of tumor biology that integrates properties intrinsic to carcinomas with tissue environmental modulators of behavior is needed. In no aspect of tumor progression is this more evident than the acquisition of cell motility that is critical for both escape from the primary tumor and colonization. In this overview, we discuss how this behavior is modified by carcinoma cell phenotypic plasticity that is evidenced by reversible switching between epithelial and mesenchymal phenotypes. The presence or absence of intercellular adhesions mediate these switches and dictate the receptivity towards signals from the extracellular milieu. These signals, which include soluble growth factors, cytokines, and extracellular matrix embedded with matrikines and

matricryptines will be discussed in depth. Finally, we will describe a new mode of discerning the balance between epithelioid and mesenchymal movement.

2. INTRODUCTION

Acquisition of a mesenchymal-like cell phenotype is one of the striking hallmarks of progression to dissemination of most all carcinomas. The one exception is ovarian carcinoma wherein spread throughout the peritoneal cavity occurs with an epithelial cell phenotype, but seeding of distant organs does coincide with cell dedifferentiation. This cancer-associated Epithelial-to-Mesenchymal Transition (EMT) has been strongly correlated with metastasis and shortened life expectancy of many carcinomas (1). As a number of interventions in animal models of tumor dissemination show, at least partially, a causal role for EMT in dissemination (2, 3), the question arises as to the cell behavior enabled. Herein, we will discuss the evidence that this EMT promotes tumor cell motility as the key event in progression.

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Carcinogenesis involves a combination of mainly genetic events that generate a tumor cell. Though the source of that cancer is still uncertain for most carcinomas—for instance, whether the cancer derives from a stem cell compartment or by alteration of a differentiated epithelial cell—but a series of mutations endows the cell with an ability to proliferate inappropriately to the situation. However, these intrinsic changes do not make the tumor cell fully autonomous. It is now appreciated that the tumor is a multicellular tissue in which non-cancer cells and the matrix modulate carcinoma behavior.

Further changes are needed for the cancer cell to disseminate from its origin. Despite many queries, the transition to this morbid and mortal stage appears not to be mutational. Rather, epigenetic events, possibly driven by the tumor microenvironment, provide the cellular changes needed for dissemination. This suggests that unlike the mutational events that mark carcinogenesis, the alterations for dissemination are potentially reversible (4). Thus, we need to review the cellular aspects that mark escape from the ectopic site.

Carcinoma cell dissemination requires the acquisition of cellular properties and behaviors that enable the cells to escape from the original site, breach the surrounding barrier basement membranes, and survive in ectopic locales. There are both qualitative and quantitative distinctions between localized invasion and distant metastasis. In the former, the cancer and support cells in the tumor may move together as a syncytium into the adnexia, providing not only for contiguous vascular support but also for a quasi-orthotopic signaling environment. However, in metastasis the cancer cells are generally accepted as solo travelers that must fully break from the primary mass and establish themselves in a truly foreign milieu; this is in addition to surviving the stresses of transiting vascular conduits. For cell intrinsic properties this dissemination requires quantitative degrees of changes.

The acquisition of the mesenchymal phenotype in carcinoma-associated EMT is a hallmark of carcinoma dissemination. Central to this is the downregulation of cell-cell adhesions mediated by E-cadherin (though N-cadherin is often found upregulated in many carcinomas allowing for cell heterotypic adhesion to endothelial cells for extravasation). This loss of cell adhesion may be partial to continue to provide syncytial behavior such as in localized invasion noted often in prostate carcinoma (5), or it may be complete to generate distant metastases such as in breast carcinomas (6).

The behaviors common to both forms of dissemination involve breachment of the basement membrane and active migration into an ectopic milieu. The initial steps involve recognition and remodeling of the matrix. While proteolytic activity is required for transmigration this barrier (7, 8), it appears not to be a wholesale degradation but rather a selective processing (9, 10). Further, most carcinoma cells, whether invasive or not, present copious levels of proteases so that the regulation appears to be more of activation or localized effect rather

than de novo production (11, 12).

What is qualitatively different is autocrine and paracrine stimulation of cell motility. The loss of E-cadherin during EMT not only allows for cells to move away from the tumor mass but also is accompanied by a breakdown in the apical-basal polarity that separated apically secreted growth factors, often those for the EGFR and c-Met receptors, from their cognate receptors presented on the basolateral faces (Figure 1). This allows for autocrine stimulation of these motogenic receptors. This intrinsic cell behavior, by and large restricted to invasive and metastatic carcinoma cells (and cells during wound repair), is the focus of our discussion of the role of cell migration in carcinoma progression and how that reciprocally ties in with phenotypic switching.

3. DISRUPTION OF CELL ADHESION IN EMT ENABLES CELL MOTILITY

One of the main distinguishing characteristics between epithelial and mesenchymal cells is that epithelial cells are linked by cell adhesion molecules to form contiguous sheets. These intercellular physical interactions not only limit motility away from the connected cells but also establish apico-basal polarity that regulates signaling between cells and with the surrounding environment. In contrast, mesenchymal cells exhibit transient and changeable front-back polarity and present loose and readily tractable intercellular contacts. This dictates that epithelioid cells act within a tissue whereas the cells in the mesenchymal state may disseminate.

There are four main types of cell-cell junctional molecules that connect epithelial cells. Tight junctions provide a barrier for solutes and small molecules along the apical surface of cells. Adherens junctions provide strong mechanical cohesion through connection to the actin cytoskeleton, but also control key signaling pathways through sequestration of catenins. Desmosomes also mediate intercellular contacts, but through anchorage to intermediate filaments. Gap junctions form intercellular junctions that allow the passage of ions and small molecules. In addition, integrins are cell-substratum adhesion molecules that are located on the basal surface of epithelial cells and facilitate interactions between the ECM and the cytoskeleton. Members of all these different families of cell adhesion molecules act in concert to contribute to a fully polarized epithelial phenotype.

3.1. EMT and aberrant regulation of adhesion molecules at the primary site

Loss of cell-cell adhesions is a critical step during EMT that allows for physical detachment of individual or groups of cancer cells from the primary tumor. This also allows for autocrine activation of signaling pathways that enable migration (Figure 2). EMT is most discernable at the invasive front of primary carcinomas and has been visualized as individual or a group of cells migrating into the surrounding tissue (13). Downregulation of cell adhesion molecules has been repeatedly documented to be associated with invasion and poor prognosis in many

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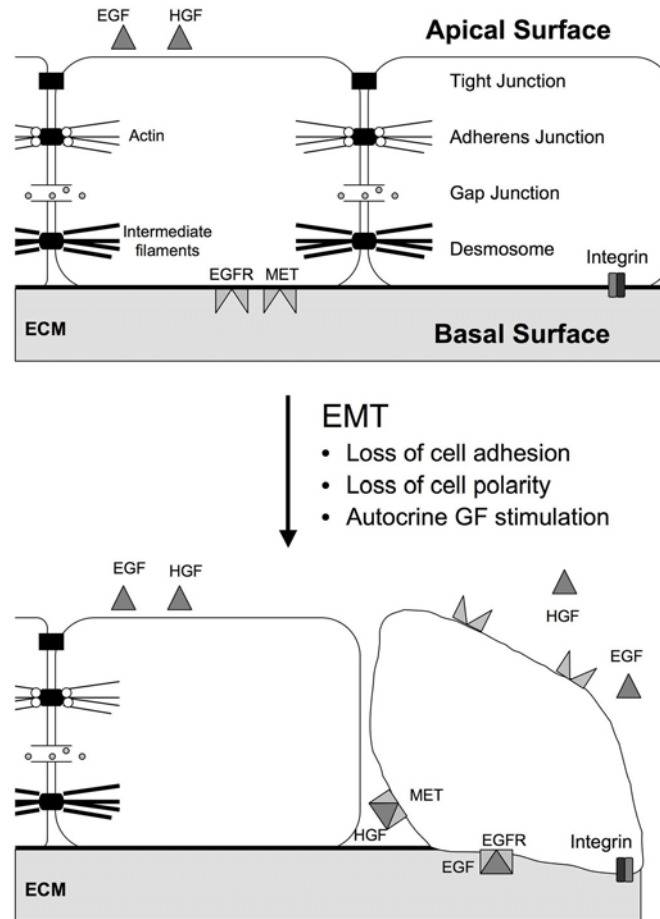


Figure 1. Transition from Epithelial to Mesenchymal Phenotype. EMT results in the loss of cell-cell adhesions allowing for the autocrine stimulation as the basolaterally-restricted receptors are no longer isolated from the apically-secreted motogenic cognate growth factors.

carcinomas. Therefore, cell adhesion molecules are an important mediator of the transformation between epithelial and mesenchymal phenotypes during EMT in metastasis. The disruption of cell adhesion and consequent induction of motility is a critical step in metastatic progression.

3.1.1. Tight Junctions

Tight Junctions maintain the apical-basal polarity of epithelial sheets. Epithelial cells are most commonly found as sheets of cells that line the surfaces of organs. An important function of epithelia is to serve as a barrier to maintain tissue homeostasis. The apical distribution of tight junctions serves as a “gate” to limit the transport of ions, pathogens and small molecules and as a “fence” to restrict lipid and membrane proteins along the apico-basolateral axis (14). The net result is that most growth factor receptors are restricted to the basolateral surfaces while the epithelial-expressed growth factors are secreted through the apical side, with the tight junctions preventing autocrine activation.

A fully polarized epithelial phenotype requires the cooperation of tight junctions, adherens junctions, and desmosomes. Dissolution of tight junctions is an early

event in EMT so it is not surprising that several tight junction components are dysregulated in cancer progression. Expression of occludin in breast cancer cells decreases invasion and migration *in vitro* and *in vivo* (15). Similarly, levels of claudins are downregulated in invasive carcinomas and exogenous introduction of claudins increases adhesion and prevents migration and invasion (16, 17). The Par complex regulates tight junction signaling and its expression is also altered in invasive carcinomas. Not only is complex member Par6 required for TGFβ-dependent EMT but also disruption of this complex perturbs apico-basal polarity and stimulates chemotactic migration by stabilizing front-back polarity (18, 19).

3.1.2. Cadherins

Cadherins are a family of transmembrane glycoproteins that mediate calcium-dependent homophilic interactions. The classical members most widely studied are E-cadherin, expressed in epithelial cells; N-cadherin, R-cadherin, P-cadherin, and OB-cadherin (cadherin-11), expressed by mesenchymal cells; and VE-cadherin, expressed by endothelial cells. The structures of these cadherins differ mainly in the extracellular domain, which is responsible for the adhesive function. However, the

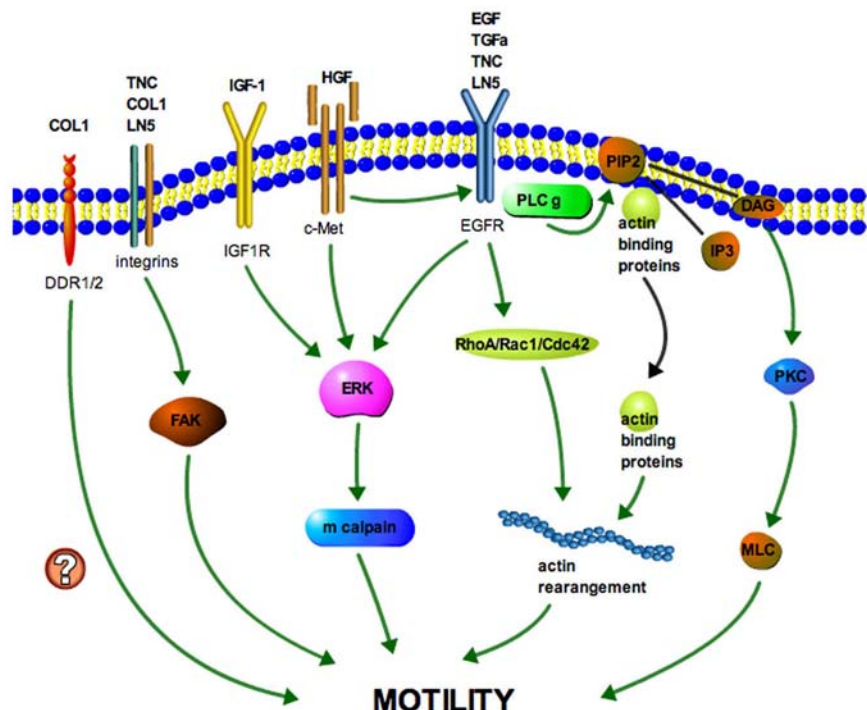


Figure 2. Key Motogenic Intracellular Signaling Pathways Emanating from Cell Surface Receptors. Shown are select receptors and the key motogenic signaling pathways. Not shown, for clarity of the schematic, are all the overlapping signals and other, less thoroughly documented pathways that have been linked to driving motility. These pathways have been demonstrated to be viable targets limiting tumor cell motility in preclinical models.

cytoplasmic domain is highly conserved and binds to beta-catenin and p120, which through binding to alpha-catenin link the cadherins to the actin cytoskeleton. Importantly, beta-catenin is a nuclear transcriptional co-activator for the mitogenic LEF/TCF family of transcription factors, so sequestration of this molecule by cadherins prevents activation of downstream signaling pathways (20).

As the only cadherin expressed by epithelial cells, E-cadherin has been described as the “caretaker” of the epithelial phenotype and thus loss of E-cadherin is central to EMT (21). Downregulation of E-cadherin expression has also been correlated to the progression of most carcinomas (22, 23). Loss of E-cadherin is sufficient to increase the metastatic behavior of noninvasive breast cancer cells and is a rate-limiting step of the transition from adenoma to invasive carcinoma (24, 25). However, in this mouse model a complete EMT was not necessary, as vimentin and other mesenchymal markers were not expressed. Furthermore, use of a dominant-negative E-cadherin that resulted in the subcellular localization and prevented intercellular contacts was sufficient to induce the invasive phenotype, but expression of a constitutively active beta-catenin was not (26).

E-cadherin is considered an invasion suppressor, as transfection of invasive E-cadherin-negative carcinoma cell lines with E-cadherin cDNA decreases invasiveness, which can be reversed after treating transfected cells with an anti-E-cadherin function blocking antibody (27).

Perturbation of E-cadherin expression can promote cell motility in several ways. Physical adhesion promoted by E-cadherin prevents the dissociation and migration of cells. Alternatively, E-cadherin down-regulation results in release of beta-catenin from the membrane, where it can then act as a transcription co-activator in signaling pathways such as Wnt. Studies using E-cadherin mutants suggest that the beta-catenin binding function and not adhesion is responsible for the invasion suppression (28). In addition, loss of E-cadherin alone is not sufficient to drive beta-catenin signaling, so it is likely that E-cadherin regulates the threshold of beta-catenin signaling (29).

Although down-regulation of E-cadherin has been shown to be sufficient to induce the changes in cell behavior downstream of EMT, in some cases expression of the mesenchymal cadherins can be sufficient or dominant. Downregulation of E-cadherin is often, but not always, accompanied by an upregulation of N-cadherin suggesting a cadherin switch in EMT (30). However, colocalization of both E-cadherin and N-cadherin has been observed (31). In addition, forced expression of N-cadherin in the absence of changes in E-cadherin has been shown to induce migration and invasiveness of cancer cells either through FGFR signaling or through interactions with N-cadherin expressed by the surrounding stromal cells. Similarly, expression of R-cadherin in BT-20 breast cancer cells leads to downregulation of E- and P-cadherins and induction of cell motility through sustained activation of Rho GTPases (32). Although seemingly contradictory, these studies suggest

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that E-cadherin and the mesenchymal cadherins may induce motility via different mechanisms intrinsic to the disparate functions of the cadherins.

3.1.3. Desmosomes

Desmosomes define the third class of cell-cell adhesion junctions. Desmosomal components are also commonly downregulated in carcinomas and associated with the presentation of distant metastases, especially in cancers of the head and neck (32). Desmosomes are intercellular adhesion molecules that are anchored to the intermediate filaments in the cytoskeleton. They are composed of the desmosomal cadherins that have an extracellular domain, which mediates cell-cell adhesion, and a cytoplasmic domain, which interacts with plaque proteins that bind to intermediate filaments. Loss of the plaque proteins plakophilin-1 and -3 has been shown to increase cell motility and metastasis of carcinoma cells (33, 34). Transfection of desmosomal components desmocollin, desmoglein, and plakoglobin into L929 fibroblasts resulted in intercellular adhesion and suppression invasion into collagen gels even in the absence of the assembly of full desmosome complexes with linkage to intermediate filaments (35). These studies suggest that desmosomes mainly act to prevent cell motility through physical cohesion.

3.1.4. Gap Junctions

Gap Junctions are cell adhesion complexes that mediate intercellular communication, rather than adhesion, through the exchange of ions and small molecules. These aqueous pores are composed of hexamers of connexins (Cx), which form a membrane-spanning pore. There are over 20 subtypes of connexins, with most variability in the subtypes occurring in the cytoplasmic domain (36). There is evidence that gap junctions may perform channel-independent functions, including effects on cell migration. Inhibition of cell motility of prostate carcinoma and melanoma cells is correlated with increased localization of Cx43 at cell-cell contacts (37). In contrast, transfection of Cx43 into HeLa and glioma cells increases invasion *in vitro* and metastasis of melanoma cells *in vivo* (38-40). There is still much controversy over whether connexin expression is pro- or anti-migratory and whether the function of gap junctions differs depending on the stage of the tumor.

3.1.5. Integrins

Integrins are cellular adhesion molecules that couple the cell to the extracellular matrix. Not only do they provide anchorage to the actin cytoskeleton but also transmit signals, based on both clustering and integrin isoforms. Integrins are composed of alpha and beta subunits that form a heterodimeric complex to determine specificity to ligands. Some integrin heterodimers exhibit great promiscuity by binding to several different ECM components while others may recognize only unique ligands. Epithelial cells typically express the beta1 subunit, which recognizes collagen and laminin, and the epithelial-specific alpha6beta4, alpha5beta3 integrins (41). The integrins are critical for providing the substratum adhesion during motility. When integrins are knocked down, motility commensurate with the mesenchymal transformation is

abrogated demonstrating the enabling function of these “adhesion” molecules.

Several studies have documented the differential expression, distribution, and ligand affinity of integrins in preneoplastic lesions and carcinomas. Expression of integrins and therefore adhesion to ECM is regulated by TGF-beta, which is a potent inducer of EMT (42). Induction of TGF-beta in carcinoma cells activates the mesenchymal gene expression profile and promotes tumor invasion and spread (43, 44). TGF-beta downstream targets Smads activate the expression of integrins and focal adhesion-associated proteins. Integrin signaling, through both alpha5, beta1 and beta5 integrins, has been shown to be necessary for TGF-beta induction of EMT in mammary epithelial cells as addition of a beta1 neutralizing antibody or beta5 siRNA prevented invasion *in vitro* (45, 46). Beta5 integrin blockade did not influence mesenchymal gene expression such as the downregulation of E-cadherin, but prevented the formation of actin stress fibers with two-point focal attachment. The formation of these stress fibers may be necessary for the generation and maintenance of tension and force needed for cell migration (46). Transformation of mammary epithelial cells with the Fas oncogene induces EMT and the upregulation of integrins alpha2, alpha3, alpha5, alpha6, and beta1 and consequently increased adhesion to matrix components collagen, fibronectin and laminin 1. EMT and integrin expression changes in these Ras transformed cells is maintained by an autocrine TGFbeta1 loop (47). Besides changes in expression level, localization of integrins can also contribute to the capacity for cell migration in EMT. For example, expression of the alpha6beta4 integrin in normal mammary epithelial cells is localized in hemidesmosomes to connect intermediate filaments with laminin in the basement membrane. However, in invasive breast carcinoma cells, alpha6beta4 is localized to the lamellipodia of invading cells (48).

3.2. Loss of cell polarity leads to dysfunctional growth factor signaling

As described in the preceding sections, besides tethering cells together to prevent the detachment and migration of individual cells, tight and adherens junctions also serve to establish tissue polarity. This is a critical regulatory mechanism, as most all epithelial cells secrete growth factors from their apical surfaces, and also express the cognate receptors, but on their basolateral surfaces (Figure 1). When cell adhesion molecules are disrupted in carcinoma-associated EMT, this organization is lost, and the growth factor receptors that are located basolaterally can now come into unrestricted contact with their ligands. Furthermore, the growth factors now have access to the basement membrane, and stromal compartment, and can affect changes in the tumor microenvironment to further promote motility. For example, induction of EMT through TGFbeta1 expression normally leads to increased ECM production and deposition and reformation of the basement membrane that stops the autocrine loop. However, in tumors this feedback loop is disrupted and TGFbeta1 is produced continuously.

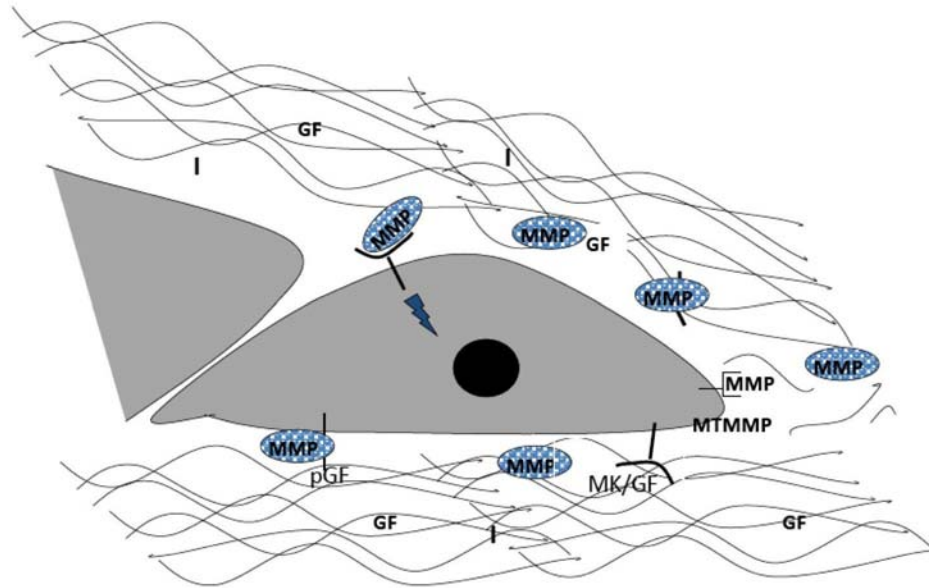


Figure 3. Matrix-Embedded and Encoded Signals Liberated by Proteolysis. Extracellular matrix is not only recognized by adhesion and other (DDR, etc) receptors but also contains predeposited soluble factors, encoded factors, and cryptic signaling elements. Matrix metalloproteinases (MMP) cause proteolysis of the matrix, degrade invasion inhibitors (I) and release and uncover ECM-embedded signals (GF – growth factors, MK – matricines). Invadopodial-localization of such activity can be accomplished by membrane-tethered MMP. Adapted from (56).

4. MOTILITY IN ESCAPE FROM PRIMARY SITE

Following the loss of cellular adhesion that allows for detachment from the primary tumor mass, tumor cells must penetrate through the surrounding tissue and basement membrane in order to disseminate. Intravital imaging has revealed that within primary tumors there are two categories of cells—very motile single cells and slower collectively moving cells—and the mode of the cell motility determines spread through the blood or lymphatic system (49). Induction of EMT leads to a program of epigenetic changes to confer a migratory and invasive phenotype (50). In addition, tumor-surrounding stroma is actively remodeled by proteolytic degradation of the extracellular matrix (ECM), which causes the release of growth factors and other molecules that provide feedback signals for further active cell migration (Figure 3). Dysregulation of matrix metalloproteinases contributes to tumor cell migration through multiple mechanisms: releasing individual cells by cleaving junction proteins, cleaving ECM to allow movement of the cells, unmasking cryptic sites with new roles in tumor cell migration, and releasing growth factors ‘deposited’ within the ECM, such as b-FGF, TGF-beta1, PDGF, HB-EGF/Amphiregulin, and IFN-gamma (51). Signaling towards motility is achieved both by receptors that modulate adhesion and provide basal traction and receptors for cytokines and growth factors (52).

4.1. Motility signaled from soluble factors

Cancer cells accumulate intrinsic/autonomous behaviors that allow for dysregulated growth, but despite these genetic mutations, they remain responsive to external signals in the form of growth factors. It appears that it is

these signals that drive both EMT and the subsequent migration leading to dissemination that occurs after neoplastic transformation generates the primary tumor growth. In cancer invasion, growth factor-induced autocrine, paracrine, and matricrine loops (Figure 3) coordinately contribute to tumor progression. Paracrine loops function in both directions, with cancer cells driving stromal cells to alter the microenvironment and subsequent signals released by the stromal cells promoting cancer cell migration. *In vitro*, a large number of growth factors alone can induce both EMT and cell migration (EGF, VEGF, TGF-beta, SDF) (53). Below, we will highlight a few growth factors and other soluble factors that are unequivocally implicated in cancer cell invasion.

4.1.1. EGF

EGF and its receptor system is the most-extensively described growth factor system for induced cell motility. Autocrine EGFR activating loops are present in most all carcinomas, with the produced ligand being EGR or TGFalpha, depending on quantitative balance between receptor and ligand (54, 55). The motogenic pathways activated by EGFR activity have been delineated (56). PLC-gamma is immediately downstream of the EGFR motogenic pathway (57) and cancer cell migration in response to EGF is promoted by PI3K and PLC dependent mechanisms (58). Phosphorylation and inactivation of FAK (59) and increase in urokinase and MMP9 are just some of the downstream effectors in this cascade.

4.1.2. HGF

HGF, mesenchymal derived cytokine and its receptor c-Met are overexpressed or amplified in many types of human cancer. This is a second receptor tyrosine

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kinase that not only drives cell scattering and induces EMT, but also actuates motility. Activation of c-Src, PI3K and PKC are crucial for HGF-induced cell motility and are accompanied by increased MMP activity (60). In breast cancer, activated c-Met receptor can even activate EGFR through c-Src activation (61). The role of HGF signaling in cancer motility is further underscored by presence of somatic c-Met mutations in metastatic carcinomas that confer a motile-invasive phenotype in cancer cells (62). Of interest, HGF is a pro-growth factor that is activated only upon cleavage in the extracellular milieu. The uPA system that activates HGF is upregulated by EGFR signaling in prostate carcinoma, suggesting a further amplification of cell dissemination (63).

4.1.3. IGF-1

The Insulin-like Growth Factor 1 (IGF-1) receptor axis promotes cell motility by activating AKT and MAPK pathways (64). In addition to the above pathways, IGF-1 also promotes cell migration by phosphorylation of FAK and paxilin. It has been shown that IGF-1 stimulates cell migration coordinately with ECM integrin stimulation: IGF-1 can bind to vitronectin, which is upregulated at the leading edge of migrating cells, and IGF-1-VN-IGF-Binding protein complexes promote cell migration by sustained activation of the PI3K/AKT pathway (65). IGF-1 induced migration is mediated by an increase in MMP-9 activity and α 5 β 1 integrin activation (66). IGF-1 induced PI3K/AKT signaling axis also promotes expression of MT1-MMP and synthesis of MMP2 and facilitates invasion of tumor cells (67).

4.1.4. TGF-beta

TGF-beta is another growth factor that alone can induce epithelial to mesenchymal transition. TGF-beta may have a role in the initial dissemination process. Fast moving single cells that are able to intravasate express high levels of TGFbeta (49). It is believed that transient high TGFbeta activity in the primary tumor enables high metastatic efficiency at the primary site and that decreased TGFbeta activity at the secondary site allows the resumption of the cell proliferation program (68). In addition, paracrine TGF-beta1 signaling induces ECM deposition (collagens, fibronectin, tenascin, elastin) by myofibroblasts, thus promoting a pro-migratory microenvironment. This signaling system is distinct from the classical growth factor receptors noted above as it signals via serine/threonine kinases and SMAD intermediaries. There are three ligand isoforms with TGF-beta1 being the isoform linked to cancer dissemination.

4.1.5. Cytokines/chemokines

Cytokines/chemokines are soluble factors most often implicated in the inflammatory response, though they also signal to and from formed elements of tissues. Many cancers show evidence of active inflammation that appears to be supportive rather than anti-neoplastic (69-71). Cytokines, small proteins originally found secreted by specific cells of the immune system, carry signals locally between cells to trigger inflammation and respond to infections, and have also been revealed to be involved in tumor initiation and progression. In this section, the

discussion will be mainly focused on migration signals from the primary site via cytokine receptors. One of the best described examples is that of the tumor associated macrophages (TAM) that appear to chemotax breast cancer cells in a reciprocal paracrine signaling with the cancer cells involving CSF-1 and EGF.

4.1.6. Tumor necrosis factor-alpha (TNF-alpha)

Tumor necrosis factor-alpha (TNF-alpha) was firstly identified as an anti-tumor cytokine by inducing immune-mediated necrosis of cancers (71). In recent years, evidence has indicated that TNF-alpha can also play an important role in promoting cancer cell migration and invasion (72). TNF-alpha is expressed in a variety of cancers, including lymphoma, breast, ovarian, pancreatic, renal, colon and prostate cancers (73-79). TNF-alpha induces breast and ovarian cancer cell invasion through activation of the NF-kappaB and JNK signaling pathways, following by elevation of MMP production in cancer cells (80, 81). Studies in ovarian cancer cells indicate that TNF-alpha also enhances cell migration and metastasis through induction of CXCR4 chemokine receptor via a NF-kappaB-dependent-manner (82). How CXCR4 regulates cancer cell migration will be discussed in the following context. Furthermore, TNF-alpha can also promote breast cancer cell trans-endothelial migration through upregulation of endothelial lectin-like oxidized-low-density lipoprotein receptor-1 (LOX-1) (83). Interestingly, both tumor- and macrophage-produced TNF-alpha play an important role in the epithelial-mesenchymal transition (EMT) via repression of E-cadherin expression (84, 85).

4.1.7. SDF-1/CXCL12

SDF-1/CXCL12, the homeostatic chemokine stromal cell-derived factor-1, is the only chemokine for the widely expressed cell surface receptor CXCR4 (86). The CXCR4-CXCL12 axis regulates the migration of cancer cells to metastatic sites in many carcinomas (87-92). Blockade of CXCR4 signals using chemical antagonists, antibodies, or interfering RNAs inhibits tumor dissemination and metastasis in animal models (87, 93-96). The expression of CXCR4 can be regulated by VEGF and TNF in many cancers (82, 97). With binding of CXCL12 to CXCR4, the receptor activates phospholipase C (PLC) and phosphoinositide-3 kinase (PI3K) and inhibits adenylyl cyclase by different G-protein subunits (98). Signaling from PI3K induces the activation of PAK, Akt and RhoGTPase, which play important roles in cell polarization and actin polymerization involved in cell migration. On the other hand, PLC activates calcium release and protein kinase C (PKC), followed by Erk activation leading to cell migration (99-101). In addition, CXCR4-CXCL12 signals also direct invasion of human basal carcinoma cells and prostate cancer cells by the up-regulation of MMP-13 and MMP-9 respectively (102, 103). Cancer cell survival signals induced by CXCR4-CXCL12 axis will not be discussed here.

4.2. Motility signaled from the matrix

The functional connection between properties of the extracellular matrix (ECM) and normal cell behavior in tissue homeostasis is well documented (104). Though

cancer cells are mutated and their responses dysregulated, they remain responsive to these same signals. Tumor progression is characterized by changes in ECM structure and composition and these changes influence the type of cell migration by providing ligands and a structural frame (105). Apart from growth factors embedded within the ECM, modulation of migrational mode is achieved by varying the expression of adhesive and anti adhesive ECM proteins and proteolytic cleavage of the present ECM components.

Various ECM proteins with potential pro-migratory roles are upregulated in cancerous tissues: collagens I and IV, laminins, tenascin C, fibronectin and vitronectin. The ability to alter the stromal microenvironment correlates with the tumor invasive potential (106, 107). Cancer cells induce gene expression changes in fibroblast and other stromal cells to produce ECM molecules that promote tumor migration and increase MMP production to loosen the stiffness of the matrix (108,109). Depending on the cell surface receptors present, cancer cells may utilize different ECM components to improve migration (110, 111). In addition, proteolytically cleaved fibronectin, laminin and collagen compete with their non-cleaved counterparts for adhesion sites and facilitate cell detachment (112).

While ECM signaling through integrins provides a sensor for the mechanical properties of the ECM and basal adhesion and traction upon which growth factor pro-migratory signaling can function, another class of ECM molecules – matrikines and matricryptines – has emerged as crucial to cancer motility (113). Matrikines possess low binding affinity for growth factor receptors but are often present in high valency, which increases avidity for the receptor and enables signaling. These domains, to-date, are found in collagen, laminin, decorin and tenascin C and they enable persistent non-degradable signals. Presentation of growth factor-like sequences within constrained ECM results in unique signaling with preferentially activating pro-migratory cascades compared to soluble ligands (114).

Below, we will highlight a few of the ECM proteins that are dysregulated during epithelial to mesenchymal transition and contribute to cell motility signaling through both integrin signaling and tyrosine kinase receptors.

4.2.1. Collagen I

Collagen I is one of the main structural components of ECM, but fibrillar collagen not only serves as a substratum for integrins but also signals via DDR receptors. Up-regulation of collagen I in metastatic adenocarcinomas is mainly derived from the tumor stroma (115) and contributes to the increased stiffness of the tissue (116). Cancer cells tend to migrate toward the regions of the increased stromal stiffness (117), but loosening of the fibrillar collagen enables cancer invasion (118). MT1-MMP expression levels, the matrix-metalloproteinase mainly responsible for collagen I degradation, correlate with tumor invasiveness. The enhanced migration of cultured tumor cells in the presence of collagen degradation products and

not intact collagen suggests a role for collagen fragmentation in tumor invasion (119, 120). This dual nature of collagen function is still to be understood, but it likely relates to quantitative balances of signals.

Collagen I can promote migration via $\alpha 1\beta 2$, $\alpha 5\beta 3$ or $\alpha v\beta 3$ integrin signaling and via discoidin domain receptors (DDR), a class of tyrosine kinase receptors (121, 122). DDRs are upregulated in many types of cancer (123) and *in vitro* overexpression of DDRs increases migration of cancer cells (124, 125). It has been shown that collagen I overexpression promotes motility by upregulation of N-cadherin expression through $\alpha 2\beta 1$ and DDR1 signaling, which underlies the role of collagen I in EMT (126, 127).

4.2.2. Laminin 5

Laminin 5 (Ln-5) is one of the major components of the basement membrane. While epithelial cell adhesion to basement membrane occurs via integrin adhesion to laminin-5 (Ln-5), cleavage of Ln-5 by MT1-MMP (128) and MMP-2, both upregulated in tumors, reveals cryptic pro-migratory sites (129). These pro-migratory sites were shown to be EGF-like repeats that stimulate breast cancer cell migration in an EGFR-dependent manner (130). Upregulation of Ln-5 was observed in many carcinomas, especially at the invasive front (131), which further supports role of the Ln-5 in dissemination from the primary site.

4.2.3. Tenascin C

Tenascin C (TN-C) upregulation in invasive carcinomas recalls the similarities of cancer with embryogenesis and wound repair (onco-fetal-wound connection). In normal physiological TN-C establishes interactions between the epithelium and the mesenchyme during embryonic development, tissue differentiation and wound repair and its expression is transient and strictly regulated (132). Persistent high levels of TN-C are present in various tumor tissues, including brain, bone, prostate, intestine, lung, skin, and breast (133) and are produced by both epithelial tumor and stromal cells (134). TN-C expression can be induced by various growth factors and cytokines (EGF, TGF- β , TNF- α , IFN- γ , IL) and by mechanical stress and hypoxia, all present in the tumor environment and its upregulation coincides with situations requiring either proliferation or migration (135).

TN-C is a multidomain molecule, and FNIII-like repeats of TN-C can interfere with integrin signaling, thereby enhancing cell proliferation (136), but soluble TN-C induces loss of focal adhesions and increase in cell migration by binding to annexin II on cell surface through an alternatively spliced FNIII-like domain (137). In glioma cells, tenascin C promotes migration via $\alpha 2\beta 1$ integrins and has a positive effect on cell migration on fibronectin (138). Another way in which TN-C promotes invasion is by stimulating the production of matrix metalloproteinases: in chondrosarcoma, breast cancer and glioblastoma exogenous addition of the large splice variant of TN-C or induction of its endogenous expression increases production of matrix metalloproteinases and

invasion in *in vitro* assays (139-141).

Most recently, it has been shown that tenascin C possesses a novel mode of matricrine signaling via cryptic growth factor receptor ligands in its epidermal growth factor (EGF)-like repeats that are able to bind to the epidermal growth factor receptor (EGFR) (142, 143) as well as subsequently activate EGFR-signaled pro-migratory cascades in fibroblasts (144). Unlike in the case of soluble EGFR ligands where binding induces internalization and degradation upon binding to receptor (145), the EGF-like repeats of TN-C cannot be internalized and constantly signal from the cell membrane (144). EGF-like domains of TN-C can be released by MMP cleavage (146), and in the face of increased MMP activity during epithelial to mesenchymal transition TN-C mediated EGFR motogenic signaling is very possible.

4.3. Avoidance of stop signals

The migration of tumor cells from their primary mass to ectopic sites not only requires positive signals as noted above, but also avoidance of inhibitor signals. This takes two steps. The first of which is to down-regulate the molecules/structures that maintain organ structure through EMT. However, this just sets the stage for motility and the question remains of whether there are 'stop motility' signals that need to be overcome.

Recently, a 'stop motility' axis has been described for physiological cell migration during wound healing (147-149). This operates via the CXCR3 receptor for the family of ELR-negative CXC chemokines. CXCR3 is activated by specific binding of the ligands, CXCL4/PF4, CXCL9/MIG, CXCL10/IP10, CXCL11/IP9/I-TAC, and the activation induces diverse cellular responses, including chemotactic migration and cell proliferation, or inhibition of migration and even endothelial death depending on the cell type (150). CXCL9, CXCL10, CXCL11 can be induced by INF while CXCL4 is released from alpha-granules of activated platelets during platelet aggregation (86). There are two splice variants of CXCR3: CXCR3A and CXCR3B, with CXCR3B containing a longer extracellular domain at N-terminus (151). CXCR3A mainly functions in promoting cell proliferation and motility (151, 152). However, CXCR3B, primarily found expressed on fibroblasts, endothelial and epithelial cells, inhibits cell growth and migration (151, 153). Some studies suggest that CXCR3A and CXCR3B play reciprocal roles through different G-protein coupling and lead distinct signaling transduction pathways (151, 154-156). Chemokines CXCL9, CXCL10 and CXCL11 bind to both CXCR3 isoforms, while CXCL4 only associates with CXCRB variant, possibly due to the extended extracellular domain of CXCR3B (151).

CXCR3 expression has been shown in melanoma, breast, colorectal and renal carcinomas (157-165). Several groups have reported that CXCR3 promotes breast, colon and melanoma cell metastasis, but has no effect on tumor growth in murine models (161, 164-166), suggesting that CXCR3 plays a more important role in tumor metastasis than in localized expansion in these types

of cancers. However, how CXCR3 regulates tumor growth and metastasis remains unclear. Since the CXCR3B isoform was identified recently, only a few studies have focused on the functions of two CXCR3 splice variants in cancers. Renal cells treated with calcineurin inhibitors developed bigger tumors in nude mice by downregulation of CXCR3B, the expression of which correlates with tumor necrosis in renal cell carcinoma indicating that CXCR3A and CXCR3B may differentially influence cancer progression *in vivo* (160, 162). The studies of CXCR3 isoforms in keratinocytes, fibroblasts and endothelial cells suggest that in CXCR3 pathways, both CXCR3A and CXCR3B activate PLCbeta by G proteins. PLCbeta hydrolyzes the highly phosphorylated lipid phosphatidylinositol 4,5-bisphosphate (PIP2), generating two products: inositol 1,4,5-trisphosphate (IP3), a universal calcium-mobilizing second messenger; and diacylglycerol (DAG), an activator of protein kinase C (PKC). IP3 induces intracellular calcium flux, which activates mu-calpain and results in cell motility induction. In addition to PLCbeta activation, there is another unique signal transduction path via CXCR3B through an accumulation of cAMP. With CXCR3B signals, PKA, known as cAMP-dependent protein kinase, is activated which inhibits m-calpain activation and blocks cell migration (151, 153-155, 167). Therefore, in these cells, CXCR3A is likely to play a role of pro-migratory and CXCR3B signals as an anti-migratory signal for cell migration. However, how these two CXCR3 splicing variants regulate cell migration and invasion in cancers remains unclear. Our recent results suggest that prostate cancer cells increase CXCR3A and reduce CXCR3B expressions to subvert a stop signal to a promotion signal in cell motility and regulation of invasion (168) (Figure 4).

5. MOTILITY AND PHENOTYPE AT THE TARGET ORGAN

Extravasation from the vascular conduit and ectopic seeding are necessary for metastatic dissemination. Both of these steps require not only cell motility or at least transmigration but also cell-cell interactions that enable the cancer cell to interact with its new and foreign milieu. Carcinoma cells, unlike hematopoietic cells, are arrested due to size prior to extravasation, allowing more time for interactions and juxtacrine signaling that enables the carcinoma cell to squeeze between the endothelial lining. Further, once the vascular wall is breached, carcinoma cells have been noted to move towards the post-capillary spaces of the tissue, as if seeking a lower oxygen environment. This may reflect the glycolytic metabolism of carcinomas. The mesenchymal phenotype of disseminating carcinomas promotes all these steps. However, ectopic seeding may be a unique situation that will be discussed below.

5.1. Expression of adhesion molecules during extravasation

Once a cancer cell has undergone EMT to enable migration and dissemination from the primary tumor, a new set of challenges must be overcome in order to establish metastatic foci at a secondary organ site. Although mechanical entrapment of circulating tumor cells occurs,

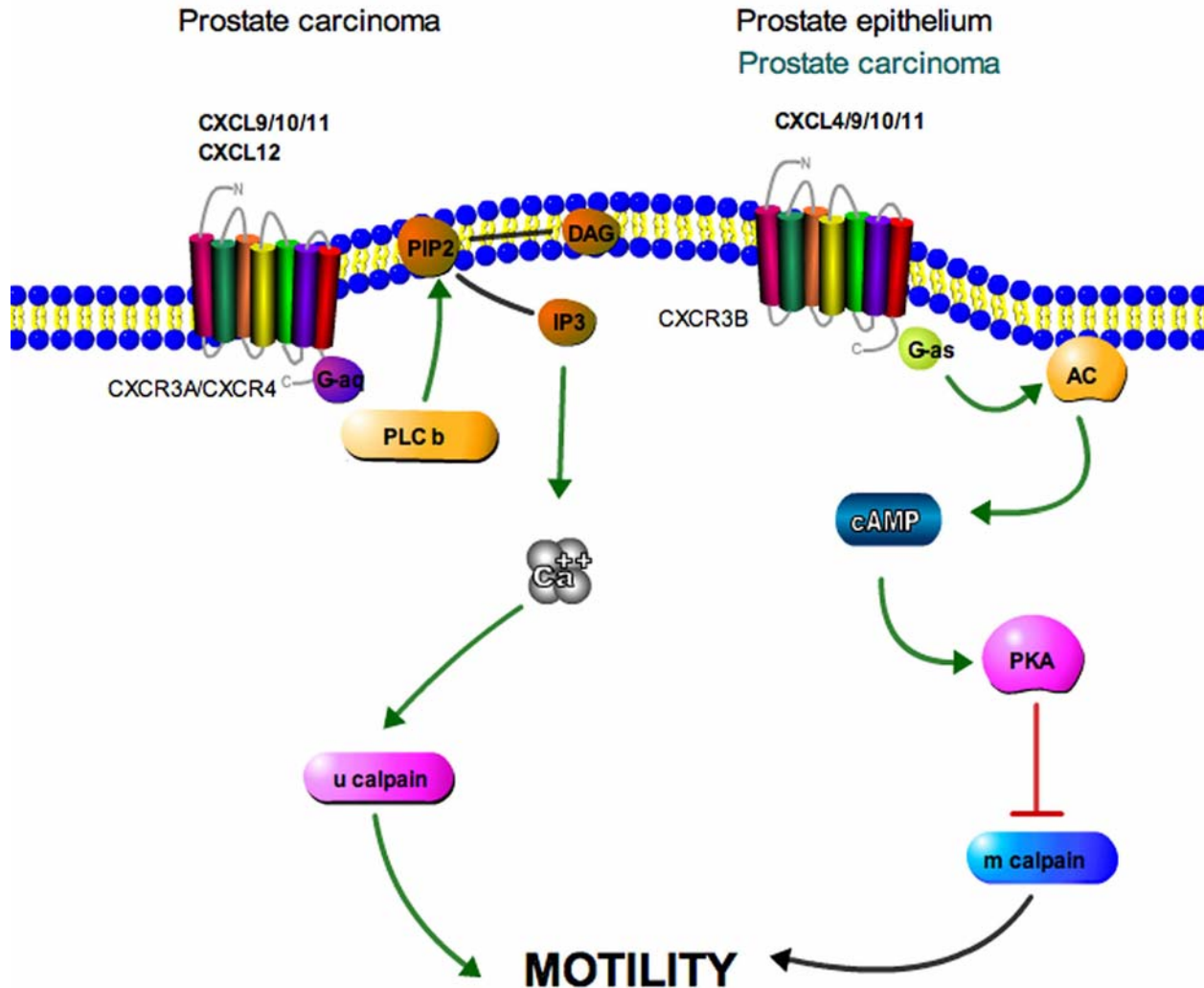


Figure 4. CXCR3 Isoforms Modulate Motility in Opposing Directions. CXCR3A signaling mainly via Galphaq subunits activates phospholipase C-beta (PLC-b) to initiate calcium influx; activation of mu-calpain (calpain 1) at the membrane shifts the adhesion regiment to a more permissive state to facilitate motility. CXCR3B, while also signaling via Galphaq subunits, more strongly initiates Galphas subunits that trigger protein kinase A (PKA) to inhibit m-calpain (calpain 2) a prevent rear release during motility. In normal prostate epithelial cells, only CXCR3B is expressed, but in prostate carcinoma cells, both isoforms are present at roughly equivalent levels.

tumor cells must then actively adhere to the vascular and extravasate, or migrate through the endothelium into organ parenchyma. The process of extravasation is similar to diapedesis, or transendothelial migration, exhibited by leukocytes in inflammation. During diapedesis, leukocytes adhere to and roll along the vasculature and then migrate between endothelial cells. The initial attachment of cells to the endothelium is mediated by a class of cell adhesion molecules called selectins, followed by stronger adhesions facilitated by immunoglobulin adhesion molecules, integrins and cadherins. Expression of many of these cell adhesion molecules are necessary for extravasation of disseminated carcinoma cells (169). These are not merely attachments, as signals between the endothelial and extravasating cells also direct retraction of endothelial cells that allows for active movement of invading cells through

the vascular lining.

Selectins are a family of adhesion receptors that bind to carbohydrate ligands. E-selectins are expressed primarily by endothelial cells, P-selectins by platelets, and L-selectins by leukocytes. Presentation of selectin ligands on cancer cells is believed to be critical to extravasation. Interactions of circulating cancer cells with platelets and leukocytes via P- and L-selectins may support tumor cell embolic arrest and immune evasion in the vasculature (170). Cancer cell binding to E-selectin on endothelial cells is critical to the extravasation of colon cancer cells in metastatic colonization of the liver. Attachment of cancer cells to the endothelium and subsequent formation of liver metastases can be inhibited by addition of antibodies against E-selectin (171). Furthermore, selectin-dependent

adhesion to endothelial cells results in morphology changes, reorganization of the cytoskeleton, and tyrosine phosphorylation, suggesting that these interactions are not limited to adhesion and may have downstream signaling effects (172). Differential expression of selectin ligands can also influence the site of metastatic colonization and account for organotropism (173).

Following the attachment initiated by selectin binding, adhesion between cancer cells and endothelial cells may be further strengthened by other adhesion molecules. Expression of immunoglobulin cell adhesion molecule (IgCAM) family members ICAM and VCAM has been observed in distant metastases of colorectal cancer but not in benign lesions, suggesting that these adhesion molecules are part of the EMT. The attachment of metastatic cells to endothelial cells and to extracellular matrix has been shown to be necessary for metastasis (174). The cadherin switch that occurs during EMT results in the down-regulation of E-cadherin and the upregulation of N-cadherin. Endothelial cells have been shown to express N-cadherin, so this switch may facilitate the heterotypic binding of cancer cells to endothelial cells. Indeed, N-cadherin has been shown to mediate attachment of MCF-7 breast cancer cells to endothelial monolayers as well as the transendothelial migration of melanoma cells (31, 175). Exogenous expression of Cx43 into MDA-MET, a breast cancer cell line variant that is highly metastatic to bone, results in increased adhesion to endothelial cells. Others have also shown similar heterophilic binding between cancer cells and endothelial cells in melanoma and lung cancer (39, 176). Finally, engagement of integrins expressed on cancer cells also contributes to adhesion to the microvasculature, as antibodies against beta1, alpha2, and alpha6 integrins inhibit adhesion to and migration through sinusoids in colorectal metastases to the liver (177). Although cancer cells may arrest in capillaries due to size-restriction, these studies show that adhesion to endothelial cells is nonetheless a required step of extravasation.

5.2. Adhesion molecules during colonization

Despite the wealth of studies describing EMT in carcinoma cells *in vitro*, and the strong clinical association between loss of expression of adhesion molecules and invasion and poor prognosis, metastases often present a well-differentiated, epithelial phenotype, bringing into question whether EMT is reversible. It is well described that signals from the primary tumor microenvironment greatly contribute to induction of EMT at the primary tumor, so dissemination not only removes cancer cells from these signals but also exposes them to new ones at the secondary organ site. Furthermore, post-extravasation survival has been shown to be the rate-limiting step of metastasis (178) and most cancers seem to display a propensity to metastasize to a set of organs that cannot be explained by circulation alone. Just as adhesion impacts the intravascular survival of a circulating cancer cell, intercellular adhesion between cancer cells and parenchymal cells can influence survival at the ectopic site (179).

While the mesenchymal phenotype that results

from EMT may promote invasion and dissemination, there is evidence that metastatic colonization favors an epithelial phenotype. In bladder carcinoma, cell lines selected *in vivo* for increasing metastatic ability reacquire epithelial morphology and gene expression. When these cells are injected orthotopically, they show a decreased ability to colonize the lung when compared to the more mesenchymal parental cell line. However, when they are injected via intracardiac or intratibial inoculation, they show an increased ability to colonize the lung compared to the parental cell line (180). Therefore, while induction of EMT through loss of E-cadherin may promote tumor invasion and dissemination, MET through E-cadherin re-expression may allow the metastatic cancer cell to complete the last steps of the metastatic process and survive in the new organ (4, 6). When queried by pathology, a number of studies have shown that E-cadherin-expression metastases may derive from dedifferentiated, E-cadherin-negative primary carcinomas (181-185). Similarly, changes in beta-catenin localization have been documented (186) and a study of breast cancer found increased expression of Cx26 and Cx46 in metastatic lymph nodes compared to the primary tumors, with even positive foci originating from connexin-negative primaries (187).

The question remains whether the well-differentiated phenotype observed in metastases is the result of an expansion of epitheloid cells or from reversion of EMT – a transition back to an epithelial phenotype from a mesenchymal state (MErT). As E-cadherin down-regulation in invasive carcinomas is largely the result of promoter methylation and transcriptional repression, cancer cells can easily switch between epithelial and mesenchymal phenotypes. Promoter hypermethylation leading to E-cadherin suppression is dynamic and reversible and therefore re-expression in response to changes in the microenvironment is possible (188). As evidence of the phenotypic plasticity of cancer cells, PC3 prostate cancer cells cultured in 3D Matrigel form cell-cell contacts, tight junctions, and decrease in mesenchymal gene expression, suggesting that a change in tissue architecture is enough to induce such morphological changes (189). Work in our lab has shown that coculture of breast and prostate carcinoma cells with hepatocytes results in the re-expression of E-cadherin (6). *In vivo*, mice inoculated with E-cadherin-negative MDA-MB-231 cells also form E-cadherin-positive lung metastatic foci (190, 191). The basement membrane component laminin-1 may participate in re-expression of E-cadherin at the metastatic site (192). While these studies show that reversion through MErT is possible, they do not rule out the possibility of expansion of epithelial cells that have detached from the primary tumor.

Selective cellular adhesion may account for some of the organotropism exhibited by cancers. For example, breast cancer typically metastasizes to the lung, liver, bone, and brain, while colorectal cancer may metastasize to a different set of organs. Mechanical entrapment in the first capillary bed encountered does not explain the characteristic pattern of metastases (169, 193). E-cadherin re-expression could explain the propensity for breast cancer cells to metastasize to lung and liver, both lined with

epithelia. Aberrant expression of osteoblast cadherin, also known as OB-cadherin and cadherin-11, on breast and prostate cancer cells, increases metastases to the bone by increasing migration and intercalation with osteoblasts (194, 195). Furthermore, there may be changes in integrin profiles of metastatic cancer cells to adapt to the new ECM compositions of the target organ. One group has shown that human melanoma cells express α 5 β 3 integrins to adhere to lymph node vitronectin, while breast carcinoma cells utilize α 3 β 1 integrins to bind lymph node fibronectin (196). The α 5 β 3 integrin combination when expressed in breast and prostate cancer cells also contributes to bone-specific metastasis (197, 198). While selective growth and chemotactic honing are also critical mechanisms that contribute to site-specific metastasis, selective adhesion facilitated by these cell adhesion molecules is certainly important.

6. MIGRATION IN MESENCHYMAL AND EPITHELIAL PHENOTYPES

It is generally considered that EMT is associated with a significant gain in cell motility, due to loss of stable E-cadherin-based cell-cell junctions (199-201). However, disparities in motility behavior between epithelial and mesenchymal forms of a carcinoma tissue may be more nuanced and context-dependent than this dichotomous notion. Epithelia can exhibit efficient migratory behavior even while maintaining integrity of cell-cell interactions (202), and some invasive carcinomas penetrate adjacent connective tissue as multi-cellular aggregates (203). Thus, the issue at hand likely more concerns quantitative differences in key characteristics and molecular regulation of motility behavior for mesenchymal versus epithelial phenotypes rather than residing in a qualitative “on/off” motility switch. One interesting model, in fact, suggests that cooperation between epithelial and mesenchymal subpopulations of a tumor enhances distal metastasis because contributions of motility from both are needed in order to meet diverse challenges inherent in intravasation and extravasation (3).

The mechanistic basis for mesenchymal and epithelial motility must emphasize different balances among the underlying biophysical processes of membrane (lamellipod, filopod, and/or invadopod) protrusion, cell/matrix attachment formation, cytoskeletal contraction, cell deformation, and cell/matrix detachment, along with cell/cell adhesive interactions. Cell/cell adhesive interactions are more important for coordinated epithelial cell aggregate motility, whereas lamellipod protrusion should have greater influence on individual mesenchymal cell motility; in both cases, nonetheless, net cell locomotion can only arise from an appropriate balance of forces associated with the cohort of biophysical processes involved. Indeed, computational models have been proposed for purposes of quantifying the relevant balance of forces generating net locomotion in each of the categories (204, 205), although a direct comparison has not yet been undertaken.

With respect to particularly vital processes,

invadopodia associated with focal proteolysis of the extracellular matrix are believed to be generally vital for tissue penetration and highly prevalent in mesenchymal cells (206, 207). Nonetheless, at least some mesenchymal cell types appear to undertake locomotion in an amoeboid manner independent of matrix proteolysis under certain circumstances (208). Detailed quantitative biophysical and biochemical analyses of cell and matrix properties are beginning to elucidate the conditions under which proteolysis is critical or not (10, 207, 209). As with motility *per se*, the role of proteolysis in mesenchymal versus epithelial migration may not be categorical, with it contributing to both kinds of invasion (210). A very recent study has identified a set of pseudopod-specific proteins associated with metastatic tumor cell lines, with a subset (AHNAK, septin-9, eIF4E, S100A11) found to be essential for actin polymerization and pseudopod protrusion related to *in vitro* invasiveness (211). More established promoters of carcinoma invasion and metastasis involved in control of lamellipod and invadopod formation, at least in breast tumors, include a splicing isoform of the Ena-VASP protein Mena (212, 213), cofilin (214), and cortactin (215), among others. Motility and invasion can be governed not only by processes occurring at the cell front, of course, but also by other processes transpiring at the cell rear; calcium-independent calpain-mediated deadhesion of cell/matrix attachments has been found to be a process rate-limiting for migration and invasion of prostate tumor cells (216). It should be noted that calpain activity may also be involved in another biophysical processes involved in invasive motility, by regulation of invadopodia (217).

Coordination of the underlying biophysical processes to produce cell migration depends intricately on integration of receptor-mediated signals distributed across multiple pathways in both a temporal and spatial manner (218), so it can be expected that mesenchymal and epithelial motility modes should exhibit diverse dependencies on various intracellular signals. Unfortunately, there are few literature reports bearing on this issue. We note that the question of signals driving motility of mesenchymal cells versus epithelial cells is not the same as the question of signals inducing epithelial-to-mesenchymal transition; the latter question has been intensely investigated (e.g., (200, 219)) whereas the former question has seen little address to date. An intriguing clinical observation highlighting the point is the resistance of mesenchymal carcinomas to tyrosine kinase inhibitors of EGFR in contrast to the sensitivity of epithelial carcinomas to this set of drugs (53, 220-222). Since EGFR remains substantively expressed on the mesenchymal tumor cells, a major challenge is to determine how the signaling network governing motility becomes “rewired” during EMT. An analogous challenge for HER2-overexpressing breast epithelial cells has been addressed using quantitative phospho-proteomic measurement across multiple signaling pathways coupled with computational modeling to ascertain the key network differences between normal and overexpressing cells (223). A number of differences were elucidated, and computational modeling showed that quantitative combinations of a subset of pathway activities could predict the change in motility behavior as well as in

response to various kinase inhibitors (224, 225). Some of us (AW, DL) have recently applied a similar approach to explore differences in motility-related signaling in mesenchymal versus epithelial forms of human mammary epithelial cells with EMT induced by the transcription factor Twist. This study has found that at least a half-dozen intracellular kinase pathway activities are differentially influential for mesenchymal versus epithelial motility across stimulation by a variety of growth factors including EGF, HRG, HGF, IGF, and PDGF, demonstrating the complexity of signaling network “rewiring” downstream of EMT induction (HD Kim, A Wells, FB Gertler, DA Lauffenburger; unpublished data).

7. FUTURE DIRECTIONS

It is too early in our investigations of how the microenvironment interacts with carcinoma intrinsic changes to dictate tumor behavior and progression to propose interventions. Due to limitations of focus and space we have discussed only cell migration, which has been shown to be a critical step in the stage of tumor dissemination. We have not delved into proliferation or death (apoptotic, necrotic, or autophagic) as the cancer-associated dysregulation of these behaviors arises well before dissemination at the earliest stages of carcinogenesis. Also, the foregoing, while quite extensive, did not deal with critical aspects of immune response, systemic hormonal/cytokine signaling, and angiogenesis. Each of these whole organism responses impact upon tumor outcome in nuanced and situation-dependent manners, and hold avenues for successful interventions. Still, we posit that better examination of the local tumor microenvironment, at both the primary and ectopic sites, can highlight key regulatory, and possible targetable, events in the transition to dissemination.

It is evident that more systematic approaches to these questions are needed. The variety of signals and possibilities of behavioral outcomes make it evident that no one signal is required and thus models must account for redundancy. Further, quantitative aspects will dictate the resultant behavior; a glaring example of this is collagen, which provides substratum traction for migration with higher concentrations providing a stiffer matrix that promotes mesenchymal phenotypic behaviors, yet collagen can also serve as a barrier to dissemination. Further, the systematic approaches need to account for higher levels of regulation. Tissue- and site-specific signals determine which cell behaviors promote tumor progression. For instance, EMT, resulting in mesenchymal-like single cells, enables escape from the primary tumor mass but a reversion to epithelioid syncytial properties may be critical for ectopic survival once a distant site is involved. Further, invasion into adjacent tissues may be accomplished as a mass of mixed carcinoma and orthotopic stroma whereas distant metastases most likely involve isolated cells adapting to the foreign environment.

Even within such consideration of multiple levels of control, and quantitative and nuanced analysis of the data, there remain areas for reductionist examination. One

aspect that is only now becoming approachable is how the tumor cells escape from the physiological controls that maintain differentiation and prevent epithelial mislocalization. While avoidance of the immune response has been appreciated for half a century, the signaling focus for the past decades has mainly highlighted acquisition of signaling capabilities that promote carcinoma progression. Yet, these carcinoma cells must overcome the physiological ‘stop’ signals that efficiently prevent the transient EMT of wound repair or embryogenesis from leading to dysplasia, and even in the most aggressive appearing carcinomas make dissemination a rare event at the cellular level. Yet, this is not likely to simply be avoidance by negation, but rather a switch in receptivity, as noted by the well-described dual nature of TGF-beta signaling, a paradigm being seen again in early studies on the chemokine receptor CXCR3.

In sum, the understanding of carcinoma cell events that lead to the migration that enables dissemination is ripe for explorations at system and reductionist levels. These studies will likely yield not only fundamental insights into the multicellular and multicompartiment tissue we refer to as a tumor, but also suggest avenues for interventions that target distinct stages in carcinoma progression to dissemination.

8. ACKNOWLEDGEMENTS

We thank members of the Wells and Lauffenburger laboratories for discussions that enriched this review. The concepts behind this were supported currently and over the years by grants from the Veterans Administration, DoD Congressionally Directed Medical Research Programs in Breast and Prostate Cancer, and the National Institutes of Health (USA).

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Key words: Epithelial Mesenchymal Transition, EMT, Mesenchymal Epithelial reverting Transition, MErT, Growth Factor Receptors, Tumor Microenvironment, Migration, Tumor Dissemination, Review

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