Journal of Cellular Biochemistry 101:816–829 (2007)

Mechanisms of Metastasis: Epithelial-to-Mesenchymal Transition and Contribution of Tumor Microenvironment

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Abstract Every year about 500,000 people in the United States die as a result of cancer. Among them, 90% exhibit systemic disease with metastasis. Considering this high rate of incidence and mortality, it is critical to understand the mechanisms behind metastasis and identify new targets for therapy. In recent years, two broad mechanisms for metastasis have received significant attention: epithelial-to-mesenchymal transition (EMT) and tumor microenvironment interactions. EMT is believed to be a major mechanism by which cancer cells become migratory and invasive. Various cancer cells—both in vivo and in vitro—demonstrate features of epithelial-to-mesenchymal-like transition. In addition, many steps of metastasis are influenced by host contributions from the tumor microenvironment, which help determine the course and severity of metastasis. Here we evaluate the diverse mechanisms of EMT and tumor microenvironment interactions in the progression of cancer, and construct a rational argument for targeting these pathways to control metastasis. J. Cell. Biochem. 101: 816–829, 2007.

Key words: cancer; metastasis; epithelial to mesenchymal transition; EMT; mesenchymal to epithelial transition; MET; tumor microenvironment; extracellular matrix

Metastasis is a fatal step in the progression of cancer, with death from metastases representing 90% of all human cancer mortalities [Sporn, 1996]. Most cancer patients die from metastases rather than from their primary tumors; therefore, it is critical to study the molecular mechanisms of metastasis and elucidate therapeutic targets to prevent the spread of cancer.

Aberrant control of epithelial proliferation and angiogenesis underlie the initiation and growth of primary carcinomas [Hanahan and Weinberg, 2000]. However, additional steps of action must be completed for the successful establishment of a metastatic tumor [Woodhouse et al., 1997; Chambers et al., 2002] (Fig. 1). First, cancer cells from the primary tumor must gain access to the circulatory system, a process typically aided by angiogenesis and remodeling of the basement membrane [Folkman, 1992]. The intravasated cancer cells must then survive the shear forces of circulation and home to distant metastatic sites. At the exit point, extravasation of cancer cells requires recognition and adhesion to endothelial cells followed by matrix degradation. Lastly, the cancer cells must invade the secondary tissue and reestablish organizational growth as a solid secondary tumor.

One mechanism that may enhance the dissemination of cancer is epithelial-to-mesenchymal transition (EMT) (Fig. 2). It is believed that the migratory characteristics acquired by the transition to a mesenchymal-like state enable the invasive capabilities of the cancer cell. Many researchers observe some loss of epithelial characteristics paired with a gain of mesenchymal markers in the invasive front of various

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Received 25 October 2006; Accepted 30 October 2006
DOI 10.1002/jcb.21215

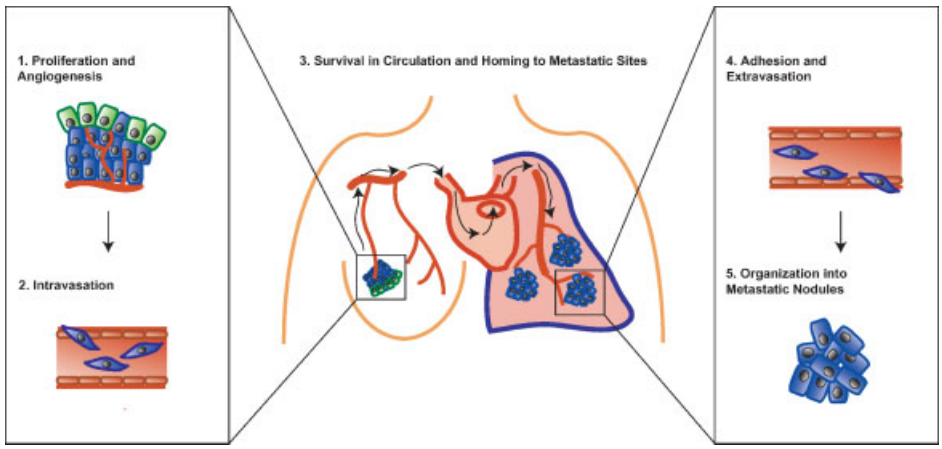

Fig. 1. Metastatic progression of cancer. Progression from normal epithelium to invasive carcinoma and the establishment of metastatic nodules in secondary organs requires several steps. (1) The uncontrolled proliferation of cancer cells is perpetuated by mutations in the normal epithelium and the nutrients supplied via angiogenesis. (2) Angiogenesis also provides the cancer cells with a path to the body's systemic circulatory system, resulting in the mobility of cancer cells throughout the body. (3) After intravasation, the cancer cells must survive the shear forces of circulation and localize to conducive metastatic site(s). (4) Upon reaching the metastatic site, cancer cells adhere to the endothelial cells and negotiate their way through the basement membrane and undergo extravasation. (5) Invasion of secondary tissues is followed by reorganization of the cancer cells into tumorigenic nodules.

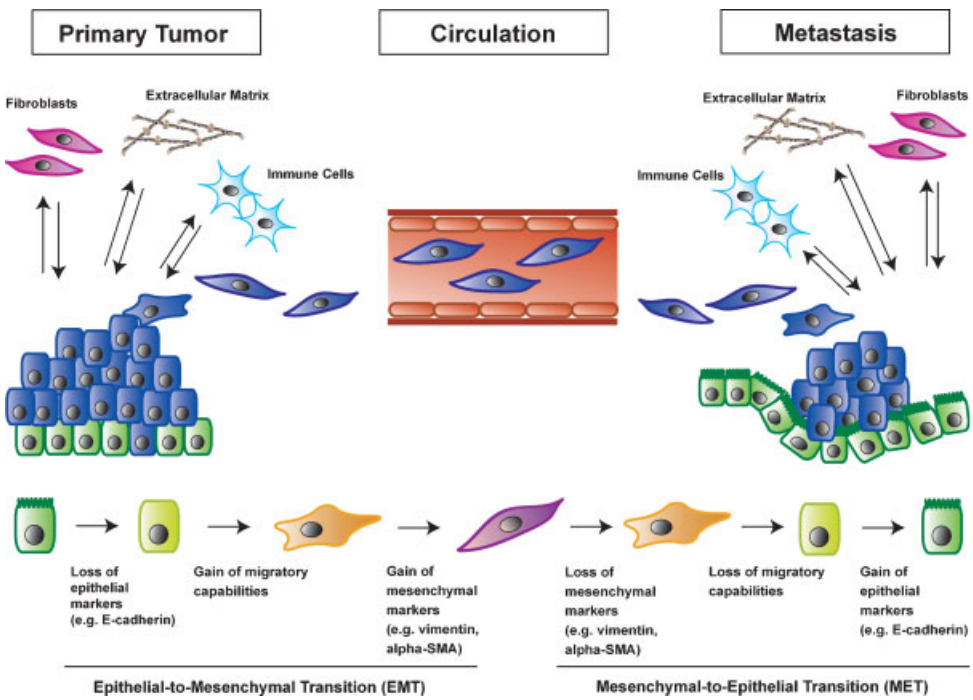

Fig. 2. Mechanisms of metastasis. Epithelial-to-mesenchymal transitions and tumor microenvironment interactions are likely associated with the progression of cancer. Carcinoma of an epithelial origin may acquire a mesenchymal-like state in order to facilitate its migration and invasion. Upon reaching their metastatic sites, the cancer cells revert back to their epithelial state to form organized tumorigenic nodules. During EMT and MET, a bimodal communication exists between the host fibroblasts, extracellular matrix/basement membranes, and also the immune cells. Interactions with host elements also influence the progression of cancer at all stages. Changing dynamics in the composition of extracellular matrix can induce new signaling pathways within the cancer cell. Immune cells may also play a role in the progression of cancer—in some cases even protecting the cancer cell from apoptosis. How host cells are usurped by cancer cells to facilitate cancer progression and metastasis is not yet clear.

cancers [Gotzmann et al., 2002]. Such observations point to a possible contribution of EMT in the acquisition of an invasive phenotype leading to metastasis.

Recent research has also emphasized the role of tumor stroma in the development of cancer [Pupa et al., 2002; Kiaris et al., 2004; Mueller and Fusenig, 2004; Kalluri and Zeisberg, 2006; Kopfstein and Christofori, 2006]. A dynamic interaction likely exists between cancer cells and the host microenvironment to support cancerous growth and spread. The limited range of metastatic sites attributed to each cancer type suggests a specific contribution by the host environment to enable metastases [Fidler, 2002], further highlighting the "seed and soil" theory postulated by Paget [1889]. Research focused on uncovering factors that define the suitability and specificity of the "soil" in aiding cancer dissemination is beginning to unravel a unique crosstalk between cancer cells and the host microenvironment, which exploits the interactive mechanisms of physiological processes.

The mechanisms involving EMT and tumor microenvironment interactions synergize to direct the progression of metastasis. Genetic changes mark the uncontrolled proliferation of cancer cells, and EMT may pair this quality with invasive properties leading to the systemic spread of cancer cells. The acquisition of genetic changes likely alters the manner in which the host microenvironment reacts to and interacts with tumorigenic cells influencing the metastatic course. Cancer cells do not appear to accumulate any additional major mutations beyond the genetic alterations acquired at the primary tumor site [Seftor et al., 2005], yet the cancer cell must execute a distinct set of tasks to complete metastasis. In this sense, the host microenvironment may provide the cancer with a misguided compensating support needed to complete the steps of metastasis. EMT most likely plays a role in the initiation of metastasis, while the importance of tumor microenvironment interactions may become more dominating in later stages.

EPITHELIAL-TO-MESENCHYMAL TRANSITION AND METASTASIS

EMT is one possible mechanism behind the initiation of cancer progression during staged metastasis [Jechlinger et al., 2002; Thiery, 2002; Xue et al., 2003], resulting in invasive cancers that possess the migratory characteristics of mesenchymal cells (Fig. 2). The evidence for EMT-associated tumor movement is supported by network signaling pathways mediated by fluctuating levels of TGFβ, EGF, PDGF, ERK/MAPK, PI3K/Akt, Smads, RhoB, β-catenin, LEF, Ras, c-Fos, integrins β4 and integrin α5, and most importantly the dissolution of cell–cell junctions mediated by Snail, Slug, SIP1, and E2a transcription factors [Jechlinger et al., 2002; Thiery, 2002; Shi and Massague, 2003].

While TGFβ is an important suppressor of primary tumorigenesis, its role as a positive modulator of late tumor progression and metastasis is also critical [Hata et al., 1998; Oft et al., 1998]. In fact, this latter role for TGFβ may be more important than its tumor suppressive effect. The early role of TGFβ in apoptosis and cell cycle arrest is microenvironment-dependent and becomes less effective as cancer progresses. TGFβ then switches to function as a potentiator of EMT. Ras-transformed hepatocytes and MDCK cells undergo TGFβ-induced EMT when compared to their wild-type counterparts [Lehmann et al., 2000; Gotzmann et al., 2002]. Ras-transformed tumor cells (EpH4 mouse mammary epithelial cell line) also undergo EMT when exposed to TGFβ via MAPK, and interestingly, PI3K inhibits induction of apoptosis by TGFβ [Oft et al., 1996; Janda et al., 2002b]. The effect of Ras mutants on EMT specifically activates either the ERK/MAPK or the PI3K-Akt/PKB pathway [Janda et al., 2002a], and this transition can be reversed by wild-type Ras or MEK1 inhibitors. Cells are protected from TGFβ-induced apoptosis when Ras-mediated PI3K/Akt pathway is active [Janda et al., 2002a], demonstrating a clear transition from the earlier role of TGFβ to this later role.

Similar studies with NMuMG mouse mammary epithelial cells reveal that autocrine TGFβ requires integrin β1 to induce EMT, and this effect is mediated by p38MAPK and RhoA [Bhowmick et al., 2001a,b]. In mice, Ras-transformed EpH4 cells progressively acquire a mesenchymal phenotype in association with autocrine production of TGFβ. Constitutive activation of Raf in MDCK cells induces EMT and is dependent on autocrine TGFβ. Here, Raf counteracts TGFβ-mediated growth inhibition and apoptosis, and at the same time enhances

the facilitating actions of TGFβ on invasiveness. Such dual properties of TGFβ have been demonstrated in mouse models of skin carcinoma and human colon cancer where a lack of TGFβ receptor confers better prognosis [Cui et al., 1996; Watanabe et al., 2001].

Compelling evidence for the role of Smad proteins in the regulation of TGFβ action has also emerged in the recent years [Miyazono, 2000; Derynck et al., 2001; Attisano and Wrana, 2002]. Depending on cell context, it is likely that Smads mediate the induction of EMT [Heldin et al., 1997], and in this regard TGFβ ALK5 receptor and Smad proteins regulate EMT in NMuMG breast epithelial cells [Piek et al., 1999]. In kidney tubular epithelia and NMuMG cells, TGFβ-induced EMT is dependent on the downregulation of E-cadherin via Smad3 [Zeisberg et al., 2003]. Smad-mediated signaling by TGF [Zeisberg et al., 2003] interfaces with other receptor kinases to obviate its tumor suppressive effect and thus facilitates motility. This is accomplished by modulating the differential effects of relevant transcription factors and cytoplasmic kinases with inhibitory Smads, and also by directly inducing autocrine production of TGFβ [Miyazono, 2000; Derynck et al., 2001; Attisano and Wrana, 2002; Ten Dijke et al., 2002]. Other signaling pathways that mediate the action of β-catenin and LEF also cooperate with Smads in creating new transcriptional complexes that induce EMT [Eger et al., 2000; Stockinger et al., 2001; Kim et al., 2002].

Epithelium forms functional units through cell–cell contacts and apical-basolateral polarity [Hay, 1995]. While activation of oncogenes along with mutations in tumor suppressor genes transform these cells to achieve significant protection against apoptosis and cell-cycle arrest, the loss of E-cadherin is pivotal to the further induction of EMT [Edelman et al., 1983; Tepass et al., 2000]. E-cadherin connects to actin microfilaments indirectly via α-catenin and β-catenin in the cytoplasm [Kim et al., 2002]. Loss of E-cadherin in cancer correlates with susceptibility to EMT and the acquisition of an invasive phenotype [Thiery, 2002]. Therefore, epigenetic control of E-cadherin and β-catenin-LEF activity as described above is relevant for establishing the metastatic potential of given subpopulations of cancer cells.

Support for this latter notion comes from several different observations. The constitutive expression of E-cadherin in normal or transformed tumor cells maintains adherens junctions and decreases their capacity to migrate or degrade extracellular matrix [Frixen et al., 1991; Vleminckx et al., 1991]. In a normal mouse mammary cell line, the induction of the c-Fos oncogene induces EMT and is associated with downregulation of E-cadherin expression [Eger et al., 2000]. Cell proliferation is inhibited and cell-adhesion complexes are reestablished, when full-length E-cadherin or just its cytoplasmic portion containing the β-catenin binding site is reintroduced to the target cell [Reichmann et al., 1992; Eger et al., 2000]. In breast cancer clones of high metastatic potential, the upregulation of the transcription factor Twist also promotes EMT with the loss of E-cadherin and the additional activation of mesenchymal markers with cell motility [Yang et al., 2004]. EMT correlates with the presence of β-catenin/LEF in the nucleus and thus sequestration of β-catenin in the cytoplasm is essential for preservation of primary tumor epithelium [Gottardi et al., 2001; Stockinger et al., 2001]. Interestingly, overproduction of β-catenin does not induce EMT but results in apoptosis [Kim et al., 2000]. Therefore, loss of E-cadherin combined with increased levels of free β-catenin is essential for cancer cells to undergo EMT and metastasize [Kim et al., 2002].

Various carcinomas in culture undergo different degrees of EMT (scatter vs. complete EMT) when exposed to exogenous growth factors, and the capacity to undergo EMT correlates inversely with levels of E-cadherin [Thiery, 2002]. E-cadherin-deficient cell lines show increased tumorigenicity and metastasis when transferred into immune incompetent mice, providing the first suggestive connection between EMT and metastasis [Birchmeier and Behrens, 1994]. E-cadherin expression varies in different human tumors and again there seems to be an inverse relationship between level of E-cadherin and grade of cancer or degree of patient survival [Hirohashi, 1998]. Mutations in the E-cadherin gene also occur in cancer cells making them more susceptible to EMT and metastasis [Muta et al., 1996; Hirohashi, 1998; Saito et al., 1999].

Several zinc-finger containing transcriptional repressors, such as Snail and Slug, and the bHLH transcriptional factors SIP1 and E12/E47-E2A have been associated with repression

of E-cadherin [Batlle et al., 2000; Cano et al., 2000; Perez-Moreno et al., 2001; Blanco et al., 2002; Nieto, 2002]. Snail and E-cadherin are inversely correlated in breast cancer and oral squamous cell carcinoma [Yokoyama et al., 2001; Blanco et al., 2002]. In several lines of cancer cells, the loss of E-cadherin is associated with high levels of Snail in the nucleus [Blanco et al., 2002]. Introduction of Snail into cell lines expressing E-cadherin leads to EMT and a metastatic phenotype [Batlle et al., 2000; Cano et al., 2000]. SIP1, another E-box-binding bHLH protein, also acts as a repressor of E-cadherin [Comijn et al., 2001; Van de Putte et al., 2003]. Snail and SIP1 bind to overlapping sites in the E-cadherin promoter and SIP1 can be induced in epithelial cells undergoing EMT on exposure to TGFβ. How SIP1 and Snail regulate their respective activities in the same carcinoma cell is yet undetermined, but both of these transcription factors can be found together [Blanco et al., 2002].

In several human epithelial cancers, tissue fibroblasts carrying p53 mutations can be found adjacent to primary cancer cells carrying the same mutation, providing suggestive evidence that adjacent fibroblasts may have originated from EMT before or during tumorigenesis [Kurose et al., 2002]. While EMT seems relevant to cancer progression [Bhowmick et al., 2001a; Janda et al., 2002a], what has been difficult is establishing an in vivo correlative of an EMT-induced metastasis. The cancer transcriptome engages a variety of proteins necessary for progression that probably have nothing to do with EMT. Therefore, agreement on what are good surrogate markers for tracking EMT in vivo is extremely important. In this regard, fibroblast specific protein 1 (FSP1), also known as S100A4 protein, fills this void [Nikitenko et al., 2000; Rudland et al., 2000]. And recent studies in transgenic mice suggest that the presence of $FSP1^+$ breast tumor cells correlates with the number of micrometastases in the lung [Xue et al., 2003].

These observations demonstrate EMT as a possible mechanism for metastasis by generating invasive cancer cells. Yet to establish fully formed metastatic nodules, the cancer cell must still complete various tasks after entering the bloodstream, which is likely aided by interactions with the tumor microenvironment and the exploitation of host mechanisms.

CANCER CELLS UTILIZE CHEMOTACTIC INTERACTIONS WITH HOST MICROENVIRONMENT FOR HOMING TO METASTATIC SITES

In their journey to preferential metastatic sites, cancer cells exploit pre-existing host mechanisms that govern the precise migration of cells during physiological processes. In particular, chemokines and chemokine receptors have emerged as pathways by which regulation of cell migration occurs [Le et al., 2004]. Chemokines and chemokine receptors were originally identified as traffic controllers of immune cells for their ability to generate chemoattractive interactions between immune cells and sites of inflammation [Oppenheim et al., 1991]. The uses for chemokines and chemokine receptors were later discovered to extend beyond such an inflammatory response. Even in development, cells utilize chemokines and their receptors for homing to their appropriate destinations. Pole cells in *Drosophila*, for example, must complete a complex migration pattern before arriving at their final destination in the gonad. This migration of the germline cells is regulated by chemotactic interactions with somatic tissues of the gonad [Jaglarz and Howard, 1994; Knaut et al., 2003]. Thus, the chemokinetic system appears to exist as a common mechanism for the directed migration of cells, depending on chemotactic contributions from both the cell and the destination tissue.

Given the general physiological use of the chemokinetic system for directed cell migration, it is not surprising that cancer cells utilize the various chemokines for homing to metastatic sites [Kakinuma and Hwang, 2006]. A correlation exists between the chemokine ligands and receptors expressed at the site of metastasis and those expressed by the cancer cell. For example, the chemokine receptors CXCR4 and CCR7 are highly expressed in human breast cancer cells, while their corresponding ligands CXCL12/SDF-1α and CCL21/6kine exhibit peak expression in the preferred organs for breast cancer metastasis [Muller et al., 2001]. Similar reciprocal expressions of chemokines and chemokine receptors have also been observed in metastases of gastric carcinoma and prostate cancer [Mashino et al., 2002; Taichman et al., 2002]. Furthermore, changes in the chemokine expression of an organ can affect its metastatic potential. Chemokine receptors expressed by

the stromal cells at a potential metastatic site can dictate the rate of metastasis [van Deventer et al., 2005]. Most importantly, cancer cells will not migrate to expected sites of metastasis that no longer express the corresponding chemokine ligands and/or receptors [Jones et al., 2006].

Mechanistically, it may appear that chemokines and chemokine receptors act only as signaling molecules to direct the migration of cancer cells to potential metastatic sites. However, experiments have demonstrated that chemokines and chemokine receptors may also aid in the extravasation of cancer cells. Expression of certain chemokine ligands and receptors increases tumor cell adhesion to endothelial cells [Burger et al., 2003; Cardones et al., 2003; Engl et al., 2006]. The chemoattraction produced by chemokines and their receptors may slow cancer cells in circulation to allow adherence to endothelial cells for extravasation. Chemokinetic activity between cancer cells and endothelial cells has also been shown to stimulate the degradation of endothelial basement membrane, thereby allowing transendothelial migration of cancer cells [Bartolome et al., 2004; Lee et al., 2004]. Such observations begin to reveal how cancer cells might utilize the chemokinetic network to modulate host microenvironment for their own progression.

Recent studies suggest that even before cancer cells exit the primary site, chemokines and growth factors might instigate a series of events that will prepare the future metastatic beds for possible tumorigenesis [Kaplan et al., 2005]. Evidence suggests that the secreted factors of a cancer induce migration of bone-marrow-derived cells to pre-metastatic sites and aid in the arrival of tumor cells. Most interestingly, the secreted factors are tumor-specific in determining which organs become prepped pre-metastatically, as the swapping of serum between cancers with different metastatic potentials will change the metastatic pattern to that of the other cancer. However, it is still unclear how the bone-marrow-derived cells specifically migrate to these pre-metastatic sites and the identity of host cells that facilitate creation of appropriate environment for metastasis remains unknown. It seems likely that the factors released by the primary tumor are able to bookmark pre-metastatic sites, which can then be recognized by the bone-marrow-derived cells. One might speculate a role for long-range chemokinetic interaction in this situation to induce susceptibility within the "soil" for establishing metastasis.

VARIATIONS IN THE HOST BACKGROUND ALTER SUSCEPTIBILITY OF THE "SOIL" TO INDUCE METASTASIS

Distinct metastatic patterns of cancer types are partially due to the cell type from which the cancer originates in combination with oncogenic transformations in the cancer cells themselves. The expression profile of the cancer cells determines the components that may create specific interactions with host microenvironment. Additions or subtractions to the genotype-derived composition of compatible systems can alter the metastatic potential of a cancer. However, it should be kept in mind that cancer-host interactions are reciprocative events.

The reciprocal partnership must possess functional counterparts for productive interactions. The composition of corresponding signaling components in the host microenvironment is likely crucial to the metastatic range of a given cancer, as exemplified by the chemokinetic interactions discussed above. While appropriate signaling components are necessary for cancer and host to initiate interactions, other compatibility factors likely play into the sustainability of metastatic spread and growth. Keeping the host environment constant, cancers that possess more aggressive oncogenic properties establish metastases better than cancers with less aggressive oncogenic properties [Qian et al., 1989; Glinsky and Glinsky, 1996; Yang et al., 2004]. This has been demonstrated by experiments in which the transformation of poorly metastatic cell lines with oncogenic components, such as Ras signaling members, ERK/MAPK constituents, adhesion mediators, and various matrix metalloproteinase, increase invasiveness and metastasis [Bernhard et al., 1994; Tsunezuka et al., 1996; Clark et al., 2000; Hazan et al., 2000; Welch et al., 2000; Ala-Aho et al., 2002; Tester et al., 2004]. Alternatively, the host environment can substitute for oncogenic components by becoming more conducive to tumorigenesis. Therefore, the relative compatibility between cancer cells and host microenvironment appears to determine the metastatic susceptibility of the "soil."

Distinct metastatic patterns of various cancers reveal the effect host microenvironment

has on metastatic potential. Therefore, variations in host microenvironment between individuals can also affect the degree of metastasis. Studies have demonstrated the growing importance of an individual's genetic background in expression variability of oncogenic and anti-oncogenic elements and the propensity for metastasis [Yang et al., 2005; Crawford and Hunter, 2006]. In the future, genetic profiles may be able to identify susceptible individuals and prescribe tailored therapies accordingly.

In addition to the genetic component behind host microenvironment composition in metastasis, disease pathologies also contribute to the creation of an environment that is more susceptible to metastatic growth. In particular, disease associated with chronic injury and fibrosis has often been correlated with the advent of cancer and metastasis [Yashiro et al., 1996; Farazi et al., 2006]. The increased proliferation of fibroblasts and their deposition of extracellular matrix proteins is a commonality between inflammatory diseases and cancer. Cancer-associated fibroblasts have been demonstrated to aid the progression of cancer and metastasis [Kalluri and Zeisberg, 2006], while the accumulation of fibroblasts has been observed in the inflammatory response to various diseases [Jordana et al., 1994]. The fibrotic environment induced by pathological conditions may confer increased susceptibility to the formation of tumors due to the potential recruitment of existing fibroblasts to aid the progression of cancer.

Fibroblastic contribution to cancer likely lies in the prominent production of extracellular matrix in this cell type [Kalluri and Zeisberg, 2006]. Interestingly, fibroblasts activated during injury and disease exhibit an increased deposition of extracellular matrix [Castor et al., 1979; Muller and Rodemann, 1991]. Excessive accumulation of extracellular matrix in the liver due to fibrosis induces metastasis of liver carcinoma to the lung [Sawada et al., 2001]. However, the specifics underlying the contribution of a fibrotic environment to metastatic progression remain to be investigated.

THE IMPORTANCE OF EXTRACELLULAR MATRIX IN METASTASIS

Interactions governing cancer progression extends beyond simple cellular interactions. The emergence of fibroblasts as major players in cancer and metastasis highlights the role of extracellular matrix in these processes. Matrix components can be laid down by both cancer cells and various host cells, but fibroblasts are the prominent source of extracellular matrix in the body. Many of the effects fibroblasts have on cancer progression are likely to be mediated by its deposition of extracellular matrix and generation of growth factors. The increased proliferation of cancer-associated fibroblast and the resulting change in matrix composition may have prominent effects on metastasis.

Extracellular matrix composition may determine whether a particular organ site is conducive to metastatic growth [Chung et al., 1988]. For example, the establishment of pre-metastatic niches coincides with an increased deposition of fibronectin [Kaplan et al., 2005], suggesting that matrix composition may be one of the bookmarks recognized by the circulating cancer cells. Experiments have also demonstrated that metastasis is inhibited by the ectopic overexpression of tissue inhibitors of metalloproteinases (TIMPs) at sites of metastasis, demonstrating that changes in the matrix composition can disrupt metastatic potential [Kruger et al., 1997, 1998].

The modulation of extracellular matrix by matrix metalloproteinases and its inhibitors play a large role in cancer progression, as the expression level of specific matrix modulators have been observed to coincide with the metastatic potential of a cancer. However, different cancers appear to have varying dependencies on the palette of matrix modulators available at its disposal. The metastatic potential of transformed rat cell lines was found to correlate with the expression levels of MMP-3 and -10 but not MMP-2 and -9 [Sreenath et al., 1992]. And while expression of MMP-2 did not exhibit significant correlation with metastasis in squamous cell carcinoma, there was a metastatic correlation with MMP9 levels [Hong et al., 2000]. The importance of host genetic background in cancer and metastasis is further supported by the degree of metastasis associated with polymorphisms in the matrix metalloproteinase promoters [Ye, 2000].

In addition to a source of secreted matrix metalloproteinases [Stetler-Stevenson et al., 1993; Sternlicht et al., 1999; Boire et al., 2005], fibroblasts also stimulate the secretion of matrix metalloproteinases from cancer cells. Through the extracellular glycoprotein thrombospondin-1 (TSP-1), fibroblasts can upregulate

the expression of MMP-9 in breast cancer [Wang et al., 2002]. Additionally, fibroblasts may indirectly activate MMP-9 through its deposition of fibronectin via the Mek-1/MAPK and PI3K/Akt pathways in ovarian cancer [Thant et al., 2000].

Tenascin-C also induces expression of MMP-9 [Ilunga et al., 2004]. Tenascin-C is an extracellular adhesion molecule that is produced by both cancer cells and stromal cells [Hanamura et al., 1997; Yoshida et al., 1997; De Wever et al., 2004]. The presence of tenascin-C has been demonstrated to affect various steps associated with cancer, including proliferation, migration, invasion, and angiogenesis [Orend and Chiquet-Ehrismann, 2006]. Its production can be stimulated by various cytokines [Chiquet-Ehrismann and Chiquet, 2003], as well as Ras/MAPK and Wnt signaling pathways [Ruiz et al., 2004]. Tenascin-C is typically absent in normal adult tissues, but expression is markedly increased in pathological conditions, including inflammation and cancer. During injury and disease, tenascin-C functions in various positive feedback loops [Dang et al., 2004; Ruiz et al., 2004], likely aiding in cancer invasiveness. In development, the loss of tenascin-C expression correlates with the acquisition of polarity by the mammary epithelium [Wirl et al., 1995]. Thus, the gain of tenascin-C expression during injury and cancer may correspondingly reflect epithelial dedifferentiation. This parallels well with the effects of matrix modifications by metalloproteinases during cancer progression as described above. Changes in the matrix structure may result in the release of previously confined growth factors and other signaling molecules as well as allow cancer cells to access cryptic adhesion sites to activate signaling pathways for metastasis. Most importantly, the degradation of extracellular matrix may facilitate the migration and invasion of cancer cells by relieving structural tissue barriers.

MESENCHYMAL-TO-EPITHELIAL TRANSITION (MET) AND THE FORMATION OF METASTATIC TUMORS

While host cells including stromal fibroblasts and matrix components may create the necessary environment for establishing metastatic nodules, the cancer cells must also organize themselves for nodule formation upon arrival at the metastatic site. Blood borne migratory capabilities of cancer cells are believed to be achieved through an EMT as discussed previously. And while metastatic transitions produce a mobile cancer with mesenchymal characteristics, secondary nodules formed at distant sites within the body typically resemble the primary tumor phenotype (Fig. 2). It is necessary for cancer cells to reestablish their epithelial identity at the site of metastasis in order to establish metastatic nodules through proliferation [Brabletz et al., 2001].

The migratory phenotype reversal cannot be solely explained by genetic alterations [Hynes, 2003]. The metastasized cancer cells must also change the epigenetic and molecular cues of the microenvironment in which a traveling primary tumor cell finds itself [Bissell et al., 2002; Jechlinger et al., 2002; Thiery, 2002]. During metastasis of primary melanoma, changes in gene expression were observed to be transient, as microarray analysis revealed no significant genomic changes between non-metastasizing melanocytes and metastasizing melanocytes, highlighting the ability of the microenvironment to induce invasiveness of melanocytes independent of genetic mutations [Seftor et al., 2005]. The mechanism by which cancer cells return to a state of differentiated epithelium may resemble a mesenchymal-to-epithelial transition, complementary to the initial EMT (Fig. 2).

MET does occur in various regions of embryonic development and other physiological processes. It is particularly well studied in the development of the kidney. In renal development, nephric tubules are formed via MET, which is governed by its interactions with surrounding stromal cells. It was recently demonstrated that bone morphogenic protein-7 (BMP-7) is capable of inducing MET of renal fibroblasts to facilitate regeneration of the injured kidney and renew the nephric tubules [Zeisberg et al., 2005]. This may be similar to how BMP-7 induces MET of mesenchymal cells to form epithelial tubes during renal development. In addition, hepatocyte growth factor (HGF) expressed by the mesenchymal cells prior to MET also appears to play a role in MET through its interaction with the met receptor on surrounding stromal cells [Woolf et al., 1995]. It has been demonstrated that both bone morphogenetic proteins and HGF play a role in the formation of metastases [Feeley et al.,

2005; Ono et al., 2006], implying the possibility of MET during metastasis.

The most compelling evidence lies in the molecular differences between primary tumors and metastatic tumors, illustrated by the phenotypic conversions of a carcinoma as it mobilizes from the invasive front of the primary tumor to its metastatic destination. Changes in β-catenin localization have been observed in colorectal carcinoma in which β-catenin appears cytoplasmic in the central primary tumor, nuclear at the invasive front, and then cytoplasmic again in the metastatic nodule at the lymph node [Brabletz et al., 2001].

In the cytoplasm, β-catenin provides a structural role in stabilizing E-cadherin junctions, as E-cadherin connects to actin microfilaments indirectly via α- and β-catenin [Kim et al., 2002]. In the nucleus, β-catenin activates the transcription of the DNA binding proteins LEF-1/TCFs [Behrens et al., 1996] to stimulate various signaling pathways required to express mesenchymal markers for a likely EMT [Eger et al., 2000; Stockinger et al., 2001; Kim et al., 2002]. The overexpression of LEF-1 can compete with E-cadherin for the same binding sites on β-catenin, resulting in further depolarization of epithelial cells [von Kries et al., 2000]. In addition, the β-catenin/LEF-1 complex binds to the promoter region of E-cadherin, suggesting a potential role in the downregulation of E-cadherin transcription [Huber et al., 1996]. Thus, when β-catenin relocalizes from the nucleus to the cytoplasm, it can reverse the migratory characteristics of the cell by reestablishing interactions with E-cadherin and producing cell–cell adhesion. The downregulation of beta-catenin signaling via LEF-1/TCFs has been linked to colonic epithelial cell differentiation [Mariadason et al., 2001]. Furthermore, the suppression of β-catenin and LEF-1/TCFs can restore epithelial polarity in colorectal cancer [Naishiro et al., 2001]. Components of the extracellular matrix may play a role in the molecular changes accompanying the epithelial-mesenchymal transitions, as the activation of integrin-linked kinase has been shown to result in the nuclear accumulation of β-catenin and transcriptional repression of E-cadherin [Tan et al., 2001]. Interestingly, a corresponding transient loss of basement membrane has been observed at the invasive front of the primary tumor that is not observed in distant metastases [Spaderna et al., 2006]. This implicates a potential role for the exposure of cryptic matrix adhesion sites or release of embedded signaling molecules via the degradation of basement membrane leading to an activation of integrin-linked kinase and induction of β-catenin-related pathways.

CONCLUDING REMARKS

Here we summarize evidence for mechanisms considered responsible for metastasis. We divide the mechanism into three broad categories: the migration of cancer cell from primary tumor into systemic circulation, localization of cancer cells to a future metastatic site, and finally the establishment of metastatic nodules generating self-perpetuating secondary tumors (Fig. 1). While EMT is a likely mechanism for creating invasive cancers, it is not the sole mechanism responsible for metastasis. There is evidence for metastasis without the incurrence of EMT [Pinkas and Leder, 2002]. In addition, cellular phenotype changes are likely plastic [Tarin et al., 2005; Christiansen and Rajasekaran, 2006].

The changing characteristics of cancer cells during epithelial-mesenchymal transitions are likely associated with aberrant host microenvironment interactions that dictate the course of metastasis (Fig. 2). Evidence continues to accumulate for the fact that cancer cells cannot act alone for the generation of metastasis. Cancer pathology is a parasite dependent on host mechanisms and a premier example of host environment interactions usurped for self-serving purposes. It is now appreciated that cancer is a disease of accumulative mutations. And with recent research illuminating the involvement of stromal cells and matrix microenvironment in cancer progression, a more comprehensive view of how cancer spreads in a specific manner will likely emerge in the near future.

ACKNOWLEDGMENTS

The research work in laboratory of the authors is supported by DK 55001, DK 62987, DK 61688, and AA 13913 and program funds to the Division of Matrix Biology from BIDMC. We would like to thank Daniel J. O'Connell for his helpful comments and for editing the manuscript. We thank Dr. Michael Zeisberg for his help with contribution to the schematic illustrations.

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