Reassessing Epithelial to Mesenchymal Transition as a Prerequisite for Carcinoma Invasion and Metastasis

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Abstract

For most carcinomas, progression toward malignancy is accompanied by loss of epithelial differentiation and a shift towards a mesenchymal phenotype. This process, referred to as epithelial to mesenchymal transition (EMT), exacerbates motility and invasiveness of many cell types and is often considered a prerequisite for tumor infiltration and metastasis. However, there are numerous examples of advanced carcinomas that adopt some mesenchymal features, yet retain characteristics of well-differentiated epithelial cells. We provide a review of these reports and describe mechanisms to explain the morphologic and molecular heterogeneity and plasticity of malignant carcinoma cells, including incomplete EMT, reversion to an epithelial phenotype, and collective migration. We suggest that these mechanisms can manifest in a series of independent and reversible steps and that EMT represents just one mechanism in the global metastatic carcinoma development process. (Cancer Res 2006; 66(17): 8319-26)

Introduction

Carcinoma is by far the most prevalent form of cancer, with >90% of all human malignancies derived from epithelial origin. The thin layers of epithelia that line external surfaces and internal cavities of the body are composed of highly specialized cells with unique morphologic properties. Well-differentiated epithelial cells possess extensive junctional networks that physically separate the plasma membrane into apical and basolateral domains, promote adhesion, and facilitate intercellular communication, thus restricting motility, preserving tissue integrity, and permitting individual cells to function as a cohesive unit (1).

Epithelial and mesenchymal cells represent distinct lineages, each with a unique gene expression profile that imparts attributes specific to each cell type. During the progression of carcinoma, advanced tumor cells frequently exhibit a conspicuous down-regulation of epithelial markers and a loss of intercellular junctions, resulting in a loss of epithelial polarity and reduced intercellular adhesion. The loss of epithelial features is often accompanied by increased cell motility and expression of mesenchymal genes. This process, referred to as epithelial to mesenchymal transition (EMT), can promote hallmark features of carcinoma, including loss of contact inhibition, altered growth control, and enhanced invasiveness (Fig. 1*A*; ref. 2). Molecular and morphologic features indicative of EMT correlate with poor histologic differentiation, destruction of tissue integrity, and metastasis. Therefore, EMT is often presumed to be absolute and indispensable for tumor invasion and metastasis. However, such assumptions discount the remarkable heterogeneity and plasticity inherent among cancer cells. In fact, tumors are a highly diverse population of cells that display a remarkable range of phenotypes. Although some carcinoma cells may undergo a complete transition to a mesenchymal phenotype, many of the defining characteristics of EMT do not present themselves in all invasive or metastatic tumor cells.

In this review, we investigate many of the molecular mechanisms that promote phenotypic changes associated with invasion and metastasis. We also provide evidence of malignant carcinoma cells capable of metastasizing despite the retention of a well-differentiated epithelial morphology. These observations belie the assumption that a complete transition to a mesenchymal phenotype is required for invasion and metastasis of carcinoma cells. Furthermore, we propose potential mechanisms by which metastatic cells retain certain epithelial characteristics.

Epithelial to Mesenchymal Transition

EMT provides mechanisms for epithelial cells to overcome the physical constraints imposed on them by intercellular junctions and adopt a motile phenotype. The process was originally identified during specific stages of embryonic development in which epithelial cells migrate and colonize different embryonic territories during regulated events (3, 4). Many of the molecular mechanisms involved in EMT are now being elucidated; however, an unambiguous definition of this process remains elusive. This is compounded by the relative difficulty in identifying this phenomenon within individual carcinoma cells *in vivo*. Currently, EMT is commonly defined relative to the suppression or appearance of molecular or morphologic end points specific to epithelial or mesenchymal cells, respectively.

Morphologic features and molecular markers of epithelial and mesenchymal cells. E-cadherin is a transmembrane protein localized to the adherens junctions and basolateral plasma membrane. E-cadherin represents the best-characterized molecular marker expressed in epithelial cells. During epithelial morphogenesis, E-cadherin regulates the establishment of the adherens junctions, which form a continuous adhesive belt below the apical surface. Whereas the extracellular domain of E-cadherin mediates calcium-dependent homotypic interactions with E-cadherin molecules on adjacent cells, the intracellular domain binds cytosolic catenins and provides a link to the actin cytoskeleton (Fig. 1*A*; ref. 5). In contrast to well-differentiated epithelial cells, mesenchymal cells do not establish stable intercellular junctions. Dissolution of adherens junctions imparts E-cadherin-negative cell lines with a higher propensity to detach in

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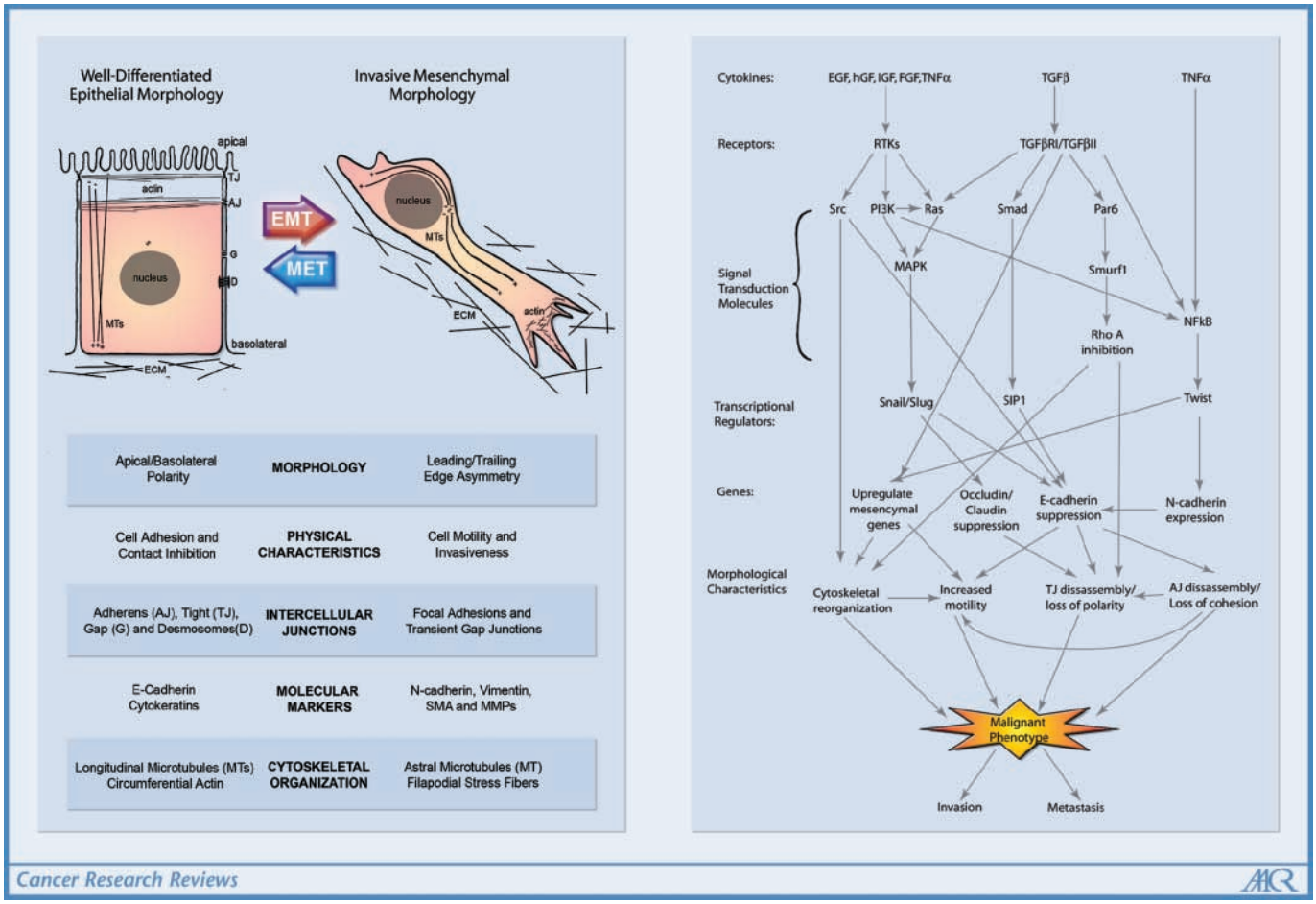

Figure 1. Morphologic, phenotypic, and molecular differences between well-differentiated epithelial cells and highly invasive carcinoma cells and the signaling pathways involved in mediating the transition between the two types of cells. *Left panel,* well-differentiated epithelial cells posses a highly polarized morphology with distinct apical and basolateral plasma membrane domains. Epithelial cells form extensive junctional complexes, express various molecular markers, and have a unique cytoskeletal organization. During the process of EMT, well-differentiated epithelial cells acquire an invasive mesenchymal morphology. Mesenchymal cells do not form extensive intercellular junctions. These cells possess different cytoskeletal arrangements and express a different profile of genes, relative to epithelial cells. Mesenchymal cells can revert to an epithelial phenotype by a process of mesenchymal to epithelial transition (*MET*). *Right panel,* the transition from a well-differentiated epithelial cell to a highly invasive carcinoma cell involves diverse signal transduction cascades. These signaling pathways are activated in response to various cytokines and involve a number of downstream effector molecules. These pathways converge to initiate genetic and epigenetic changes that promote cell motility, invasiveness, and metastasis.

response to low shear forces analogous to those encountered within lymphatic vessels and venules (6). The observed decrease in adhesive force presumably facilitates dispersion of carcinoma cells from the primary tumor mass. In addition to promoting passive dissemination of carcinoma cells, loss of E-cadherin function can also promote cell invasiveness. Methods to abolish E-cadherin function promote epithelial cell invasion into a variety of substrates, as determined in numerous *in vitro* and *in vivo* experimental systems (7–9).

The tight junctions are situated just above the adherens junctions at the apical side of the lateral membrane in epithelial cells. Transmembrane proteins, including claudins, occludins, and junctional adhesion molecules, form a network of interconnected strands that seal intercellular spaces and form permeability barriers, which prevent the flow of molecules across the epithelial layer and restrict the lateral diffusion of the apical and basolateral plasma membranes (10, 11). These functions are critical for plasma membrane polarity and greatly influence the environment to which a particular cell surface is exposed (12). In the absence of tight junctions, mesenchymal cells lack an apical-basolateral polarity. Instead, these cells possess an elongated morphology with front-back asymmetry that facilitates motility and locomotion (Fig. 1*A*). Filapodial extensions at the leading edge of the mesenchymal cells are enriched with integrin family receptors that interact with the extracellular matrix (13) and also contain matrix metalloproteinases (MMP) that digest basement membranes and promote invasion (14).

In addition to the dissolution of junctions and the down-regulation of epithelial proteins, progression to a malignant phenotype is also accompanied by increased expression of mesenchymal proteins, such as the intermediate filament protein vimentin. Expression of vimentin is observed in mesodermal cells during specific stages of embryonic development (15) and is associated with a highly invasive cellular phenotype (16). In addition to vimentin, other cytoskeletal proteins are up-regulated in mesenchymal cells, including smooth muscle actin, γ-actin, β-filamin, and talin, as are extracellular matrix components such as fibronectin and collagen precursors (17). Up-regulation of these proteins can facilitate pseudopod formation and cytoskeletal remodeling. Other proteins up-regulated during EMT, including

Src kinase, integrin-linked kinase, integrin β-5, and MMP-11, MMP-12, and MMP-14, induce cytoskeletal remodeling and promote cell motility (17).

Molecular mechanism of EMT. The diverse molecular mechanisms that contribute to EMT (Fig. 1*B*) have been the subject of many reports and exhaustive reviews (18, 19). Many of these mechanisms involve growth factors that promote various signaling cascades through their cognate receptor tyrosine kinases (2, 20). Downstream kinases, such as Ras, Src, phosphatidylinositol 3-kinase, and mitogen-activated protein kinase (MAPK), have all been implicated in promoting a malignant phenotype (21–23). Activation of Src can increase invasiveness (24) and promote the degradation of E-cadherin (25, 26), whereas downstream targets of Ras and MAPK include Snail and the related protein Slug (23). These multiple zinc finger–containing proteins inhibit expression of genes with conserved E-boxes in their promoter regions, including E-cadherin (27, 28), tight junction proteins such as occludin (29) and claudin (30), as well as important regulators of tight junction assembly such as Na,K-ATPase β-subunit (31). Snail and Slug also directly inhibit transcription of the epithelial markers such as cytokeratin-8 and cytokeratin-19 (32), desmoplakin, and mucin-1 (33), as well as induce RhoB, a small GTPase-associated with increased motility (34).

Signaling through transforming growth factor β (TGF-β) offers another potent mechanism to promote the phenotypic changes of EMT. Activation of TGF-β receptors results in signal propagation through various pathways implicated in EMT (35), including Ras-dependent (36) and Smad-dependent mechanisms. For the Smad-dependent pathway, TGF-β signaling promotes translocation of a Smad complex into the nucleus, where it activates the transcriptional corepressor SIP-1 (37). Like Snail and Slug, SIP-1 is a zinc finger–containing protein that binds consensus E-box sequences (38). Furthermore, TGF-β1 control elements have been identified within the promoter regions of mesenchymal proteins, such as α-smooth muscle actin, which is induced by TGF-β signaling (39, 40). A novel Smad-independent pathway has also recently been implicated in TGF-β-mediated transition to a malignant phenotype. TGF-β binding initiates Par6 phosphorylation and activation of the E3-ubiquitin ligase Smurf-1. Activated Smurf-1 promotes degradation of RhoA (41), resulting in tight junction dissociation, inhibition of cell adhesion, and F-actin polymerization (42, 43).

Nuclear factor κB (NF-κB) has also been implicated in EMT by promoting expression of the basic-helix-loop-helix transcription factor Twist (44). Like Snail, Slug, and SIP-1, Twist also binds to E-box sequences and down-regulates E-cadherin (45). However, the mechanism by which Twist promotes EMT is poorly understood, as Twist is believed to function as a transcriptional activator when bound to E-box sequences (46). Twist may promote the mesenchymal characteristics by activating N-cadherin (45), which down-regulates E-cadherin and induces mesenchymal morphology in mammary tumor cell lines (47). In addition, activation of NF-κB may promote expression of mesenchymal proteins independently of Twist, as NF-κB binds regulatory sequences within the promoter of vimentin (48).

Contradictions to Classic EMT as a Universal Feature in Tumor Invasion and Metastasis

By adopting a mesenchymal phenotype, individual carcinoma cells can infiltrate adjacent tissues, cross endothelial barriers, and enter the circulation through blood and lymphatic vessels. Although EMT represents a fundamentally important process conducive to tumor dissemination and metastatic spread, there are several lines of evidence to suggest that many invasive and metastatic carcinomas have not undergone a complete transition to a mesenchymal phenotype or even lack signs of EMT. Many advanced carcinomas possess molecular and morphologic characteristics indicative of well-differentiated epithelia, including high levels of E-cadherin expression, the presence of epithelial junctions, and apical-basolateral plasma membrane asymmetry.

Well-differentiated epithelial morphology in invasive and metastatic carcinomas. There are several reported examples of carcinoma cells within primary and metastatic lesions with well-differentiated epithelial morphology. Histologic examination of pelvic lymph nodes removed from patients having undergone radical prostatectomy for what was presumed to be organ-confined prostate cancer identified abundant low-volume metastatic lesions. In most cases, these early secondary tumors maintained a glandular appearance indicative of a well-differentiated epithelial morphology (49).

These observations are consistent with identification of regions of well-differentiated epithelial morphology within prostate cancer lymph node metastases. Areas within infiltrating tumors contained distinct glandular/acinar structures with identifiable lumenal spaces. The surrounding tumor cells displayed morphologic characteristics reminiscent of those observed in benign prostatic tissues and well-differentiated adenocarcinoma. Immunohistochemical analysis revealed that the plasma membrane marker, prostate-specific membrane antigen, was similarly restricted to the apical surface in both well-differentiated tumor cells and benign polarized epithelial cells, thus confirming the presence of epithelial junctions and plasma membrane asymmetry. These results indicate that prostate cancer cells can possess a well-differentiated morphology, even within secondary metastatic lesions (50).

Additional studies have also identified well-differentiated epithelial morphology in other invasive tumors. Analysis of primary and metastatic tissue samples taken from patients suffering from mammary ductal carcinoma showed that many tissue sections contained tumor micropapillae that resembled small acinar structures with a central lumenal space. These structures contained tight junctions, adherens junctions, and desmosomes that were abundantly evident by electron microscopy (51). Tan et al. (52) also found epithelial polarity among invasive tumors of the breast. Tumor tissue specimens derived from patients suffering grade 1 invasive ductal carcinoma were evaluated for histologic features indicative of epithelial polarity. The majority of tumor samples from the 149 patients examined possessed extensive tubule formation, and most cells displayed morphologic characteristics of apical-basolateral polarity. Furthermore, the presence of epithelial polarity in invasive carcinoma cells failed to prognosticate presence of metastatic lesions or overall patient outcome (52). These results suggest that loss of epithelial morphology is not required for invasion and metastasis of carcinoma.

Well-differentiated cells with intact tight junctions have also been reported in epithelial tissue formations devoid of a central lumenal space, including squamous cell carcinomas derived from a variety of origins including the esophagus, oral epithelium, lung, and cervix. Tumor tissue specimens representing a range of pathologic grades were subject to immunohistochemical analysis. Regions of cell-cell contact in both primary and metastatic tumor

samples exhibited reactivity for tight junction proteins, such as occludin, claudins, ZO-1, and cingulin (53). Highly polarized cells within invasive carcinoma and secondary tumors have also been observed in salivary neoplasms. These tumors exhibited a diverse range of cellular architecture that failed to correlate with disease stage (54).

Expression of epithelial markers in metastatic carcinoma. Down-regulation of E-cadherin is a common feature in many forms of carcinoma and can often predict invasiveness and metastatic potential (55–57). Although an inverse correlation between E-cadherin and invasiveness has been firmly established for many forms of carcinoma, abundant examples exist that are inconsistent or contrary to this general assumption.

Whereas normal Madin-Darby canine kidney (MDCK) cells are nontumorigenic in nude mice, infection with the Harvey murine sarcoma virus causes these cells to become invasive and metastatic. Although infection initially results in down-regulation of E-cadherin, populations of cells were identified that had reverted to express abundant E-cadherin. Despite the expression of E-cadherin, these revertant cells were still invasive and metastatic in nude mice, and gave rise to primary and metastatic tumors that exhibited both E-cadherin immunoreactivity and morphologic characteristics indicative of well-differentiated epithelial tissue (58).

The sustained synthesis of key epithelial markers has also been shown in invasive colon cancer cells. Despite the progression of an invasive phenotype, various molecular components of the adherens junctions and desmosomes were readily detected. Even within metastatic lesions, E-cadherin continued to complex with catenins and desmosmal structures continued to form (59).

In situ analysis of carcinoma also shows an imperfect relationship between E-cadherin expression and invasiveness. Results from one comprehensive immunohistochemical study revealed minimal correlation between E-cadherin expression and cell polarity or glandular organization among salivary gland carcinomas. E-cadherin expression was strong and uniformly present both in benign salivary epithelial tissues and in the vast majority of cells in all tumors across the malignant spectrum (54). Results from another study recapitulate these findings among 413 cases of gastric carcinoma. Although the histologic type and the level of epithelial morphology exhibited by the primary tumor sections were both associated with E-cadherin expression, no correlation was observed between E-cadherin levels and depth of invasion, the presence of lymph node metastasis, or vascular invasion (60). These observations indicate that expansion of carcinoma cells into surrounding tissues is not prevented by the presence of E-cadherin.

Similar observations are also described in mammary carcinomas. Expression of three epithelial markers, E-cadherin, α-catenin, and β-catenin, were observed in the majority of invasive breast carcinomas examined (61). Expression levels of these markers were comparable to those in benign tissues in almost all cases of organ-confined intraductal breast carcinoma and in ~70% of invasive ductal carcinoma. These invasive tumors were further classified as having either a scattered or solid morphology. Results showed that there was no correlation between tumor morphology and E-cadherin, with 67% (10 of 15) and 69% (18 of 26) of scattered and solid tumors expressing normal levels of E-cadherin, respectively. Furthermore, metastatic status was not correlated with expression of E-cadherin, α-catenin, or β-catenin, as node-negative and node-positive tumors showed similar expression of these three proteins. E-cadherin was preserved in 55% (21 of 38) of ductal breast carcinomas negative for lymph node metastasis and in 63% (10 of 16) of lymph node–positive tumors (61). These results indicate that detachment from the primary tumor site is not prevented by adhesion between carcinoma cells.

Other investigations have also failed to correlate E-cadherin expression with increasing malignancy in breast carcinoma. An investigation of 208 breast cancer biopsies revealed that the majority of carcinoma cells expressed E-cadherin. The status of E-cadherin expression was not correlated with either nodal status or the presence of metastasis at the time of diagnosis (62). Additional investigations have recapitulated these findings and showed that E-cadherin status correlated poorly with disease recurrence, distant metastases, vascular invasion, or other prognostic factors (63–65). Taken together, these observations suggest that a complete transition to a mesenchymal phenotype is not required for invasion and metastasis.

Reconciling the Paradox of Well-Differentiated Malignant Carcinoma

The presence of well-differentiated epithelial characteristics within invasive and metastatic carcinoma occurs with unexpected frequency given the presumed role of EMT in cancer progression. There are several potential explanations to resolve these seemingly contradictory observations. For example, malignant carcinoma cells may initiate a partial transition to a mesenchymal phenotype, revert from a mesenchymal to an epithelial phenotype at sites of distal metastasis, or use alternative modes of infiltration and metastasis, such as collective migration (Fig. 2). Here we examine these processes in detail and investigate potential molecular mechanisms that may contribute to the phenotypic changes in cancer cells.

Incomplete EMT. The process of EMT involves diverse signal transduction cascades that contribute to a mesenchymal phenotype. However, it is important to consider that the initiation of signal transduction cascades can manifest in disparate outcomes in different cell types. For example, exposure to TGF-β was recently shown to exact disparate effects among a host of 20 different human and murine cell lines. Whereas a few cell lines underwent some phenotypic and morphologic alterations associated with EMT, including formation of stress fibers at the cell periphery, acquisition of an elongated morphology, or loss of junctional complexes, the majority of cell lines did not adopt any mesenchymal properties in response to prolonged TGF-β exposure. Only 2 of 20 cell lines underwent a complete transition to a mesenchymal phenotype, as defined by the loss of E-cadherin, dissolution of tight junctions, and adoption of an elongated spindle-shaped morphology (66).

The disparate responses to identical stimuli underscore the molecular differences between cell types and insinuate that transition to an aggressive malignant phenotype is not an "all or nothing" event, but rather manifests in phenotypic changes over a broad spectrum, from purely epithelial to purely mesenchymal. Differences in cellular responses could be ascribed to partial inactivation or blockade of a particular signal transduction cascade. For example, inactivating mutations in Smad proteins have been identified in lung and colorectal carcinomas, where they are presumed to mitigate the growth inhibitory effects of TGF-β signaling (67, 68). The inactivation of Smad-dependent TGF-β signaling would also affect some aspects of EMT, such as

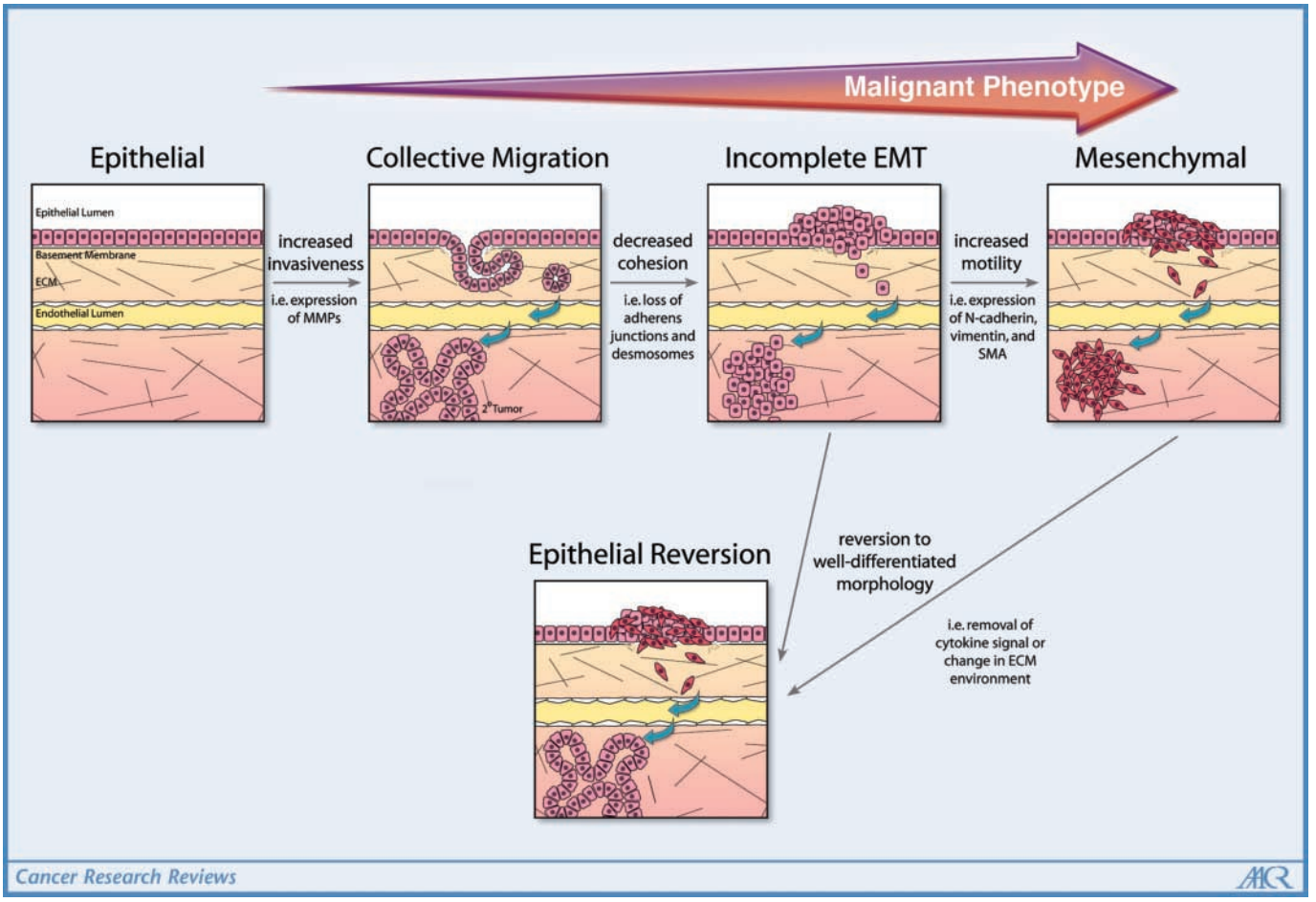

Figure 2. Epithelial to malignant transition encompasses a wide range of metastatic phenotypes. During the progression of invasive and metastatic carcinoma, normal epithelial cells can adopt increased invasiveness yet retain well-differentiated morphology and cohesiveness. These cells can invade surrounding tissue and metastasize by collective migration. Loss of intercellular cohesion via incomplete EMT would increase metastatic potential, as would a full conversion to a mesenchymal phenotype. Following invasion or distal metastasis, cells that have undergone progressive steps of epithelial to malignant transition can also revert to a well-differentiated epithelial phenotype.

SIP-1-mediated down-regulation of E-cadherin, yet allow other Smad-independent molecular changes to occur. Such a phenomenon has been described in skin carcinomas, which frequently exhibit decreased TGF-β receptor II expression. Expression of a dominant negative form of TGF-β receptor II within the epidermal cells of a transgenic mouse model abrogated Smad-dependent signaling in response to TGF-β. Epidermal cells retained expression of E-cadherin, which was complexed with catenins at the plasma membrane. In spite of E-cadherin expression and EMT abrogation, these cells achieved an invasive and metastatic phenotype associated with TGF-β-dependent modulation of RhoA and activation of MAPK (69).

Furthermore, molecular alterations that result in constitutive activation of downstream effectors in a signal transduction cascade may promote some properties of EMT without eliciting a complete transition to a mesenchymal phenotype. Activation of downstream MAPK signaling can enhance the motility and invasiveness of the EpH4 cell line. These cells are polarized and nontumorigenic with many morphologic and physiologic characteristics of normal mammary epithelial cells. However, EpH4 cells expressing constitutively activated MEK1 produced highly invasive and vascularized tumors that frequently metastasized when implanted into mice. These changes involved expression of proliferation genes and MMPs, which are up-regulated in several solid tumors. Whereas these cells exhibited many changes associated with EMT, such as remodeling of the actin cytoskeleton, increased motility, and redistribution of β-catenin and ZO-1 away from sites of cell-cell contact, there was no reduction in the levels of E-cadherin or keratin-18 expression, nor was there any induction of mesenchymal markers, such as smooth muscle actin or vimentin (70).

Virally encoded oncogenes such as v-Src and v-Ras may also activate signal transduction pathways that elicit phenotypic changes associated with partial EMT. Expression of these oncogenes in MDCK cells resulted in some mesenchymal properties (71). Interestingly, expression of v-Src in a colorectal carcinoma–derived cell line resulted in anchorage-independent growth and increased motility and invasiveness; however, these cells required activation of a second signaling pathway to obtain a complete mesenchymal phenotype. Incubation with tumor necrosis factor α exacerbated the motility and invasiveness of these cells and resulted in cell scattering and the down-regulation of E-cadherin (72).

RhoA is another potential target that could promote some mesenchymal characteristics. The temporal and spatial regulation of RhoA can mediate actin cytoskeleton organization, filapodia formation, and cell polarity (43, 73). Aberrant regulation of RhoA

can promote cell invasiveness and the loss of epithelial polarity; however, in the absence of other EMT-associated signaling events, affected cells still retain epithelial markers and do not express mesenchymal markers (74).

Reversion to an epithelial morphology. After migrating to new embryonic territories, mesenchymal cells can regain epithelial morphology by a phenomenon known as mesenchymal to epithelial transition. Following the colonization of distal sites, metastatic tumor cells may initiate mesenchymal to epithelial transition and reestablish E-cadherin expression and epithelial junctions (75).

The transition from a mesenchymal to an epithelial morphology may be ascribed to changes in the extracellular environment, such as removal of a cytokine signal or interactions with extracellular matrix. PC3 cells are a highly invasive cell line derived from metastatic prostate adenocarinoma. Consistent with cells that have undergone EMT, PC3 cells do not express E-cadherin and express high levels of vimentin. PC3 cells do not form appreciable epithelial junctions and are nonpolarized when cultured in planar dishes. However, when PC3 cells were grown in a three-dimensional matrigel culture, they formed hollow acinar spheroids with abundant cell-cell contacts and tight junctions evident by electron microscopy. These spheroids were polarized, with microvilli and secretory vesicles at the apical surface. These morphologic changes were accompanied by expression of prostate-specific markers and a decrease in vimentin, implicating the ability of microenvironmenal factors to dictate cell phenotype and function (76).

Promoter hypermethylation is an efficient mechanism to suppress the expression of genes. Hypermethylation of the E-cadherin promoter was shown to occur frequently in mammary carcinoma cell lines and was associated with decreased E-cadherin levels and fibroblastic morphology (77). Therefore, promoter hypermethylation and demethylation may also represent a reversible mechanism to preserve the well-differentiated phenotype of metastatic carcinoma cells. Although reversion to an epithelial phenotype following metastatic spread may technically support a role for EMT in carcinoma progression, mesenchymal to epithelial transition represents an important concept that may increase understanding of cell morphology in cancer.

Collective migration. Although increased motility is an important factor for invasion and metastasis, malignant cells need not disseminate from primary tumors solely as individual mesenchymal-like cells. Invasive carcinoma may also invade surrounding tissues as multicellular aggregates or clusters in a process known as "collective" or "cohort" migration (78). Collective migration has been described in detail and occurs within the framework of normal development (79, 80). Using a three-dimensional collagen matrix and time lapse video microscopy, Friedl et al. (81) showed that clusters of cells derived from a variety of tumors could detach from the site of the primary tumor and migrate as independent aggregates within the adjacent extracellular matrix. This type of migration has also been observed among colorectal and breast tumor cells, which migrated as protruding sheets and tubules connected to the primary tumor (82, 83).

The significance of collective migration has also been shown using murine xenograft models. Whereas MDCK cells fail to form palpable tumors when injected into adult nude mice, ectopic expression of membrane-type-1 MMP was sufficient to induce active tumor invasion and infiltration into surrounding muscular tissue. These tumor xenografts formed extensive, organized tubular structures reminiscent of well-differentiated glandular epithelial tissue. These structures had a clearly defined lumenal space surrounded by a single layer of cells with obvious polarity, as evidenced by restricted localization of Na,K-ATPase at the basolateral surface. Furthermore, MDCK cell aggregates were able to enter lymphatic and blood vessels, which is consistent with observations that clusters of metastatic cells from a variety of tumors can be detected in the circulation (84).

Perspectives

The transition from a well-differentiated epithelial phenotype to an invasive mesenchymal phenotype may involve diverse molecular mechanisms that may independently enhance motility and invasiveness without inducing a complete conversion of cellular identity. Thus, metastatic tumor cells are likely to exhibit a wide range of phenotypes by adopting some properties of mesenchymal cells while retaining other epithelial characteristics (Fig. 2). This is consistent with several reports that show well-differentiated epithelial cells within metastatic lesions and those that fail to correlate expression of epithelial or mesenchymal molecular markers with invasive and metastatic potential. In fact, a recently published review questions the role of EMT in malignant carcinoma, citing a lack of evidence of this phenomenon *in vivo*. This review further suggests that carcinoma cells do not need to undergo a dramatic conversion in cell identity to achieve all the morphologic and phenotypic changes necessary for metastasis (85).

Phenotypic differences among carcinoma cells could have strong implications for tumor behavior during metastasis. Carcinoma cells could potentially metastasize either as individual "mesenchymal" cells or as a multicellular aggregation of cells with a more epitheliod morphology. Whereas mesenchymal cells exhibit a highly motile phenotype and can readily traverse basement membranes, interstitial spaces, and endothelial barriers, the strong cohesive forces associated with multicellular aggregates could offer potential advantages for tumor survival. Collective migration could allow specialization and synergy among cells. For example, motile cells with a high propensity for invasion could work in concert with rapidly dividing or apoptosis-resistant cells to achieve a highly malignant secondary tumor (78). Furthermore, cells located at the interior of a multicellular aggregate would be buffered from the external environment and thus protected from immunologic attack and high shearing forces within the vasculature. Multicellular tumor aggregates may also facilitate establisment of micormetastatic lesions. Although conventional models of metastasis typically envision a fundamental role for extravasation of individual tumor cells at the site of metastasis, such events were reported to be a rare phenomenon. Instead, metastatic cells attached to vessel walls of arterioles and capillaries, where they proceeded to multiply within the vasculature (86). Likewise, aggregates of cohesive cells would presumably get trapped within the narrow microvascular lumen, where subsequent proliferation would eventually rupture capillary walls.

Epithelial junctions in well-differentiated metastatic carcinomas can form physical barriers that restrict access of drugs or antibodies to the sites of tumors (12). The epithelial junctions can significantly limit the perfusion of these agents to the outermost layers of multicellular aggregates, thus severely diminishing the efficacy of such therapeutic modalities (87). Additionally, the presence of intact tight junctions in secondary tumors may have a significant negative effect on therapy. Although

the basolateral surface of polarized epithelial cells would be readily accessible to the underlying vasculature, antigens on the apical plasma membrane would be inaccessible to i.v. administered agents due to the gate function of the tight junctions (50). Malignant cells with a well-differentiated morphology would be relatively resistant to targeted therapies directed against apical antigens, such as monoclonal antibodies against carcinoembryonic antigen or prostate-specific membrane antigen (50, 88, 89).

Although metastasis is the most important event leading to cancer death, this process is among the most poorly understood. This unfortunate lapse in conceptual understanding is partially due to difficulties inherent to direct observation of this phenomenon. Clearly, techniques to facilitate such real-time *in vivo* observations will greatly enhance the understanding of metastasis and answer many nagging questions about the role of EMT in this process. Technologies such as intravital microscopy may represent a powerful tool to study fluorescently labeled proteins within individual tumor cells in animal models (90).

The term EMT insinuates that carcinoma cells invariably adopt a mesenchymal phenotype to invade surrounding tissues and metastasize. However, compelling evidence suggests that carcinoma cells do not necessarily require dramatic changes in cell identity to achieve a metastatic or invasive phenotype. Carcinoma cells may invade or metastasize without losing epithelial morphology or molecular markers, and without inducing expression of mesenchymal genes. Thus, the broad use of the term EMT may not always be appropriate for describing the diverse processes associated with invasion and metastasis. Rather, EMT may represent just one, albeit important, potential mechanism that can contribute to advancing malignancy in carcinoma. A more comprehensive appreciation for the heterogeneity and plasticity inherent to carcinoma cells would emphasize the need to assess the differentiation status of tumor lesions to predict pathologic course and to devise ways to accommodate therapeutic strategies accordingly.

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