

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

Jason J. Christiansen¹ and Ayyappan K. Rajasekaran²

¹Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, and ²Molecular Biology Institute and Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles, California

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. 1A; 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. 1A; 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

Requests for reprints: Ayyappan K. Rajasekaran, Department of Pathology and Laboratory Medicine, Room 13-344 CHS, University of California, Los Angeles, Los Angeles, CA 90095. Phone: 310-825-1199; Fax: 310-267-2410; E-mail: arajasekaran@mednet.ucla.edu.

©2006 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-06-0410

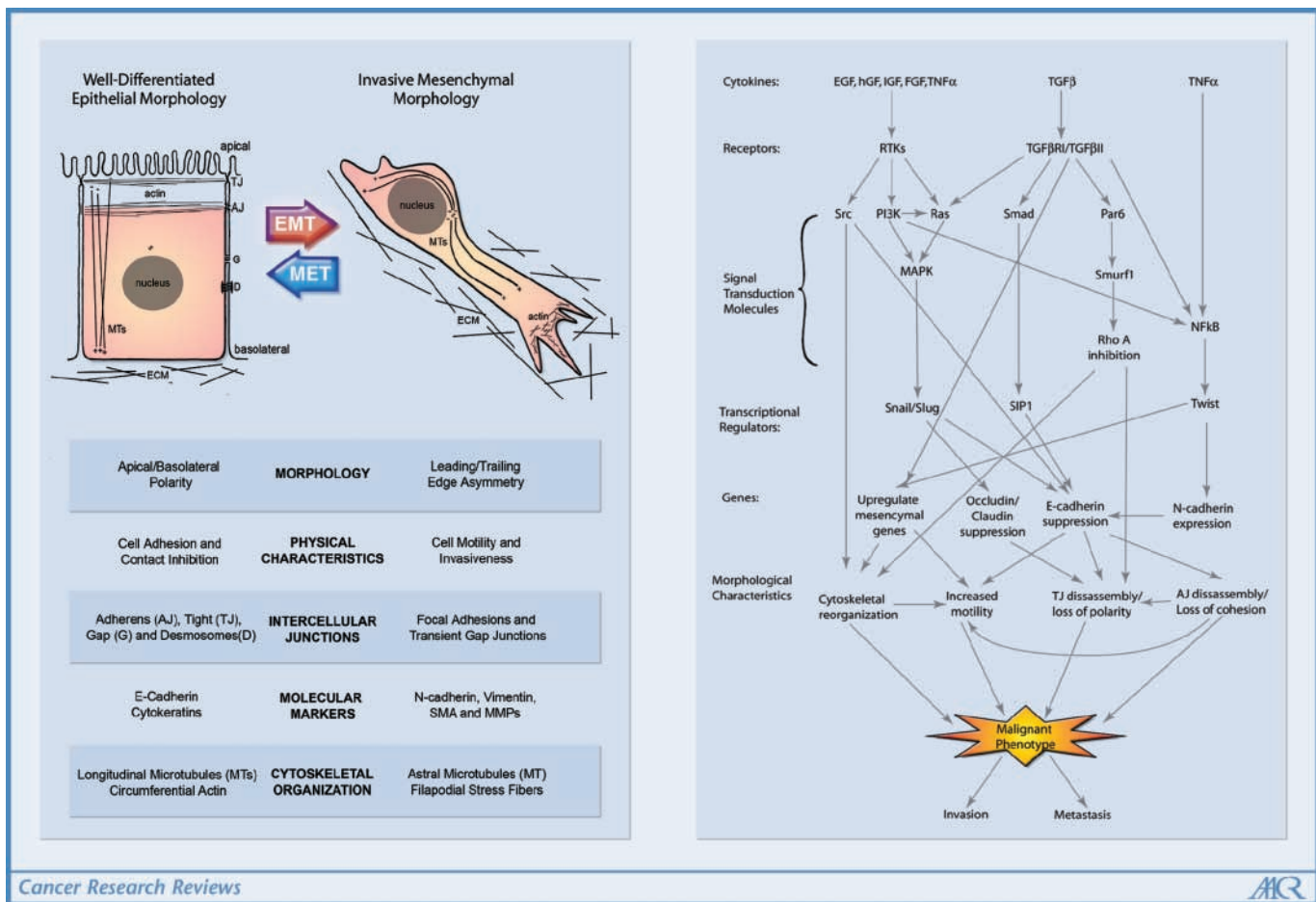


Figure 1. Morphologic, phenotypic, and molecular differences between well-differentiated epithelial cells and highly invasive carcinoma cells and the signaling pathways involved in mediating the transition between the two types of cells. *Left panel*, well-differentiated epithelial cells possess a highly 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. 1A). Filopodial 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. 1B) 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- β 1 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 κ B (NF- κ B) 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- κ B may promote expression of mesenchymal proteins independently of Twist, as NF- κ B 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 luminal 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 luminal 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 luminal 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 desmosomal 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

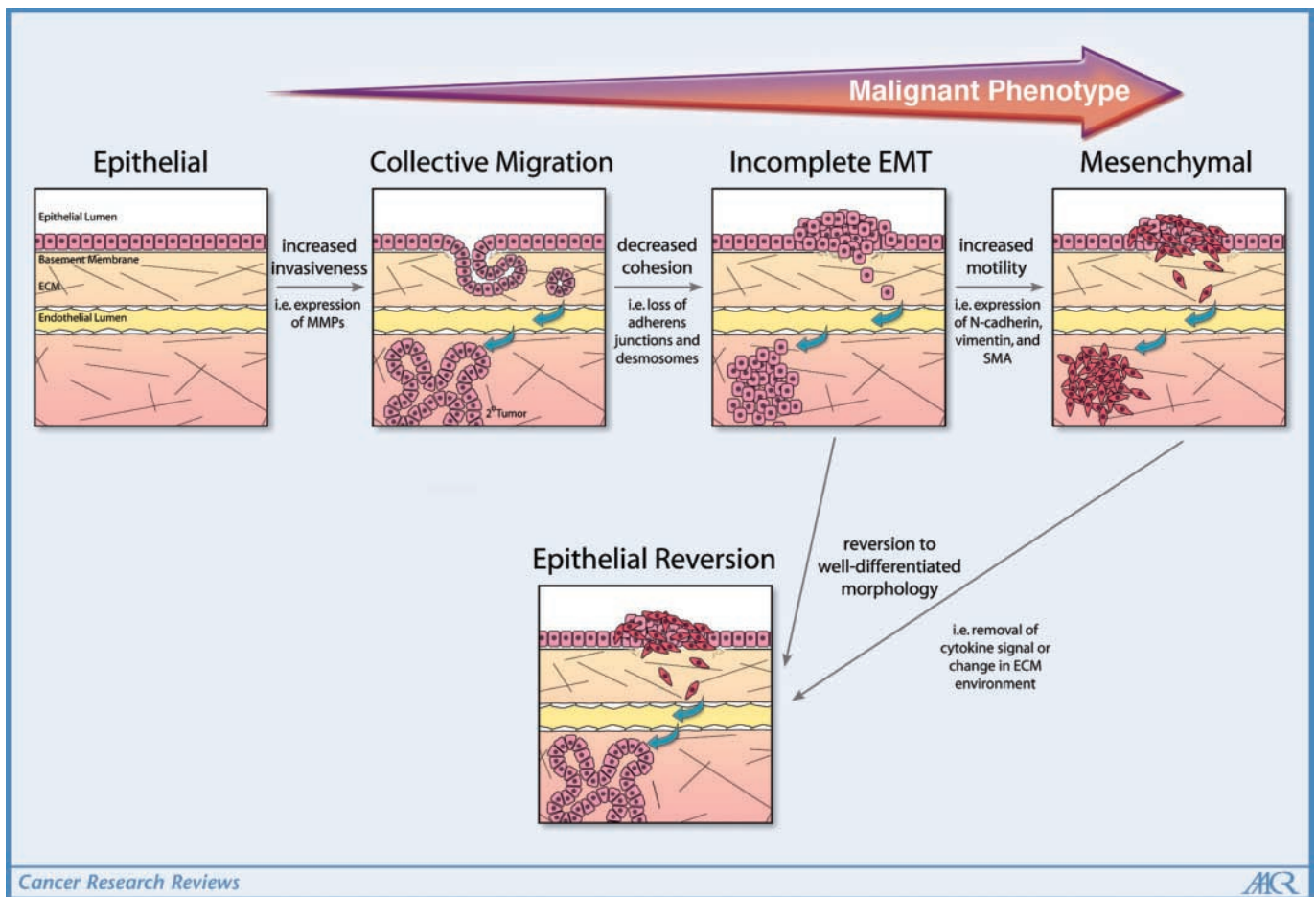


Figure 2. Epithelial to malignant transition encompasses a wide range of metastatic phenotypes. During the progression of invasive and metastatic carcinoma, normal epithelial cells can adopt increased invasiveness yet retain well-differentiated morphology and cohesiveness. These cells can invade surrounding 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, filopodia 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 adenocarcinoma. 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 microenvironmental 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 luminal 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 epithelial 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 establishment of micrometastatic 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 carcino-

ma 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.

Acknowledgments

Received 2/1/2006; revised 5/9/2006; accepted 5/26/2006.

Grant support: NIH grants DK 56216, W81XWH-04-1-0132, and W81XWH-04-1-0113, and National Research Service Award Training Grant NIH-NCI-T32CA09056-30 (J.J. Christiansen).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

- Farquhar MG, Palade GE. Junctional complexes in various epithelia. *J Cell Biol* 1963;17:375-412.
- Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol* 2003;15:740-6.
- Burdsal CA, Damsky CH, Pedersen RA. The role of E-cadherin and integrins in mesoderm differentiation and migration at the mammalian primitive streak. *Development* 1993;118:829-44.
- Nieto MA. The early steps of neural crest development. *Mech Dev* 2001;105:27-35.
- Kemler R, Ozawa M. Uvomorulin-catenin complex: cytoplasmic anchorage of a Ca²⁺-dependent cell adhesion molecule. *BioEssays* 1989;11:88-91.
- Byers SW, Sommers CL, Hoxter B, et al. Role of E-cadherin in the response of tumor cell aggregates to lymphatic, venous and arterial flow: measurement of cell-cell adhesion strength. *J Cell Sci* 1995;108:2053-64.
- Behrens J, Mareel MM, Van Roy FM, et al. Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *J Cell Biol* 1989;108:2435-47.
- Le PU, Nguyen TN, Drolet-Savoie P, et al. Increased β -actin expression in an invasive Moloney sarcoma virus-transformed MDCK cell variant concentrates to the tips of multiple pseudopodia. *Cancer Res* 1998;58:1631-5.
- Zheng ZH, Sun XJ, Zhou HT, et al. Analysis of metastasis suppressing function of E-cadherin in gastric cancer cells by RNAi. *World J Gastroenterol* 2005;11:2000-3.
- Anderson JM, Van Itallie CM. Tight junctions and the molecular basis for regulation of paracellular permeability. *Am J Physiol* 1995;269:G467-75.
- van Meer G, Simons K. The function of tight junctions in maintaining differences in lipid composition between the apical and the basolateral cell surface domains of MDCK cells. *EMBO J* 1986;5:1455-64.
- Christiansen J, Rajasekaran AK. Biological impediments to monoclonal antibody-based cancer immunotherapy. *Mol Cancer Ther* 2004;3:1493-501.
- Li S, Guan JL, Chien S. Biochemistry and biomechanics of cell motility. *Annu Rev Biomed Eng* 2005;7:105-50.
- Seiki M. Membrane-type 1 matrix metalloproteinase: a key enzyme for tumor invasion. *Cancer Lett* 2003;194:1-11.
- Duprey P, Paulin D. What can be learned from intermediate filament gene regulation in the mouse embryo. *Int J Dev Biol* 1995;39:443-57.
- Lang SH, Hyde C, Reid IN, et al. Enhanced expression of vimentin in motile prostate cell lines and in poorly differentiated and metastatic prostate carcinoma. *Prostate* 2002;52:253-63.
- Lagamba D, Nawshad A, Hay ED. Microarray analysis of gene expression during epithelial-mesenchymal transformation. *Dev Dyn* 2005;234:132-42.
- Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* 2006;7:131-42.
- Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr Opin Cell Biol* 2005;17:548-58.
- Weidner KM, Arakaki N, Hartmann G, et al. Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc Natl Acad Sci U S A* 1991;88:7001-5.
- Boyer B, Roche S, Denoyelle M, et al. Src and Ras are involved in separate pathways in epithelial cell scattering. *EMBO J* 1997;16:5904-13.
- Liu H, Radisky DC, Bissell MJ. Proliferation and polarity in breast cancer: untying the gordian knot. *Cell Cycle* 2005;4:646-9.
- Peinado H, Quintanilla M, Cano A. Transforming growth factor β -1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions. *J Biol Chem* 2003;278:21113-23.
- Behrens J, Vakaet L, Friis R, et al. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/ β -catenin complex in cells transformed with a temperature-sensitive v-SRC gene. *J Cell Biol* 1993;120:757-66.
- Warren SL, Nelson WJ. Nonmitogenic morphoregulatory action of pp60v-src on multicellular epithelial structures. *Mol Cell Biol* 1987;7:1326-37.
- Palacios F, Tushir JS, Fujita Y, et al. Lysosomal targeting of E-cadherin: a unique mechanism for the down-regulation of cell-cell adhesion during epithelial to mesenchymal transitions. *Mol Cell Biol* 2005;25:389-402.
- Leptin M. twist and snail as positive and negative regulators during *Drosophila* mesoderm development. *Genes Dev* 1991;5:1568-76.
- Nieto MA, Sargent MG, Wilkinson DG, et al. Control of cell behavior during vertebrate development by Slug, a zinc finger gene. *Science* 1994;264:835-9.
- Ohkubo T, Ozawa M. The transcription factor Snail down-regulates the tight junction components independently of E-cadherin down-regulation. *J Cell Sci* 2004;117:1675-85.
- Ikenouchi J, Matsuda M, Furuse M, et al. Regulation of tight junctions during the epithelium-mesenchyme transition: direct repression of the gene expression of claudins/occludin by Snail. *J Cell Sci* 2003;116:1959-67.
- Espinada CE, Chang JH, Twiss J, et al. Repression of Na,K-ATPase β 1-subunit by the transcription factor snail in carcinoma. *Mol Biol Cell* 2004;15:1364-73.
- Tripathi MK, Misra S, Chaudhuri G. Negative regulation of the expressions of cytokeratins 8 and 19 by SLUG repressor protein in human breast cells. *Biochem Biophys Res Commun* 2005;329:508-15.
- Guaita S, Puig I, Franci C, et al. Snail induction of epithelial to mesenchymal transition in tumor cells is accompanied by MUC1 repression and ZEB1 expression. *J Biol Chem* 2002;277:39209-16.
- del Barrio MG, Nieto MA. Overexpression of Snail family members highlights their ability to promote chick neural crest formation. *Development* 2002;129:1583-93.
- Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF- β family signalling. *Nature* 2003;425:577-84.
- Xie L, Law BK, Chytil AM, et al. Activation of the Erk pathway is required for TGF- β 1-induced EMT *in vitro*. *Neoplasia* 2004;6:603-10.
- Verschueren K, Remacle JE, Collart C, et al. SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5'-CACCT sequences in candidate target genes. *J Biol Chem* 1999;274:20489-98.
- Comijn J, Berx G, Vermassen P, et al. The two-handed E box binding zinc finger protein SIP1 down-regulates E-cadherin and induces invasion. *Mol Cell* 2001;7:1267-78.
- Tomasek JJ, McRae J, Owens GK, et al. Regulation of α -smooth muscle actin expression in granulation tissue myofibroblasts is dependent on the intronic CArG element and the transforming growth factor- β 1 control element. *Am J Pathol* 2005;166:1343-51.
- Xu G, Bochaton-Piallat ML, Andreutti D, et al. Regulation of α -smooth muscle actin and CRBP-1 expression by retinoic acid and TGF- β in cultured fibroblasts. *J Cell Physiol* 2001;187:315-25.

41. Ozdamar B, Bose R, Barrios-Rodiles M, et al. Regulation of the polarity protein Par6 by TGF β receptors controls epithelial cell plasticity. *Science* 2005;307:1603–9.
42. Rajasekaran SA, Palmer LG, Quan K, et al. Na,K-ATPase β -subunit is required for epithelial polarization, suppression of invasion, and cell motility. *Mol Biol Cell* 2001;12:279–95.
43. Begum R, Nur EKMS, Zaman MA. The role of Rho GTPases in the regulation of the rearrangement of actin cytoskeleton and cell movement. *Exp Mol Med* 2004;36:358–66.
44. Kang Y, Massague J. Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* 2004;118:277–9.
45. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004;117:927–39.
46. Castanon I, Baylies MK. A Twist in fate: evolutionary comparison of Twist structure and function. *Gene* 2002;287:11–22.
47. Islam S, Carey TE, Wolf GT, et al. Expression of N-cadherin by human squamous carcinoma cells induces a scattered fibroblastic phenotype with disrupted cell-cell adhesion. *J Cell Biol* 1996;135:1643–54.
48. Lilenbaum A, Paulin D. Activation of the human vimentin gene by the Tax human T-cell leukemia virus: I. Mechanisms of regulation by the NF- κ B transcription factor. *J Biol Chem* 1993;268:2180–8.
49. Rubin MA, Putzi M, Mucci N, et al. Rapid (“warm”) autopsy study for procurement of metastatic prostate cancer. *Clin Cancer Res* 2000;6:1038–45.
50. Christiansen JJ, Rajasekaran SA, Inge L, et al. N-glycosylation and microtubule integrity are involved in apical targeting of prostate-specific membrane antigen: implications for immunotherapy. *Mol Cancer Ther* 2005;4:704–14.
51. Ng WK. Fine-needle aspiration cytology findings of an uncommon micropapillary variant of pure mucinous carcinoma of the breast: review of patients over an 8-year period. *Cancer* 2002;96:280–8.
52. Tan DS, Potts HW, Leong AC, et al. The biological and prognostic significance of cell polarity and E-cadherin in grade I infiltrating ductal carcinoma of the breast. *J Pathol* 1999;189:20–7.
53. Langbein L, Pape UF, Grund C, et al. Tight junction-related structures in the absence of a lumen: occludin, claudins and tight junction plaque proteins in densely packed cell formations of stratified epithelia and squamous cell carcinomas. *Eur J Cell Biol* 2003;82:385–400.
54. Economopoulou P, Hanby A, Odell EW. Expression of E-cadherin, cellular differentiation and polarity in epithelial salivary neoplasms. *Oral Oncol* 2000;36:515–8.
55. Oka H, Shiozaki H, Kobayashi K, et al. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res* 1993;53:1696–701.
56. Madhavan M, Srinivas P, Abraham E, et al. Cadherins as predictive markers of nodal metastasis in breast cancer. *Mod Pathol* 2001;14:423–7.
57. Zhou YN, Xu CP, Han B, et al. Expression of E-cadherin and β -catenin in gastric carcinoma and its correlation with the clinicopathological features and patient survival. *World J Gastroenterol* 2002;8:987–93.
58. Mareel MM, Behrens J, Birchmeier W, et al. Down-regulation of E-cadherin expression in Madin Darby canine kidney (MDCK) cells inside tumors of nude mice. *Int J Cancer* 1991;47:922–8.
59. Kartenbeck J, Haselmann U, Gassler N. Synthesis of junctional proteins in metastasizing colon cancer cells. *Eur J Cell Biol* 2005;84:417–30.
60. Gabbert HE, Mueller W, Schneiders A, et al. Prognostic value of E-cadherin expression in 413 gastric carcinomas. *Int J Cancer* 1996;69:184–9.
61. Hashizume R, Koizumi H, Ihara A, et al. Expression of β -catenin in normal breast tissue and breast carcinoma: a comparative study with epithelial cadherin and α -catenin. *Histopathology* 1996;29:139–46.
62. Lipponen P, Saarelainen E, Ji H, et al. Expression of E-cadherin (E-CD) as related to other prognostic factors and survival in breast cancer. *J Pathol* 1994;174:101–9.
63. Parker C, Rampaul RS, Pinder SE, et al. E-cadherin as a prognostic indicator in primary breast cancer. *Br J Cancer* 2001;85:1958–63.
64. Kovacs A, Dhillon J, Walker RA. Expression of P-cadherin, but not E-cadherin or N-cadherin, relates to pathological and functional differentiation of breast carcinomas. *Mol Pathol* 2003;56:318–22.
65. Gonzalez MA, Pinder SE, Wencyk PM, et al. An immunohistochemical examination of the expression of E-cadherin, α - and β / γ -catenins, and α 2- and β 1-integrins in invasive breast cancer. *J Pathol* 1999;187:523–9.
66. Brown KA, Aakre ME, Gorska AE, et al. Induction by transforming growth factor- β 1 of epithelial to mesenchymal transition is a rare event *in vitro*. *Breast Cancer Res* 2004;6:R215–31.
67. Yanagisawa K, Uchida K, Nagatake M, et al. Heterogeneities in the biological and biochemical functions of Smad2 and Smad4 mutants naturally occurring in human lung cancers. *Oncogene* 2000;19:2305–11.
68. Salovaara R, Roth S, Loukola A, et al. Frequent loss of SMAD4/DPC4 protein in colorectal cancers. *Gut* 2002;51:56–9.
69. Han G, Lu SL, Li AG, et al. Distinct mechanisms of TGF- β 1-mediated epithelial-to-mesenchymal transition and metastasis during skin carcinogenesis. *J Clin Invest* 2005;115:1714–23.
70. Pinkas J, Leder P. MEK1 signaling mediates transformation and metastasis of EPH4 mammary epithelial cells independent of an epithelial to mesenchymal transition. *Cancer Res* 2002;62:4781–90.
71. Hay ED, Zuk A. Transformations between epithelium and mesenchyme: normal, pathological, and experimentally induced. *Am J Kidney Dis* 1995;26:678–90.
72. Kawai N, Tsuji S, Tsujii M, et al. Tumor necrosis factor α stimulates invasion of Src-activated intestinal cells. *Gastroenterology* 2002;122:331–9.
73. Bruewer M, Hopkins AM, Hobert ME, et al. RhoA, Rac1, and Cdc42 exert distinct effects on epithelial barrier via selective structural and biochemical modulation of junctional proteins and F-actin. *Am J Physiol Cell Physiol* 2004;287:C327–35.
74. Nawshad A, Hay ED. TGF β 3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. *J Cell Biol* 2003;163:1291–301.
75. Bukholm IK, Nesland JM, Borresen-Dale AL. Re-expression of E-cadherin, α -catenin and β -catenin, but not of γ -catenin, in metastatic tissue from breast cancer patients [see comments]. *J Pathol* 2000;190:15–9.
76. Lang SH, Sharrard RM, Stark M, et al. Prostate epithelial cell lines form spheroids with evidence of glandular differentiation in three-dimensional Matrigel cultures. *Br J Cancer* 2001;85:590–9.
77. Lombaerts M, van Wezel T, Philippo K, et al. E-cadherin transcriptional down-regulation by promoter methylation but not mutation is related to epithelial-to-mesenchymal transition in breast cancer cell lines. *Br J Cancer* 2006;94:661–71.
78. Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* 2003;3:362–74.
79. Kolega J. The movement of cell clusters *in vitro*: morphology and directionality. *J Cell Sci* 1981;49:15–32.
80. Cooper MS, D’Amico LA. A cluster of noninvoluting endocytic cells at the margin of the zebrafish blastoderm marks the site of embryonic shield formation. *Dev Biol* 1996;180:184–98.
81. Friedl P, Noble PB, Walton PA, et al. Migration of coordinated cell clusters in mesenchymal and epithelial cancer explants *in vitro*. *Cancer Res* 1995;55:4557–60.
82. Nabeshima K, Inoue T, Shimao Y, et al. Front-cell-specific expression of membrane-type 1 matrix metalloproteinase and gelatinase A during cohort migration of colon carcinoma cells induced by hepatocyte growth factor/scatter factor. *Cancer Res* 2000;60:3364–9.
83. Nabeshima K, Inoue T, Shimao Y, et al. Cohort migration of carcinoma cells: differentiated colorectal carcinoma cells move as coherent cell clusters or sheets. *Histol Histopathol* 1999;14:1183–97.
84. Soulie P, Carrozzino F, Pepper MS, et al. Membrane-type-1 matrix metalloproteinase confers tumorigenicity on nonmalignant epithelial cells. *Oncogene* 2005;24:1689–97.
85. Tarin D, Thompson EW, Newgreen DF. The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res* 2005;65:5996–6000.
86. Al-Mehdi AB, Tozawa K, Fisher AB, et al. Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. *Nat Med* 2000;6:100–2.
87. Green SK, Karlsson MC, Ravetch JV, et al. Disruption of cell-cell adhesion enhances antibody-dependent cellular cytotoxicity: implications for antibody-based therapeutics of cancer. *Cancer Res* 2002;62:6891–900.
88. Ahnen DJ, Nakane PK, Brown WR. Ultrastructural localization of carcinoembryonic antigen in normal intestine and colon cancer: abnormal distribution of CEA on the surfaces of colon cancer cells. *Cancer* 1982;49:2077–90.
89. Tobioka H, Isomura H, Kokai Y, et al. Polarized distribution of carcinoembryonic antigen is associated with a tight junction molecule in human colorectal adenocarcinoma. *J Pathol* 2002;198:207–12.
90. Condeelis JS, Segall JE. Intravital imaging of cell movement in tumours. *Nat Rev Cancer* 2003;3:921–30.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

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

Jason J. Christiansen and Ayyappan K. Rajasekaran

Cancer Res 2006;66:8319-8326.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/66/17/8319>

Cited articles This article cites 90 articles, 36 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/66/17/8319.full#ref-list-1>

Citing articles This article has been cited by 75 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/66/17/8319.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/66/17/8319>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.