Carcinoma Invasion and Metastasis: A Role for Epithelial-Mesenchymal Transition?

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Carcinogenesis involves the accretion of unprogrammed genetic and epigenetic changes, which lead to dysregulation of the normal control of cell number. But a key clinical turning point in carcinoma progression is the establishment by immigrant cells of secondary growth sites (i.e., metastasis). The metastatic “cascade” comprises numerous steps, including escape from the primary tumor site, penetration of local stroma, entry of local vascular or lymphatic vessels (intravasation), aggregation with platelets, interaction with and adhesion to distant endothelia, extravasation, recolonization, and expansion (1), all the time avoiding effective immune clearance and being able to survive in these multiple contexts.

The epithelial-mesenchymal transition (EMT) is a phenotype switch clearly recognized for many decades in developmental biology as instrumental in effecting rapid morphogenetic changes in Metazoan embryos (2). The EMT, which is marked by complex and coordinated set of molecular changes leading to cell behavioral changes, is a portmanteau concept that can be applied to the metastatic behavior of carcinoma cells at a number of junctures.

To appreciate this, it is important first to have a clear idea of what the terms epithelium and mesenchyme mean in general, and what they do not mean. Second, it is important to understand what specific features or functions are always, sometimes, or never associated with these states. This, of course, extends to molecules and genes whose expression can be used as markers signifying the epithelial or mesenchymal state of the cells in question.

An epithelium is a collection of cells forming a relatively thin sheet or layer due to the constituent cells being mutually and extensively adherent laterally by cell-to-cell junctions. The layer is polarized, the two sides showing nonidentical properties so that the sides can be defined as, say, inside or outside, or more precisely, apical and basal. This is reflected in the individual cells that all show an identical apicobasal polarity, an extension of which is the presence on the basal surface of a complete or nearly complete layer of specialized extracellular matrix (ECM), the basal lamina.

Cell-to-cell adhesion molecules typically involve (but are not restricted to) members of the cadherin axis, which are distributed widely but with a particular aggregation complex usually as a circumferential belt at the lateral border. The principal ECM adhesion sites (involving integrin complexes) are strongly biased to the basal face, mediating adhesion to basal lamina molecules, such as laminin. The actin cytoskeleton is also strongly apicobasally polarized, in part mirroring the circumferential cell-to-cell adhesion complexes. Intermediate filaments typically include cytokeratin types.

This arrangement gives an overall impression of strong regimentation of the cells, and it is often assumed that the cells in an intact epithelium are virtually immobile. Despite this, there is a repertoire of epithelial plasticity. In some circumstances, cells in an epithelial layer can alter shape, such as change from flat to columnar, or pinch in at one end and expand at the other. However, these tend to occur in cell groups rather than individually, and their effect is to produce the compaction/expansion and foldings that are the mainstay of morphogenetic movements such as neurulation. In these cases, the epithelial integrity is maintained and there seems to be broad preservation of cellular neighbors, at least in the short term. However, movement relative to adjacent landmarks can occur. This often occurs with the cells remaining largely associated with their original neighbors, as in epibolic spreading (i.e., the movement is relative to features outside the particular epithelium). However, real movement in the sense that cells in an epithelium change their nearest epithelial neighbors without disrupting the integrity of the layer do occur (see ref. 3).

Mesenchyme cells form a relatively diffuse tissue network: There is no complete cellular layer, and the cells typically have only points on their surface engaged in adhesion to their neighbors. These adhesions may also involve cadherin associations (i.e., with molecular family similarity to those of epithelial cells). Adhesion sites to ECM (also involving integrins) are widely distributed at points all around the cells, as is the ECM that is a meshwork structurally unlike the basal lamina and that typically involves interstitial collagens and fibronectin. The actin cytoskeleton is not apicobasally polarized and does not show circumferential organization. Instead, the actin may form a cortical network and perhaps trans-cytoplasmic actin bundles; these may have a provisional polarity termed “front-back” by Hay (4), and this polarity may be approximately aligned between neighboring cells, especially when the mesenchymal cell population is moving in a concerted manner. The intermediate filament make-up typically includes vimentin.

Mesenchyme gives the impression of much more relaxed organization, and this suggests flexibility, individualism, and motile propensities. In many cases, mesenchyme cells do participate in cell migrations. However, many other mesenchyme cells show poor ability to move. Sclerotomal mesenchyme cells are motile in vitro, but they normally undergo limited local population-based shifts and expansion in vivo. In addition, these cells have little migratory potential in vivo, as shown by grafting them into neural crest mesenchyme migration pathways (5). Moreover, cells may move collectively (see ref. 6), and even in the archetypal “individualistic” migrating mesenchyme, that of the neural crest, recent time-lapse observations revealed that the cells move as contacting groups (“chains”) in vivo, although there is often rapid neighbor exchange at the level of individual cells (7). Indeed, migration has a population requirement because isolation of neural crest cells from their fellows leads to rapid cessation of movement, as shown recently by direct time lapse imaging (8).

Thus, the core definitions of epithelium and mesenchyme depend, in our view, on the following:

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(a) the presence (epithelial) or absence (mesenchymal) of cellular polarity that allows the definition of apical, basal, and, hence, lateral faces. This is evidenced by the arrangement of actin and the distribution of cell-to-cell and cell-to-ECM adhesion molecules.

(b) the extent of cell-to-cell junctions as a lateral belt (epithelial) or only as points (mesenchyme).

The presence (epithelial) or absence (mesenchyme) of a basal lamina (although this need not be complete) is a typical correlate, but new mesenchyme generated by EMT may transiently retain basal lamina fragments (9). Many of the other molecular signatures depend as much on location as on the specific molecule and its abundance, and so are actually reflections of polarity. Nevertheless, differences are frequently seen, such as in cadherin type and level, or cytokeratin/vimentin ratio. As with the basal lamina, these differences in type and amount may be less obvious in new mesenchyme.

As mentioned, capacity to move, individually or in groups, are not absolute defining characteristics for epithelia and mesenchymes, although, in general, the latter seem more dynamic and plastic. Indeed, as regards motility, the "typical" epithelium and the "typical" mesenchyme might be viewed as two expanded ends of a continuum. Indeed, the creation of the mesodermal mesenchyme in frog embryos involves an involution of a cell layer, albeit a plastic and dynamic layer, whereas the same outcome in many other species is via a clear EMT. Epithelial and mesenchymal modes of assembly are found in many derivatives of different germ layers, so they are better thought of as cellular states, and not as cell lineages per se. This correlates with their interconvertibility seen normally and experimentally via EMT and MET (see below).

The defining hallmarks of developmental EMT include derangement of apicobasal polarity and cell-to-cell adhesive architecture and function, lack of basal lamina integrity, and cell shape plasticity (10). Often, but not always, the immediate consequence is to generate a cell type with considerable translocation (migration) ability, with the cell exiting the epithelium of origin via the basal surface. In addition, the post-EMT behavior of the cell may include the reverse transition, a mesenchymal-epithelial transition (MET) as epitomized by the formation of nephric tubules from intermediate mesenchyme (11).

Classes of molecules that change in expression, distribution, and/or function during the EMT, and that are causally involved, include growth factors [e.g., transforming growth factor (TGF)-β, wnts], transcription factors (snails, SMAD, LEF, and nuclear β-catenin), molecules of the cell-to-cell adhesion axis (cadherins, catenins); and of the cell-to-ECM adhesion axis (integrins, focal contact proteins, ECM proteins), cytoskeletal modulators (Rho family), and extracellular proteases (matrix metalloproteinases, plasminogen activators). Other molecular changes seem to occur after the initial behavioral change; for example, there is often a trend to replace cytotkeratin intermediate filaments with other types, typically vimentin (12).

These same elements, histologic, molecular, and transcriptional, are commonly associated with carcinoma progression, leading to the obvious possibility of EMT as a part of the metastatic process. However, the execution of a development-like EMT by cancer cells is only one hurdle in achieving metastatic "success," so one cannot expect sure and immediate metastasis even when the primary tumor shows signs of EMT. In addition, primary tumors are heterogeneous, and usually only a very small proportion, sometimes called the "invasive front," shows the histologic and molecular EMT-like signature. Nonetheless, EMT-like attributes accompany heightened metastatic potential in many systems.

The likelihood of carcinoma EMT is further supported by a host of studies in various cellular systems. For example, breast (Fig. 1A; ref. 13) and prostate carcinoma cell lines derived from different patients can be classified as predominantly epithelial or mesenchymal. Zachowksi et al. (14) used gene array to define an expression signature of 24 gene products that predicted invasiveness in human breast cancer cell lines, and the great majority of these were classic epithelial (noninvasive) or mesenchymal (invasive) proteins. Also, numerous examples exist where epithelial and mesenchymal states of the same cell line show dramatically different cancer activities (Fig. 1B; ref. 15 and reviewed in ref. 16). Invariably, the mesenchymal state will be more motile and invasive in vitro, more tumorigenic, and often selectively metastatic. This is true in mouse, rat, and human systems from multiple organs sites, such as bladder, cervix, mammary, colon, bronchial, prostate, kidney, and squamous cell carcinoma (reviewed in ref. 16). Similar aggressiveness can be induced in the epithelial state by disrupting E-cadherin or other molecules in the cadherin axis, and conversely functional restitution of this axis restores the cells to a less aggressive state. Although not all molecular correlates of EMT have been investigated in every tumor, taken together, these molecular/cellular characteristics are representative of that seen during developmental EMT. The developmental EMT provides a consistent and reproducible framework upon which the somewhat more variable observations in cancer systems can be hung (17).

It is important to point out that all EMTs are not identical, as evidenced in the embryo (2). Often, however, the molecular differences involve members of the same family (e.g., E-cadherin or N-cadherin, TGF-β or BMP-4, slug or snail, etc.). The cells produced by EMT, although usually considered invasive and motile, are not always so, even in development. The example of sclerotome mesenchyme cells being unable to exploit neural crest pathways has been referred to above (5). Pathologically, fibroblast cells are produced by an EMT-like mechanism in fibrotic kidney, and these cells remain local (18, 19). In any case, cells leaving an epithelial tumor probably need to transit a very short distance through stromal tissue before encountering a vessel. Also, it should be remembered that long distance translocation is not necessarily effected by highly individualistic cells. Indeed, the extent of cell dissociation required for translocation of cells can vary (6), with examples such as the border cell migration in the Drosophila ovary toward the oocyte, where a tight cell cluster migrates with polarized loss of epithelial characteristics at the front margin only (20). Thus, exactly what form and degree of EMT that we should expect in carcinoma is unclear, and is likely variable.

Evidence of EMT in clinical cancer specimens include loss or delocalization of junctional E-cadherin (21), switch to other cadherins (e.g., N-cadherin replaces E-cadherin in prostate cancer; ref. 22), degradation of cell-to-cell adhesion, apicobasal polarity and tissue architecture, pleiotropic cell shape, nuclear β-catenin, Snail or Slug expression (17), and the otherwise unexpected expression of mesenchymal markers such as the intermediate filament protein vimentin (12). These changes often associate with poor prognosis. This is true in cervical carcinoma where vimentin positivity correlates with lymph node metastasis; however, the lymphatic metastases lack vimentin, suggesting reversion by MET (23). Focal vimentin expression has been reported variably in ~15% of invasive breast cancer studies (Fig. 1C; ref. 12), and associated with aggressive parameters in some but not all studies, suggesting that vimentin may not be a clear indicator of EMT and could be spuriously expressed outside of the EMT. Other usually stromal/mesenchymal indicators have also been detected in breast cancer, such as stromelysin-3 in metaplastic breast carcinoma (24), and nuclear β-
Figure 1. A, Human breast carcinoma cell lines show full spectrum of morphology and markers. MCF-7 cells exhibit a cobblestone morphology in culture, and express abundant cytokeratin and negligible vimentin. MDA-MB-231 cells exhibit some stellate morphology under phase contrast and coexpress lower levels of cytokeratin and vimentin. MDA-MB-435 cells are highly individualistic in culture, completely lack cytokeratin staining with this antibody, and abundantly express vimentin. These cells were recently shown to better resemble melanoma cells through a large panel of markers, perhaps through lineage plasticity. B, the PMC42-LA human breast cancer cell line is initially predominantly cytokeratin-positive (red stained), with a small proportion (10-15%) of the cells expressing vimentin (green stained). Upon treatment with epidermal growth factor for 3 days, the cells adopt vimentin expression and continue to express low levels of keratins. C, two different sections from the same human breast cancer show regions where the vimentin is exclusively in the surrounding stroma and vascular structures (LHS, brown staining), or clearly in the tumor parenchyma.
catten in colon/gastric cancers (25). Vimentin is among a group of so-called “basal markers” identified by gene array studies to predict a poorer prognosis in breast cancer than tumors with luminal features (26), and is differentially expressed in invasive breast carcinoma compared with ductal carcinoma in situ (27). Again, it is possible that these changes arise through EMT activity and/or signal EMT capability in these tumors. The most extreme and telling case is the rare but distinct metaplastic breast cancers and carcinomas, where carcinomatous (epithelial) and sarcomatous (mesenchymal) regions can be easily delineated.

Functional evidence for the in vivo carcinoma EMT associated with metastasis has been long sought after, and is starting to accrue. The best examples we are aware of to date are indeed compelling. Xue et al. (28) showed that carcinoma cells leaving HER-2/neu transgenic primary tumors selectively expressed a GFP transgene, driven by the mesenchymal-specific promoter from fibroblast-specific protein-1 (FSP-1; S100A4). Furthermore, transgenic mice showed dramatically reduced metastasis where this promoter drove a suicide construct, consistent with EMT leading to ablation of the metastatic cells. Similarly low metastasis was found from tumors derived from FSP-1 null mice. These data provide the first mechanistic evidence for the important role of EMT in carcinoma spread.

The cellular social behavioral changes leading to break up of an epithelium in model systems has been classified as “true” EMT or “scattering”. The two are differentiated by irreversibility for true epithelium in model systems has been classified as “true” EMT or “scattering”. The two are differentiated by irreversibility for true EMT and reversibility (i.e., via MET) for scattering, as well as differences in signal transduction pathways (29). However, in development, classic EMTs are often seemingly reversed; for example, in the progression from epithelium to primary mesenchyme to somite to sclerotome. Experimentally, migratory neural crest mesenchyme grafted into the neural tube epithelium can revert to the neural tube state, although they never do so normally (30). Therefore, using the definitive developmental examples as our yardstick, we do not regard reversibility as a defining characteristic of EMT. Another aspect of metastasis that aligns well with the reversibility of embryonic EMT process is the recent recognition that metastases can undergo not only growth but also startling morphogenesis (presumably via MET) and cell differentiation to resemble the originating epithelium (31). This has been studied by Brabletz et al. (25) in colon carcinoma, specifically in relation to the epithelial nature of the metastasis compared with cells at the invasive front of the primary, or indeed in transit. On a more general level, a host of gene array studies comparing breast carcinoma at the primary site and corresponding metastases indicated a high concordance of the two (31).

Finally, one has to ask: Why not EMT in carcinoma progression? If carcinomas have hijacked every other useful aspect of Metazoan developmental cell biology, why not this one which has such obvious parallels to the process of metastasis? As counterpoint, cancers in plants (galls) grow locally and not by metastatic cell seeding. It is significant that EMT is not in the normal developmental morphogenetic repertoire of plants. Presumably, if you have not got it ontologically, you cannot flaunt it oncologically.

The EMT remains for us a very likely candidate process for carcinoma metastasis, with opportunities for diagnosis, prognosis, and treatment to be explored.

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The survey provided in the companion article, in this pair of polemical reviews, does not provide any additional information to counteract the conclusion that EMT is an unfortunate misconception resulting from erroneous interpretation of pathologic data. For example, vimentin is normally expressed in breast myoepithelium and the contradictory evidence cited does not constitute pathologically valid proof of epithelial-mesenchymal conversion in tumors. Additionally, the absence of EMT in plant tumors, mentioned in the last paragraph of the article is not, as implied, an explanation why plant tumors are non-metastatic. In fact the absence of metastasis in plants is primarily because the vascular channels (phloem and xylem) are not open and continuous. They are obstructed at intervals by cell walls with small perforations (see http://www.sirinet.net/~jgjohnso/phloem.jpg). Hence tumor cells cannot be disseminated along them to distant parts of the plant. We conclude that the molecular changes attributed to EMT do not integrate into the known biology and pathology of development, healing, and neoplasia and need to be reconsidered.

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