The Fallacy of Epithelial Mesenchymal Transition in Neoplasia

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Abstract

Epithelial mesenchymal transition has been postulated as a versatile mechanism which facilitates cellular repositioning and redeployment during embryonic development, tissue reconstruction after injury, carcinogenesis, and tumor metastasis. The hypothesis originates from parallels drawn between the morphology and behavior of locomotory and sedentary cells in vitro and in various normal and pathologic processes in vivo. This review analyzes data from several studies on embryonic development, wound healing, and the pathology of human tumors, including work from our own laboratory, to assess the validity of the proposal. It is concluded that there is no convincing evidence for conversion of epithelial cells into mesenchymal cell lineages in vivo and that the biological repertoire of normal and malignant cells is sufficient to account for the events and processes observed, without needing to invoke radical changes in cell identity. (Cancer Res 2005; 65(14): 5996-6001)

Setting the Stage

It is becoming increasingly common to read of the concept of epithelial mesenchymal transition (EMT) in neoplasia (1–4). The time is therefore ripe to consider exactly what is meant by this term and whether there is any factual evidence to support the idea of a change of identity of cells from one differentiated lineage into another during tumor formation and progression. It needs to be understood at the outset of the discussion that epithelium is a term for a cellular phenotype which performs surface-barrier and secretory functions, whereas the term mesenchyme refers to cells and intercellular materials that serve scaffolding and anchoring roles. Hence, the hypothesized occurrence of EMT implies a radical and fundamental change in function, developmental fate and character of a cell lineage. It is not easy to find precise definitions of the postulated process but most descriptions refer to changes in tumor cell morphology, such as assumption of a fusiform or stellate cell appearance and gradual loss of intercellular adhesions to other epithelial cells. It is also often claimed that tumor cells which have these properties are more migratory and invasive in vitro and the extrapolation is often made that tumor cells which seem to be scattered singly in the component tissues of naturally arising tumors have undergone an epithelial-mesenchymal transition. From this, it has been inferred that the transition event may be mechanistically important in the emergence of the invasive and metastatic phenotype (3, 4). It has also been extrapolated by proponents of this theory that tumors which display histopathologic features including loose, scattered cancer cells and sometimes spindle cell cytology, are more aggressive, as judged by grade, stage, and clinical outcome and this is claimed to support the misconception that EMT is mechanistically significant (5). The invocation of EMT, by many investigations and articles, as a defining aspect of malignant conversion and progression, makes it important to assess whether there is convincing evidence of its occurrence during the pathogenesis of human and animal tumors.

The concept of EMT emerged mainly from investigations on embryologic development and from observations on tumor cells in vitro and it is useful background to consider the data from these. The embryologic examples most often cited include the change in phenotype and behavior seen in cells invaginating from the surface into the interior of the embryo to form the mesoderm in gastrulation (3, 5), the changes observed during renal organogenesis and the origin and fate of the neural crest (6). The observations on cultured cells mainly concern the scattering behavior and spindling of cells when exposed to various extracts and factors in the surrounding medium (7, 8) and from observations on explanted embryonic tissues (9). These events are supposed to be the counterpart of the loosening and dissemination of tumor cells characterizing some histopathologic types of human tumors.

Presenting the Data

Taking first the embryologic phenomena on which the concept of EMT is based: the change in phenotype during gastrulation of surface cells to form the mesoderm is not really an EMT because neither of these components has yet been “determined” at this developmental stage to produce specific cell lineages, tissues or organs. The surface ectoderm is not yet a differentiated epithelium or neural plate and the mesoderm is not yet committed to make mesenchymal cell lineages. Both the embryonic ectoderm and the mesoderm are still plastic and each can, as shown by grafting experiments (10), be induced to substitute for portions of the other germ layer. Moreover, as seen in sagittal (Fig. 1A) and coronal sections (Fig. 1B) through the gastrula, the invaginating mesodermal cells are not spindle-shaped or stellate, nor have they lost cohesion with each other. Another example claimed as EMT is the formation, by the endothelial lining, of the heart valves in the developing heart (11). This error results from a misunderstanding of cell lineage because the vascular endothelium is not an epithelium, never becomes an epithelium, and has a different developmental history and function to epithelia. On the other hand, there are numerous examples in the development of the kidney, pancreas, liver, and other organs of the induction of mesenchymal to epithelial transitions. In the absence of the appropriate inductive stimulus, the target cells continue on the pathway to specialize as mesenchymal lineages, indicating that a true change in their developmental pathway is elicited by the inductive stimulus. However, once the mesenchymal to epithelial transition occurs, it is fixed and irreversible, so as to maintain vital organ function and avoid anarchy, in contrast to the proposed reversibility of EMT in tumor progression and metastasis.
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(see below). Perhaps the most interesting evidence of possible EMT in embryonic development is the conversion of cells at the edges of the neural plate into noncohesive spindle-shaped cells which migrate over large distances in the embryo, infiltrate many organs and differentiate into a large variety of mesenchymal derivatives including nerve sheaths, cranial bones, pigment cells, autonomic neurons, etc. Calling this phenomenon an EMT assumes that the progenitor cells were already a differentiated epithelium in the first place, which is open to argument. Even so, it is unconvincing to extrapolate and generalize from this highly ordered series of events, reproducibly followed by a specific cell lineage in vertebrate embryos, to the disorganized series of events during tumor progression in many different adult organs. Thus, the conclusions about EMT, derived from comparisons with embryologic data, are based upon imagined similarities to some microscopic features of human tumors. They are conceived without clinical or diagnostic pathologic experience and, in some cases, upon misidentification of cell lineages. This group of suppositions is, therefore, collectively weak, as a substantiation of cases, upon misidentification of cell lineages. This group of suppositions is, therefore, collectively weak, as a substantiation of the existence of EMT in mature organisms.

Secondly, the data for EMT, derived from observations in vitro, refer to cells in a highly artificial two-dimensional environment in which there is no dynamic vascular, endocrine or neurologic contribution. Studies on cells in three-dimensional matrices also do not recapitulate the internal body environment although they do increase knowledge of what cells can do in investigator-specified conditions. The identification of cells in vitro as epithelial or mesenchymal, on the basis of shape and morphology is notoriously subjective and unreliable. Even in studies where epithelial and mesenchymal cell markers are used, the evidence for a switch from one differentiated cell lineage to the other is thin. Firstly, this is because they rely upon relating a marker to a morphologic phenotype, which itself is claimed to transmute into another cell type and the identification process therefore becomes a circular, self-fulfilling argument. Secondly, it is rare for more than one marker to be used in such claims and the phenomenon of inappropriate gene expression in tumors is well known. This encompasses, for example, the secretion of adrenocorticotropic hormone by lung carcinomas and no clinician contends that this is evidence of pituitary cells in the lungs. Such inappropriate expression in genetically unstable cells could be responsible for shifts in single markers of lineage, such as the claims of FSP1 expression in epithelial cells. Clearly, the reliability of this marker depends upon how certain it is that only fibroblasts ever express this gene and, because it is difficult for scientists to define a cell as a fibroblast with confidence, on morphologic criteria alone, the issue again becomes a circular argument. The gain or loss of one or a few markers is insufficient to conclude that a cell population has implemented whole-scale gene expression reprogramming to abandon one life-style and adopt another which is totally different. Evidence from experiments in vitro, therefore, are unsatisfying as evidence that EMT is a real phenomenon in vivo.

Having considered the precise definition and origin of the term EMT, it is appropriate to evaluate whether there is convincing evidence of EMT in clinical samples of human and animal tumors. If this is a real entity, with significant mechanistic implications, it should be seen frequently in surgical pathology specimens sent for diagnosis and be a well-recognized feature, used for prognostic evaluation. In >40 years experience of reporting on the pathology of many thousands of tumor samples, the author has not seen convincing evidence of EMT (i.e., a true, stable conversion of one differentiated cell lineage into another) at any stage of neoplasia, from the conversion of in situ into invasive carcinoma, nor during progression to the most advanced stage IV lesions with systemic metastases. Moreover, it has not been recognized and documented extensively by other surgical pathologists and is not used clinically in any of the published schemata for evaluating the grade, stage, or prognosis of a cancer. As the main focus of their work is to recognize features that relate to clinical outcome for individual cancer patients, it seems intrinsically unlikely that experienced clinical practitioners of tumor histopathology would have overlooked or misinterpreted such a phenomenon, if it were a significant event in carcinogenesis and tumor progression. Histologic appearances which superficially seem to resemble the descriptions of EMT do sometimes occur in human tumors but do not withstand close scrutiny. Disorderly differentiation, loss of cell polarity and loss of lineage specific or tissue specific cytologic features are defining aspects of carcinomas. The degree of disorder could progress to extreme pleomorphism and anaplasia and it is understandable that these features might be misinterpreted by unwary observers to assume that the cells have changed into a separate differentiated (mesenchymal) lineage. However, it is wise to exercise caution in constructing any hypotheses regarding mechanisms of tumor behavior in vivo on this assumption. The examples most often cited as evidence of EMT in naturally occurring neoplasms, namely (a) scattered single cell infiltration by lobular carcinomas of the breast (4) or by diffuse (or signet-ring) carcinomas of the stomach (12); (b) spindle cell differentiation in squamous carcinomas; and (c) blending of sheets of carcinomatous cells into a highly cellular, minimally fibrous, (i.e., desmoplastic) stroma without a clear line of demarcation, represent misapprehensions of the histopathology of the lesions. These will be discussed sequentially below: diffusely infiltrating cells in lobular carcinomas retain their epithelial identity. Although this class of tumors is defined by the tendency of its cells to invade as single units, often in Indian file arrangement (Fig. 1C), their epithelial lineage is clearly hallmark by their characteristic appearance as seen in Fig. 1C. Often the single infiltrating epithelial cells contain large intracellular mucin vacuoles (signet ring cells), a quintessential feature confirming their epithelial (nonmesenchymal) identity. The metastases of lobular carcinoma of the breast are also not difficult to recognize as being carcinomatous and are distinct from sarcomatous metastases. Thus, the idea that lobular carcinoma of the breast exemplifies transition from epithelial to mesenchymal lineages is spurious. Next, spindle cell differentiation in squamous carcinomas and adenocarcinomas (Fig. 1D) is a well recognized but relatively infrequent occurrence. This change in the appearance of the epithelial cells occurs in the context of their anatomic position and architectural arrangement within the tissues of the organ and rarely confuses experienced pathologists. It can be seen in Fig. 1D that the fusiform cells show marked variation in size shape and staining (pleomorphism) indicative of malignancy but are still recognizably epithelial. The features of this entity do not support a conclusion that the malignant epithelial cells have converted into a mesenchymal cell lineage, with new potential for specializing into stromal (mesenchymal) derivatives such as cartilage, bone, muscle, or tendon. Lastly, the blending of neoplastic epithelial cells into stimulated stromal cells of a desmoplastic reaction in the stroma (Fig. 1E), although sometimes difficult to visually unravel into distinct cell populations is not firm, objective evidence of a transition, via intermediate forms, of one cell type into another.
Figure 1. A, sagittal section through the dorsal lip (asterisk) of the amphibian gastrula showing curving inward migration (curved arrow) of mesodermal cells (M) under the surface ectoderm (E). Straight arrows denote boundary between the layers. Note that there is no loss of cohesion or spindling of the invaginating cells. Inset, whole embryo with arrows denoting the plane of histologic sectioning. B, coronal section across the dorsum of a slightly older gastrula, Note that the ectodermal (E) and invaginating mesodermal cells are still tightly aggregated although the early outlines of the notochord (N) and somites (S) are emerging. Arrows, ectomesodermal boundary. No EMT visible. C, lobular carcinoma of the breast showing Indian filing, signet ring cells (plain arrow) and single cell infiltration (bobbed arrows). Malignant cells are clearly epithelial and show no EMT. D, fascicles of spindle-shaped malignant epithelial cells (arrows) masquerading as EMT adjacent to adenocarcinoma clumps (asterisk). E, desmoplastic reaction of benign stromal cells between carcinoma cells distinguishable from appearances shown in (D) by absence of malignant epithelial features in the fusiform cells. No EMT seen. F, invading clumps of adenocarcinoma glands with intervening desmoplastic stromal cell reaction. No EMT seen. G, invading clumps of squamous carcinoma cells making keratin (pink whorls). The malignant cells show no signs of EMT.
On the contrary, carcinomas most often invade as groups of malignant glands (adenocarcinomas) sharply delineated from the surrounding desmoplastic reaction as in Fig. 1F, or clumps of clearly squamous epithelial cells (Fig. 1G). This desmoplastic reaction of the nonneoplastic stroma of the organ, which is induced by the presence of the invading tumor cells, is fascinating evidence of tumor-host interactions and recent data from our laboratory has shown that xenografted human cancer cells and neighboring mouse cells alter each other's gene expression patterns. The desmoplastic reaction is a manifestation of this cross-talk and is not to be confused with a conversion of one cell type into another. Evidence of EMT is conspicuously lacking and it, therefore, seems unlikely that it is an essential or important event in the progression to malignancy. The onus for proof of such lineage metamorphosis, therefore, rests with the proponents of EMT and remains unfulfilled. Interestingly, the morphologic changes such as spindling of tumor cells and blurring of boundaries between malignant and nonneoplastic cell populations (Fig. 1D and E) seems more common in tumors in transgenic animals than in human clinical specimens. However, even in such models, careful studies (13) do not report a high incidence of transitional histologic features. This information should prompt some caution in extrapolation of conclusions from the animal to the human tumors.

Although bone and cartilage are occasionally seen in carcinomas, these are examples of metaplasia of nonneoplastic host stromal components within the tumor and not of lineage change among the malignant cell population. Metaplasia is a phenomenon which is worth reviewing briefly in the context of EMT. It involves a change of differentiation of a specialized cell type of a particular lineage into another cell type, of the same lineage. Examples include squamous metaplasia in bronchial epithelium, which is normally columnar, columnar metaplasia in cervical epithelium, which is normally squamous and the formation of cartilage and bone in loose connective tissue. Although metaplasia sometimes precedes neoplasia in a tissue, it is not an example of a fundamental change of one lineage into another.

Carcinosarcoma is another entity, which although very rare, is pertinent to a discussion of whether EMT occurs in naturally occurring cancers. In these tumors, both the epithelial and the stromal components show the features of high-grade malignancy, namely nuclear and cytoplasmic pleomorphism, many mitoses, bizarre mitotic figures, severe histologic disorganization, and infiltrating margins. Carcinosarcomas are usually very aggressive, both locally and systemically, resulting in short survival of the patients. These tumors are poorly understood, mainly because of their rarity and the consensus view among pathologists is that they represent simultaneous malignant transformation in both epithelial and stromal components of the tissues of an organ or a collision of two adjacent malignant tumors. This is not a fully satisfying explanation of the origin of such tumors, because they do tend to occur in certain organs more than in others. These neoplasms are difficult to investigate further, because animal models are unavailable, and human samples are scarce and not amenable to experimental manipulation. However, EMT is not a favored interpretation of this type of tumor and its very infrequent occurrence excludes it from being adduced as supporting EMT being an essential or important pathway in tumor progression and metastasis.

The metastatic spread of cancer from the primary site to form secondary tumors in distant organs constitutes the most stringent test of the EMT hypothesis. One of the most intrinsic and characteristic aspects of metastasis is that the secondary tumors often recapitulate deformed but recognizable histologic features typical of the original primary tumor. This close similitude of histopathology of secondary tumors to their primary progenitor neoplasms is often used in clinical practice to predict and locate the site of occult primary tumors. Thus, if EMT is truly a critical event in malignant conversion and progression, it has to be postulated that the disseminating cells undergo a reverse mesenchymal to epithelial transition in the target organ as part of the process of secondary tumor formation (5). There is no convincing and corroborated description of this process in the metastasis or clinical histopathologic literature. A recent study of breast tumors in genetically engineered mice reported evidence suggesting such reversible transitions occur (14), but depended on acceptance that the dsp1 gene is a truly specific marker of fibroblasts and on intravenous inoculation of tumor cells to simulate metastasis. Intravenous inoculation is in fact only a lung colonization assay, which has its uses, but cannot substitute for spontaneous metastasis. The transitions that are claimed to occur in this model are not convincing as a paradigm for the real process and, moreover, did not occur in a temporal continuum, within primary tumors and their metastases, in the same animals. Although technically inventive and interesting, this study, therefore, does not provide compelling evidence of reversible transitions between epithelial and mesenchymal phenotypes during metastasis. Accordingly, in the absence of corroborated data, this proposed series of reversible transitions during metastatic dissemination and colonization is difficult to sustain in the face of thousands of clinical and research observations to the contrary on tumor metastasis. Unfortunately, this conclusion undermines otherwise good molecular biological and biochemical studies, which have accepted EMT as a basis to investigate mechanisms of tumor progression and metastasis, because the core premise is weak. It is important to be clear, however, that this skepticism about EMT in malignancy in no way challenges the role of epithelial mesenchymal interactions in development (15), healing and remodeling (16), carcinogenesis (17, 18), and metastasis (19–21), for which there is substantial evidence. The skepticism is directed towards transmutation of one lineage into another, not towards biologically important interactions between them.

Interpreting the Meaning

The crux of the issue of whether EMT has a significant role in neoplasia and tumor progression rests upon whether it is a true entity in adult organisms. The mature metazoan organism is composed of numerous distinct and separate cell lineages which, at least in vertebrates and other higher species, must maintain their identity throughout the lifetime of the individual for the efficient function of complex organs. Clearly, random or unpredictable transdifferentiation of cells into different lineages would cause malfunction of vital structures. It is, therefore, theoretically possible that the disturbed function of tumor tissues is the result of EMT, but the progressive disorderly histologic architecture of tumors has...
been sequentially studied (22–24) in detail with optical and electron microscopes many times (see Tarin 25 for review) and the observed facts do not support the postulated involvement of EMT in induced and naturally occurring tumors. Nor do detailed sequential ultrastructural studies of wound healing (26, 27) support claims (28) that EMT is important in tissue healing and repair.

The many biochemical and molecular biological investigations founded upon the hypothesis of EMT have produced substantial amounts of interesting data, but the fundamental premise that EMT occurs in real cancers is seriously in doubt and the interpretation of the molecular observations needs to be revisited. According to histopathologic criteria, these findings seem unlikely to be directly informative about the mechanisms underlying the dynamic progression of human and animal tumors in vivo, but may still provide information on the molecular options available to cancer cells in vitro. This story is a classic example of the need to incorporate histopathologic expertise on human and animal cancers into modern cancer research, in order to make basic mechanistic investigations relevant to the clinical management of neoplasia.

Acknowledgments

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Response

We appreciate the opportunity to engage in debate over the issue of EMT in carcinoma progression. We can only assume that we must agree to disagree, by definition of being invited to provide the opposite perspectives on this topic, and thus our comments are unlikely to lead to a major change in opinion or text on the part of the author, nor do we expect our comments are unlikely to lead to a major change in opinion or text on the part of the author, nor do we expect

order to provide for relaxation of the epithelium. This then reverts to the semantics issue mentioned above.

There is also a tendency to refer to Epithelia and Mesenchyme as lineages, when in fact we don’t consider this in the embryologic sense, rather that these are phenotypic states within a lineage. We extrapolate this to the carcinoma scenario.

The specific example of endothelium is an interesting case in point. No-one can deny the mesodermal origin of these cells, however, in the vessels, they constitute an epithelium in relation to apico-basal polarity and junctional specialisations. In order to sprout they must undergo trans-endothelial migration (TEM), and parallels between, EMT and TEM with respect to overcoming adhesion within the monolayer and engaging migrational machinery have been made.

The authors comments on the validity of ascribing EMT to cellular changes in vitro are rigorous in a way which does push for better characterisation, however, in our view, may be too narrow. As detailed in our counterpoint article, a number of studies collectively point to a very large number of coordinated molecular changes (rather than a smaller number implied by the author) between Epithelial and Mesenchymal states. Furthermore, when these molecules are introduced ectopically, an EMT can be induced. These EMTs are occurring through regulation by specific pathways, and can be blocked by specific pathway blockers such as NFKb and SMADs. The tight regulation and commonality among these pathways in numerous system, does in our view support a programmatic change such as EMT.

We also concede in our manuscript that in vivo proof of the existence and importance of EMT in carcinoma progression is very hard to get, and the points of view provided by the author again enrich the process of scientific exploration. The lack of such proof is balanced, in our view, by a growing mass of compelling correlative data, and only full molecular analysis on individual cells in actual tumours will answer this question. For the reasons outlined above in relation to the many guises that EMT may take in vivo, evidence of EMT may well escape histological analysis, consistent with this not having been noticed by the author over the 40 years of pathological analysis. We would anticipate that careful marker analysis will be required to answer the question.

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