Epithelial to mesenchymal transition: The doorway to metastasis in human lung cancers

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Lung cancer remains a disease characterized by early metastasis and poor 5-year survival. Lung cancers are almost exclusively derived from epithelial tissues, and most tumor cells retain epithelial characteristics even as the tumor progresses. Small numbers of cells, however, are thought to undergo a process of epithelial to mesenchymal transition in which the malignant cells acquire a fibroblastlike morphology, lose intracellular adhesions, and become mobile. This process represents a crucial event in cancer invasion and metastasis. These mesenchymal cells may subsequently revert to an epithelial phenotype, allowing clinically relevant growth of metastases. Ongoing studies are required to determine ways in which the process of epithelial to mesenchymal transition can be exploited in patients with lung cancer for screening, diagnostic studies, and therapeutic options.

Lung cancer remains the most common cause of malignancy-related death among both men and women in America with more people dying of lung cancer than of melanoma, colorectal, breast, and prostate cancers combined.[1] The overall 5-year survival among patients with non–small cell lung cancer (NSCLC) remains a dismal 15%, primarily from development of distant metastatic disease. Despite the expectation that curative surgical resection of early stage NSCLC leaves patients disease free, 40% to 60% of these patients still have metastases develop and die of lung cancer. Efficacy of the best available chemotherapeutic agents to control metastasis has reached a plateau, and further advancement of cytotoxic therapy is unlikely. More recently, targeted agents have attracted interest after the introduction of antibodies and small molecule inhibitors of epithelial growth factor receptor and vascular endothelial growth factor. Novel agents delivered against these receptors provide survival benefits only among select patients, however, and the application of targeted agents to the overall population of patients with lung cancer provides only modest benefits.[2,3]

Recently, the concept of epithelial to mesenchymal transition (EMT) has been proposed as a putative mechanism for tumor invasion and metastasis in numerous types of solid tumors, including NSCLC, on the basis of clinical observations and laboratory investigations.[4] EMT is a normal physiologic process that is essential for development as embryos progress from single-layered to multilayered organisms. EMT remains critical during development for the migration of some cells, such as neural crest cells, throughout the body. Even in adulthood, the induction of EMT remains a natural and necessary component of the inflammatory process and normal wound healing.[5] The phenomenon of EMT has therefore been subdivided into 3 separate classes: class 1 EMT is involved with embryologic development, class 2 EMT is involved with wound healing, and class 3 EMT is involved with cancer cell invasion and migration.

Expression of mesenchymal markers among small clusters of cells within epithelium-derived tumors has been well described and is thought by some to represent a crucial event in malignancy that is required for local invasion and establishment of distant metastasis.[6] Markers for EMT in human tissue specimens have been correlated with increased risk of cancer recurrence, the presence of metastasis, and decreased survival, supporting the concept that EMT events represent an aggressive biologic transition. This review provides an overview of our current understanding of the process of EMT in lung cancer and highlights the need for further investigations in this area by clinician–scientists. The goal is to identify ways in which EMT can be exploited for therapeutic benefits.

Evidence supporting the EMT hypothesis includes the observation of malignant cells with a mesenchymal phenotype in areas of carcinomas located at the advancing tumor front, where malignant cells infiltrate surrounding normal tissues. In addition, the ability to manipulate carcinoma cells in vitro into adopting mesenchymal phenotypes by the forced overexpression of EMT-linked transcription factors provides convincing evidence that EMT does occur. The clinical relevance of EMT in lung and other cancers is supported by the prognostic implications of mesenchymal markers within the primary tumor. Importantly, the presence of EMT cells within tumors has been correlated with the existence of metastasis,[7] an increased risk of cancer recurrence,[8] and decreased patient survival.[9,10]

Despite evidence supporting the hypothesis that EMT conveys enhanced invasiveness and metastasis, the concept remains controversial for several reasons. Most importantly, the EMT phenomenon does not conform to the canonical

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Abbreviations and Acronyms

E47	= basic helix-loop-helix transcription factor (*E2A* gene product)
ECM	= extracellular matrix
EMT	= epithelial to mesenchymal transition
GSK-3β	= glycogen synthase kinase-3β
IL	= interleukin
MET	= mesenchymal to epithelial transition
MMP	= matrix metalloproteinase
MT1-MMP	= membrane type 1 matrix metalloproteinase
NSCLC	= non–small cell lung cancer
SMAD	= family of proteins originally identified as class of proteins related to Sma and Mothers against decapentaplegic protein
TGFβ	= Transforming growth factor β

belief that cancers evolve by progressively acquiring genetic mutations that convey an increasingly aggressive phenotype. Instead, EMT events are transient changes driven by signals derived from the extracellular matrix (ECM) and inflammatory cells recruited to the advancing tumor front. Therefore, mesenchymal cells revert back to an epithelial form when signals driving the mesenchymal transition dissipate. The transient nature of EMT events makes scientific investigation of this phenomenon difficult for 2 reasons. First, transformed mesenchymal cells in human tumor specimens represent only a small percentage of the entire tumor, and therefore they can only be reliably identified by examining entire tissue slides, rather than representative samples in tissue microarrays. Similarly, because large segments of tumors do not uniformly undergo EMT, it is impossible to capture large numbers of transformed mesenchymal cells in vivo, as in frozen tumor samples, for investigation. Much of what has been learned about the EMT phenomenon has therefore come through in vitro investigations that have been correlated with clinical observations in tumor specimens.

EMT AS A MARKER FOR POOR PROGNOSIS

Malignant tumor cells with a mesenchymal phenotype appear clinically relevant, because the presence of EMT cells within primary tumors has been associated with worse clinical outcomes. The presence of transformed mesenchymal cells within tumors correlates with an increased risk of cancer recurrence and negatively relates to long-term survival among patients with numerous different types of solid tumor malignancies, including colorectal cancer,[7] gastric cancer,[11] and NSCLC,[8,9] and is related to recurrent disease among patients with bladder cancer.[8] Furthermore, expression of transcription factors driving the mesenchymal morphology has been linked to chemoresistance in pancreatic cell lines and in human specimens.[12] In addition to its apparent effect on traditional cytotoxic agents, the mesenchymal phenotype also correlates with resistance to the novel epidermal growth factor receptor inhibitor erlotinib.[13] This clinical information validates the understanding that EMT represents an aggressive transition of carcinomas into a more strongly pathologic phenotype.

The presence of EMT cells has also been associated with an increased rate of metastasis. In colorectal cancers, small clusters of EMT cells have been described as tumor budding that can be visualized penetrating normal surrounding tissues in histologic specimens. Tumor buds are highlighted with immunohistochemical stains for pancytokeratin. By means of receiver–operator curves, Prall and colleagues[14] defined a cutoff value of 25 tumor buds/high-power field to discriminate a patient population with early-stage colorectal cancer at significantly greater risk for tumor metastasis and decreased survival than patients with fewer than 25 tumor buds/high-power field. This clinical observation further supports the concept that EMT contributes to tumor metastasis in patients with solid tumors.

Numerous biochemical markers for the induction of EMT have been described. The marker most tightly linked to EMT is the loss of E-cadherin expression, and the loss of this marker has been has been associated with an increased rate of metastasis among patients with breast cancer.[15] Similarly, EMT markers have also been correlated with worse outcomes among patients with lung cancer. The expression in NSCLC tumor specimens of Slug, a transcription factor known to downregulate E-cadherin expression, correlates with an increased rate of cancer recurrence and decreased survival.[10] Furthermore, forced overexpression of Slug in lung cancer cell lines used for the creation of xenografts in mice demonstrated markedly diminished E-cadherin expression, significantly enhanced expression of matrix metalloproteinase (MMP) 2, and enhanced propensity for metastases to develop in vivo.[10] Collectively, these data support the concept that EMT events lead to tumor invasion and metastasis and decreased survival among patients with any of a multitude of solid tumor malignancies.

Currently, the working model suggests that EMT events allow malignant cells to disseminate throughout the body. The histology of established tumor metastases recapitulates the tissues of origin, however, rather than cells with a mesenchymal phenotype. Models of EMT as a prominent mechanism of metastasis suggest that migratory mesenchymal cells are able to revert back to an epithelial form through a process of mesenchymal to epithelial transition (MET), which could explain the epithelial morphology of cells in metastatic foci of lung cancers and other carcinomas (Figure 1). Ongoing studies will be required to determine whether the EMT cells are actually the cells responsible