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Epithelial-Mesenchymal Transitions: Twist in Development and Metastasis

Minireview

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Epithelial-mesenchymal transitions (EMT) are vital for morphogenesis during embryonic development and are also implicated in the conversion of early stage tumors into invasive malignancies. Several key inducers of EMT are transcription factors that repress *E-cadherin* expression. A recent report in *Cell* (Yang et al., 2004) adds Twist to this list and links EMT to the ability of breast cancer cells to enter the circulation and seed metastases.

Tumors are often viewed as corrupt forms of normal developmental processes. Indeed, genes that are important in embryonic development are frequently found to be culprits in cancer. Conversely, genes discovered for their oncogenic role are often found to be key players in embryogenesis. This trend applies to the steps that initiate tumor formation. It also applies to the steps that mediate tumor progression, including local invasion, spread through the circulation and, most devastatingly, metastasis. In this context, EMT, a process first appreciated by developmental biologists, is attracting increasing attention from oncologists. Genes implicated in EMT during embryogenesis are turning up, one after the other, in tumorigenesis. The recent addition of *Twist* to this list (Yang et al., 2004) provides not only a new player but also some of the best evidence to date for a specific role of EMT in the movement of cells from a primary tumor into the circulation.

EMT is a process whereby epithelial cell layers lose polarity and cell-cell contacts and undergo a dramatic remodeling of the cytoskeleton (Thiery, 2002). Concurrent with a loss of epithelial cell adhesion and cytoskeletal components, cells undergoing EMT acquire expression of mesenchymal components and manifest a migratory phenotype. This type of conversion occurs during many critical steps of metazoan embryogenesis. The morphogenic movements underlying gastrulation and the subsequent formation of various tissues and organs such as the neural crest, heart, musculoskeletal system, craniofacial structures, and peripheral nervous system all rely on EMT. EMT is also implicated in tissue repair in the adult.

EMT was recognized as a feature of embryogenesis in the early 1980s (reviewed in Thiery [2002]). Before the decade was over, EMT had been proposed to be relevant in cancer as well. This idea was initially met with skepticism. Pathologists could not say conclusively that EMT was apparent in human tumor samples. Today, however, a growing body of evidence strongly suggests that EMT is an important, if transient, event in the progression of many carcinomas, and it may be a permanent feature in tumors that have a mixed carcinoma/sarcoma appearance (Thiery, 2002).

A hallmark of EMT is the loss of *E-cadherin* expression. E-cadherin is a central component of cell-cell adhesion junctions and is required for the formation of epithelia in the embryo and to maintain epithelial homeostasis in the adult. Loss of E-cadherin is consistently observed at sites of EMT during development and cancer. This loss increases tumor cell invasiveness in vitro and contributes to the transition of adenoma to carcinoma in animal models (Thiery, 2002). The expression level of E-cadherin often is inversely correlated with tumor grade and stage. Furthermore, inactivating mutations in *E-cadherin* have been found in about 50% of breast lobular carcinomas. Individuals who inherit one mutant allele of *E-cadherin* are at high risk of developing diffuse gastric carcinoma. By these criteria at least, *E-cadherin* is a tumor suppressor gene. Although mutational inactivation of the second allele has not been observed, E-cadherin expression is frequently inhibited by various epigenetic mechanisms (Thiery, 2002). Promoter hypermethylation accounts for the loss of *E-cadherin* expression in some tumors cells. In many other cases, *E-cadherin* expression is inhibited by transcriptional repression.

Several developmentally important genes that induce EMT have been shown to act as *E-cadherin* repressors. The first of these is the zinc finger protein Snail, a DNA binding factor that recognizes E box motifs in target promoters, including the *E-cadherin* promoter (reviewed in Nieto [2002]). In *Drosophila*, *Snail* is specifically expressed in invaginating mesodermal cells just before they undergo EMT. *Snail* null flies have severe defects in mesoderm formation. In vertebrates, *Snail* and the closely related gene *Slug* are essential for gastrulation and neural crest emergence from the neural tube. Snail has been shown to be a transcriptional repressor of *E-cadherin* and a mediator of EMT in mouse and human invasive carcinoma cells (Nieto, 2002). Another repressor of *E-cadherin* and inducer of EMT is the zinc finger protein SIP1 (ZEB-2) (Comijn et al., 2001). SIP1 and Snail bind to partly overlapping E boxes in the *E-cadherin* promoter, with similar repressor effects. SIP1 is highly expressed in several E-cadherin-deficient human carcinoma cell lines. When overexpressed, Snail and SIP1 abrogate E-cadherin-mediated intercellular adhesion and promote tumor cell invasion. The findings of Yang et al. (2004) add Twist to this class of *E-cadherin* repressors and inducers of EMT. Furthermore, their findings implicate Twist in tumor cell intravasation, or entry into the circulation to seed metastases.

Metastasis is thought to be a multistep process requiring the concerted actions of multiple genes. Specific genes allow tumor cells to overcome barriers to local invasion, intravasation, survival in circulation, arrest in capillaries, extravasation, and finally outgrowth to produce macrometastases at distant organs (Chambers et al., 2002; Fidler, 2003). Mouse models coupled with tran-

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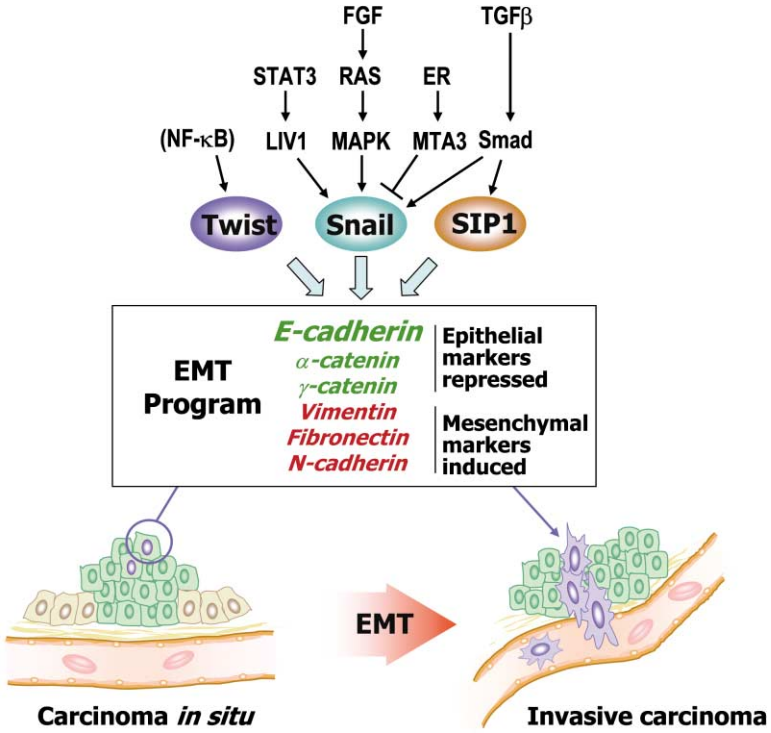

Figure 1. Drivers and Mediators of EMT

Early stage tumor cells (green) maintain epithelial properties similar to the neighboring normal epithelium (brown). The accidental overexpression of master regulators of EMT, such as Twist, Snail, and SIP1, in cancer cells (shown with purple nuclei) leads to dramatic changes in gene expression profile and cellular behavior. Twist, Snail, and SIP1 repress the expression of *E-cadherin* via E boxes in its promoter and trigger expression of an entire EMT transcriptional program through as yet unknown mechanisms. Several pathways are known to regulate Twist, Snail, and SIP1 expression in tumor cells while others (shown in parentheses) do so at least in developmental contexts.

scriptomic profiling technology are proving instrumental in identifying metastasis genes for each of these steps. Using mice as a "cell sorter" to select for cells with high metastatic ability, it has been possible to identify genes that enhance lung metastasis by mouse melanoma cells (Clark et al., 2000) and a set of genes that mediate metastasis to the bone by human breast cancer (Kang et al., 2003). These studies were designed to screen for genes that facilitate the formation of metastases to these organs by tumor cells inoculated directly into the circulation, not genes that would facilitate the entrance of tumor cells into the circulation in the first place.

Yang et al. (2004) sought to identify genes in the latter class by profiling gene expression in a series of isogenic tumor cell lines previously derived from a spontaneous mouse breast tumor. These cell lines, when implanted orthotopically in the mammary fat pads of syngeneic mice, had different abilities to generate lung metastases. Among the genes overexpressed in the metastatic tumors, transcriptional factor *Twist* was chosen for detailed study. Through a series of in vivo and in vitro experiments, Twist was shown to specifically enhance the intravasation step of metastasis, while having no significant effect on the survival, extravasation, or growth rate of the tumor cells. Elimination of *Twist* expression by RNAi in highly metastatic cells dramatically decreased the efficiency of lung metastasis from the mammary gland and specifically decreased the number of tumor cells that could be recovered from the circulation. No decrease was observed in the ability of Twist-depleted cells to grow in suspension, arguing that Twist confers intravasation capacity rather than resistance to cell death by suspension.

Clues about the mechanism mediating these effects of Twist came from its role in development. In multicellular organisms ranging from *Drosophila* to vertebrates, Twist acts as a basic helix-loop-helix transcription factor in a signaling cascade that initiates mesoderm development during gastrulation (Castanon and Baylies, 2002). Throughout this process, the invaginating precursors of the mesoderm lose their epithelial characteristics and gain mesenchymal properties, dispersing and spreading out along the ectoderm to form the mesoderm layer. This epithelial to mesenchymal switch has the features of classic EMT. Indeed, Yang et al. demonstrated that ectopic expression of Twist potently induces EMT in dog kidney epithelial cells and immortalized human mammary epithelial cells in culture. The morphological transition of these cells from an epithelial to a fibroblastic appearance was accompanied by scattering and directional migration toward serum factors, a gain of mesenchymal cell markers (fibronectin, vimentin, smooth muscle actin, and N-cadherin), and a loss of epithelial markers (E-cadherin, and α- and γ-catenin) (Yang et al., 2004) (Figure 1). Twist represses transcription from the *E-cadherin* promoter via the E boxes that are also targeted by Snail and SIP1 (Comijn et al., 2001; Nieto, 2002). Twist does not induce Snail expression (Yang et al., 2004), but it remains to be determined if the interaction of Twist with these E boxes is direct or through a mediator.

Downregulation of E-cadherin alone is not sufficient for EMT, as ectopic expression of E-cadherin could not restore the epithelial phenotype in cells overexpressing Twist (Thiery, 2002; Yang et al., 2004). Indeed, loss of E-cadherin expression and E-cadherin-mediated cell adhesion alone do not constitute EMT. The EMT program also includes the acquisition of mesenchymal functions. It will be important to identify in the future the other key downstream targets of Twist that are essential for initiating EMT and keeping cells in that state

afterwards. Identification of these genes could lead to development of new tumor markers as well as new molecular targets for anticancer drug development.

During embryonic development, Twist and other master regulators of EMT are expressed under strict spatial-temporal control (Castanon and Baylies, 2002). How *Twist* is activated during tumorgenesis in mouse tumors and human cancers remains unknown. The identification of upstream activators of Twist could provide valuable information about additional factors that may be important in tumor progression. HGF/SF (hepatocyte growth factor/scatter factor), FGF (fibroblast growth factor), and EGF (epithelial growth factor), acting through RAS and the mitogen-activated protein kinase (MAPK) pathway or phosphoinositide 3-kinase (PI3K) pathway, can potently induce EMT in certain epithelial cells in culture (Grunert et al., 2003; Thiery, 2002) (Figure 1). The TGFβ (transforming growth factor β)-SMAD pathway synergizes with RAS to induce EMT in culture and metastasis in mouse models. Remarkably, most of these signals converge on Snail expression (Nieto, 2002). The TGFβ-SMAD pathway additionally activates SIP1 expression independently of its effect on Snail (Thiery, 2002). In adult mammary epithelium, Snail is repressed by MTA3 (metastasis-associated gene 3), a Mi-2/NuRD histone deacetylase subunit (Fujita et al., 2003), which is induced by estrogen signaling. Loss of MTA3 function in estrogen receptor (ER) negative cancer cells results in EMT and metastatic progression. And recently, the zinc transporter LIV1 has been reported to mediate STAT3-dependent activation of Snail during gastrulation in zebrafish (Yamashita et al., 2004). Both LIV1 and MTA3 had been implicated in metastatic progression of breast cancer before their role in controlling Snail expression and EMT was discovered (Fujita et al., 2003; Yamashita et al., 2004). This exemplifies, yet again, the convergence of studies on development and cancer.

Some of these pathways might also participate in the activation of Twist expression. In *Drosophila*, however, *Twist* is a downstream target of Dorsal, a NF-κB-like transcription factor (Castanon and Baylies, 2002), and this connection is also present in vertebrates (Sosic et al., 2003). NF-κB activation in response to inflammatory cytokines and growth factors is frequently observed in metastatic breast cancer cells. NF-κB has been shown to be essential for EMT and metastasis in a model of breast cancer progression (Huber et al., 2004). One wonders whether the elevated expression of Twist in these cells is a response to a hyperactive NF-κB pathway.

Thus, *Twist* appears to be a bona fide metastasis gene that specifically promotes tumor cell metastasis with no apparent benefit to the growth of the primary tumor. Similarly, *Snail* promotes tumor progression (Nieto, 2002) but attenuates proliferation, rather than promoting growth (Vega et al., 2004). Arguments that no such genes may exist (Bernards and Weinberg, 2002) therefore seem muted by the findings of Yang et al. (2004) and those of others. Overexpression of *Twist*, *Snail*, and *SIP1* correlates with loss of *E-cadherin* expression and gain of *N-cadherin* in human gastric cancer (Rosivatz et al., 2002). An inverse correlation between *Twist* and *E-cadherin* expression is also observed in invasive lobular carcinomas (Yang et al., 2004). On the other hand, poor-prognosis gene expression signatures identified by large-scale trancriptomic profiling of human tumor samples have not yielded genes known to be involved in EMT (Ramaswamy et al., 2003; van't Veer et al., 2002). A possible explanation for this is that EMT may be a transient process that affects only a small fraction of the tumor cell population at any given time. Therefore, the transcriptomic contribution of such a population would be diluted by the whole. Carcinoma metastases often present an epithelioid phenotype, raising the possibility that metastatic tumors regain this phenotype after they reach a target organ. Furthermore, carcinoma cells that have undergone EMT may be misidentified as stromal components and excluded from analyses (Petersen et al., 2003). Further studies including immunohistochemical detection of EMT master regulators like Twist in the invasive front of human tumor samples may help establish the true magnitude of the EMT problem in cancer.

Selected Reading

Bernards, R., and Weinberg, R.A. (2002). Nature *418*, 823.

Castanon, I., and Baylies, M.K. (2002). Gene *287*, 11–22.

Chambers, A.F., Groom, A.C., and MacDonald, I.C. (2002). Nat. Rev. Cancer *2*, 563–572.

Clark, E.A., Golub, T.R., Lander, E.S., and Hynes, R.O. (2000). Nature *406*, 532–535.

Comijn, J., Berx, G., Vermassen, P., Verschueren, K., van Grunsven, L., Bruyneel, E., Mareel, M., Huylebroeck, D., and van Roy, F. (2001). Mol. Cell *7*, 1267–1278.

Fidler, I.J. (2003). Nat. Rev. Cancer *3*, 453–458.

Fujita, N., Jaye, D.L., Kajita, M., Geigerman, C., Moreno, C.S., and Wade, P.A. (2003). Cell *113*, 207–219.

Grunert, S., Jechlinger, M., and Beug, H. (2003). Nat. Rev. Mol. Cell Biol. *4*, 657–665.

Huber, M.A., Azoitei, N., Baumann, B., Grunert, S., Sommer, A., Pehamberger, H., Kraut, N., Beug, H., and Wirth, T. (2004). J. Clin. Invest., in press.

Kang, Y., Siegel, P.M., Shu, W., Drobnjak, M., Kakonen, S.M., Cordon-Cardo, C., Guise, T.A., and Massagué, J. (2003). Cancer Cell *3*, 537–549.

Nieto, M.A. (2002). Nat. Rev. Mol. Cell Biol. *3*, 155–166.

Petersen, O.W., Nielsen, H.L., Gudjonsson, T., Villadsen, R., Rank, F., Niebuhr, E., Bissell, M.J., and Ronnov-Jessen, L. (2003). Am. J. Pathol. *162*, 391–402.

Ramaswamy, S., Ross, K.N., Lander, E.S., and Golub, T.R. (2003). Nat. Genet. *33*, 49–54.

Rosivatz, E., Becker, I., Specht, K., Fricke, E., Luber, B., Busch, R., Hofler, H., and Becker, K.F. (2002). Am. J. Pathol. *161*, 1881–1891.

Sosic, D., Richardson, J.A., Yu, K., Ornitz, D.M., and Olson, E.N. (2003). Cell *112*, 169–180.

Thiery, J.P. (2002). Nat. Rev. Cancer *2*, 442–454.

van't Veer, L.J., Dai, H., van de Vijver, M.J., He, Y.D., Hart, A.A., Mao, M., Peterse, H.L., van der Kooy, K., Marton, M.J., Witteveen, A.T., et al. (2002). Nature *415*, 530–536.

Vega, S., Morales, A.V., Ocana, O.H., Valdes, F., Fabregat, I., and Nieto, M.A. (2004). Genes Dev. *18*, 1131–1143.

Yamashita, S., Miyagi, C., Fukada, T., Kagara, N., Che, Y.S., and Hirano, T. (2004). Nature *429*, 298–302.

Yang, J., Mani, S.A., Donaher, J.L., Ramaswamy, S., Itzykson, R.A., Come, C., Savagner, P., Gitelman, I., Richardson, A., and Weinberg, R.A. (2004). Cell *117*, 927–939.