Exploring a New Twist on Tumor Metastasis

Jing Yang, Sendurai A. Mani, and Robert A. Weinberg

Abstract

Unraveling the genetic programs that drive metastasis may offer insights into how to limit or prevent this deadly aspect of cancer progression. Our recent studies indicate that tumor cell metastasis involves the activity of the transcription factor, Twist, which regulates epithelial-mesenchymal transition and early embryonic morphogenesis. Here, we review the Twist signaling pathway during normal development and discuss how the transcription factor Twist and the epithelial-mesenchymal transition program impinge their biological functions during tumor metastasis. (Cancer Res 2006; 66(9): 4549-52)

Introduction

Metastasis is a complex, multistep process, during which tumor cells spread from the primary tumor mass to distant organs. The metastasis cascade is thought to consist of the following steps: local invasion, intravasation into the systemic circulation, survival during the transport, extravasation, and establishment of micrometastases in distant organs, and colonization of macroscopic metastases. Because metastasis occurs at the late stage during tumor progression, genetic instability and the resulting heterogeneity within the tumor cell population can mask the identity of the small number of metastatic carcinoma cells. Extensive interactions between tumor cells and surrounding tissues during their dissemination further complicate the efforts to dissect the signaling events during this cascade. Because of the complex nature of tumor metastasis, our current understanding of the process remains very descriptive. The most pressing questions in metastasis are to pinpoint the rate-limiting steps during metastasis and to define the genetic and epigenetic changes conferring such behaviors.

To address these issues, it is important to use experimental metastasis models that can recapitulate, at least in outline, the physiologic and pathologic conditions observed in human cancer patients. In the past, extensive effort has been devoted to introduce human metastatic carcinoma cells into mice. Surprisingly, although most such cell lines were isolated from metastases in human patients, they rarely spread from the implanted primary tissues (such as the s.c. site, the mammary gland, and the prostate) to distant organs in mice. As a result, most popular experimental mouse metastasis models rely on the introduction of tumor cells directly into the systemic circulation (such as the tail vein, portal vein, or by intracardiac injection) to establish metastases in distant organs. These approaches bypass some initial steps of metastasis, including local invasion and intravasation. In addition, simultaneous injection of large numbers of tumor cells into the circulation may not fully mimic the gradual, low-level dissemination of carcinoma cells in vivo.

Gene Expression Profiles Associated with Metastatic Progression

Given the above considerations, we choose to explore a mouse mammary tumor metastasis model. This system consists of four mouse mammary tumor cell lines, which were originally derived from a single spontaneous BALB/c mouse mammary tumor. Although all four derived cell lines could form primary tumors at very similar rates when implanted in mouse mammary glands, they are capable, to differing extents, of completing various steps of the invasion-metastasis cascade (1). In this system, implantation of breast tumor cells in their orthotopic organ—the mammary gland allows one to examine the entire metastasis process in vivo.

We took an unbiased gene expression profile approach to search for the signaling molecules involved in metastasis in this mouse mammary tumor metastasis system. By comparing the gene expression profiles of primary tumors derived from the four cell lines, we identified a list of specific genetic and epigenetic changes associated with their respective metastatic abilities. Several of the candidate genes identified are known players during tumor progression, and importantly, their activation indicates that the corresponding biological pathways might contribute to tumor metastasis. For example, the expression of CXCR3, a member of the CXCR chemokine receptor family, is associated with increased metastatic abilities, suggesting that the activation of chemokine signaling is essential to elicit chemotactic and invasive responses in carcinoma cells (2). Higher levels of matrix metalloproteinase-9 in metastatic cells indicates that remodeling and degradation of the extracellular matrix can promote tumor invasion and metastasis (3). Up-regulation of MENA, a protein of the Ena/VASP family that controls cell motility (4), highlights the importance of activation of cellular migratory pathways in metastatic tumor cells.

Identification of the Transcription Factor Twist as a Key Regulator of Tumor Metastasis and an Inducer of Epithelial-Mesenchymal Transition

One of the most differentially expressed genes on the list is the Twist transcription factor, which regulates cell movement and tissue reorganization during early embryogenesis. We found that suppression of Twist expression in highly aggressive 4T1 mammary carcinoma cells specifically inhibits their ability to metastasize from the mammary gland to the lung (5). In contrast, the ability of these cells to form primary mammary tumors was not affected. Furthermore, loss of Twist expression hindered metastatic tumor cells from intravasating into the blood circulation. These results showed that Twist plays an essential role in the efficient execution of several steps of the invasion-metastasis cascade by such tumor cells.

Like other known players in tumorigenesis, Twist is most likely to exert the same biological activities during tumor metastasis as it does during normal development. The Twist gene was originally identified as being required for mesoderm induction in Drosophila (6, 7). In vertebrates, Twist is predominantly expressed in neural
crest cells. Twist mutation in mice causes failure in cranial neural tube closure, indicating its role in proper migration and differentiation of neural crest and head mesenchymal cells (8, 9). Both mesoderm formation and neural crest development engages a cellular event termed the epithelial-mesenchymal transition (EMT), which involves the conversion of a sheet of tightly attached epithelial cells into highly mobile mesenchymal or neural crest cells. We examined the ability of Twist to induce EMT by

Figure 1. A reversible EMT model in tumor metastasis. Upon expression of the Twist transcription factor, carcinoma cells activate the EMT program to achieve local invasion and dissemination into the systemic circulation. Once distant organs are reached, these mesenchymal tumor cells reverse to an epithelial identity via mesenchymal-epithelial transition (MET) to regain the ability to proliferate.
expressing Twist in kidney and mammary epithelial cells. Indeed, ectopic expression of Twist resulted in the loss of E-cadherin-mediated cell-cell adhesion, activation of mesenchymal markers, and gain of cell motility (5). These results indicate that Twist can contribute to invasion and metastasis by promoting this latent EMT developmental program.

Our finding that Twist can induce EMT to promote tumor metastasis does not exclude the possibility that Twist might also activate additional cellular functions during tumor progression. Two studies reported that the expression of Twist could inhibit Myc-induced apoptosis in mouse embryo fibroblasts (10) and neuroblastoma cells (11). Because Twist, functioning as a basic helix-loop-helix transcription factor, could homodimerize or heterodimerize with other basic helix-loop-helix proteins, it may activate or suppress diverse downstream targets including apoptosis genes. For example, loss of cell-cell adhesions by epithelial cells normally results in apoptosis. During EMT, Twist may need to activate antiapoptotic programs in order to allow epithelial cells to convert to a mesenchymal fate while avoiding cell death.

The Involvement of Twist in Malignant Human Tumors

We examined the expression of Twist mRNA by microarray in a group of invasive human breast tumors of three subtypes, specifically ductal carcinoma, lobular carcinoma, and mixed ductal/lobular type. Twist expression was highest in the group of invasive lobular carcinomas (5). This observation is very intriguing because invasive lobular carcinoma cells exhibit many similarities with cells that have undergone an EMT induced by Twist. Invasive lobular carcinoma is composed of single tumor cells or single-file rows of cells that have lost the typical epithelial tissue structures found in mammary ducts and in ductal carcinomas. More interestingly, in gastric cancers, Twist expression was shown to be specifically up-regulated in the diffuse-type of gastric cancer, when compared with the intestinal type (12). The histopathologic similarity and the E-cadherin loss in both lobular carcinoma and diffuse-type gastric cancer suggest that Twist may contribute to the infiltrative growth and loss of E-cadherin expression in both types of carcinomas. Previous studies showed that inactivating mutation (13) and promoter methylation of the E-cadherin gene (14) contributed to E-cadherin loss in a number of lobular breast cancers and diffuse-type gastric cancers. In order to understand the multiple causes of E-cadherin down-regulation in these two tumor types, a comprehensive study is needed to examine E-cadherin RNA and protein expression, inactivating mutation, loss of heterozygosity at the E-cadherin locus, promoter methylation, and transcriptional suppression by Twist concurrently in individual tumors.

In the past year, several studies have also reported the expression of Twist in other human tumor types. High Twist expression was observed in metastatic melanoma and can serve as an independent marker to predict poor outcome in melanoma patients (15). Up-regulation of Twist was also shown to be associated with the more aggressive subtype of neuroblastomas with N-Myc amplification (11). Both melanoma and neuroblastoma are directly derived from neural crest cells. Because in vertebrates Twist functions primarily in the neural crest, it is conceivable that melanoma and neuroblastoma are very capable of reactivating Twist to achieve motility and invasiveness.

The Twist Signaling Pathway during Normal Development and Tumor Metastasis

Given the importance of Twist expression in human tumors, one key question to be answered is how this developmental program is reactivated in cancer cells. The regulatory networks controlling Twist expression during embryogenesis might give us some clues. In Drosophila embryos, Twist expression is induced by an interleukin-1-like TOLL receptor through nuclear factor κB activation during mesoderm formation (16). In vertebrates, Twist expression in cranial neural crest tissues is essential for correct patterning of the neural tube (8, 9). The nuclear factor κB pathway has not been shown to be involved in mesoderm formation or neural crest development in vertebrates. Instead, the BMP, Wnt, and fibroblast growth factor pathways are known to modulate vertebrate neural crest development (17), therefore, they are potential inducing signals of Twist expression in carcinoma cells.

In addition to Twist, several proteins, including transforming growth factor-β (18) and the transcription factors Snail (19, 20), Slug (21), and Sip1 (22), have also been shown to induce the EMT program. Are these EMT-inducing signals acting individually or in a cooperative fashion to promote the EMT program? Again, their molecular actions during normal embryogenesis sheds some light. During mesoderm formation in Drosophila, Twist induces the expression of the transcription factor Snail to allow invagination and mesoderm differentiation (7). During neural crest development in vertebrates, expression of Snail and Slug occurs at the neural plate border where Twist is also expressed, and all three transcription factors play critical roles in neural crest formation (17). Based on these developmental data, it is very plausible that a number of such EMT-promoting molecules may act together as an EMT signaling network to invoke tumor invasion and metastasis.

EMT and Tumor Metastasis

A reversible EMT model has been proposed to describe the dynamic changes that carcinoma cells experience during tumor metastasis. In this model, carcinoma cells undergo EMT to achieve local invasion and dissemination to distant organs. Once those organs are reached, these mesenchymal cells may need to reverse to an epithelial identity via a mesenchymal-epithelial transition in order to regain the ability to proliferate (Fig. 1).

Expression of several EMT-inducing genes, including TGF-β (18) and the transcription factors Snail (23), Slug (21), and Twist, have been shown to be up-regulated at the mRNA level in more aggressive human tumors. However, it remains a challenge to observe EMT in human carcinomas. One major difficulty is caused by the transient, reversible nature of EMT during carcinoma invasion and metastasis. Because only a small minority of carcinoma cells may be invasive and undergo an EMT in primary tumors, the alteration of gene expression in such cells can be masked by the bulk of nonmetastatic cells. In the case of Twist, however, we and others have been able to successfully detect Twist mRNA up-regulation in invasive lobular breast tumors (5) and the diffuse-type gastric cancers (12), in which the entire population of carcinoma cells undergo an essentially permanent EMT. It is very likely that Twist is also induced in a small number of invading cells in invasive ductal carcinomas and intestinal-type gastric cancers. Detecting such transient cells with sensitive antibodies will be critical to assess the contribution of EMT to the behavior of high-grade carcinomas. Indeed, increased expression of Twist and Slug...
was recently reported to be associated with breast cancer patients with poor survival and occurrence of distant metastases (24).

Another major challenge in such studies is to identify reliable molecular markers to define cells that are undergoing EMT in human tumors. Currently, the most commonly used markers for EMT are adherens junction proteins and mesenchymal markers such as vimentin. To date, the best report of EMT in human cancers is the detection of EMT cells at the invading front of colorectal carcinomas using loss of E-cadherin expression and nuclear β-catenin as markers (25). Because the main consequence of EMT is the acquisition of invasiveness and motility, it will be very useful to score EMT through the presence of motility-associated genes that are activated in human cancers. Indeed, the identification of the downstream effector pathways that are activated during EMT holds the promise of revealing new diagnostic markers of advanced malignancy and, quite possibly, novel targets for antimetastasis therapeutics.

Acknowledgments

Received 10/24/2005; revised 2/8/2006; accepted 3/3/2006.

We apologize to our many colleagues whose work cannot be cited due to space limitations. J. Yang is supported by a NIH NRSA fellowship (1 F32 CA101507). R.A. Weinberg is an American Cancer Society Research Professor and a Daniel K. Ludwig Cancer Research Professor.

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Cancer Res 2006; 66: (9). May 1, 2006

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