ISSN 0025-7680

International Symposium
NEW DIRECTIONS IN CANCER MANAGEMENT
Academia Nacional de Medicina
Buenos Aires, 6-8 June 2007

MEDICINA (Buenos Aires) 2007; 67 (Supl. II): 3-5

KEYNOTE ADDRESS

DETERMINANTS OF INVASION AND METASTASIS

ROBERT A. WEINBERG

Whitehead Institute for Biomedical Research; Department of Biology, M.I.T., Cambridge MA, USA

The process of multi-step tumorigenesis, itself quite complicated, often ends with high-grade malignancies, more specifically the growths that invade locally and metastasize to distant sites. The mechanisms underlying the processes of invasion and metastasis have, until recently, been relatively obscure. However, over the past five years, a variety of experimental procedures have shed new lights on the biological and genetic determinants that govern whether invasion and metastasis occur.

In our laboratory, we have been interested in developing experimental models of breast cancer in which the genetic elements responsible for creating the cancer cells have been well defined. To do so, we developed a new type of cell culture medium, termed WIT, that allows the outgrowth of normal human mammary epithelial cells (MECs) that have a more luminal phenotype that contrasts with the more basal phenotype associated with normal human MECs that are cultured in standard media. When these cells were transformed through the introduction of a defined set of genes (*hTERT,* SV40 early region, *ras*), they yielded invasive ductal carcinomas, in contrast to the squamous cell carcinomas that arose when the identical set of genes was introduced into human MECs propagated in the standard medium.

Importantly, the transformed human MECs derived from the WIT medium generated metastases when implanted in a subcutaneous site in host mice, while the transformed MECs derived from the standard medium did not. This provided one indication of an important determinant of metastasis: the differentiation program of the normal cell-of-origin that existed prior to the transformation event. While most depictions of invasion and metastasis imply that these phenotypes are acquired as a consequence of events occurring late in multi-step tumor progression, this evidence indicates that metastasis is already strongly influenced by processes occurring before the onset of transformation.

Additional evidence of important determinants of invasion-metastasis came from comparing the gene expression profiles of a series of mouse mammary carcinoma cell lines that had been derived by others. One of these formed primary tumors that did not complete the initial step of the invasion-metastasis cascade –local invasion and entrance of cells into the circulation, the latter step being called “intravasation”. Another variant cell line released cells into the circulation, but these cells were unable to escape from the circulation and invade into nearby tissue parenchyma –the step termed “extravasation”. Yet another formed micrometastic deposits in distant tissues but these did not grow into macroscopic metastases –the step termed “colonization”. A fourth cell line completed all of these steps.

When the gene expression profiles of these four lines were compared, one gene, termed Twist, stood out as being overexpressed in the more malignant variant cancer cells. Twist was of special interest because of its involvement in an early step of *Drosophila* embryogenesis. More specifically, Twist is involved in specifying the invasion of cells from the ectoderm into the interior of the embryo, this being associated with the process of gastrulation. Such movement requires the embryonic cells to undergo an epithelial-mesenchymal transition (EMT), in which they shed their epithelial markers, such as E-cadherin

- - - -
e-mail: weinberg@wi.mit.edu

and cytokeratins, and assume instead mesenchymal markers, such as N-cadherin, fibronectin, and vimentin. Indeed, when we expressed Twist in mammalian epithelial cells, they underwent an EMT.

The EMT imparts to cancer cells many of the traits of cells associated with high-grade malignancies. This caused us to determine whether the highly metastatic mouse breast cancer cells that expressed Twist depended on Twist for their metastatic powers. Indeed, when Twist was shut down in these cells through the use of an siRNA, their proliferation *in vitro* and *in vivo* as primary tumors was unaffected. However, their metastatic powers, as represented by metastases in the lungs, were reduced by 85%, and those metastases that continued to form all exhibited ongoing Twist expression. Together, these experiments indicated that Twist was required in order for these mouse breast cancer cells to metastasize. They did not prove, however, that Twist expression was sufficient, on its own, to enable invasion and metastasis in previously non-metastastic cancer cells –a point that remains unproven.

In fact, Twist, as well as other similarly acting EMT-inducing transcription factors, can impart cell motility, invasiveness, and increased resistance to apoptosis. These are precisely the cell-biological traits that cancer cells require in order to execute all of the steps of the invasion-metastasis cascade outlined above except for one: the last step of colonization. This provides an important insight into how the last steps of tumor progression occur. Rather than requiring an extensive series of additional mutations, cancer cells can acquire many of the traits required for metastatic dissemination simply by activating a pleiotropically acting transcription factor like Twist. Hence, such cancer cells can act opportunistically to reactive a normally latent embryonic program.

Another important insight came from our study of the histopathology of the engrafted, genetically engineered invasive ductal carcinoma cells described above. When these xenografts were stained for cytokeratin and human vimentin, we discovered a striking pattern of gene expression. The carcinoma cells in the interior of epithelial cell island expressed cytokeratins, as is expected of such cells. However, the cells in the outer perimeters of these islands, which were in direct contact with the adjacent host stroma, had shut down cytokeratin expression and instead expressed vimentin. This could be observed in many of the islands of carcinoma cells. Such behavior taught us yet another lesson: that carcinoma cells can be provoked to undergo an EMT by contextual signals that they receive from their microenvironment, specifically from the stromal cells that they have recruited into their midst.

The nature of these heterotypic signals is still obscure. Nonetheless, this phenomenon indicates that activation of the EMT does not necessarily require any additional mutations beyond those that cancer cells have acquired during their evolution to form primary tumors. Presumably, the differentiation programs of their cells-of-origin (see above) together with acquired somatic mutations are the factors that determine whether or not carcinoma cells will be responsive to heterotypic signaling from the nearby tumor-associated stroma.

In related work, we investigated the actions of another embryonic transcription factor termed Goosecoid, which is involved in the Spemann organizer at the site of the blastopore lip of the *Xenopus laevis* embryo. It too programs an EMT when ectopically expressed in mammalian epithelial cells. When ectopically expressed in weakly metastatic human breast cancer cells, it can strongly enhance their metastatic powers.

Our attempts at transforming various types of human cells revealed that, in general, such transformation yielded cells that were strongly tumorigenic. Nonetheless, such cells were weakly metastatic or not metastatic at all. An exception came from transforming normal human melanocytes with the identical set of introduced genetic elements (see above). These cells also formed nicely growing primary tumors at subcutaneous sites of implantation. However, when the internal organs of tumor-bearing mice were observed, they were found to contain hundreds of metastatic deposits. This reinforced a lesson learned from study of the experimental transformed human breast cancer cells. Once again, the differentiation

program of the normal cell-of-origin is a strong determinant of the eventual metastatic powers of cells following their transformation.

Melanocytes originate in the primitive neural crest and undergo an EMT as they delaminate from the crest and subsequently disperse throughout the body of the embryo. They are known to depend on the Slug transcription factor to undertake this dispersion. We found that the transformed melanocytes expressed Slug at levels 1,000-fold higher than transformed MECs. When Slug was shut down in the experimental melanomas, they lost >95% of their metastatic powers. This shows directly that the metastatic powers of these transformed melanocytes, and by extension of naturally arising melanoms, derive from reactivating a transcription factor that is involved in allowing the embryonic precursors of melanocytes to disseminate throughout the body.

The expression array screen that identified Twist yielded a second embryonic transcription factor, termed FOXC2, which is normally involved in programming mesenchymal cell fate in the embryo. Unlike other EMT-inducing embryonic transcription factors, FOXC2 is rather weak at repressing epithelial cell markers, while it is very potent at inducing mesenchymal gene expression. Significantly, FOXC2 expression is induced by other transcription factors, such as Twist and the Slug-related Snail transcription factor. This suggests another dimension of complexity: transcription factors like Twist and Snail repress epithelial gene expression and induce FOXC2 expression in order to elicit the mesenchymal portion of the EMT program.

Lately we have discovered yet another apparent determinant of metastasis by implanting strongly and weakly growing transformed human breast cancer cells in opposite (contralateral) flanks of host mice. The growth of the weakly growing tumor cells is strongly stimulated ("instigated") by the distantly located strongly growing tumor cells, an interaction that does not involve the metastatic seeding of the weakly growing tumors by the contralaterally located, vigorously growing tumor cells. This instigation depends on the ability of the vigorously growing tumor cells to activate the bone marrow of the tumor-bearing host. The bone marrow, in turn, responds by releasing various types of mesenchymal cells that are recruited by and that stimulate growth of the otherwise slowly growing tumor cells. This suggests a more general interaction that occurs in tumor-bearing organisms, in which a primary tumor can foster the growth of its metastatic derivatives by activating the bone marrow and mobilizing bone marrow cells that can then be recruited into the stroma of metastases, facilitating their growth.

- - - -

We dance round in a ring and suppose
But the Secret sits in the middle and knows

Bailamos en círculos y presumimos
Pero el Secreto sentado en el medio sabe

Robert Frost (1874-1963)