

CLINICAL UPDATE

Translating Scientific Advances into Clinical Practice

Epithelial-Mesenchymal Transition in Cancer Progression

Andrew J. Armstrong, MD
Department of Medicine
The Duke Prostate Center
Duke Cancer Institute
Duke University Medical Center
Durham, North Carolina

H&O What is epithelial-mesenchymal transition (EMT)?

AA EMT is thought to be a reversible core biologic process related to the ability of an epithelial cell to switch its phenotype to one that is more mesenchymal.¹ This biology has been described in several contexts in normal physiology. One example is found in embryology: cells of an embryo undergo multiple EMTs in order to form organs and allow the embryo to develop. The heart, stomach, liver, skin, and bones are constructed by cells that undergo several phenotypic transitions between epithelial cells and mesenchymal cells during their journey into organ development. This plasticity is a fundamental process of stem-like cells that is very important in early development but also plays an important role in wound healing and in the body's response to injury as well as during cancer progression.²

H&O What is the role of EMT in cancer progression?

AA EMT or epithelial plasticity has emerged as a fundamental process during cancer metastasis in many preclinical cancer models, particularly genetically engineered mouse models. Robert Weinberg, PhD, at the Whitehead Institute for Biomedical Research, has led many of these studies investigating the role of EMT in breast cancer.³ In several preclinical studies, EMT has been linked to the acquisition of stem-like properties in

cancer cells and to the acquisition of metastatic tendencies in these cancer cells. The induction of an EMT, or introduction of the genes that cause an EMT, prompt cancer cells to become more aggressive and spread, as well as to acquire a stem-like state and become resistant to common therapies.⁴ This provides a link between the ability of a cell to convert between epithelial and mesenchymal states and the ability of a cell to have cancer stem-like properties and to be aggressive.

In patients, however, evidence for this association has been lacking, largely because pathologists do not see strong evidence of this transition in the tumors of primary cancers. The metastases of these patients also resemble the original primary tumor, meaning that they do not necessarily appear mesenchymal, but they take on the phenotype of the original primary tumor cells. This is not surprising. EMT is thought to occur at the invasive front of cancer cells—as the cancer cells enter the blood stream—so these cells are very isolated in nature and difficult to appreciate in the primary tumor.

In our recent study published in *Molecular Cancer Research*, my colleagues and I sought to identify the existence of EMT in circulating tumor cells (CTCs) in patients with castration-resistant prostate cancer (CRPC) or metastatic breast cancer.⁵ We thought that the CTC would be an ideal source of tumor tissue in which to investigate whether EMT was important to human cancer.

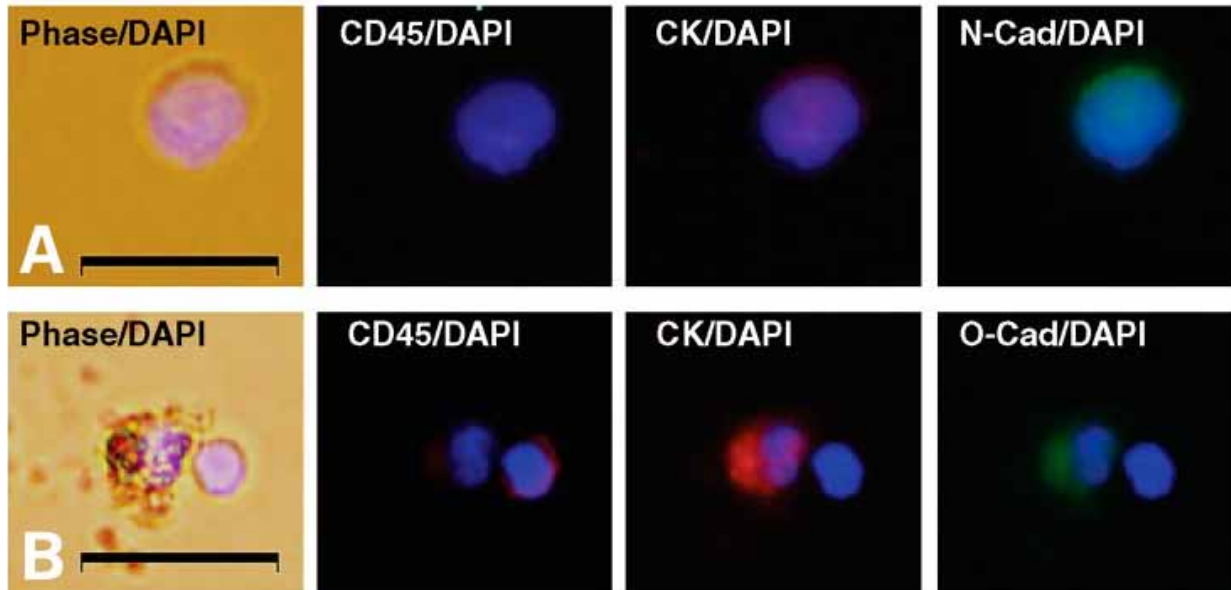


Figure 1. Images of circulating tumor cells (CTCs) from men with metastatic castration-resistant prostate cancer. Row A shows a CTC with N-cadherin (CAD) expression. Row B shows a CTC with O-cadherin expression and a nearby leukocyte. DAPI=4',6-diamidino-2-phenylindole. Reprinted with permission from Armstrong AJ et al. Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers. *Mol Cancer Res.* 2011;9:997-1007.

H&O What was the design of the study?

AA It was a prospective, clinical study that enrolled men with CRPC (n=41) and women with metastatic breast cancer (n=16). All patients had progressive disease. We collected the blood of these patients and tested it with the CellSearch assay (Veridex, LLC), the standard, CTC assay cleared by the US Food and Drug Administration that uses the epithelial cell adhesion molecule (EpCAM) to isolate these cells. We also performed some additional analyses of the CTCs.

CTCs are captured using an antibody ferrofluid, which is an iron-containing antibody that allows separation of epithelial cells from non-epithelial cells in the bloodstream with the use of a magnet. The number of cells found in a tube of blood has been shown to be prognostic and to correlate with survival in many cancer types. One of the interesting things about this process is that these cells are epithelial, which provides the basis for the test. Our hypothesis was that if EMT were important in cancer, there would be other cells that may be more mesenchymal in the spectrum of plasticity, or that the epithelial cells would have a more transitional phenotype and express both epithelial and mesenchymal markers. We profiled the CTCs for a host of other stem cell and mesenchymal markers. The mesenchymal markers included CD133, N-cadherin, O-cadherin, and vimentin. The epithelial markers included EpCAM, cytokeratin, and E-cadherin. Most of these cells lacked CD45 and were not leukocytes.

H&O What were the study results?

AA The majority of the CTCs that were captured using the CellSearch method co-expressed epithelial and mesenchymal markers. In the men, for example, more than 80% of the CTCs co-expressed both epithelial proteins, such as EpCAM and E-cadherin, and mesenchymal proteins, such as vimentin, N-cadherin, and O-cadherin (Figure 1). More than 80% of the men with CRPC also expressed CD133, which has been linked, although controversially, to a prostate cancer stem cell phenotype. In approximately 75% of the women, CTCs also co-expressed these epithelial and mesenchymal markers. Evidence of EMT was found in a high frequency of cells from the patient samples through direct immunofluorescent imaging, which permits the visualization of these proteins as they are co-expressed in an individual cell. These findings support the importance of EMT in patients who have metastatic disease.

H&O What are the clinical implications of the study findings?

AA Although this study does not prove the existence of EMT or plasticity, it provides strong evidence that EMT markers are present during metastatic progression. This study used CTCs to look for these markers, and we think that CTCs are important for seeding

metastasis. Showing that these markers are expressed on these important cells, including the stem cell markers, provides a translational link from the striking preclinical findings in our laboratory, the Weinberg laboratory, and many others. These translational studies were led by both myself and my collaborator, Mariano A. Garcia-Blanco, MD, PhD, a basic scientist, an RNA biologist, and Director of the Duke Center for RNA Biology. This study reflects a unique combination of both preclinical data and observations from basic science, and we think that translation of these findings will have implications for both therapy and technology development.

The primary clinical implication is that the current FDA-cleared CTC test may be missing cells that have undergone a further EMT, in which they have lost the epithelial markers altogether. We have ongoing research plans to modify and further develop the circulating tumor assay so that it can identify a broader variety of CTC phenotypes. This would allow the collection of more cells and a broader phenotypic representation of these cells, which should lead to the identification of important cancer-driving biomarkers and development of therapeutic strategies to kill these cells and prevent them from metastasizing. A limitation of current CTC assays is that they do not find many of these cells, which makes it difficult to perform broad genomic or proteomic profilings. Any improvements on these technologies that would allow isolation of these cells would be helpful.

Many have called this approach a liquid biopsy. It is a minimally invasive, low-risk test; it is just a simple blood draw. But if it allows access to the patient's tumor biology, it may avoid the harms of a metastatic biopsy and may allow profiling of the tumor over time, not just at one point. Tumors change; they are highly plastic. Therapy could be tailored depending on the resistance or sensitiv-

ity of an individual patient, so these findings may have implications for personalized medicine as well.

H&O What are some areas of future research?

AA Ongoing research in our laboratory aims to improve the assay to see if non-epithelial cells really exist. Another area of research is to use this assay in conjunction with other techniques that employ single-cell sequencing to isolate the DNA and RNA proteins in these cells in order to develop personalized therapy. The implications are not just for breast or prostate cancer. These CTCs have been found in almost all epithelial cancers, and this EMT process has been linked to metastatic spread in almost all epithelial cancers. We are interested in broadening this approach to include colon cancer, lung cancer, and other cancer subtypes.

Acknowledgment

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