Epithelial to Mesenchymal Transition in Lethal Forms of Cancer and Fibrotic Disease

By John Leavitt, Ph.D., Nerac Analyst

Over the last two years I’ve paid close attention to the role of epithelial-to-mesenchymal transition (EMT) in pulmonary fibrosis. I found skepticism about the idea that an epithelial cell could become a myofibroblast which caused this disease. If untreated, pulmonary fibrosis proceeds to kill the victim in three to five years, a worse prognosis than most forms of cancer. It turns out that EMT plays a lethal role in cancer as well. This cellular phenomenon was first described by Boyer et al., 1989, at the German Cancer Research Center in Heidelberg, Germany; but interest in EMT grew slowly until recent years. There is now compelling evidence that EMT of epithelial tumor cells (carcinomas) plays an important role in metastasis which is considered to be the main cause of cancer mortality. Physiologically EMT mucks up our anatomical organization whether it be in the form of fibrosis (scarring of organs due to over expression of collagen) or metastasis (invasion to disrupt resident and distant organ function). The main difference that I can see between these two diseases is that the fibroblast-like cells causing fibrosis are not immortalized like the cells arising in tumors; instead, the fibroblast-like cells are continuously replaced by the EMT process.

The figure below shows how EMT research has mushroomed in the last 10 years.

Since the beginning of 2005 the number of journal articles on the relationship of EMT to cancer has grown about 20-fold and in 2014 at least 65 percent of those papers discussed the role of EMT in metastasis or tumor invasiveness. Likewise the role of EMT in pulmonary fibrosis has grown about 13-fold based upon number of research papers published for the same 10-year span. Cancer is a much larger problem and area of investigation under the auspices of the National Cancer Institute (576,685 people—about 1,580 people a day—died of cancer in the United States in 2011) whereas
pulmonary fibrosis, which is almost 100% fatal, causes about 17,000 deaths in the US annually, a statistic that appears to be on the rise.

The plasticity of epithelial cells is well established in that they undergo MET (mesenchymal-to-epithelial transition) when migratory stem cells form epithelial layers in developing organs and then epithelial cells can undergo EMT to later differentiate into multiple cell types (Nieto, 2013). Recent research has also established that primary epithelial tumors undergo EMT to circulate in the blood stream and spread to distant organs during the process of metastasis (Sugimachi et al, 2014).

Both MET and EMT are characterized by dramatic changes in architectural protein expression and rearrangement in the cellular cytoskeleton due to differential expression of intermediate filament proteins (keratins, vimentin, and syncoilin) and the microfilament system (actin isoforms, tropomyosin isoforms, plastin isoforms and other actin binding/regulatory proteins). One common feature of EMT is the induction of the myofibroblast phenotype due to expression of alpha-smooth muscle actin (one of six actin isoforms) and down-regulation of keratin intermediate filaments which are replaced with mesenchymal vimentin-based intermediate filaments. These filamentous architectural proteins are the most abundant proteins of all cells which determine cell shape, motility, and hence cellular function. The architectural changes in EMT are thought to determine at least three major components of the cellular phenotype: invasion and metastasis, stemness and clonogenicity, and resistance towards death stimuli of chemotherapeutic drugs.

The actin filament bundling protein, T-plastin (PLS3), has recently been linked casually to EMT in blood-circulating colon cancer cells. These circulating tumor cells in venous “tumor-drainage” and peripheral blood are selected as the result of increased Xq23 chromosomal copy number leading to over-expression of T-plastin from that locus, an aberration that may lead to enhanced adhesiveness, metastasis, and poor prognosis (Sugimachi et al, 2014). Furthermore, mutations in both L- and T-plastin promote significantly faster re-growth (recurrence) of colon carcinomas following surgical resection of these tumors and chemotherapy (Ning et al, 2014).

On October 15, 2014, Susana Lechuga et al at Virginia Commonwealth University published a truly surprising finding related to EMT of human lung epithelial cells that lends support to the proposal that normal lung epithelium can transform into myofibroblasts which appear to mediate development of pulmonary fibrosis. In this current study, investigators began examining the inhibition of expression using isoform specific siRNAs of either of the two highly abundant cytoplasmic actins, beta- and gamma-actin (expressed at a ratio of 0.55 to 0.45 respectively) in A549 human lung epithelial cells (these acts amount to about 10% of the total cellular protein). Previously, my colleagues and I demonstrated that over-expression of recombinant beta-actin or recombinant gamma-actin in human fibroblasts led to proportionate down-regulation (auto-regulation) of the other actin isoform (Leavitt et al, 1987; Ng et al, 1988). In this current study, investigators were interested in the effect that silencing of expression of either cytoplasmic actin isoform would have on gene expression of other cytoskeletal proteins. The surprising finding was that inhibition of gamma-actin synthesis, but not inhibition of beta-actin synthesis, resulted in induction of alpha-smooth muscle actin, one of two smooth muscle actin isoforms, and a key marker of all myofibroblasts associated with development of organ fibrosis.
Silencing of gamma-actin synthesis in A549 cells led to a dramatic induction of other EMT markers (in addition to alpha-smooth muscle actin) such as SM-22, L-caldesmon, calponin-1, and tropomyosin and these changes were associated with up-regulation of their respective messenger RNAs; beta-actin depletion did not stimulate expression of these contractile/cytoskeletal proteins. This effect was achieved with six different gamma-actin-specific siRNAs and in other types of epithelial cells such as 293HEK (kidney), PANK1 (pancreatic), and SW13 (thyroid) cells.

This study went on to show that the induction of contractile proteins associated with silencing of gamma-actin expression was regulated by serum response factor (SRF; a multifunctional transcription factor) and SRF coactivators, myocardin-related transcriptional factor A (MRTF-A) and MRTF-B. MRTF transcription factors are known to localize in the cytoplasm and are transported to the nucleus during differentiation of muscle cells. Based upon immunofluorescence experiments loss of cytoplasmic gamma-actin triggered a considerable translocation of endogenous MRTF-A and MRTF-B into the nucleus.

Furthermore, a monoclonal antibody that selectively bound the gamma-actin isoform co-precipitated MTRF-A together with gamma-actin; this was not found with a monoclonal antibody that precipitated beta-actin. Likewise, an SRF antibody co-precipitated gamma-actin, but not beta-actin. The up-regulation of alpha-smooth muscle actin in this fashion confirms that epithelial cells can, indeed, transform into myofibroblast-like cells.

How does tumor heterogeneity caused by EMT affect sensitivity to chemotherapeutic drugs? The Table on the left shows that about 1/4th of the papers addressing the relationship between EMT and cancer also discussed the impact of EMT on sensitivity or resistance to anti-cancer drugs. As mentioned above epithelial tumor cells that pass through EMT exhibit enhanced invasive properties, metastatic properties, clonogenicity, and resistance towards cytotoxic chemotherapeutic drugs.

One important mechanism of drug resistance acquired during EMT was reported on November 28, 2014, by Keitel et al at the University of Goettingen in Germany. These investigators showed both in vitro and in vivo that breast carcinoma cells that undergo EMT acquired resistance to multiple standard chemotherapeutic drugs such as cisplatin, carboplatin, and doxorubicin, and that this acquired chemo-resistance was associated with the induction of anti-apoptotic Bcl-xL.
expression which accompanied EMT. This reduction in chemo-sensitivity was reversed by Bcl-xL inhibitors. This finding along with others strongly suggest that drugs targeting the EMT process used in combination with standard anti-cancer drugs may improve outcomes with the more difficult to treat carcinomas such as breast, ovarian, pancreatic, and colorectal cancers. Indeed, inhibitors of EMT have proved efficacious in treating 100% lethal idiopathic pulmonary fibrosis (King et al., 2014; ASCEND trials on recently approved pirfenidone).

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About the Analyst

John Leavitt, Ph.D.

Analyst John Leavitt, Ph.D., helps life science companies develop solutions to critical problems and pursue novel business strategies. Dr. Leavitt applies his expertise in the biotech fields of diagnosis and treatment of human diseases, genetics, and cell and molecular biology to help companies make informed decisions.

John’s academic career as a molecular and cell biologist started as a graduate student in the Department of Biochemistry at the University of Pittsburgh, School of Medicine, then as a postdoctoral fellow at Johns Hopkins University in cancer research. He was a Senior Fellow at the National Institutes of Health and a career civil servant with CBER, a part of the FDA located on the NIH campus involved with regulation of vaccines and biologic drugs. While working for the FDA, John conducted research related to improvement of the influenza and polio vaccines and development of the cytomegalovirus (CMV) vaccine. Later, as a senior scientist at the Linus Pauling Institute in Palo Alto, Calif., John cloned and characterized several important human gene families linked to the development of cancer. His discovery of mutations in the human beta-actin gene associated with a human tumor cell has since been repeated by other cancer researchers, and his discovery of a new family of cancer biomarkers and mediators, called plastins, has been extended to a wide variety of human malignancies.

John’s research was supported with grants and contracts from the National Cancer Institute, American Cancer Society, the U.S. Air Force, and private foundations. John has published over 60 research papers. He also has three patents, one of which Stanford University successfully licensed to the biotech industry for the full duration of patent protection.

Academic Credentials

- Senior Fellow, National Institutes of Health (FDA)
- Postdoctoral Fellow, Johns Hopkins University
- Ph.D., Biochemistry, University of Pittsburgh School of Medicine
- B.S., Chemistry, Bethany College
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