Molecular & Cellular Oncology 1:2, e961822; April/May/June 2014; Published with license by Taylor & Francis Group, LLC

The endoplasmic reticulum may be an Achilles' heel of cancer cells that have undergone an epithelial-to-mesenchymal transition

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Keywords: EMT, ER stress, UPR

Abbreviations: ECM, extracellular matrix; EMT, Epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; UPR, unfolded protein response.

In a recent report published in Cancer Discovery we identified a novel vulnerability of cancer cells that have undergone an epithelial–mesenchymal transition (EMT) and established that the PERK branch of the unfolded protein response is constitutively activated upon EMT. In this commentary, we summarize and provide context for our findings.

Scientists have known for decades that cancer cells can become invasive and metastatic by undergoing an epithelial-to-mesenchymal transition (EMT).[1] More recently, researchers have discovered that the EMT also confers resistance to radiation and a wide spectrum of chemotherapy drugs, including DNA-damaging agents and targeted inhibitors of specific kinases.[2,3] Moreover, cancer cells that undergo an EMT are, in many cases, functionally indistinguishable from cancer stem-like cells.[4,5] These observations have revealed that, by merely changing their differentiation state, cancer cells can gain the key malignant traits responsible for most cancer-related deaths.

In light of this surprising fact, there is significant interest in finding ways to treat tumors by targeting the EMT. One major focus has been to delineate the ligands, receptors, and downstream signaling proteins that, when activated, induce cells to undergo an EMT; inhibiting these factors could suppress tumor progression by either preventing cancer cells from undergoing an EMT, or by reversing EMT in cells that have already undergone the transition.[6] A second area of focus has been to delineate the molecular mechanisms by which the EMT causes cells to acquire either invasiveness or drug resistance;[7] while it would not prevent or reverse EMT, inhibiting these mechanisms could provide a therapeutic benefit by suppressing the malignancy of cells that have undergone an EMT.

A more direct approach to targeting the EMT would be to search for agents that are selectively lethal to cells that have undergone an EMT. In a high-throughput screen of over 300,000 compounds, we succeeded in identifying a few small molecules with strong EMT-selective toxicity.[8] The discovery of these EMT-selective compounds was not a foregone conclusion because EMT cells were resistant to all test compounds they had been exposed to before the actual screen, and suggested that such agents were exploiting unique vulnerabilities acquired by cells upon EMT. At the time, however, we did not know what any of these vulnerabilities actually were.

In our recent publication, we show that 2 of the EMT-selective compounds identified in the above chemical screen selectively activate endoplasmic reticulum (ER) stress pathways – collectively termed the unfolded protein response (UPR). By contrast, closely related structural variants of these compounds that are not toxic to EMT cells do not activate ER stress signaling. We further show that EMT sensitizes cells to 4 molecules that are established perturbagens of ER function and to reductions in expression of the ER chaperone BiP. Taken together, our findings identify the first known vulnerability of EMT cells: sensitivity to agents that perturb the function of the ER (**Fig. 1**).[9]

This Achilles' heel of cells that have undergone EMT appears to be a consequence of physiological changes that occur in cells when they migrate and invade. Invading cells remodel the extracellular matrix (ECM). Upon EMT, cells significantly upregulate the synthesis and secretion of pro-migratory ECM components; this, in turn, significantly increases the protein load within their ER. In our recent publication we show that the increased sensitivity of EMT cells to ER stress is a consequence of this increased ER load, since inhibiting ECM synthesis reduces

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Submitted: 07/23/2014; Revised: 07/28/2014; Accepted: 07/29/2014
http://dx.doi.org/10.4161/23723548.2014.961822

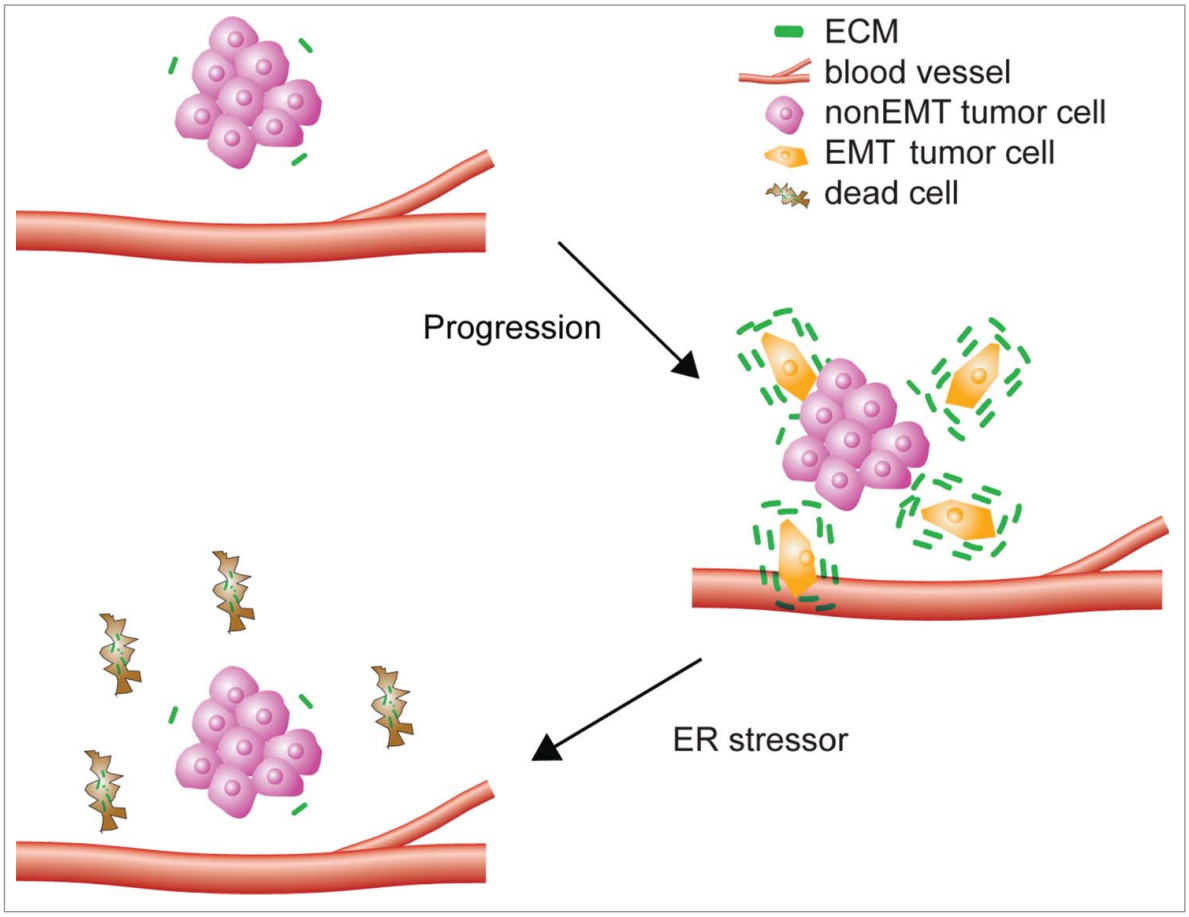

Figure 1. Epithelial-to-mesenchymal transition sensitizes cancer cells to endoplasmic reticulum stress.

the sensitivity of EMT cells to ER stress. Inhibiting ECM synthesis, however, also prevents EMT cells from migrating. Because ECM synthesis is essential for invasion, our findings suggest that increased sensitivity to ER stress may be a general feature of metastatic cancer cells.

Because some highly secretory cell types appear to selectively utilize UPR pathways,[10] we examined whether there was any evidence of this occurring upon EMT. We found that the highly secretory EMT cells specifically activate the PERK branch of the UPR at low levels, even in the absence of any treatment with exogenous ER stressors. In contrast, there was no detectable activation of the IRE1 or ATF6 branches of the UPR upon EMT. Treatment with ER stressors greatly increased the level of PERK signaling in EMT cells, while also strongly activating both IRE1 and ATF6 signaling.

These findings led naturally to the question of the role of PERK signaling in EMT cell biology. The PERK branch of the UPR pathway is critical for the function of many secretory cells including osteoblasts and β cells. PERK loss of function causes reduced secretion in osteoblasts and cell death in β cells, manifesting in animal models as decreased bone density and diabetes, respectively. Although PERK inhibition did not affect the survival or growth of EMT cells, we found that inhibiting PERK activity increased the sensitivity of EMT cells to ER stress. Moreover, we found that EMT cells required PERK signaling to form tumorspheres and to migrate.

The observation that the EMT program leads to selective activation of the PERK branch of the UPR was supported by analysis of gene expression data from uncultured patient tumors. Analysis of expression data from 800 tumors – spanning a range of tumor types including breast, colon, gastric, and lung cancer – revealed a strong positive correlation between the expression of EMT genes and PERK pathway genes. In contrast, no significant correlation was observed between EMT genes and IRE1 pathway genes in the same set of tumor expression data.

Our findings have two implications for the treatment of invasive tumors. First, they suggest that agents that promote ER malfunction should be explored for their potential to selectively eradicate cancer cells that have undergone an EMT. Second, they suggest that PERK pathway inhibitors may prove useful for suppressing several of the malignant traits associated with EMT.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Tom DiCesare for assistance with graphical design.

Funding

This research was supported by grants from the Richard and Susan Smith Family Foundation and the Breast Cancer Alliance (to PB Gupta), and the National Science Foundation Graduate Research Fellowship (Grant No. 1122374; to ES Sokol).

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