Commentary on "Epithelial–to–Mesenchymal Transition Contributes to Drug Resistance in Pancreatic Cancer"

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See related article by Arumugam et al., Cancer Res 2009;69: 5820–8.

First described in the early 1980s, epithelial-to-mesenchymal transition (EMT) is a process wherein epithelial cells lose their differentiated characteristics, such as cell–cell adhesion and apical–basal polarity, and acquire a more motile mesenchymal phenotype (1, 2). EMT is essential in regulating embryonic development and maintaining adult tissue homeostasis; however, aberrant activation of this program contributes to numerous pathologic conditions, such as fibrosis and cancer (2). By the mid-2000s, EMT was becoming established as an important driver of invasion and metastasis in various solid tumors, including breast, colorectal, prostate, and pancreatic cancers (2, 3). Here, we highlight the key findings of Arumugam and colleagues that supported an additional role of EMT in mediating drug resistance in pancreatic cancer (4).

Overcoming intrinsic and acquired drug resistance is an ongoing battle in the treatment of cancer. Advanced stage pancreatic ductal adenocarcinoma (PDAC) is frequently refractory to first-line chemotherapy, contributing to poor patient survival. Accordingly, Arumugam and colleagues sought to better understand molecular mechanisms underlying multidrug resistance in PDAC (4). Although previous studies in PDAC had mostly focused on single-agent resistance, Arumugam and colleagues utilized a panel of PDAC cell lines with differential sensitivity to gemcitabine, 5-fluorouracil, and cisplatin to better define broadly active resistance mechanisms (4, 5). Using microarrays to monitor global gene expression, they made the central observation that drug resistance correlated strongly with expression patterns consistent with EMT. Most notably, an inverse correlation was observed between expression of the epithelial marker E-cadherin and its transcriptional repressor Zeb1. A causative link between these observations was confirmed when knockdown of Zeb1 in resistant PDAC cells increased E-cadherin expression and drug sensitivity. A negative correlation between E-cadherin and Zeb1 was confirmed in additional PDAC cell lines and in human tumor samples by IHC. Arumugam and colleagues concluded that Zeb1–mediated EMT played a critical role in multidrug resistance, providing a rationale for therapeutic targeting of EMT in PDAC (4).

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doi: 10.1158/0008-5472.CAN-16-3022

Cancer Stem Cells, Desmoplasia, and Drug Resistance

Concurrent and subsequent studies involving Zeb1–mediated EMT in PDAC demonstrated a strong association with the induction of a stem cell–like phenotype. Wang and colleagues found that acquisition of an EMT phenotype, including Zeb1 upregulation, in gemcitabine-resistant PDAC cells was due to activation of Notch signaling, a pathway involved in normal stem cell development (6). Further work by several groups identified that Zeb1, in addition to activating EMT, was necessary to maintain a stem cell–like phenotype in cancer cells via repression of the stemness-inhibiting miR-200 family (7, 8). As part of the migrating cancer stem cell hypothesis, Brabletz and colleagues proposed that EMT could contribute to the establishment of cancer stem cells (CSC) and that EMT-induced drug resistance could be attributed to the intrinsic properties of CSCs, including decreased proliferation, increased DNA repair capability, and avoidance of apoptosis (9). However, the interdependency of EMT and the generation of CSCs is far from straightforward and remains a topic of much debate.

PDAC tumors are pathologically characterized by an excessive fibrotic response of the surrounding tumor stroma, which is collectively referred to as desmoplasia. Although briefly mentioned by Arumugam and colleagues, desmoplasia is now also recognized as a significant contributor to drug resistance in PDAC. In a proof-of-principle study, interruption of Hedgehog signaling in the stroma of a PDAC mouse model caused stromal depletion, increased intratumoral vascular density, and normalization of interstitial fluid pressure, which together enhanced drug uptake and cytotoxicity (10). In addition to acting as a physical barrier to chemotherapy, others have demonstrated the tumor stroma, and specifically, pancreatic stellate cells secrete a milieu of factors capable of inducing EMT in neighboring cancer cells (11). Together, these studies suggest that EMT may contribute to chemotherapy resistance via two distinct but related mechanisms in PDAC. Recent studies involving pancreas-specific deletion of the EMT-inducing transcription factors Snail or Twist in a mouse model of PDAC support these findings. Although the number of metastases to other organs was not reduced, suppression of EMT reduced tumor progression and increased survival following treatment with gemcitabine (12).

Targeting EMT in Pancreatic Cancer

As evidence supporting the link between EMT and drug resistance has grown, attention has focused on the identification and clinical development of compounds capable of modulating EMT and thereby potentially increasing the effectiveness of chemotherapy and targeted agents. In PDAC, attenuation of EMT has followed three main strategies: (i) inhibition of EMT regulators; (ii) inhibition of key EMT and stem cell pathways; and (iii) modulation of the tumor microenvironment. As Zeb1 represses

E-cadherin expression through recruitment of histone deacetylases (HDAC) to the promoter, Arumugam and colleagues postulated that the use of HDAC inhibitors could reverse the EMT phenotype and restore drug sensitivity (4). Indeed, *in vitro*, the HDAC inhibitor mocetinostat inhibited the expression of Zeb1 by restoring miR-203, reversing the EMT phenotype, and sensitizing cells to docetaxel (13). Clinical trials assessing the effectiveness of HDAC inhibitors in combination with gemcitabine in PDAC are ongoing (NCT02349867, NCT00948688). Inhibition of developmental pathways shared between EMT and CSCs with small-molecule inhibitors and antibodies targeting components of Notch (RO4929097) and Wnt (ipafricept, vantictumab) signaling have showed promising response rates in combination with gemcitabine in early-phase trials in metastatic PDAC (14, 15). Whereas inhibition of Hedgehog signaling has the potential of targeting both EMT and the tumor stroma, the enhanced delivery of gemcitabine to tumor and prolonged survival seen in preclinical studies could not be replicated in the clinic (10, 16). The clinical failure of Hedgehog inhibitors may be explained by a subsequent study, which demonstrated that Hedgehog pathway inhibition promoted tumor progression in PDAC models by reducing the tumor-restraining properties of surrounding stroma (17).

Modulation of the tumor microenvironment may not only inhibit cancer cell EMT, but could also disrupt stromal-mediated mechanisms of chemoresistance. PEGPH20 is a pegylated recombinant human hyaluronidase that works by enzymatically depleting hyaluronic acid (HA), which is very abundant in desmoplastic stroma and is thought to contribute to high interstitial fluid pressure (18). In a phase II study combining chemotherapy and PEGPH20 in PDAC, there was a significant improvement in progression-free survival in those patients with high levels of HA, and a global randomized phase III trial has been launched (19). Similarly, improvements in PDAC patient survival have been observed with the addition of albumin-bound paclitaxel (Nab-paclitaxel) to standard chemotherapy (20). Several studies have demonstrated that Nab-paclitaxel may selectively accumulate in pancreatic stroma and deplete it through binding to the extracellular matrix protein secreted protein acidic and rich in cysteine (SPARC; refs. 20, 21). However, a follow-up study by Neesse and colleagues showed that the effects of Nab-paclitaxel were largely dose dependent and that drug accumulation in PDAC tumors was not influenced by levels of SPARC expression (22). These results suggest that the microenvironment associated with the tumor–stromal interaction may be an alternative target for modulating EMT and chemoresistance in PDAC.

Conclusions

In the 7 years since their article, Arumugam and colleagues' observations have been validated in numerous publications. EMT clearly plays a key role in drug resistance but is a complex process that has not been fully elucidated. Although current clinical trials are encouraging, it is important to acknowledge the heterogeneity and plasticity of the EMT phenotype and to consider that targeting of specific inducers of EMT, such as Zeb1, may be necessary for an effective cancer treatment. Nevertheless, the significant correlation between EMT, CSCs, and drug resistance provides a rationale for therapeutic targeting of EMT in combination with chemotherapy in pancreatic cancer and other solid tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received November 4, 2016; accepted November 4, 2016; published OnlineFirst December 15, 2016.

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Cancer Res 2016;76:7075-7077.