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Previews

EMT and MET in Metastasis: Where Are the Cancer Stem Cells?

Thomas Brabletz[1,*]
[1]Department of General and Visceral Surgery, Comprehensive Cancer Center and BIOSS Centre for Biological Signalling Studies, University of Freiburg Medical Center, Hugstetter Str. 55, 79106 Freiburg, Germany
*Correspondence: thomas.brabletz@uniklinik-freiburg.de
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Activation of epithelial-mesenchymal transition (EMT) is important for cancer cell dissemination. Two papers in this issue of *Cancer Cell* (Ocaña and colleagues and Tsai and colleagues) support the concept that the reversal of EMT is necessary for efficient metastatic colonization. Moreover, although EMT has been associated with stemness properties, one study indicates that they are not necessarily linked.

Metastasis is responsible for more than 90% of cancer associated mortality; therefore, the clinical need to prevent or target metastasis is great. For distant metastasis, primary tumor cells must invade, disseminate through blood vessels, seed at the distant site, and colonize to macrometastases. De-differentiation through aberrant activation of the embryonic program "epithelial-mesenchymal transition" (EMT) was shown to strongly enhance cancer cell motility and dissemination (Thiery et al., 2009). Moreover, gene expression patterns in human cancers indicated that de-differentiated cancer cells combine EMT properties with a stem-cell like phenotype, leading to the concept of "migrating cancer stem cells" as the basis of metastasis (Brabletz et al., 2005). A direct molecular link between EMT and stemness was demonstrated by seminal findings that EMT activators, such as Twist1, can co-induce EMT and stemness properties (Mani et al., 2008; Morel et al., 2008), thereby linking the EMT and cancer stem cell concept (Dalerba et al., 2007). However, metastases of the most common human cancers (well- to moderately-differentiated carcinomas) often show a re-differentiation in the sense of a mesenchymal-epithelial (re-)transition (MET). Consequently, transient de-differentiation (EMT)- re-differentiation (MET) processes were proposed to be a driving force of metastasis (Brabletz et al., 2001). But why do metastases re-differentiate? Invasive, de-differentiated cancer cells were shown to be growth arrested, and proliferation was detected in re-differentiated metastasis, leading to the proposal that EMT must be reversed in order to allow growth and colonization (Brabletz et al., 2001). This is supported by the fact that EMT-inducing transcription factors can directly inhibit proliferation (Thiery et al., 2009). Although many clinical reports fostered the concept of transient EMT-MET switches in metastasis, there are only a few experimental proofs (e.g., Chaffer et al., 2006; Korpal et al., 2011). Two papers in this issue of *Cancer Cell* support the role of an EMT in dissemination and the need of a MET for efficient metastasis.

In the first report, Tsai et al. (2012, in this issue of *Cancer Cell*) used an elegant mouse model for skin cancer in which metastatic squamous cell carcinomas were induced by topic application of the carcinogens DBMA and TPA and the expression of Twist1 was selectively induced in keratinocytes by docycycline. Oral application of doxycycline induced Twist1 in all cancer cells, irrespective of their localization (primary tumor, circulating or disseminated tumor cells, or metastasis), therefore modeling "irreversible" Twist1/EMT activation. In contrast, topical application of doxycycline only induced Twist1 in the primary skin tumors, and Twist1 expression is shut down in disseminated tumor cells ("reversible" Twist1/EMT activation). Twist1 activation in both conditions (compared to uninduced controls) increased the number of circulating tumor cells and tumor cells extravasated to the lung, supporting the role of EMT in dissemination. However, the number of metastases in the "reversible" Twist1-model was higher than that in the "irreversible" Twist1-model. Moreover, the authors demonstrated that downregulation of Twist1 in metastases was associated with increased proliferation and reversal of an EMT-associated growth arrest. In summary, this study clearly supports the role of an EMT in dissemination and the necessity of a subsequent MET for colonization and macrometastasis (Figure 1A). Twist1 downregulation was shown to be important to overcome EMT-associated growth arrest, but reactivation of proliferation is likely not the only reason for a MET in metastasis. Recently, it was shown that, while re-differentiation induced by expression of miR-200 is required for metastatic colonization in a xenograft model, miR-200 also directly targets SEC23A, which stimulates the secretion of metastasis-suppressive proteins (Korpal et al., 2011).

The second study by Ocana et al. (2012, in this issue of *Cancer Cell*) also supports the role of EMT for dissemination and the necessity to revert EMT for metastasis. But, surprisingly, the features of the newly discovered EMT activator "paired-related homeobox transcription factor 1" (Prrx1) make the underlying molecular links more complex. In contrast to other EMT-activators, Prrx1 suppresses stemness traits, raising again the questions of where and which are the cancer stem cells. The authors detected Prrx1 as an additional EMT inducer activating delamination from the primitive streak in chicken embryos. Prrx1 is coexpressed and cooperates with Twist1 in inducing all EMT features relevant for

dissemination, such as migration and invasion. In a xenograft model using human BT-549 breast cancer cells (coexpressing Prrx1 and Twist1), a knockdown of both factors (but not of Twist1 alone!) increased lung metastasis after tail vein injection, but not in an orthotopic setting. Importantly, in contrast to the study by Tsai et al. (2012), depletion of Twist1 alone had no effect, indicating that Prrx1 is not only cooperating with Twist1, but is also dominating its function. The big surprise came when the authors analyzed stemness and tumor-initiating features: Prrx1 decreased stemness features and knockdown of Prrx1 in BT-549-increased mammosphere formation, self renewal capacity, and the fraction of $CD24^{low}/CD44^{high}$ cancer stem cells. Of note, increased stemness was associated with maintained proliferation capacity. This is in contrast to stemness induced by other EMT activators (Twist1 alone, Snail1, Snail2, and ZEB1), which are associated with a growth arrest. Strikingly, the presence of Twist1 was not necessary for the stemness features, because combined depletion of both Prrx1 and Twist1 had the same effect. Conversely, overexpression of Prxx1 in another undifferentiated breast cancer cell line MDA-MB-231 (expressing ZEB1 but neither Prrx1 nor Twist) also suppressed stemness features, indicating that ZEB1-associated stemness can also be inhibited. Finally, by analyzing published data sets, the authors could show that high expression of Prrx1 (often associated with Twist1 expression) in breast and the squamous type of lung cancer is associated with a good prognosis and increased metastasis-free survival. These results are of high relevance for cancer biology because they not only support the model of an EMT/MET switch in metastasis, but they also identify a potentially new mechanism allowing metastatic colonization by uncoupling stemness from EMT and growth arrest in favor of a parallel maintenance of a stemness, MET, and proliferation phenotype (Figure 1B). In this context, the study mechanistically supports a concept where cancer stem cells either can be embedded in the epithelial mass of benign precoursors, primary tumors, or metastases (stationary cancer stem cells) or linked to EMT/motility in invading, disseminating, growth-arrested tumor cells (migrating cancer stem cells) initially proposed from the analyses of colon cancer (Brabletz et al., 2005) (Figure 1).

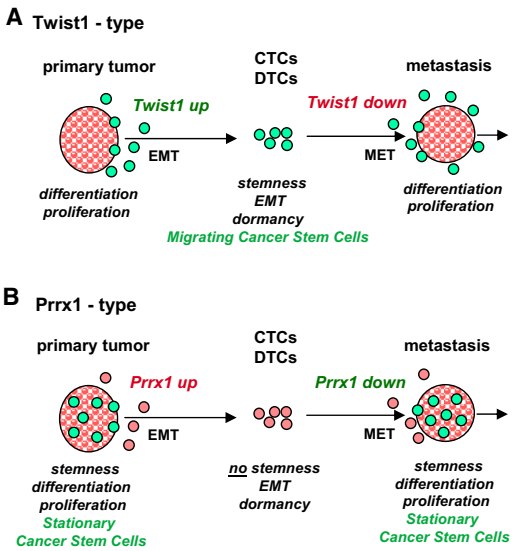

Figure 1. EMT and MET in Metastasis: Where Are the Cancer Stem Cells?

Models and consequences deduced from the papers by Tsai et al. (2012) and by Ocana et al. (2012) are shown.

(A) Data by Tsai et al. (2012) support the concept that upregulation of an EMT activator (e.g., Twist1) in invasive cells of the primary tumor induces dissemination. A downregulation of the EMT inducer and a subsequent redifferentiation (MET) at the distant site is necessary to allow colonization and macrometastasis. Because Twist1 also induces stemness properties and a growth arrest, putative cancer stem cells are mobile but nonproliferating (migrating cancer stem cells; green indicates stemness phenotype and activation).

(B) The EMT activator Prrx1, newly identified by Ocana et al. (2012), suppresses stemness properties in the EMT and dissemination state. Prrx1 must be downregulated to activate stemness properties and allow colonization. Thus, putative cancer stem cells are not mobile but embedded in the epithelial tumor mass both in the primary tumor and metastases (stationary cancer stem cells). Both types of metastasis require an EMT for dissemination and a MET for colonization. The most important difference is that in the Prrx1-type EMT, growth arrest and stemness are uncoupled, favoring the parallel maintenance of a MET, proliferation, and stemness phenotype.

However, these data on Prxx1 raise a lot of new questions, particularly in the context of publications on Twist1, e.g., by Tsai et al. (2012).

How do Twist and Prrx1 interact at a molecular level and how does Prrx1 inhibit the stemness-inducing but not the EMT-inducing function of Twist1 (and potentially other EMT inducers like Snail1 and ZEB1)?

How does the loss of Prrx1 induce stemness in (re-)differentiated epithelial cancer cells, which also downregulate Twist1?

What is the role of Prxx1 in physiological stem cell biology? Does it distinguish between stationary and migrating stem cells? Are these mutually exclusive modes of stemness? Is the Twist1-mode or the Prrx1-mode (Figure 1) more relevant for human cancer metastasis?

In which (cancer) cells and tissues is Prrx1 expressed and potentially controlling other EMT inducers (see BT549 versus MDA-MB-231; different areas during primitive streak delamination)?

In summary, both papers experimentally support the need of a re-differentiation (MET) for the colonization and metastasis of differentiated carcinomas and show that one reason is the EMT-associated growth arrest. This has a clinical impact for future therapeutic strategies against metastasis. Inducing differentiation and targeting EMT alone might be counterproductive by activating proliferation of disseminated cells; it should be combined with therapy against cycling cells, e.g., with a standard chemotherapy. In addition, inhibiting MET, thereby maintaining dormancy and/or directly targeting the stem cell phenotype, wherever it is located, could be a promising strategy.

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Disputed Paternity: The Uncertain Ancestry of Pancreatic Ductal Neoplasia

Anirban Maitra[1,3] and Steven D. Leach[2,3,*]
[1]Department of Pathology
[2]Department of Surgery and the McKusick-Nathans Institute of Genetic Medicine
[3]The Sol Goldman Center for Pancreatic Cancer Research
Johns Hopkins University, Baltimore, MD 21205, USA
*Correspondence: stleach@jhmi.edu
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In this issue of *Cancer Cell*, Kopp and colleagues report that pancreatic ductal cells are largely refractory to the induction of pancreatic neoplasia. Whereas a rare ductal subpopulation may still prove capable of neoplastic transformation, these findings refocus attention on acinar and other non-ductal cell types as initiators of this deadly neoplasm.

While malignant tumors of the pancreas can display a variety of histologic forms, the term "pancreatic cancer" is usually synonymous with a pathological diagnosis of pancreatic ductal adenocarcinoma (PDAC). As its name implies, PDAC has long been presumed to arise from pancreatic ductal epithelial cells. Along with its noninvasive precursor, pancreatic intraepithelial neoplasia (PanIN), these tumors typically display a distinctly duct-like histology, and express markers of ductal differentiation. As demonstrated for other tumor types, however, tumor histology is often misleading in determining tumor lineage, and work from Kopp et al. (2012) published in this issue of *Cancer Cell* reinforces the disputed paternity of pancreatic "ductal" neoplasia.

Initial clues suggesting that non-ductal cells might serve as effective cells of origin for pancreatic ductal neoplasia were provided by studies involving transgenic misexpression of individual oncogenes under the regulation of non-ductal promoter elements, in which a subset of resulting tumors displayed histologic resemblance to adult ductal epithelium (Sandgren et al., 1991). However, these similarities were ultimately proven to be only skin-deep, as additional studies of PanIN and PDAC revealed activation of transcriptional programs typically observed in embryonic pancreatic epithelium, but not in differentiated duct cells (Miyamoto et al., 2003; Park et al., 2011).

With the advent of autochthonous mouse models of pancreatic neoplasia, more recent studies have interrogated individual pancreatic cell types for the ability to generate PanIN, based upon Cre/lox-mediated activation of oncogenic *Kras*. Initial seminal work in this arena utilized either *Pdx1*^{Cre} or *Ptf1a*^{Cre} alleles to activate *Kras* in embryonic pancreatic progenitor cells (Aguirre et al., 2003; Hingorani et al., 2003). While these studies demonstrated that embryonic activation of oncogenic *Kras* effectively initiated pancreatic ductal neoplasia, they provided considerably less information regarding the capacity of individual adult cell lineages to similarly serve as effective cells of origin. Based on the availability of appropriate Cre driver lines, this adult capacity was first interrogated in pancreatic acinar cells. Using either a Nestin-Cre driver to activate oncogenic *Kras* in exocrine progenitor cells and their acinar cell descendants (Carrière et al., 2007) or a variety of inducible Cre lines to activate *Kras* in adult acinar cells (De La O et al., 2008; Guerra et al., 2007; Habbe et al., 2008), these studies provided strong evidence that acinar cells could indeed serve as effective biologic parents for pancreatic ductal neoplasia. In these studies, the ability of adult acinar cells to generate PanIN was dramatically accelerated in the context of associated pancreatitis, a known risk factor for the human disease. Additional studies suggested that a permissive inflammatory microenvironment could broadly bestow PanIN-parenting capabilities, as even insulin-expressing cells