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Review

The epithelial–mesenchymal transition under control: Global programs to regulate epithelial plasticity

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ABSTRACT

The epithelial to mesenchymal transition or EMT has become one of the most exciting fields in cancer research. Nevertheless, its relevance in tumor biology and the metastatic process still faces some controversy. Clarification may arise when considering the EMT as a reversible and often incomplete process, essentially a manifestation of strong epithelial plasticity. Transient cellular states are generated to fulfill specific requirements in each and all the steps of the metastatic process, from primary tumor cell detachment to dissemination and colonization. Opposing multiple cellular programs that promote or prevent EMT, thereby destabilizing or reinforcing epithelial integrity, play a central role in the inherent cellular dynamics of cancer progression. These cell biology programs not only drive cells towards the epithelial or the mesenchymal state but also impinge into multiple cellular and global responses including proliferation, stemness, chemo and immunotherapy resistance, inflammation and immunity, all relevant for the development of the metastatic disease.

1. The EMT canonical concept

The EMT was defined as the process by which epithelial cells change their phenotype to mesenchymal-like, involving a complex cell and genetic program that leads to the loss of cell–cell interactions and of epithelial apico-basal polarity with the concomitant acquisition of mesenchymal markers and a marked migratory behavior. The EMT was initially identified in embryos as a fundamental process for the generation of tissues and organs [1]. In the last 12–15 years, the EMT has also been recognized by researches from different fields as a key process in several physiological and pathological situations, including wound healing, cancer and organ fibrosis. Remarkably, the EMT in tumor cells has been envisioned as a way to facilitate the metastatic cascade at the steps that require active migration to disseminate tumor cells from the primary tumor like during invasion, intravasation and/or extravasation [2]. EMT in tumors is a dynamic and transient process that can be spatio-temporally regulated as it occurs during embryonic development, with the reversion to the epithelial phenotype contributing to metastasis formation [3,4]. In contrast, the EMT associated to organ fibrosis is a progressive unidirectional process which can lead to organ failure.

The EMT has been described on different cell culture and developmental systems. The activation of the so-called EMT transcription factors (EMT-TFs: Snail, Twist, Zeb and others) together with the loss of both *E-cadherin* transcription and apico-basal polarity are considered important hallmarks of the process [4,5]. Despite sharing most phenotypic features, EMTs occurring during development, in tumor cells or during organ fibrosis also reveal differences including the ability to disseminate (embryonic and tumor cells) or the occurrence of associated inflammation (cancer and fibrosis), leading to the definition of three types of operationally different EMT [6]. In this review we discuss (i) the potential reasons for the controversy around the EMT in pathology, and (ii) the EMT as an intrinsically dynamic process resulting from the execution of global cellular programs that control epithelial plasticity. Finally, we enunciate the different biological processes and molecular mechanisms that will be further developed in the next chapters in relation to the EMT together with the emergence of new EMT connections including the response to viral infection or obesity.

2. The EMT: success and debate

Last year was an interesting year for the EMT field. While inheriting an unprecedented success in terms of publications, with 20% of all original articles on EMT published in 2010 [4], it also witnessed a revitalized discussion on its relevance in cancer, and in particular on the EMT as a driver of the metastatic disease [7]. While the success was well regarded by the EMT community, it also added a

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note of caution on the use (and sometimes abuse) not only of the term EMT but also of the implications for in vivo tumor biology, which at least in part explains the vivid discussion. With respect to the seemingly ever increasing publication rate, less of a problem, as 2011 saw a very modest 5% increase in EMT original publications. Regarding the discussion on the relevance of the EMT in tumor biology, more fuel was added from the field of organ fibrosis [8], and therefore, a reflection on the concept of EMT and the origins of the controversy is warranted.

Inherent to the dynamics and transient nature of EMT in physiological contexts, the inverse mesenchymal to epithelial transition process or MET, is required for the formation of organs in the final destinations of embryonic migratory cells. Similarly, in tumors, MET has been proposed as required for establishment of overt metastasis at distant sites [3]. Indeed, pathologists have for a long time recognized that distant metastatic lesions mainly reproduce the histological pattern of the primary tumor, thus meaning that in the case of carcinomas their metastasis manifest the "epithelial phenotype". In agreement with this concept, the EMT favors local invasion but may inhibit the growth of distant metastasis [9]. Thus, a high degree of epithelial plasticity probably better reflects the in vivo situation both in tumors and in embryos, where several rounds of EMT and MET processes occur during organogenesis [10]. Therefore, signs of the EMT process or markers associated with cells that have undergone EMT should not necessarily be present in metastases. The question is how and when is the EMT evident and functionally relevant during the whole process. Solving this issue will require to monitor the fate of individual cells during all the steps of the metastatic cascade, which is not plausible in patients. However, analysis of circulating tumor cells (CTCs) and disseminated tumor cells indicate that the presence of signs of EMT and mesenchymal markers predicted worse prognosis [11,12]. One potential problem in the interpretation of data from CTCs is that epithelial markers have been typically used to purify them, which have surely dismissed an important fraction of the CTCs if they have undergone the EMT [13,14]. Animal models are already providing solid data reinforcing the EMT concept. Transplants of human breast tumor cells in mice have shown spontaneous EMT in vivo associated with the ability to intravasate and generate CTCs [15]. Visualization in the living animal has shown the delamination of individual cells from primary tumors that when analyzed depicted expression profiles compatible with EMT [16,17] and very importantly, that EMT and dissemination can be a very early event, as recently shown to occur in pancreatic carcinoma [18].

The EMT in primary tumors is a focal event that can be overlooked in histological sections or diluted in global expression analyses of tumors. The bottom line is that EMT has been very difficult to observe in primary tumors, making it reasonable that some pathologists refrain from accepting a concept that they have not observed in their analysis of biopsies [19]. There is also the misconception that EMT should entail a fundamental change in cell identity. First of all, the EMT does not involve a change in cell fate but rather a change in motility and behavior. Moreover, an incomplete EMT can occur in tumors, with cells acquiring some mesenchymal properties without undergoing the full EMT as it also occurs in embryos, where intermediate phenotypes have been described in different contexts [4]. A full mesenchymal phenotype might only be overtly manifested in some specific tumor types in which the process is not a focal event for cell migration but rather a characteristic of the tumor type. Indeed, significant expression of EMT markers occur in subtypes of breast carcinomas, like basal cell carcinomas and claudin-low subtypes [20–23]. Expression of EMT markers has been also observed in carcinosarcomas and, interestingly, some of the markers are already detected in the epithelial component [20] supporting that even in tumors with an overt mesenchymal component a partial EMT can occur on cells in their way to acquire a full mesenchymal phenotype.

Animal models are also helping to understand other steps of the progression to the metastatic disease. Intravascular migration and extravasation of tumor cells transplanted in the zebrafish have shown that Twist expression is associated with morphological changes in the tumor cells compatible with EMT [24]. These findings support the original proposed role of Twist in intravasation of breast carcinoma cells [25].

If the reversion to the epithelial phenotype has likely been one of the problems to visualize the EMT during cancer progression, this should not be the case during organ fibrosis, as the progression of the fibrotic disease does not imply a MET. The main issue in fibrosis has been the unequivocal identification of the origin of the collagen-producing interstitial cells. Compelling studies describing their origin in the renal epithelial or endothelial cells through a process of EMT or EndMT (see for instance Refs. [26,27]), have failed to convince part of the nephrologists community. Conflicting data emerging from fate analyses in different experimental models of renal fibrosis (reviewed in Ref. [8]) raised some doubts on the specificity of particular markers and pointed out the difficulty in detecting cells in the interstitium that express epithelial markers. The latter might be explained again by the disappearance of the epithelial phenotype after the EMT at late stages of the disease, but nonetheless intermediate states have been shown in renal, liver and cardiac fibrosis [27–29] and during the development of fibrodysplasia ossificans progressiva [30]. These intermediate phenotypes reinforce the idea of an EMT process during organ fibrosis, as it also does the overt attenuation of the disease observed in a model of liver fibrosis developed in mice defective for the EMT inducer Snail in hepatocytes [31].

Going back to EMT in cancer, it is important to note that some carcinomas invade as collective cell masses in which most of the epithelial characteristics are maintained and only clearly loosened at migratory margins, reflecting epithelial dynamics that do not involve EMT, or even a partial EMT, independent of the regulation of E-cadherin expression ([32–34]; reviewed in Ref. [4]). Interestingly, breast tumor cells that invade through collective migration can disseminate into lymph nodes but cannot give rise to blood-borne metastasis. The migratory behavior can switch to individually migrating cells after transient pulses of the potent EMT inducer TGF-beta, making them competent to intravasate into blood vessels [35]. Recent data show that platelet-tumor cell interactions activate the TGF-beta pathway promoting EMT during intravascular transit of tumor cells [36]. These data suggest that although invasion does not necessarily require an EMT-like process, it is important for intravasation and vascular dissemination. However, it is also worth noting that tumors have an abnormal vasculature and malignant cells can escape through leaky vessels [37]. Interestingly, recent data indicate that vessel-associated pericytes in the tumor microenvironment are important regulators of tumor progression. While favoring tumor growth, pericytes protect from hypoxia, EMT and Met receptor activation, therefore attenuating metastasis formation [38].

Although a firm evidence for the relevance of EMT in cancer biology and metastasis is still missing, we believe that as: (i) the EMT concept by definition implies the existence of intermediate states, (ii) the EMT is reversible, and (iii) compelling evidences supporting the EMT coming from animal models and analyses of CTCs from patients are accumulating, doubts will be soon relieved. Opposing views consider that rather than a change in cellular identity, the metastatic process is initiated by genetic changes in tumor cells that, among other programs, can also target cell–cell adhesion properties (see Ref. [7]). In essence, the end result is very similar to that occurring after the EMT, with the advantage that EMT was implemented in evolution whenever epithelial cells required