**1. Introduction**

1.1. Epithelial plasticity during carcinoma progression and metastasis

Since the beginning of the present century, a plethora of *in vitro* studies have firmly established that activation of the EMT program promotes tumor cell invasion and metastasis and have defined the molecular players, environmental cues, and signaling pathways implicated in EMT induction (Chaffer *et al.*, 2016; De Craene and Berx, 2013; Lambert *et al.*, 2017; Lamouille *et al.*, 2014; Nieto, 2013; Nieto and Cano, 2012; Nieto *et al.*, 2016; Peinado *et al.*, 2007; Thiery, 2002; Thiery *et al.*, 2009; Yang and Weinberg, 2008).

EMT is envisioned as the loss of epithelial status and apico-basal polarity sustained on cell–cell adhesion molecules in order to gain mesenchymal traits. This transition entails the up- and downregulation of different proteins responsible for a profound cellular reorganization resulting in the acquisition of enhanced migratory and invasive properties (Nieto *et al.*, 2016; Thiery *et al.*, 2009). Context-dependent signaling transduction pathways and microenvironmental

**Abbreviations**

CFP, cyan fluorescent protein; CRC, colorectal cancer; CTC, circulating tumor cell; eDTCs, early disseminated tumor cells; EMT, epithelial/mesenchymal transition; EMT-TF, epithelial/mesenchymal transition transcription factor; EpCAM, epithelial cell adhesion molecule; FSP1, fibroblast-specific protein 1; GFP, green fluorescent protein; HCC, hepatocellular carcinoma; HER2/Neu/ERBB2, HERB2 receptor tyrosine kinase 2; HNSCC, head and neck squamous cell carcinoma; ILK, integrin-linked kinase; MET, mesenchymal/epithelial transition; MMP, metalloproteinase; NCID, Notch intracellular cytoplasmic domain; NSCLC, non-small-cell lung cancer; PCA, prostate cancer; PDAC, pancreatic ductal adenocarcinoma; RFP, red fluorescent protein; SCC, squamous cell carcinoma; TCGA, The Cancer Genome Atlas; TGF-β, tumor growth factor beta; WNT, wingless; YFP, yellow fluorescent protein.
signals, such as hypoxia, oxidative stress, nutrient deprivation, or inflammation, impinge on particular EMT transcription factors (EMT-TFs) such as Snail1/ Snail2, ZEB1/ZEB2, and Twist1, responsible to induce and sustain the mesenchymal phenotype (De Craene and Berx, 2013; Nieto and Cano, 2012; Peinado et al., 2007). The EMT process is tightly controlled in normal tissues through a complex regulation of EMT-TFs, with interconnected regulatory networks operating at different transcriptional and post-translational levels (i.e., alternative splicing, noncoding RNAs, epigenetic regulatory mechanisms, protein stability) (De Craene and Berx, 2013; Diaz-Lopez et al., 2014; Nieto, 2011; Nieto and Cano, 2012; Peinado et al., 2007; Tam and Weinberg, 2013; Yang and Weinberg, 2008). On the other hand, EMT-TFs control gene expression programs that are far beyond the acquisition of mesenchymal traits and influence multiple cellular processes through the direct or indirect transcriptional regulation of numerous genes (Nieto and Cano, 2012; Nieto et al., 2016; Thiery et al., 2009). The pleiotropic effects of the EMT program, when activated in tumor cells, favor the acquisition of a compendium of cellular abilities intimately linked to tumor progression and metastasis besides influencing tumor evolution and response to therapeutic treatments (Lambert et al., 2017; Nieto et al., 2016). Furthermore, the EMT process within the tumor context is highly dynamic, implying transient and reversible states, thus resembling embryonic development where multiple rounds of EMT and the reverse mesenchymal/epithelial transition (MET) processes occur as necessary steps for early embryogenesis and morphogenesis (Nieto, 2013; Nieto et al., 2016; Thiery et al., 2009). This reversibility might be indeed an essential feature of the metastatic cascade. As repeatedly appreciated by pathologists, distant metastases arising from carcinomas usually present the same histology as the primary tumor, indicating the maintenance or reacquisition of the epithelial morphology by disseminated tumor cells at distant sites (Brabletz, 2012a; Brabletz et al., 2005). In fact, the requirement of MET for the establishment of macrometastases was promptly enunciated at the emergence of the EMT field (Thiery, 2002), and supporting evidence has been recently provided (Korpai et al., 2011; Ocaña et al., 2012; Stankic et al., 2013; Tsai et al., 2012).

One of the misconceptions, widely extended in the field until recently, is that EMT implies a complete transdifferentiation from a functional epithelial cell into a mesenchymal-like cell. However, numerous reports suggest that complete EMT is restricted to some types of tumors (i.e., carcinosarcomas) and that intermediate EMT states coexist in most tumors, with cells acquiring some mesenchymal properties without undergoing a full EMT (Nieto and Cano, 2012). Recent observations support that EMT and MET processes represent two final endpoint states of dynamic epithelial plasticity routes. Thus, intermediate states between epithelial (E) and mesenchymal (M) phenotypes might occur at different steps of the metastatic cascade with cells transitioning through hybrid E/M states during tumor dissemination (Chaffer et al., 2016; Diepenbruck and Christofori, 2016; Lambert et al., 2017; Nieto et al., 2016; Yeung and Yang, 2017).

Despite the overwhelming information accumulated on EMT and cell plasticity in tumor biology, at least two key questions are still unresolved: (a) the requirement of EMT/MET processes for metastasis to occur in vivo and (b) the actual relevance of EMT in the clinical practice. In the following sections, we provide an update of the evidence on the first issue and discuss recent insights into EMT translational opportunities presently and in the near future.

2. Epithelial plasticity is required for metastasis: lessons from mouse cancer models

To outline the in vivo functional contribution of EMT/ MET processes to cancer progression, we will discuss the recent, and sometimes controversial, evidences obtained using genetically engineered mouse cancer models. Most of the current genetic models are based on the manipulation to knock-out/knock-in key regulators of EMT (i.e., EMT-TF genes) and/or in EMT lineage tracing models (Fig. 1).

2.1. Mouse models of EMT-TFs

Regarding genetic modulation of EMT-TF genes, two recent reports used Snail1 or Twist1 to control EMT in breast or squamous cell carcinoma (SCC) cancer models, respectively (Tran et al., 2014; Tsai et al., 2012) (Fig. 1A). In the first study, the authors characterized several mouse models of breast cancer engineered to express an inducible Snail1 transgene in combination with endogenous Snail1 reporter and conditional Snail1 knockout. Their results supported that endogenous expression of Snail1 was restricted to primary tumors and was required and sufficient for breast cancer metastasis. Additionally, the authors showed that Snail1 expression during breast cancer metastasis was transient and they hypothesized that Snail1 continuous expression blocked the MET process required
for metastasis to grow (Tran et al., 2014). In line with these findings, upregulation of Twist1 expression in a spontaneous SCC mouse model promoted invasive carcinoma progression and dissemination into the bloodstream (Tsai et al., 2012). Importantly, the authors show that Twist1 silencing at distant sites allowed the reversion of EMT to MET, which was a requisite for disseminated tumor cells to grow and form detectable metastases (Tsai et al., 2012). These data are consistent with other in vitro studies in breast cancer cell lines in which silencing of both Twist1 and PRRX1, another EMT-TF, was required for efficient metastatic outgrowth at distant sites (Ocaña et al., 2012).

Although the above-mentioned studies support an important role for dynamic EMT/MET in the development of metastasis in vivo, other recent reports controversially suggest that EMT is dispensable for invasion and metastasis but key to chemoresistance (Fischer et al., 2015; Zheng et al., 2015). Using genetic mouse models of pancreatic cancer reproducing the course of human pancreatic tumors, Zheng et al. (2015) reported that genetic suppression of Snail1 or Twist1 in primary pancreatic ductal adenocarcinomas (PDACs) did not alter tumor progression or metastatic outgrowth (Fig. 1A), thus concluding that EMT was dispensable for metastasis. A similar conclusion was derived from an EMT lineage tracing system in breast cancer models (Fischer et al., 2015; see below). One likely explanation for the unanticipated results obtained by Zheng et al. (2015) is that the deletion of...
Snail1 or Twist1 might be compensated by other EMT-TFs as has been described in cell culture models (Diaz-Lopez et al., 2015) or, alternatively, that EMT-TFs expression is tumor specific, being Snail1 or Twist1 dispensable for pancreatic cancer metastasis. In support of the latter hypothesis, ZEB1 knock-out in the mouse model of pancreatic cancer reduces the metastatic burden to about 30% without affecting the expression of Snail1 or other EMT-TFs (Krebs et al., 2017). Interestingly, this study also showed that the deletion of ZEB1 freezes or halts pancreatic tumor cells in an epithelial state in which invasion, stemness, and metastatic colonization are dramatically suppressed, being cells in addition unresponsive to EMT-inducing signals. This report thus emphasizes the non-redundant actions of different EMT-TFs in metastasis generation in vivo. Although not directly addressing the metastatic potential, another transgenic breast cancer model based on the overexpression of Twist1 in the context of H-Ras activation led to highly undifferentiated invasive tumors (Fig. 1A) with a claudin-low phenotype (Morel et al., 2012) that exhibited intrinsic EMT features (Taube et al., 2010). The specific action of EMT-TFs and their context dependence are also supported by lineage tracing experiments to evaluate tumor initiation in different genetically engineered knock-in reporter breast cancer mouse lines, which demonstrated that the EMT program mediated by Snail2 (Slug) is required for normal mammary stem cells, while Snail1-induced EMT accounts for the acquisition of stemness and tumor-initiating properties of neoplastic lesions (Ye et al., 2015).

2.2. Lineage tracing mouse models

An additional concern regarding some mouse models genetically modified for EMT-TFs expression is that they rely on the alteration of a particular EMT-TF, underestimating the contribution of epithelial plasticity and hybrid E/M states to the metastatic cascade. To overcome this issue, several mouse lineage tracing models have been developed to follow the fate of epithelial cells during in vivo tumor progression without disturbing EMT-TFs expression to better evaluate the requirement of EMT for metastasis (Fig. 1B).

One of the first animal model described, based on the mouse model of PDAC, allowed the detection of migrating and invading tumor cells by the yellow fluorescent protein (YFP) tracer (Rhim et al., 2012). Migrating tumor cells (YPF⁺) were detected already in initial premalignant pancreatic intraepithelial neoplasia (PanIN) lesions, presenting features of undergoing EMT (E-cadherin⁻/N-cadherin⁺) and, noticeably, showing high ZEB1 expression and being able to disseminate in the bloodstream (Fig. 1B). Interestingly, isolated YPF⁺/E-cadherin⁻ cells from PanIN lesions displayed tumor-initiating capacity and generated heterogeneous tumors containing E-cadherin⁻ and E-cadherin⁺ cells (Rhim et al., 2012). This study supports that the EMT program occurs early in pancreatic tumor dissemination and that plasticity between epithelial and mesenchymal states during tumor development indeed arises in vivo; however, it did not address whether early disseminated cells have metastatic initiating abilities.

A similar approach was used in a mouse model for intestinal tumors conditionally activating the NOTCH receptor (NCID-GFP: NOTCH cytoplasmic intracellular domain-green fluorescent protein) and inactivating p53 within the intestine (Fig. 1B) (Chanrion et al., 2014). This model recapitulates many features of aggressive colorectal cancer (CRC), including lymph node and liver metastasis in addition to peritoneal carcinomatosis. GFP⁺ cells showing a gradient of epithelial to mesenchymal phenotypes were detected at the invasive and desmoplastic regions, indicating the plasticity of tumor cells during invasion. Expression of several EMT-TFs such as Snail1, Snail2, Twist1, and ZEB1 was found in the desmoplastic area, while ZEB1 expression could be seen in the nuclei of invasive GFP⁺ cells that have undergone a partial or complete EMT, thus confirming previous observations on ZEB1 expression at the invasive front of human CRC samples (Spaderna et al., 2006). Results from in vivo GFP⁺ tumor cell lineage tracing and ex vivo imaging analyses of NCID/p53 tumor sections by two-photon microscopy allowed the identification of individual mesenchymal-like GFP⁺ cells as well as clusters of GFP⁺ cells at the invasive regions. Although mesenchymal-like GFP⁺ cells were also detected at metastatic sites, the contribution of single EMT-like cells migrated from the primary tumor could not be traced using the NCID/p53 mouse model. Noticeably, examination of human CRC samples revealed that activation of NOTCH in the context of p53 downregulation is significantly associated with metastatic CRC, supporting the validity of this NCID/p53 genetic model for further studies on epithelial plasticity (Chanrion et al., 2014).

Other novel EMT lineage tracing studies derive from breast cancer transgenic mouse models. Transgenic MMTV-PyMT or MMTV-Neu mice were engineered to express Cre recombinase in cells of mesenchymal lineage (Fsp1-Cre) and a constitutive Cre-switchable fluorescent marker (lox-RFP-STOP-lox-GFP). Thus, RFP⁺ (red fluorescent protein⁺) epithelial tumor cells undergoing a full EMT permanently convert into...
GFP+ cells (Fischer et al., 2015) (Fig. 1B). The authors showed that only a small proportion of tumor cells underwent EMT, whereas lung metastases were mainly composed of non-EMT/RFP+ cells that maintained their epithelial phenotype, concluding therefore that EMT is dispensable for breast cancer metastasis. Besides, the authors also uncovered that EMT cells were responsible for recurrent lung metastasis after chemotherapy (Fischer et al., 2015), in line with the results obtained using the pancreatic cancer model discussed above (Zheng et al., 2015). The major limitation of the models generated by Fischer et al. (2015) is that they allow the detection of cells undergoing a full EMT, due to the restricted expression of the Fsp1 (fibroblast-specific protein 1) promoter to mesenchymal cells (Bhowmick et al., 2004), but might underestimate the detection of intermediate E/M states during the course of tumor cell dissemination. The ubiquitous stromal expression of GFP imposes an additional limitation for the accurate detection of the low population of GFP+ tumor cells in vivo, leaving open the question of their potential implication in the metastatic cascade.

An additional study used a similar lineage tracing model system allowing the tracking of converted RFP+ to GFP+ cells upon the activation of the Fsp1 promoter in the context of MMTV-PyMT breast cancer mouse model (Fig. 1B) (Zhao et al., 2016). Upon RFP+ cell isolation and orthotopic injection, the authors monitored EMT through the detection of GFP+ cells during tumor formation combining intravital imaging of live mice and tumor sections. RFP+ cells transforming to GFP+ mesenchymal-like cells were detected and localized preferentially to blood vessels. These data clearly demonstrate that EMT indeed occurs in cells migrating from primary PyMT tumors; however, the use of the Fsp1 promoter may favor the detection of tumor cells primed to acquire a full mesenchymal phenotype but precluding the detection of tumor cells in dynamic plastic states as those undergoing intermediate E/M transitions. This limitation was partially overcome in another approach also based on the breast cancer MMTV-PyMT mouse model. This study combined the analysis of YFP+ tumor cells and endogenous E-cadherin fused to mCFP (mouse cyan fluorescent protein) (E-cadherin-CFP+)-expressing cells (Fig. 1B) (Beerling et al., 2016). Cell lineage tracing with high-resolution intravital imaging allowed the detection of a small population of cells undergoing partial EMT (defined as E-cadherin-low: Ecadlo or CFPlo). Importantly, the Ecadlo population had the abilities to invade, migrate, intravasate, and extravasate besides reaching metastatic organs such as the liver. Rapid conversion of Ecadlo cells to Ecadhi (E-cadherin-high and thus epithelial) occurred at metastatic sites as early as upon two cell divisions (Fig. 1B), supporting the high plasticity potential of tumor cells at distant organs. Noticeably, a rare population of Ecadlo cells could be detected in human breast tumors expressing a gene signature similar to that defined for mouse Ecadlo cells. This study supports both the existence of EMT and epithelial plasticity in vivo and, remarkably, the interconversion between mesenchymal and epithelial states as soon as tumor cells reach the metastatic organ (Beerling et al., 2016).

Moreover, detection of early disseminated tumor cells (eDTCs) suffering a partial EMT (Ecadlo/Twisthi) was recently reported using the MMTV-Her2 breast cancer mouse model (Harper et al., 2016). Interestingly, the partial EMT detected in eDTC Her2+ cells seemed mediated by canonical WNT signaling and was associated with the inhibition of MAPK p38 activity promoting eDTCs survival during dissemination. In addition, eDTCs were even more invasive than primary tumor cells and able to reach distant metastatic sites where they remained dormant and eventually generated metastasis. Intriguingly also, mammospheres obtained from eDTCs exhibited higher metastatic potential than those derived from the bulk of the primary tumor, suggesting that their higher intrinsic plasticity conferred advantages for metastatic growth (Harper et al., 2016). However, whether the metastatic outgrowth of eDTCs required the shutdown of Twist1 expression and reacquisition of an epithelial phenotype (i.e., a MET process) is not yet clarified. This study, together with the previous report on pancreatic cancer models (Rhim et al., 2012), strongly supports the existence of EMT and plasticity processes in vivo at very early stages of tumor progression, even though their translation to human tumors is yet unclear. As recently discussed by others (Gomis and Gawrzak, 2016; Lambert et al., 2017), how disseminated tumor cells from early lesions acquire the additional genetic and epigenetic changes, including microenvironment adaptation, necessary for leaving dormancy and promoting metastasis outgrowth, is still far from understanding.

2.3. Models of intermediate E/M states

On the other hand, the emerging concept that intermediate or hybrid E/M states are more relevant to metastasis than fixed epithelial or mesenchymal phenotypes suggests that such a hybrid E/M status confers a transient metastable phenotype. Thus, the hybrid E/M status would facilitate transitions between different stages of epithelial plasticity and allow rapid adaptation and
EMT clinical applications

response to diverse environmental cues (Chaffer et al., 2016; Diepenbruck and Christofori, 2016; Nieto et al., 2016). Although a clear evidence for this premise is still lacking, recent works suggest the existence of phenotypic stability factors, such as OVOL (ovo-like zinc finger) and GRHL2 (grainyhead-like transcription factor 2), that can contribute to stabilize the hybrid E/M phenotype in vivo during mammary branch morphogenesis as well as in cancer (Jia et al., 2015; Jolly et al., 2016; Watanabe et al., 2014). These findings are also in agreement with proposals from mathematical models suggesting that a ‘fixed’ hybrid E/M state is certainly sufficient to promote metastatic progression (Hong et al., 2015; Jolly et al., 2016). Whether these observations can be extended to human tumors awaits the characterization of markers accurately defining the E/M status. In contrast to these proposals, other studies favor that switching across the spectrum of different cell phenotypes underlying dynamic and plastic E/M states might be indeed advantageous for metastatic progression. Intriguingly, a gene expression signature derived from E6.5 mouse embryos, representing a high cellular plasticity state due to embryonic spatiotemporal cellular dynamics, predicts the metastatic behavior of breast cancer cells and has prognostic value in the clinical setting (Soundararajan et al., 2015).

Although cell tracing experiments along with intravital imaging have provided further support for the occurrence of EMT process in vivo, additional interrogations remain such as whether the plasticity processes are relevant for all tumor subtypes. Due to the limited information attained from available in vivo models, so far restricted to specific carcinoma mouse models (SCC, breast, pancreas, CRC), generalizations cannot be made. Indeed, some studies support that both plasticity-dependent and plasticity-independent mechanisms can operate in different cancer contexts (Brabletz, 2012b; Diepenbruck and Christofori, 2016; Somarelli et al., 2016). Therefore, additional studies using innovative genetically engineered mouse models to reliable trace and analyze tumor cells responsible for seed metastasis, together with implementation of higher-resolution in vivo intravital imaging microscopy, would certainly contribute to better understand the biological relevance of EMT and plasticity processes to metastasis in different tumor contexts.

3. EMT in human tumors: translation to clinical diagnosis and prognosis

Despite the accumulated evidence on EMT involvement in metastasis supported by recent evidences from preclinical models, its translation into the clinical setting is still challenging. This is not only due to the difficulty to ascertain EMT in pathological analyses of human biopsies (Tarin et al., 2005) but also to the heterogeneity inherent to tumors, the diverse metastatic behavior associated with different cancer contexts and the leap that entails translating results from homogenous cell lines in culture and preclinical models to tumors developed within persons. Moreover, the dynamic and transient nature of EMT comprising a broad spectrum of intermediate phenotypes, which might be represented in the primary tumor at spatially and temporarily distinct ratios (Lambert et al., 2017; Nieto et al., 2016), adds further complexity to the use of tumor EMT status as an indicator of diagnosis and/or prognosis. Thus, one of the pending challenges is the characterization of a few number of genes or proteins that could be studied in human samples to predict the establishment or acquisition of EMT or hybrid E/M features, even at early tumor stages, along with the detection of the reverse MET process linked to the micro- to macrometastasis formation (Lambert et al., 2017; Nieto et al., 2016). In this regard, upon intense efforts, several groups have focused on the identification of genetic or protein EMT signatures able to differentiate the epithelial versus mesenchymal phenotypes in cancer cells (Pasquier et al., 2015; Steinestel et al., 2014; Zeisberg and Neilson, 2009).

3.1. Detection of EMT markers in tumor samples

Since the initial characterization of the hallmark markers linked to the EMT process (Kalluri and Weinberg, 2009; Moreno-Bueno et al., 2009; Thiery, 2003; Thiery et al., 2009), changes in the expression of several EMT-associated genes and/or proteins have been used to assess EMT status in human tumor samples in an effort to establish an association with clinical significance (Pasquier et al., 2015; Steinestel et al., 2014). However, this is still a debated question among pathologists being some of them reluctant to accept the biological significance of the EMT process in tumor development (Tarin et al., 2005). In fact, cells that have undergone EMT are not frequently observed in tumor samples probably because biopsies represent a precise moment during tumor development, whereas cells suffering EMT could be shifting among hybrid E/M states and appear at different time points during tumorigenesis. Additionally, cells acquiring a full mesenchymal phenotype (endpoint EMT) would resemble stromal cells surrounding the tumor, making its clinical assessment by conventional histopathological techniques difficult (Ledford, 2011; Tarin et al., 2005).

Indeed, the expression of some EMT-TFs, such as
Snail1, has been detected not only in tumor cells but also in activated stromal fibroblasts favoring invasiveness and metastasis (Alba-Castellon et al., 2016; Franci et al., 2006; Rowe et al., 2009), adding complexity to the histological determination of EMT status based on single EMT markers.

Although the number of studies that include tumor analyses of EMT markers is considerable, most common alterations studied within human tumor samples are related to the loss or aberrant expression of proteins required to maintain the epithelial phenotype and usually involved in cell–cell adhesion (Steinestel et al., 2014), being E-cadherin one of the strongest markers routinely used in the clinic for cancer diagnosis or progression (Pasquier et al., 2015). E-cadherin downregulation is considered a hallmark of EMT together with the concomitant overexpression of specific mesenchymal markers such as N-cadherin and vimentin, among others (Kalluri and Weinberg, 2009; Moreno-Bueno et al., 2009; Nieto, 2011; Thiery, 2002; Thiery and Sleeman, 2006; Yang and Weinberg, 2008). Apart from diffuse gastric and lobular breast carcinomas where germine mutations and somatic inactivation of the E-cadherin (CDH1) locus occurs (Berx et al., 1996; Fitzgerald and Caldas, 2004; Guilford et al., 1998; Peinado et al., 2004), different studies have supported E-cadherin detection as a diagnostic marker in several tumor types. Immunohistochemically detected E-cadherin status correlates with the differentiation grade and histological type in breast carcinomas (Acs et al., 2001; Gamallo et al., 1993; Moll et al., 1993) and E-cadherin expression is also used for the differential diagnosis of papillary thyroid carcinoma (Ceyran et al., 2015). Besides these tumors, where E-cadherin status supports diagnosis, its aberrant expression has also been associated with diverse clinicopathological features in various carcinomas such as colon, lung, ovary, esophagus, prostate, or cervix, among others (reviewed in Steinestel et al., 2014). However, some studies indicate that N-cadherin overexpression, rather than E-cadherin loss, is a predictive marker of lymph node metastasis in gastric tumors (Okubo et al., 2017) as well as a promoter of thyroid tumorigenesis (Da et al., 2017).

Considering that loss of cell–cell adhesion and apico-basal polarity are hallmarks of EMT, the alterations in key molecular players conferring those cell properties, apart from cadherins, have also been used as markers of EMT in tumor samples. The relocalization to the cytoplasm and/or nucleus of β-catenin, an architectural membrane protein in adherens junctions, has been associated with CRC progression (reviewed in Schmalhofer et al., 2009) and similar relocalization of p120 catenin has been detected in breast tumors and carcinosarcomas (Sarrio et al., 2004, 2008). In this line, downregulation or loss of function of proteins involved in epithelial homeostasis such as tight junction components [claudins, occludins, ZO-1 (zonula occludens-1)] is frequently observed in several carcinomas (Steinestel et al., 2014; Zeisberg and Neilson, 2009), whereas the expression shift from epithelial keratins to mesenchymal vimentin, required for cell migration and invasion, is also commonly assessed in diverse types of tumors (Sarrio et al., 2008; Satelli and Li, 2011; Zeisberg and Neilson, 2009). Some specific protein signatures including few epithelial and mesenchymal markers have also been reported. In this regard, a study with 29 markers in around 500 breast tumors revealed that EMT-related proteins were normally overexpressed in basal-like breast carcinomas and in carcinosarcomas (Sarrio et al., 2008), although its diagnostic potential remains to be explored. In addition, one study of four genes [E-cadherin, MMP9 (matrix metalloproteinase-9), TCF3 (transcription factor 3)/E47 and ID2 (inhibitor of differentiation 2)] has validated their prognostic value, in terms of overall survival, in a large cohort of hepatocellular carcinomas (HCC) (Kim et al., 2010). Besides, deregulated expression of canonical EMT-TFs has been associated with several tumor types, especially those displaying EMT such as breast, cervix, ovary, or colon (reviewed in De Craene and Berx, 2013; Peinado et al., 2007; Polyak and Weinberg, 2009; Steinestel et al., 2014). Furthermore, Snail1 nuclear expression was found in invasive ductal carcinomas (Blanco et al., 2002) and in basal-like breast tumors contexts (Becker et al., 2007; Geradts et al., 2011) and correlated with lymph and cervical node and distant metastasis of breast and head and neck squamous cell carcinoma (HNSCC) (Yang et al., 2007), or even in stromal cells close to invasive areas (Franci et al., 2006). Also, ZEB1 expression was associated with prostate cancer (PCa) progression and metastasis (Putzke et al., 2011), while Twist1 plus ZEB2 expression was related to early disease recurrence in HCC (Yamada et al., 2014). However, the individual protein status of Snail1, Twist1, or ZEB1 is not regularly used for diagnostic purposes, whereas, as discussed later, their expression is most commonly linked to patient outcome and/or therapy resistance (Nieto et al., 2016; Pasquier et al., 2015; Steinestel et al., 2014; Yeung and Yang, 2017). Recently, one score determination of EMT-related splicing factors was also proposed as an EMT index with potential prognostic value in a small sample of breast carcinomas (Fici et al., 2017), although its clinical application awaits further studies in larger series and additional
Many clinical studies have demonstrated that distinct EMT signatures predict poor prognosis, tumor aggressiveness, and, in general, worse patient outcome in different types of cancer (Steinestel et al., 2014; Yeung and Yang, 2017). However, a review of the literature suggests that there are caveats in the numerous reported associations of EMT gene signatures and prognosis, reducing their clinical impact. Besides, discrepancies establishing a link between EMT gene profiles and overall survival in different types of tumors have been reported as well (Steinestel et al., 2014; Taube et al., 2010). The underlying cause for these controversies may arise from the fact that some EMT gene signatures were deduced from cell lines and their impact on tumor samples is thus limited.

Additionally, there is ample variability in the published studies regarding sample size, clinical evaluation of patients, methods used to assess the expression of EMT-associated markers, and cutoff values established, rendering unreliable prognostic information in many cases (Pasquier et al., 2015). The fact that clinical samples are obtained from tumors with inherent heterogeneity in which hybrid E/M states are expected to be restricted in time and space to specific tumor areas as well as influenced by the tumor microenvironment just adds another layer of difficulty to translate EMT signatures to patients’ prognosis. In spite of this, several EMT gene signatures support tumor subtype classification linked to clinical outcome in different types of cancer (Pasquier et al., 2015; Steinestel et al., 2014; Yeung and Yang, 2017). For instance, in breast cancer, the basal-like and claudin-low subtypes, characterized by poor prognosis, present a clear EMT signature (Hennessy et al., 2009; Sarrio et al., 2008; Taube et al., 2010). As expected, most downregulated genes found in those profiles were classified as epithelial markers involved in cell-cell adhesion and apicobasolateral polarity, including E-cadherin, desmosommal [DSG3 (desmoglein 3), DSP (desmoplakin)], and tight junction components [CLDN4/7 (claudin 4/7)] among others, whereas mesenchymal genes such as vimentin and MMP2/9 were upregulated (Chui, 2013). These EMT-related gene signatures denoted changes in the expression levels of genes involved in signaling pathways (reviewed in De Craene and Berx, 2013; Lamouille et al., 2014). In general, these gene expression changes favor the activation of intracellular signaling cascades related to tumor progression (Garg, 2013), reflecting that many regulatory networks are dynamically orchestrated during EMT (De Craene and Berx, 2013; Nieto and Cano, 2012).

The extensive data generated from different EMT gene signatures have not helped to clarify whether this information is clinically sound. However, new meta-analyses are starting to shed some light regarding this important issue. A recent meta-analysis in metastatic breast cancer including 3218 patients has revealed that the individual or combined high expression levels of the EMT-TFs, Twist1, Snail1, and Snail2, significantly correlated with poor prognosis (Imani et al., 2016). Another meta-analysis links high Twist1 or Snail1 expression with poor prognosis related to all clinical outcomes in various carcinomas such as lung and gastrointestinal tumors (Zhang et al., 2014a). In an effort to quantify the relationship between EMT and cancer progression, Tan et al. (2014) established an EMT scoring method based on EMT gene signatures obtained from ovarian, breast, bladder, lung, colorectal, and gastric carcinomas (Tan et al., 2014). When this EMT score was applied to different tumor types, it unveiled correlations between EMT status and poorer survival in ovarian and colorectal cancer, but not in breast carcinomas, and allowed to establish a connection with chemotherapeutic resistance. A recent TCGA pan-cancer analysis of EMT markers in 10 244 tumor mRNA samples representing 32 different types of cancer established a 16-gene EMT signature significantly associated with worse outcome in all tumor types (Gibbons and Creighton, 2017).

In addition to the identification of recurrent EMT profiles in different tumor contexts, the characterization of molecular gene signatures in specific tumors has been associated with cell migration, development, and/or stemness, among other aspects, and might be of clinical translational potential (Serrano-Gomez et al., 2016). Indeed, these specific signatures could be used as new tools for understanding cell plasticity in tumor biopsies as well as for diagnosis and prognosis (Schoenhals et al., 2009). In this sense, it is noteworthy to mention that the gene signature obtained from E6.5 embryonic development, denoting a gene expression pattern associated with considerable cellular plasticity, shows a higher significant predictive value in terms of relapse and distant metastasis-free survival in comparison with gene signatures associated with the expression of several EMT-TFs (Soundararajan et al., 2015). Nonetheless, the large number of genes present in the
EMT clinical applications

P. G. Santamaria et al.

E6.5 signature precludes its straight application to tumor biopsies. The refinement of such innovative gene signatures might benefit the clinical translation of tumor EMT status in the near future.

Besides, EMT-miRNA gene signatures have been associated with specific tumor contexts (reviewed in Díaz-Lopez et al., 2014; Trager and Dhayat, 2017). The miR-200 family (miR-200f), composed of tumor suppressors involved in EMT inhibition and apoptosis or proliferation (Brabletz and Brabletz, 2010; Feng et al., 2014), has been extensively characterized. In the context of human tumors, decreased expression of the miR-200f has been detected in many cancer types such as breast, CRC, HCC, non-small-cell lung cancer (NSCLC), renal clear cell carcinoma, and PCa (reviewed in Zaravinos, 2015). Additionally, in endometrial carcinosarcomas, miR-200f expression is restricted to the carcinoma area, but absent in the sarcomatous regions (Castilla et al., 2011), validating the key role of miR-200 members in maintaining the epithelial phenotype in vivo. Moreover, a link between miRNA signatures and expression of specific EMT-TFs has also been described in carcinosarcomas (Diaz-Martin et al., 2014) and PCa (Sekhon et al., 2016). Significantly, in experimental cancer models, the upregulation of miR-200f influences the cancer cell secretome favoring metastasis associated with the reacquisition of epithelial traits (Korpal et al., 2011).

Although the latter findings were validated by clinical correlations, the actual utility for diagnosis and/or prognosis is still to come. Additional efforts integrating mRNA and miRNA signatures related to EMT and epithelial plasticity in different tumor contexts are required to further translate this knowledge to the clinical context.

3.3. Translational potential of EMT to liquid biopsy

A specific consideration requires the analysis of EMT features in liquid biopsies, particularly in circulating tumor cells (CTCs), currently being characterized and emerging as a new promising prognostic factor in different types of tumors (reviewed in Cabel et al., 2017). In fact, CTC detection, to different thresholds depending on the tumor type, is indicative of a poor prognosis linked to the ability to seed metastasis from the primary tumor, as in endometrial, HCC, NSCLC, CCR, and breast tumors, among others (Alonso-Alconada et al., 2014; Barbazan et al., 2014; Bidard et al., 2014; Cristofanilli et al., 2004; Janni et al., 2016; Li et al., 2013; Wang et al., 2013; Wu et al., 2015; Yu et al., 2013). Analyses of CTCs from patients have also provided a strong link between EMT and metastasis in the clinical setting (Nieto et al., 2016). CTCs from a wide range of tumor types are characterized by the expression of both epithelial and mesenchymal markers (Alonso-Alconada et al., 2014; Yu et al., 2013), indicative of EMT plasticity. The label-free isolation of CTCs in patient samples has also allowed the identification of clusters of CTCs apparently entailing higher metastatic potential than single CTCs (Aceto et al., 2014; Cheung et al., 2016; Sarioglu et al., 2015). These CTC clusters may represent collective migration of cells, some of which retain epithelial characteristics endowing phenotypic advantage to rapidly grow macrometastasis at target organs (Diepenbruck and Christofori, 2016). Alternatively, CTC clusters can correspond to the association of individually seeded CTCs together with blood platelets that are indeed emerging as key regulators of EMT and tumor cell survival during their blood dissemination (Labelle et al., 2011; Leblanc and Peyruchaud, 2016; Takemoto et al., 2017) in addition to potential therapeutic targets (Li et al., 2016a,b; Roop et al., 2013).

Nevertheless, the assessment of the actual relevance of CTC clusters in the clinical scenario requires further evaluation in larger series of samples and different stages of disease progression. Although the presence of an EMT and/or hybrid E/M phenotypes in CTCs has been correlated with several factors reflecting worse patient outcome (Nieto et al., 2016), there is again debate over the assay allowing the detection of CTCs, which are expected to have high epithelial/mesenchymal plasticity (Alix-Panabieres et al., 2017; Liu et al., 2014). Thus, the main obstacle for the clinical application of CTC detection is that CTCs are presently isolated using microfluidic separation reliant on EpCAM, an epithelial marker (Harouaka et al., 2014) whose expression is completely lost when tumor cells begin to express key mesenchymal markers such as N-cadherin and/or vimentin (Armstrong et al., 2011). Considering these aspects, EpCAM-based detection seems to be insufficient for characterizing the CTC population. In this regard, the implementation of new immunostaining methods based on a combination of different epithelial and mesenchymal biomarkers has emerged as the current best approach for CTC detection (Harouaka et al., 2014) improving the capture of cells undergoing hybrid E/M states (Barriere et al., 2014; Parisi et al., 2016). Additional parameters such as size, deformability, and other physical properties should also be incorporated for advanced detection of CTCs with plastic phenotypes (Alix-Panabieres et al., 2017).
4. Current clinical status of EMT

4.1. EMT application to the prediction of therapy resistance

EMT has been strongly related to two additional properties of disseminating tumor cells, most likely intrinsically related, the acquisition of stemness properties (Lambert et al., 2017; Nieto et al., 2016) and of therapy resistance (Smith and Bhowmick, 2016). Accumulated evidence has lent support to the link between EMT and stemness, implying an association between EMT and tumor- or metastasis-initiating capabilities either in cell lines or in different types of tumors (Brabletz, 2012a; Mani et al., 2008; Morel et al., 2008). Further studies in in vivo breast cancer mouse models corroborated Snail1 expression associated with the acquisition of stem-like features in neoplastic cells (Ye et al., 2015). On the other hand, several studies support that EMT induction confers resistance to chemo- and radiotherapeutic treatments (Gupta et al., 2009; Kajita et al., 2004; Kurrey et al., 2009; Perez-Losada et al., 2003) as also reinforced with the preclinical models discussed above (Fischer et al., 2015; Zheng et al., 2015). However, the present knowledge does not support that EMT association with resistance is necessarily linked to cell stemness. While acquired resistance has been progressively connected to EMT and more specifically, the increase in some EMT-TFs has been related to a reduction in the therapeutical response in several preclinical models (Du and Shim, 2016), the link between EMT and cell stemness properties is not as clear (Brabletz, 2012a). Several recent studies have depicted distinct situations in which uncoupling EMT from stem cell-like properties is observed. This is the case for PRRX1 whose expression in breast carcinoma cells inhibits stemness, while its silencing, leading to MET, is required for metastasis outgrowth associated with stem-like properties (Ocaña et al., 2012). The opposite is true for Twist1, and EMT-TF inducing cell stemness (Mani et al., 2008), whose expression should be turned off at distant sites to allow metastatic growth in breast and SCC tumors (Ocaña et al., 2012; Tsai et al., 2012). Interestingly, Twist1 silencing in breast cancer cells can be mediated by ID1 (inhibitor of differentiation 1) promoting a stem-like phenotype while maintaining epithelial properties at distant sites (Stanick et al., 2013). Uncoupling EMT and stem cell-like potential linked to metastasis has also been described in prostate and bladder cancer cell models (Celia-Terrassa et al., 2012), even if the detailed involvement of individual EMT-TFs has not yet been defined.

Besides, recent data from in vivo breast cancer mouse models also support that metastasis-enhancing stem cell capacity might be independent of epithelial plasticity (Beerling et al., 2016), adding further complexity to the current situation. Overall, the present knowledge suggests that EMT and stemness might not be necessarily coupled, and this association might depend on specific EMT-TFs and the type of tumor (Brabletz, 2012a,b; Celia-Terrassa and Kang, 2016; Nieto, 2013).

Regarding therapeutic resistance, increasing evidence supports the association of EMT with chemoresistance, particularly favoring the multidrug resistance (MDR) phenotype (da Fonseca et al., 2016) but also with radioresistance (Smith and Bhowmick, 2016). Lately, numerous studies have tried to comprehend the resistance mechanisms in cells undergoing EMT in several tumor types (reviewed in Du and Shim, 2016; Smith and Bhowmick, 2016) in addition to the development of targeted therapeutic strategies to halt the EMT process based on blocking directly or indirectly specific related pathways (Du and Shim, 2016; da Fonseca et al., 2016). However, most of these studies have only revealed the low efficiency of these treatments, probably associated with the existence of alternative pathways able to induce and/or regulate the transient and plastic EMT phenotype (Zhou et al., 2017).

The first report linking EMT to resistance dates from 1996, when specific antibodies against TGF-β, one of the strongest EMT inducers, restored drug sensitivity in resistant tumors to alkylating compounds (Teicher et al., 1996) (Fig. 2). Up until now increasing findings support that EMT favors resistance (reviewed in Du and Shim, 2016) although the underlying molecular mechanisms remain partly unsolved. Indeed, a study of specific therapeutic agents in different preclinical models has revealed that increased expression levels of some EMT-TFs are strongly associated with a low therapy response rate (Du and Shim, 2016). To mention some of them, Snail1 expression has been related to cisplatin and 5-fluorouracil resistance in HNSCC and in NSCLC (Hsu et al., 2010) along with breast tumor cells (Zhang et al., 2012), respectively. Furthermore, cells undergoing EMT overexpress some specific ABC transporters (da Fonseca et al., 2016) involved in resistance mechanisms. In fact, the promoters of ABC transporters contain several binding sites for EMT-TFs (Saxena et al., 2011). Moreover, the resistance phenotype observed in NSCLC patients treated with c-MET and EGFR (EGF receptor) tyrosine kinase inhibitors is associated with ZEB1 expression (Della Corte et al., 2015; Rastogi et al., 2016).
Noticeably, in lung cancer cells in which treatment with EGFR inhibitors induced EMT, a differential sensitivity between epithelial and mesenchymal cells was observed (Yauch et al., 2005). On the other hand, an association with EMT has also been reported in gemcitabine-resistant highly invasive PCa cells, in platinum-resistant CCR cells as well as in post-ionizing radiation-associated metastasis in patients with advanced lung cancer (Du and Shim, 2016).

Finally, regarding liquid biopsy, CTC isolation and characterization have been recently addressed as a straightforward method to evaluate clinical response (Alix-Panabieres et al., 2017). Patients with refractory breast cancer presented higher levels of mesenchymal CTCs (Yu et al., 2013), while CTC changes during chemotherapy treatment were significantly associated with progression-free survival and overall survival. These facts support CTC-based models as prognostic tools for considering therapy, and/or to stratify patients and adjust therapeutic factors in clinical trials (Bidard et al., 2014). These results support the clinical application of the characterization of EMT-associated markers in patient-derived CTCs to predict treatment response (McInnes et al., 2015).

4.2. Are anti-EMT therapies feasible?

Although much interest was raised in the past decade in regard to EMT as a potential therapeutic target, the development of specific novel drugs against EMT or EMT-related signaling pathways constitute a tremendous challenge of current oncology and at present there are only a few studies based on treatments directly targeting EMT (Fig. 2).

An example is the inhibition of ILK, an integrin-linked kinase involved in AKT pathway activation leading to EMT (Jiang et al., 2015). Treatment with emodin, a (1,3,8-trihydroxy-6-methylanthraquinone) (Bruney et al., 2016), promotes a MET process by targeting ILK in ovarian cancer (Lu et al., 2016) and endometrial sarcoma cells (Zheng et al., 2016), in addition to reducing EMT through the ILK/AKT/mTOR signaling pathway in breast cancer cells (Ma et al., 2016) (Fig. 2). Besides, it has been proposed that emodin is able to inhibit Twist1-depended EMT in HNSCC cells by inhibiting the WNT/β-catenin and AKT pathways (Way et al., 2014). Interestingly, an artemisinin–melphalan conjugate drug (ARS4, an antimalarial agent) is highly toxic in ovarian cancer cells, but not in normal cells (Li et al., 2016c). In this context, ARS4 induced phenotypic changes resembling a MET process and promoted cell cycle arrest and apoptosis (Li et al., 2016c) (Fig. 2). Additionally, the small molecule cyclopamine, a steroidal alkaloid, and its semisynthetic analogue, IPI-269609, have shown efficacy against pancreatic cancer metastasis through the inhibition of Hedgehog signaling, downregulation of Snail1, and upregulation of E-cadherin in cells undergoing EMT (Katoh and Katoh, 2009). More recently, metformin, a well-tolerated treatment for type 2 diabetes mellitus which inhibits hepatic glucose production while...
increasing glucose uptake as well as reducing insulin resistance in peripheral tissue and gluconeogenesis (Goodwin et al., 2009; Hundal et al., 1992), has shown promise in cancer treatment (Barriere et al., 2013). In combination with chemotherapeutic agents, metformin seemed effective against PCa cell viability by repressing vimentin and N-cadherin while inducing E-cadherin expression (Zhang et al., 2014b) (Fig. 2).

Interestingly, the emergence of targeted therapies against signaling regulators of EMT might lead toward clinical benefits due to the specific targeting of cancer cells undergoing EMT. In this line, dasatinib, a SRC kinase inhibitor, decreased lung cancer cell growth upon EMT induction (Wilson et al., 2014). Additionally, the combination of dasatinib with erlotinib, another SRC inhibitor, has emerged as a promising treatment to prevent EMT-induced resistance (Sesumi et al., 2017) (Fig. 2). Moreover, high-throughput screenings aimed at identifying anti-EMT-related drugs have been recently developed (Arai et al., 2016; Carmody et al., 2012; Gupta et al., 2009). In one of them, 19 compounds were initially chosen as potential EMT and stemness inhibitors, uncovering a synthetic compound, ML245 (BRD-K59019422-001-01-3), that restrained cancer cell progression although additional studies are necessary to clarify the exact mechanism of action (Carmody et al., 2011). Another massive screening targeted toward specific cancer cell states and stemness properties identified salinomycin, a potassium ionophore, with potential effects on undifferentiated breast carcinoma cells (Gupta et al., 2009). Interestingly, two additional drugs, SB525334 and SU9516, targeting TGFβR1 (TGFβ receptor 1) and CDK2 (cyclin dependent kinase 2), respectively (Fig. 2), were identified in another high-throughput screening showing EMT inhibitory activity in lung cancer cells (Arai et al., 2016).

On the other hand, recent therapeutic strategies have been focused on EMT targeting through RNA interference, miRNA, and agomiRs/antagomiRs. However, their clinical application is still very limited due to unresolved issues such as target organ delivery and immune response circumvention (Shah and Calin, 2014). Despite these disadvantages, several studies showed their success in the downregulation of specific EMT mediators, advocating for their use as novel therapies or as sensitizers to radio- or chemotherapies able to block not only the EMT process but also metastasis (de Jong et al., 2015; Smith and Bhowmick, 2016) (Fig. 2). Accordingly, EMT might be neutralized using miR-875-5p, which mediates EGFR downregulation in PCa cells, promoting a radiosensitizing effect (El Bezawy et al., 2017). Additionally, the inhibition of VEGFR (VEGF receptor) expression using three artificial miRNA in PCa cells and xenograft mouse models showed a synergistic effect with standard chemotherapy and was associated with EMT inhibition (Huang et al., 2017). Indeed, it has been recently described that the combination of vaccines with miR-200 agomiRs or shZEB1 significantly inhibited EMT features in melanoma cells and prompted an immune response that blocked melanoma growth and metastasis in mouse models (Wang et al., 2016). Recent advances in the delivery of oligonucleotides, approved by the FDA for other pathologies like several nervous system disorders (Khorkova and Wahlestedt, 2017), pave the way for future advances in the application of oligonucleotide-based therapies targeting EMT and metastasis.

Finally, based on the epigenetic regulation of EMT (reviewed in Serrano-Gomez et al., 2016), it has been postulated that selected EMT-targeting epigenetic drugs can be used alone or in combination with conventional chemotherapies to overcome drug resistance (Sun and Fang, 2016). In this line, specific epigenetic inhibitors, such as sorafenib or mocetinostat, could also serve as powerful tools to target epigenetic pathways regulating EMT in vivo (Kiesslich et al., 2013) (Fig. 2). Sorafenib reverses histone modifications related to EMT in lung carcinoma cells (Zhang et al., 2013), and mocetinostat is a histone deacetylase (HDAC) inhibitor able to reverse the EMT phenotype and sensitize resistant PCa cells to docetaxel by restoring miR-203 expression and promoting ZEB1 inhibition (Meidhof et al., 2015).

4.3. Clinical trials for EMT inhibition

Recent reviews have summarized the current status of clinical studies designed to understand the role of specific EMT suppressor treatments (reviewed in Chaffer et al., 2016; Marcucci et al., 2016). Actual advances for targeted anti-EMT therapies to the clinical setting obviously require the development of clinical trials. Currently, there are some randomized clinical studies (https://clinicaltrials.gov/) in which one of the main selection criteria is the expression of specific EMT markers and the final aim is the direct or indirect EMT inhibition using novel molecular-based personalized therapies (Table 1). Most of these clinical trials are focused on testing the effects of different therapies on CTCs displaying specific EMT features. This is the case for the clinical trial based on the aspirin treatment for metastatic breast and colorectal CTCs with EMT features (NCT02602938, clinical trial identifier) (Table 1). Interestingly,
previous analyses reported the effectiveness of aspirin as a chemoprevention agent in some tumor types (Santilli et al., 2016). Other clinical trials focus on isolation and detection methods of CTCs based on EMT markers (Table 1).

Ongoing EMT-related clinical trials also include the use of known cancer targeted therapies, immunotherapies, and/or novel compounds. An example of conventional cancer therapy is the randomized phase II assay in patients with PCa (NCT01990196) using hormone therapy (androgen receptor inhibition) with and without chemotherapy against SRC or MEK (degarelix, enzalutamide, trametinib, or dasatinib) in terms of EMT inhibition (Table 1). Regarding therapies based on the use of antibodies, a phase I clinical trial with humanized monoclonal antibody AB-16B5 (NCT02412462) in advanced solid tumors is ongoing (Table 1 and Fig. 2). AB-16B5 was previously characterized for the inhibition of the potent EMT inducer secretory clusterin (sCLU) (Tremblay et al., 2010), a stress-activated and apoptosis-associated chaperone that protects cells from various stresses (Shiota et al., 2012). Moreover, sCLU was also significantly upregulated during tumor progression and metastasis (Wang et al., 2012). Finally, other clinical studies are focused on novel compounds as potential EMT-interfering drugs. MK-0646 or dalotuzumab, a humanized IgG1 monoclonal antibody which binds and blocks IGF1R (IGF1 receptor) (Atzori et al., 2011), is evaluated in combination with gemcitabine, or gemcitabine plus erlotinib, in patients with advanced pancreatic cancer to test its role in progression-free and overall survival in correlation with the expression of IGFIR, AKT, and diverse EMT biomarkers (NCT00769483) (Table 1).

Already finalized clinical trials have provided valuable data confirming the potential benefit of the treatments aimed at targeting EMT. This is the case of the phase I trial performed in advanced solid tumor patients that demonstrated the effectiveness of MRX34 (Beg et al., 2017), a liposomal miR-34a mimic, which inhibits Snail1-mediated EMT and the NOTCH pathway in pancreatic cancer cell lines (Tang et al., 2017).

Despite the advances in the molecular understanding of EMT and cell plasticity processes, not only regarding invasiveness and metastasis but also in association with therapy response and stem cell-like properties, further experimental work is necessary to render EMT knowledge beneficial for clinical standard of care. An important word of caution toward therapeutic approaches aimed at blocking EMT relates to the inherent plasticity of the EMT process during tumor progression and metastasis. While some therapies could help halt tumor cell invasion and dissemination, they might be highly deleterious for metastasis outgrowth by favoring the MET program. In addition, they may not be exploited for targeting early disseminated tumor cells believed to shred from primary tumors before diagnosis. From the present knowledge, targeting the hybrid E/M phenotype

Table 1. Active EMT-related clinical trials currently in patient recruitment phasea.

| IDb | Title                                                                 | EMT-related target                                      |
|-----|------------------------------------------------------------------------|--------------------------------------------------------|
| NCT02412462 | Phase I Dose Escalation Study of AB-16B5 in Subjects With an Advanced Solid Malignancy | Secreted clusterin (sCLU)                             |
| NCT02913859 | Hormone Therapy With or Without Definitive Radiotherapy in Metastatic Prostate Cancer | N-cadherin, E-cadherin, vimentinf                          |
| NCT02602938 | Aspirin on CTCs of Advanced Breast and Colorectal Cancer (ACABC) | Number and subtype of CTCs                            |
| NCT01990196 | Neoadjuvant Phase II Study Comparing the Effects of AR Inhibition With/Without SRC or MEK Inhibition in Prostate Cancer | N-cadherin and vimentin expression                        |
| NCT02119559 | Circulating Tumor Cells as Early Predictive in Head-and-Neck Squamous-Cell Carcinoma (CIRCUTEC) | CTCs on the progression-free survival and EMT markersf |
| NCT02119559 | Isolation of Circulating Tumor Cells Using a Novel EMT-Based Capture Method (CTC-EMT) | Presence of EMT markers on the prognosisf              |
| NCT02951897 | Application of Detecting Circulating Tumor Cells in the Accurate Treatment of Early Stage Lung Adenocarcinoma (CTCs detection) | Characterization of epithelial (E) CTCs, mesenchymal (M) CTCs, and epithelial/mesenchymal (E/M) CTCs in early diagnosisf |

*aStatus March 2017. 
bID: identifier study number in clinical trials page: https://clinicaltrials.gov/. 
cAnalysis of EMT markers (N-cadherin, E-cadherin, vimentin) before radiotherapy or after radiotherapy to establish disease progression-free survival. 
dEMT markers nonspecified. 
eEMT markers: cytokeratins 8, 18, and 19, EpCAM, vimentin, and TWIST.
(Fig. 2) seems the most promising strategy for the clinical application of EMT and epithelial plasticity in the, hopefully, near future.

5. Concluding remarks and future perspectives

The EMT field has experienced an enormous progress in the last years leading to a comprehensive understanding of the molecular pathways and regulators of the process. In the context of metastasis, the demonstration of the transient and reversible nature of EMT process, the requirement of MET for metastasis outgrowth along with the report of hybrid E/M states during the metastatic cascade have been key advances. Preclinical genetic mouse models and innovative intravital imaging have also helped to demonstrate the plasticity of the process in vivo and highlighted the nonredundant and complementary functions of EMT-TFs and their specificity in distinct tumors. All these recent studies have contributed to clarify the key in vivo role of epithelial plasticity processes at different metastasis steps. Despite all this progress, EMT clinical translation is still very limited. Nevertheless, support for using EMT markers and/or EMT signatures as predictor or prognosis factors is continuously increasing. This is particularly relevant in relation to acquired resistance for which further improvements in CTC detection methods aimed at capturing different plastic E/M states are envisioned for the near future. In addition, better understanding of the contribution of the stromal component, in particular activated fibroblast and immune components, to modulate epithelial plasticity processes, will undoubtedly contribute to improve its translation potential.

It is anticipated that the continuous development of highly sophisticated preclinical models and novel imaging techniques, together with innovative high-throughput screening platforms to target hybrid E/M phenotypes, will provide new avenues to explore the clinical potential of epithelial plasticity in the near future.

Acknowledgements

We apologize to all those colleagues whose important work has not been cited. Work in our laboratories is supported by grants from the Spanish Ministerio de Economía y Competitividad SAF2013-44739-R and SAF2016-76504-R (AC and FP), the Spanish Instituto de Salud Carlos III [RETIC-RD12/0036/0007, CIBER-ONC-CB16/12/00295 (AC); PI13/00132, PI16/00134 (GMB)] (all of them partly supported from EU-FEDER funds), and Worldwide Cancer Research (WWCR, formerly AICR) WWCR 16-0295 (AC, PGS, and FP). PGS was funded by a postdoctoral contract from the Fundación Científica AECC and presently from WWCR 16-0295.

References

Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H et al. (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 158, 1110–1122.

Acs G, Lawton TJ, Rebbeck TR, LiVolsi VA and Zhang PJ (2001) Differential expression of E-cadherin in lobular and ductal neoplasms of the breast and its biologic and diagnostic implications. Am J Clin Pathol 115, 85–98.

Alba-Castellon L, Olivera-Salguero R, Mestre-Farrera A, Pena R, Herrera M, Bonilla F, Casal JI, Baulida J, Pena C and Garcia de Herreros A (2016) Snail1-dependent activation of cancer-associated fibroblast controls epithelial tumor cell invasion and metastasis. Cancer Res 76, 6205–6217.

Alix-Panabieres C, Mader S and Pantel K (2017) Epithelial-mesenchymal plasticity in circulating tumor cells. J Mol Med 95, 133–142.

Alonso-Alconada L, Muinelo-Romay L, Madissoo K, Diaz-Lopez A, Krakstad C, Trovik J, Wik E, Happangama D, Coenegrachts L, Cano A et al. (2014) Molecular profiling of circulating tumor cells links plasticity to the metastatic process in endometrial cancer. Mol Cancer 13, 223.

Arai K, Eguchi T, Rahman MM, Sakamoto R, Masuda N, Nakatsura T, Calderwood SK, Kozaki K and Itoh M (2016) A novel high-throughput 3D screening system for EMT inhibitors: a pilot screening discovered the EMT inhibitory activity of CDK2 inhibitor SU9516. PLoS One 11, e0162394.

Armstrong AJ, Marengo MS, Oliet S, Kemeny G, Bitting RL, Turnbull JD, Herold CI, Marcom PK, George DJ and Garcia-Blanco MA (2011) Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers. Mol Cancer Res 9, 997–1007.

Atzori F, Tabernero J, Cervantes A, Prudkin L, Andreu J, Rodriguez-Braun E, Domingo A, Guijarro J, Gamez C, Rodon J et al. (2011) A phase I pharmacokinetic and pharmacodynamic study of dalotuzumab (MK-0646), an anti-insulin-like growth factor-I receptor monoclonal antibody, in patients with advanced solid tumors. Clin Cancer Res 17, 6304–6312.

Barbazan J, Muinelo-Romay L, Vieito M, Candamio S, Diaz-Lopez A, Cano A, Gomez-Tato A, Casares de Cal MA, Abal M and Lopez-Lopez R (2014) A multimarker panel for circulating tumor cells detection predicts patient outcome and therapy response in
metastatic colorectal cancer. *Int J Cancer* **35**, 2633–2643.

Barriere G, Fici P, Gullerani G, Fabbri F, Zoli W and Rigaud M (2014) Circulating tumor cells and epithelial, mesenchymal and stemness markers: characterization of cell subpopulations. *Ann Transl Med* **2**, 109.

Barriere G, Tartary M and Rigaud M (2013) Metformin: a rising star to fight the epithelial-mesenchymal transition in oncology. *Anticancer Agents Med Chem* **13**, 333–340.

Becker KF, Rosivatz E, Blechschmidt K, Kremmer E, Sarbia M and Hofler H (2007) Analysis of the E-cadherin repressor Snail in primary human cancers. *Cells Tissues Organs* **185**, 204–212.

Beerling E, Seinstra D, de Wit E, Kester L, van der Velden D, Maynard C, Schafer R, van Diest P, Voest E, van Oudenaarden A et al. (2016) Plasticity between epithelial and mesenchymal states unlinks EMT from metastasis-enhancing stem cell capacity. *Cell Rep* **14**, 2281–2288.

Beg MS, Brenner AJ, Sachdev J, Borad M, Kang YK, Stoudemire J, Smith S, Bader AG, Kim S and Hong DS (2017) Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Invest New Drugs* **35**, 180–188.

Berx G, Cleton-Jansen AM, Strumane K, de Leeuw WJ, Nollet F, van Roy F and Cornelisse C (1996) E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain. *Oncogene* **13**, 1919–1925.

Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG and Moses HL (2004) TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* **303**, 848–851.

Bidard FC, Peeters DJ, Fehm T, Nole F, Gisbert-Criado R, Mavroudis D, Grisanti S, Generali D, Garcia-Saenz JA, Stebbing J et al. (2014) Clinical validity of circulating tumor cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. *Lancet Oncol* **15**, 406–414.

Blanco MJ, Moreno-Bueno G, Sarrio D, Locascio A, Cano A, Palacios J and Nieto MA (2002) Correlation of Snail expression with histological grade and lymph node status in breast carcinomas. *Oncogene* **21**, 3241–3246.

Brabletz T (2012a) EMT and MET in metastasis: where are the cancer stem cells? *Cancer Cell* **22**, 699–701.

Brabletz T (2012b) To differentiate or not–routes towards metastasis. *Nat Rev Cancer* **12**, 425–436.

Brabletz S and Brabletz T (2010) The ZEB/miR-200 feedback loop—a motor of cellular plasticity in development and cancer? *EMBO Rep* **11**, 670–677.

Brabletz T, Jung A, Spaderna S, Hluck F and Kirchner T (2005) Opinion: migrating cancer stem cells - an integrated concept of malignant tumor progression. *Nat Rev Cancer* **5**, 744–749.

Bruney L, Liu Y, Grisoli A, Ravosa MJ and Stack MS (2016) Integrin-linked kinase activity modulates the pro-metastatic behavior of ovarian cancer cells. *Oncotarget* **7**, 21968–21981.

Cabel L, Proudhon C, Gortais H, Loirat D, Coussy F, Pierga JY and Bidard FC (2017) Circulating tumor cells: clinical validity and utility. *Int J Clin Oncol* **22**, 421–430.

Carmody LC, Germain A, Morgan B, VerPlank L, Fernandez C, Feng Y, Perez J, Dandapani S, Munoz B, Palmer M et al. Identification of a selective small-molecule inhibitor of breast cancer stem cells – probe 3. (2011) Probe Reports from the NIH Molecular Libraries Program. Bethesda. https://www.ncbi.nlm.nih.gov/books/NBK143545/.

Carmody LC, Germain AR, VerPlank L, Nag PP, Munoz B, Perez JR and Palmer MA (2012) Phenotypic throughput screening elucidates target pathway in breast cancer stem cell-like cells. *J Biomol Screen* **17**, 1204–1210.

Castilla MA, Moreno-Bueno G, Romero-Perez L, Van De Vijver K, Biscuola M, Lopez-Garcia MA, Prat J, Matias-Guiu X, Cano A, Oliva E et al. (2011) Micro-RNA signature of the epithelial-mesenchymal transition in endometrial carcinosarcoma. *J Pathol* **223**, 72–80.

Celia-Terrassa T and Kang Y (2016) Distinctive properties of metastasis-initiating cells. *Genes Dev* **30**, 892–908.

Celia-Terrassa T, Meca-Cortes O, Mateo F, Martinez de Paz A, Rubio N, Arnal-Estape A, Ell BJ, Bermudo R, Diaz A, Guerra-Rebollo M et al. (2012) Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. *J Clin Invest* **122**, 1849–1868.

Ceyran AB, Senol S, Simsek BC, Sagirolu J and Aydin A (2015) Role of cd56 and E-cadherin expression in the differential diagnosis of papillary thyroid carcinoma and suspected follicular-patterned lesions of the thyroid: the prognostic importance of e-cadherin. *Int J Clin Exp Pathol* **8**, 3670–3680.

Chaffer CL, San Juan BP, Lim E and Weinberg RA (2016) EMT, cell plasticity and metastasis. *Cancer Metastasis Rev* **35**, 645–654.

Channerion M, Kuperstein I, Barriere C, El Marjou F, Cohen D, Vignejdiev D, Stimmer L, Paul-Gilloteaux P, Bieche I, Tavares Sdos R et al. (2014) Concomitant Notch activation and p53 deletion trigger epithelial-to-mesenchymal transition and metastasis in mouse gut. *Nat Commun* **5**, 5005.

Cheung KJ, Padmanaban V, Silvestri V, Schipper K, Cohen JD, Fairchild AN, Gorin MA, Verdone JE, Pienta KJ, Bader JS et al. (2016) Polyclonal breast cancer metastases arise from collective dissemination of
keratin 14-expressing tumor cell clusters. Proc Natl Acad Sci USA 113, E854–E863.

Chui MH (2013) Insights into cancer metastasis from a clinicopathologic perspective: Epithelial-Mesenchymal Transition is not a necessary step. Int J Cancer 132, 1487–1495.

Cristofanilli M, Budd GT, Ellis MJ, Stopec A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW et al. (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351, 781–791.

Da C, Wu K, Yue C, Bai P, Wang R, Wang G, Zhao M, Lü Y and Hou P (2017) N-cadherin promotes thyroid tumorigenesis through modulating major signaling pathways. Oncotarget 8, 8131–8142.

De Craene B and Berx G (2013) Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 13, 97–110.

Della Corte CM, Bellevicine C, Vicidomini G, Vitagliano D, Malapelle U, Accardo M, Fabozzi A, Fiorelli A, Fasano M, Papaccio F et al. (2015) SMO gene amplification and activation of the Hedgehog pathway as novel mechanisms of resistance to anti-epidermal growth factor receptor drugs in human lung cancer. Clin Cancer Res 21, 4686–4697.

Diaz-Lopez A, Diaz-Martín J, Moreno-Bueno G, Cuevas EP, Santos V, Olmeda D, Portillo F, Palacios J and Cano A (2015) Zeb1 and Snail1 engage miR-200f transcriptional and epigenetic regulation during EMT. Int J Cancer 136, E62–E73.

Diaz-Lopez A, Moreno-Bueno G and Cano A (2014) Role of microRNA in epithelial to mesenchymal transition and metastasis and clinical perspectives. Cancer Manag Res 6, 205–216.

Diaz-Martín J, Diaz-Lopez A, Moreno-Bueno G, Castilla MA, Rosa-Rosa JM, Cano A and Palacios J (2014) A core microRNA signature associated with inducers of the epithelial-to-mesenchymal transition. J Pathol 232, 319–329.

Diepenbruck M and Christofori G (2016) Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? Curr Opin Cell Biol 43, 7–13.

Du B and Shim JS (2016) Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules 21, 965.

El Bezawy R, Cominetti D, Fenderico N, Zuco V, Beretta GL, Dugo M, Arrighetti N, Stucchi C, Rancati T, Valdagni R et al. (2017) miR-875-5p counteracts epithelial-to-mesenchymal transition and enhances radiation response in prostate cancer through repression of the EGFR-ZEB1 axis. Cancer Lett 395, 53–62.

Feng X, Wang Z, Fillmore R and Xi Y (2014) MiR-200, a new star miRNA in human cancer. Cancer Lett 344, 166–173.

Fici P, Gallerani G, Morel AP, Mercatali L, Ibrahim T, Scarpì E, Amadori D, Puissieux A, Rigaud M and Fabbri F (2017) Splicing factor ratio as an index of epithelial-mesenchymal transition and tumor aggressiveness in breast cancer. Oncotarget 8, 2423–2436.

Fischer KR, Durrans A, Lee S, Sheng J, Li F, Wong ST, Choi H, El Rayes T, Ryu S, Troeger J et al. (2015) Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature 527, 472–476.

Fitzgerald RC and Caldas C (2004) Clinical implications of E-cadherin associated hereditary diffuse gastric cancer. Gut 53, 775–778.

da Fonseca LM, da Silva VA, Freire-de-Lima L, Previal JO, Mendonça-Previal L and Capella MA (2016) Glycosylation in cancer: Interplay between multidrug resistance and epithelial-to-mesenchymal transition? Front Oncol 6, 158.

Frangi C, Takkunen M, Dave N, Alameda F, Gomez S, Rodriguez R, Escriva M, Montserrat-Sentis B, Baro T, Garrido M et al. (2006) Expression of Snail protein in tumor-stroma interface. Oncogene 25, 5134–5144.

Gamallo C, Palacios J, Suarez A, Pizarro A, Navarro P, Quintanilla M and Cano A (1993) Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. Am J Pathol 142, 987–993.

Garg M (2013) Epithelial-mesenchymal transition – activating transcription factors – multifunctional regulators in cancer. World J Stem Cells 5, 188–195.

Geradts J, de Herreros AG, Su Z, Burchette J, Broadwater G and Bachelder RE (2011) Nuclear Snail1 and nuclear ZEB1 protein expression in invasive and intraductal human breast carcinomas. Hum Pathol 42, 1125–1131.

Gibbons DL and Creighton CJ (2017) Pan-cancer survey of epithelial-mesenchymal transition markers across The Cancer Genome Atlas. Dev Dyn. https://doi.org/10.1002/dvdy.24485.

Gemis RR and Gawrzak S (2016) Tumor cell dormancy. Mol Onc 11, 62–78.

Goodwin PJ, Ligibel JA and Stambolic V (2009) Metformin in breast cancer: time for action. J Clin Oncol 27, 3271–3273.

Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A and Reeve AE (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392, 402–405.

Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA and Lander ES (2009) Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell 138, 645–659.

Harouaka R, Kang Z, Zheng SY and Cao L (2014) Circulating tumor cells: advances in isolation and...
EMT clinical applications

P. G. Santamaria et al.

Pharmacol Ther 141, 209–221.

Harper KL, Sosa MS, Entenberg D, Hosseini H, Cheung JF, Nobre R, Avivar-Valderas A, Nagi C, Giriun N, Davis RJ et al. (2016) Mechanism of early dissemination and metastasis in Her2+ mammary cancer. Nature 540, 588–592.

Hennessey BT, Gonzalez-Angulo AM, Stemke-Hale K, Gilcrease MZ, Krishnamurthy S, Lee JS, Fridlyand J, Sahin A, Agarwal R, Joy C et al. (2009) Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res 69, 4116–4124.

Hong T, Watanabe K, Ta CH, Villarreal-Ponce A, Nie Q and Dai X (2015) An Ovol-Zeb1 mutual inhibitory circuit governs bidirectional and multi-step transition between epithelial and mesenchymal states. PLoS Comput Biol 11, e1004569.

Hsu DS, Lan HY, Huang CH, Tai SK, Chang SY, Tsai TL, Chang CC, Tseng CH, Wu KJ, Kao JY et al. (2010) Regulation of excision repair cross-complementation group 1 by Snail contributes to cisplatin resistance in head and neck cancer. Clin Cancer Res 16, 4561–4571.

Huang J, Mei H, Tang Z, Li J, Zhang X, Lu Y, Huang F, Jin Q and Wang Z (2017) Triple-amiRNA VEGFRs inhibition in pancreatic cancer improves the efficacy of chemotherapy through EMT regulation. J Control Release 245, 1–14.

Hundal HS, Ramial T, Reyes R, Leiter LA and Klip A (1992) Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. Endocrinology 131, 1165–1173.

Imani S, Hosseinifard H, Cheng J, Wei C and Fu J (2016) Prognostic value of EMT-inducing Transcription Factors (EMT-TFs) in metastatic breast cancer: a systematic review and meta-analysis. Sci Rep 6, 28587.

Janni WJ, Rack B, Terstappen LW, Pierga JY, Taran FA, Fehm T, Hall C, de Groot M, Bidard FC, Friedl TW et al. (2016) Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. Clin Cancer Res 22, 2583–2593.

Jia D, Jolly MK, Boureto M, Parsana P, Mooney SM, Pienta KJ, Levine H and Ben-Jacob E (2015) Ovol guides the epithelial-hybrid-mesenchymal transition. Oncotarget 6, 15436–15448.

Jiang X, Wang J, Zhang K, Tang S, Ren C and Chen Y (2015) The role of CD29-ILK-Akt signaling-mediated epithelial-mesenchymal transition of liver epithelial cells and chemoresistance and radioresistance in hepatocellular carcinoma cells. Med Oncol 32, 141.

Jolly MK, Tripathi SC, Jia D, Mooney SM, Celiktas M, Hanash SM, Mani SA, Pienta KJ, Ben-Jacob E and Levine H (2016) Stability of the hybrid epithelial/mesenchymal phenotype. Onco Targets Ther 7, 27067–27084.

de Jong MC, Ten Hoeve JJ, Grennan R, Wessels LF, Kerkhoven R, Te Riele H, van den Brekel MW, Verheij M and Begg AC (2015) Pretreatment microRNA Expression Impacting on Epithelial-to-Mesenchymal Transition Predicts Intrinsic Radiosensitivity in Head and Neck Cancer Cell Lines and Patients. Clin Can Res 21, 5630–5638.

Kajita M, McClinic KN and Wade PA (2004) Aberrant expression of the transcription factors snail and slug alters the response to genotoxic stress. Mol Cell Biol 24, 7559–7566.

Kalluri R and Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119, 1420–1428.

Katoh Y and Katoh M (2009) Hedgehog target genes: mechanisms of carcinogenesis induced by aberrant hedgehog signaling activation. Curr Mol Med 9, 873–886.

Khorkova O and Wahlestedt C (2017) Oligonucleotide therapies for disorders of the nervous system. Nat Biotechnol 35, 249–263.

Kiesslich T, Pichler M and Neureiter D (2013) Epigenetic control of epithelial-mesenchymal-transition in human cancer. Mol Cell Oncol 1, 3–11.

Kim J, Hong SJ, Park JY, Park JH, Yu YS, Park SY, Lim EK, Choi KY, Lee EK, Paik SS et al. (2010) Epithelial-mesenchymal transition gene signature to predict clinical outcome of hepatocellular carcinoma. Cancer Sci 101, 1521–1528.

Korporal M, Ell BJ, Buffa FM, Ibrahim T, Blanco MA, Celia-Terrassa T, Mercatali L, Khan Z, Goodarzi H, Hua Y et al. (2011) Direct targeting of Sec23a by miR-200s influences cancer cell secretome and promotes metastatic colonization. Nat Med 17, 1101–1108.

Krebs AM, Mitschke J, Lasierros Losada M, Schmalhofer O, Borries M, Busch H, Boettcher M, Mougiasakos D, Reichardt W, Bronsert P et al. (2017) The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nat Cell Biol 19, 518–529.

Kurrey NK, Jalgaardak SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY and Bapat SA (2009) Snail and Slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. Stem Cells 27, 2059–2068.

Labelle M, Begum S and Hynes RO (2011) Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 20, 576–590.

Lambert AW, Pattabiraman DR and Weinberg RA (2017) Emerging Biological Principles of Metastasis. Cell 168, 670–691.
Lamouille S, Xu J and Derynck R (2014) Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Bio 15, 178–196.

Leblanc R and Peyruchaud O (2016) Metastasis: new functional implications of platelets and megakaryocytes. Blood 128, 24–31.

Ledford H (2011) Cancer theory faces doubts. Nature 472, 273.

Li J, Ai Y, Wang L, Bu P, Sharkey CC, Wu Q, Wun B, Roy S, Shen X and King MR (2016a) Targeted drug delivery to circumventing tumor cells via platelet membrane-functionalized particles. Biomaterials 76, 52–65.

Li J, Sharkey CC, Wun B, Liesveld JL and King MR (2016b) Genetic engineering of platelets to neutralize circulating tumor cells. J Control Release 228, 38–47.

Li YM, Xu SC, Li J, Han KQ, Pi HF, Zheng L, Zuo GH, Huang XB, Li HY, Zhao HZ et al. (2013) Epithelial-mesenchymal transition markers expressed in circulating tumor cells in hepatocellular carcinoma patients with different stages of disease. Cell Death Dis 4, e831.

Li X, Zhou Y, Liu Y, Zhang X, Chen T, Chen K, Ba Q, Li J, Liu H and Wang H (2016c) Preclinical efficacy and safety assessment of artemisinin-chemotherapeutic agent conjugates for ovarian cancer. EBioMedicine 14, 44–54.

Liu W, Vivian CJ, Brinker AE, Hampton KR, Lianidou E and Welch DR (2014) Microenvironmental influence on metastasis suppressor expression and function during a metastatic cell’s journey. Cancer Microenviron 7, 117–131.

Lu J, Xu Y, Wei X, Zhao Z, Xue J and Liu P (2016) Emodin inhibits the epithelial to mesenchymal transition of epithelial ovarian cancer cells via ILK/GSK-3beta/Slug signaling pathway. Biomed Res Int 2016, 6253280.

Ma JW, Hung CM, Lin YC, Ho CT, Kao JY and Way TD (2016) Aloe-emodin inhibits HER-2 expression through the downregulation of Y-box binding protein 1 in HER-2-overexpressing human breast cancer cells. Oncotarget 7, 58915–58930.

Malek R, Wang H, Taparra K and Tran PT (2017) Therapeutic targeting of epithelial plasticity programs, focus on the epithelial-mesenchymal transition. Cells Tissues Organs 203, 114–127.

Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M et al. (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133, 704–715.

Marcucci F, Stassi G and De Maria R (2016) Epithelial-mesenchymal transition: a new target in anticancer drug discovery. Nat Rev Drug Discov 15, 311–325.

McInnes LM, Jacobson N, Redfern A, Dowling A, Thompson EW and Saunders CM (2015) Clinical implications of circulating tumor cells of breast cancer patients: role of epithelial-mesenchymal plasticity. Front Oncol 5, 42.

Meidhof S, Brabletz S, Lehmann W, Preca BT, Mock K, Ruh M, Schuler J, Berthold M, Weber A, Burk U et al. (2015) ZEB1-associated drug resistance in cancer cells is reversed by the class I HDAC inhibitor mocetinostat. EMBO Mol Med 7, 831–847.

Moll R, Mitze M, Frixen UH and Birchmeier W (1993) Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am J Pathol 143, 1731–1742.

Morel AP, Hinkal GW, Thomas C, Fauvet F, Courtois-Cox S, Wierinckx A, Devouassoux-Shisheboran M, Treilleux I, Tissier A, Gras B et al. (2012) EMT inducers catalyze malignant transformation of mammary epithelial cells and drive tumorigenesis towards claudin-low tumors in transgenic mice. PLoS Genet 8, e1002723.

Morel AP, Lievre M, Thomas C, Hinkal G, Anseiu S and Puisieux A (2008) Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS One 3, e2888.

Moreno-Bueno G, Peinado H, Molina P, Olmeda D, Cubillo E, Santos V, Palacios J, Portillo F and Cano A (2009) The morphological and molecular features of the epithelial-to-mesenchymal transition. Nat Protoc 4, 1591–1613.

Nieto MA (2011) The ins and outs of the epithelial to mesenchymal transition in health and disease. Annu Rev Cell Dev Biol 27, 347–376.

Nieto MA (2013) Epithelial plasticity: a common theme in embryonic and cancer cells. Science 342, 1234850.

Nieto MA and Cano A (2012) The epithelial-mesenchymal transition under control: global programs to regulate epithelial plasticity. Semin Cancer Biol 22, 361–368.

Nieto MA, Huang RY, Jackson RA and Thiery JP (2016) EMT: 2016. Cell 166, 21–45.

Ocaña OH, Corcoles R, Fabra A, Moreno-Bueno G, Acloque H, Vega S, Barrallo-Gimeno A, Cano A and Nieto MA (2012) Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. Cancer Cell 22, 709–724.

Okubo K, UenoSonosyo Y, Ariyami T, Yanagita S, Matsushita D, Kijima T, Amatsu T, Uchikado Y, Kijima Y, Maemura K et al. (2017) Clinical significance of altering epithelial-mesenchymal transition in metastatic lymph nodes of gastric cancer. Gastric Cancer. https://doi.org/10.1007/s10120-017-0705-x.

Parisi C, Mastoraki S, Markou A, Strati A, Chimonidou M, Georgoulia V and Lianidou ES (2016) Development and validation of a multiplex methylation specific PCR-coupled liquid bead array for liquid biopsy analysis. Clin Chim Acta 461, 156–164.
Pasquier J, Abu-Kaoud N, Al Thani H and Rafii A (2015) Epithelial to mesenchymal transition in a clinical perspective. J Oncol 2015, 792182.

Peinado H, Olmeda D and Cano A (2007) Snail, Zeb and bHLH factors in tumor progression: an alliance against the epithelial phenotype? Nat Rev Cancer 7, 415–428.

Peinado H, Portillo F and Cano A (2004) Transcriptional regulation of cadherins during development and carcinogenesis. Int J Dev Biol 48, 365–375.

Perez-Losos J, Sanchez-Martin M, Perez-Caro M, Perez-Mancera PA and Sanchez-Garcia I (2003) The radioresistance biological function of the SCF/kit signaling pathway is mediated by the zinc-finger transcription factor Slug. Oncogene 22, 4205–4211.

Polyak K and Weinberg RA (2009) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 9, 265–273.

Putzke AP, Ventura AP, Bailey AM, Akteur C, Opoku-Ansah J, Celiktas M, Hwang MS, Darling DS, Coleman IM, Nelson PS et al. (2011) Metastatic progression of prostate cancer and E-cadherin regulation by Zeb1 and SRC family kinases. Am J Pathol 179, 400–410.

Rastogi I, Rajanna S, Webb A, Chhabra G, Foster B, Webb B and Puri N (2016) Mechanism of c-Met and EGFR tyrosine kinase inhibitor resistance through epithelial mesenchymal transition in non-small cell lung cancer. Biochem Biophys Res Commun 477, 937–944.

Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH et al. (2012) EMT and dissemination precede pancreatic tumor formation. Cell 148, 349–361.

Roop RP, Naughton MJ, Van Poznak C, Schneider JG, Lammers PE, Phuard TJ, Johnson F, Eby CS and Weilbaecher KN (2013) A randomized phase II trial investigating the effect of platelet function inhibition on circulating tumor cells in patients with metastatic breast cancer. Clin Breast Cancer 13, 409–415.

Rowe RG, Li XY, Hu Y, Saunders TL, Virtanen I, Garcia de Herreros A, Becker KE, Ingvarsen S, Engelholm LH, Bommer GT et al. (2009) Mesenchymal cells reactivate Snail1 expression to drive three-dimensional invasion programs. J Cell Biol 184, 399–408.

Santilli F, Boccataonda A and Davi G (2016) Aspirin, platelets, and cancer: the point of view of the internist. Eur J Intern Med 34, 11–20.

Sarioglu AF, Aceto N, Kojic N, Donaldson MC, Zeinali M, Hamza B, Engstrom A, Zhu H, Sundaresan TK, Miyamoto DT et al. (2015) A microfluidic device for label-free, physical capture of circulating tumor cell clusters. Nat Methods 12, 685–691.

Sarrio D, Perez-Mies B, Hardisson D, Moreno-Bueno G, Suarez A, Cano A, Martin-Perez J, Gamallo C and Palacios J (2004) Cytoplasmic localization of p120ctn and E-cadherin loss characterize lobular breast carcinoma from preinvasive to metastatic lesions. Oncogene 23, 3272–3283.

Sarrio D, Rodriguez-Pinilla SM, Hardisson D, Cano A, Moreno-Bueno G and Palacios J (2008) Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. Cancer Res 68, 989–997.

Satelli A and Li S (2011) Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci 68, 3033–3046.

Saxena M, Stephens MA, Pathak H and Rangarajan A (2011) Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. Cell Death Dis 2, e179.

Schmalhofer O, Brabletz S and Brabletz T (2009) E-cadherin, beta-catenin, and ZEB1 in malignant progression of cancer. Cancer Metastasis Rev 28, 151–166.

Schoenhals M, Kassambara A, De Vos J, Hose D, Moreaux J and Klein B (2009) Embryonic stem cell markers expression in cancers. Biochem Biophys R 383, 157–162.

Sekhon K, Bucay N, Majid S, Daihya R and Saini S (2016) MicroRNAs and epithelial-mesenchymal transition in prostate cancer. Onco TARGET 7, 67597–67611.

Serrano-Gomez SJ, Maziveyi M and Alahari SK (2016) Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. Mol Cancer 15, 18.

Sesumi Y, Suda K, Mizuuchi H, Kobayashi Y, Sato K, Chiba M, Shimoji M, Tomizawa K, Takemoto T and Mitsudomi T (2017) Effect of dasatinib on EMT-mediated-mechanism of resistance against EGFR inhibitors in lung cancer cells. Lung Cancer 104, 85–90.

Shah MY and Calin GA (2014) MicroRNAs as therapeutic targets in human cancers. Wiley Interdiscip Rev RNA 5, 537–548.

Shiota M, Zardan A, Takeuchi A, Kumano M, Beraldi E, Naito S, Zoubeidi A and Gleave ME (2012) Clusterin mediates TGF-beta-induced epithelial-mesenchymal transition and metastasis via Twist1 in prostate cancer cells. Cancer Res 72, 5261–5272.

Smith BN and Bhowmick NA (2016) Role of EMT in metastasis and therapy resistance. J Clin Med 5, https://doi.org/10.3390/jcm5020017.

Somarelli JA, Schaeffer D, Marengo MS, Bepler T, Rouse D, Ware KE, Hish AJ, Zhao Y, Buckley AF, Epstein JI et al. (2016) Distinct routes to metastasis: plasticity-dependent and plasticity-independent pathways. Oncogene 35, 4302–4311.

Soundarrajan R, Paranjape AN, Barsan V, Chang JT and Mani SA (2015) A novel embryonic plasticity gene signature that predicts metastatic competence and clinical outcome. Sci Rep 5, 11766.
Spaderna S, Schmalhofer O, Hubek F, Berx G, Eger A, Merkel S, Jung A, Kirchner T and Brabletz T (2006) A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. *Gastroenterology* 131, 830–840.

Stankic M, Pavlovic S, Chin Y, Brogi E, Padua D, Norton L, Massague J and Benezra R (2013) TGF-beta-IId1 signaling opposes Twist1 and promotes metastatic colonization via a mesenchymal-to-epithelial transition. *Cell Rep* 5, 1228–1242.

Steinestel K, Eder S, Schrader AJ and Steinestel J (2014) Clinical significance of epithelial-mesenchymal transition. *Clin Transl Med* 3, 17.

Sun L and Fang J (2016) Epigenetic regulation of epithelial-mesenchymal transition. *Cell Mol Life Sci* 73, 4493–4515.

Takemoto A, Okitaka M, Takagi S, Takami M, Sato S, Tam WL and Weinberg RA (2013) The epigenetics of epithelial-mesenchymal transition. *EMBO Mol Med* 6, 1279–1293.

Tan TZ, Miow QH, Miki Y, Noda T, Mori S, Huang RY and Thiery JP (2014) Epithelial-mesenchymal transition spectrum quantification and its efficacy in deciphering survival and drug responses of cancer patients. *EMBO Mol Med* 6, 42186.

Tam WL and Weinberg RA (2013) The epigenetics of epithelial-mesenchymal plasticity in cancer. *Nat Med* 19, 1438–1449.

Tang Y, Tang Y and Cheng YS (2017) miR-34a inhibits pancreatic cancer progression through Snail1-mediated epithelial-mesenchymal transition and the Notch signaling pathway. *Sci Rep* 7, 38232.

Tarín D, Thompson EW and Newgreen DF (2005) The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res* 65, 5996–6000.

Taube JH, Herschkowitz JI, Komurov K, Zhou AY, Gupta S, Yang J, Hartwell K, Onder TT, Gupta PB, Evans KW et al. (2010) Core epithelial-to-mesenchymal transition interactome gene-expression signature is associated with claudin-low and metaplastic breast cancer subtypes. *Proc Natl Acad Sci USA* 107, 15449–15454.

Teicher BA, Holden SA, Ara G and Chen G (1996) Transforming growth factor-beta in in vivo resistance. *Cancer Chemother Pharmacol* 37, 601–609.

Thiery JP (2002) Epithelial-mesenchymal transitions in tumor progression. *Nat Rev Cancer* 2, 442–454.

Thiery JP (2003) Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol* 15, 740–746.

Thiery JP, Acloque H, Huang RY and Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. *Cell* 139, 871–890.

Thiery JP and Sleeman JP (2006) Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* 7, 131–142.

Trager MM and Dhayat SA (2017) Epigenetics of epithelial-to-mesenchymal transition in pancreatic carcinoma. *Int J Cancer* 141, 24–32.

Tran HD, Luiel K, Kim M, Zhang K, Longmore GD and Tran DD (2014) Transient SNAIL1 expression is necessary for metastatic competence in breast cancer. *Cancer Res* 74, 6330–6340.

Tremblay G, Malouin M, Grothe S, Kalbakji A, Roy S, Pagé M, Paul-Roc B, Sulea T, Lenferink A, O’Connor-McCourt M et al. (2010) AB-16B5, a therapeutic monoclonal antibody against human clusterin that blocks the epithelial-to-mesenchymal transition. *Cancer Res* 70, 1467.

Tsai JH, Donaher JL, Murphy DA, Chau S and Yang J (2012) Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* 22, 725–736.

Wang C, Jiang K, Kang X, Gao D, Sun C, Li Y, Sun L, Zhang S, Liu X, Wu W et al. (2012) Tumor-derived secretory clusterin induces epithelial-mesenchymal transition and facilitates hepatocellular carcinoma metastasis. *Int J Biochem Cell Biol* 44, 2308–2320.

Wang J, Wang K, Xu J, Huang J and Zhang T (2013) Prognostic significance of circulating tumor cells in non-small-cell lung cancer patients: a meta-analysis. *PLoS One* 8, e78070.

Wang X, Zhao F, Shi F, He X, Pan M, Wu D, Li M, Zhang Y and Dou J (2016) Reinforcing B16F10/GPI-IL-21 vaccine efficacy against melanoma by injecting mice with shZEB1 plasmid or miR200c agomir. *Biomed Pharmacother* 80, 136–144.

Watanabe K, Villarreal-Ponce A, Sun P, Salmons ML, Fullahi M, Andersen B and Dai X (2014) Mammary morphogenesis and regeneration require the inhibition of EMT at terminal end buds by Ovol2 transcriptional repressor. *Dev Cell* 29, 59–74.

Way TD, Huang JT, Chou CH, Huang CH, Yang MH and Ho CT (2014) Emodin represses TWIST1-induced epithelial-mesenchymal transitions in head and neck squamous cell carcinoma cells by inhibiting the beta-catenin and Akt pathways. *Eur J Cancer* 50, 366–378.

Wilson C, Nicholes K, Bustos D, Lin E, Song Q, Stephan JP, Kirkpatrick DS and Settleman J (2014) Overcoming EMT-associated resistance to anti-cancer drugs via Src/FAK pathway inhibition. *Oncotarget* 5, 7328–7341.

Wu S, Liu S, Liu Z, Huang J, Pu X, Li J, Yang D, Deng H, Yang N and Xu J (2015) Classification of circulating tumor cells by epithelial-mesenchymal transition markers. *PLoS One* 10, e0123976.

Yamada S, Okumura N, Wei L, Fuchs BC, Fujii T, Sugimoto H, Nomoto S, Takeda S, Tanabe KK and
Kodera Y (2014) Epithelial to mesenchymal transition is associated with shorter disease-free survival in hepatocellular carcinoma. Ann Surg Oncol 21, 3882–3890.

Yang MH, Chang SY, Chiou SH, Liu CJ, Chi CW, Chen PM, Teng SC and Wu KJ (2007) Overexpression of NBS1 induces epithelial-mesenchymal transition and co-expression of NBS1 and Snail predicts metastasis of head and neck cancer. Oncogene 26, 1459–1467.

Yang J and Weinberg RA (2008) Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14, 818–829.

Ye X, Tam WL, Shibue T, Kaygusuz Y, Reinhardt F, Ng Eaton E and Weinberg RA (2015) Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. Nature 525, 256–260.

Yeung KT and Yang J (2017) Epithelial-mesenchymal transition in tumor metastasis. Mol Oncol 11, 28–39.

Zheng Q, Xu Y, Lu J, Zhao J, Wei X and Liu P (2016) Emodin inhibits migration and invasion of human endometrial stromal cells by facilitating the mesenchymal-epithelial transition through targeting ILK. Reprod Sci 23, 1526–1535.

Zhong X, Carstens JL, Kim J, Scheible M, Kaye J, Sugimoto H, Wu CC, LeBleu VS and Kalluri R (2015) Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. Nature 527, 525–530.