Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits

Kornelia Polyak and Robert A. Weinberg‡*

Abstract | Transitions between epithelial and mesenchymal states have crucial roles in embryonic development. Emerging data suggest a role for these processes in regulating cellular plasticity in normal adult tissues and in tumours, where they can generate multiple, distinct cellular subpopulations contributing to intratumoural heterogeneity. Some of these subpopulations may exhibit more differentiated features, whereas others have characteristics of stem cells. Owing to the importance of these tumour-associated phenotypes in metastasis and cancer-related mortality, targeting the products of such cellular plasticity is an attractive but challenging approach that is likely to lead to improved clinical management of cancer patients.

**Department of Medical Oncology, Dana–Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts 02115, USA.*
‡Whitehead Institute for Biomedical Research, Ludwig MIT Center for Molecular Oncology and MIT Department of Biology, Cambridge, Massachusetts 02142, USA.
Correspondence to K.P.
e-mail: Kornelia_Polyak@dfci.harvard.edu
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Changes in cell phenotype between epithelial and mesenchymal states, defined as epithelial–mesenchymal (EMT) and mesenchymal–epithelial (MET) transitions, have key roles in embryonic development, and their importance in the pathogenesis of cancer and other human diseases is increasingly recognized[1–4]. The term EMT refers to a complex molecular and cellular programme by which epithelial cells shed their differentiated characteristics, including cell–cell adhesion, planar and apical–basal polarity, and lack of motility, and acquire instead mesenchymal features, including motility, invasiveness and a heightened resistance to apoptosis. The EMT transdifferentiation programme was first described as a cell culture phenomenon and its relevance to *in vivo* physiological processes was long debated[1–4]. However, accumulating observations of human tumours and experimental animal models have provided convincing evidence for its physiological relevance to both normal embryogenesis and carcinogenesis[5,6]. Thus, similar to embryonic development (BOX 1), both EMTs and METs seem to have crucial roles in the tumorigenic process. In particular, EMTs have been found to contribute to invasion, metastatic dissemination and acquisition of therapeutic resistance. METs — the reversal of EMTs — seem to occur following dissemination and the subsequent formation of distant metastases.

An essential difference between the embryonic and tumorigenic processes is that the tumorigenic processes involve genetically abnormal cells that progressively lose their responsiveness to normal growth-regulatory signals and possess the ability to evolve. Such evolution derives from the genetic and epigenetic instability that is inherent in most neoplastic cell types. This instability, which generates multiple distinct subpopulations of cancer cells within larger tumours, is only one source of phenotypical heterogeneity within tumours. The other derives from the cell-biological changes induced in cancer cells by signals that they receive from their stromal microenvironment; these changes include, prominently, those associated with the multifaceted EMT and MET programmes. As indicated in FIG. 1, EMT programmes can be induced by a variety of contextual signals that cancer cells may experience in diverse tissue sites throughout the body. Importantly, expression of EMT programmes has been associated with poor clinical outcome in multiple tumour types[7], ostensibly because of the aggressive cell-biological traits that these programmes confer on the carcinoma cells within primary tumours.

Since the original description of EMTs, our understanding of the molecular processes that underlie them has grown enormously, as reflected by the large number of publications in this area that have been summarized in several recent excellent reviews[1–4]. Following a brief overview of what is currently known about the molecular mechanisms activating EMT programmes, we focus here on recently identified regulatory mechanisms that govern their continued expression and additionally discuss

At a glance

- Transitions between epithelial and mesenchymal states underlie epithelial cell plasticity and contribute to tumour progression and intratumoural heterogeneity.
- The epithelial–mesenchymal transition (EMT) is triggered by a diverse set of stimuli including growth factor signalling, tumour–stromal cell interactions and hypoxia. There is a significant crosstalk among EMT-inducing signals and transcription factors that can lead to stable reprogramming of epithelial cells to mesenchymal states.
- EMT has been shown to result in cancer cells with stem cell-like characteristics that have a propensity to invade surrounding tissue and display resistance to certain therapeutic interventions.
- The mesenchymal–epithelial transition (MET) may have a role in the reversion of disseminated mesenchymal tumour cells to a more epithelial state in distant metastases.
- microRNAs have been identified as a new class of EMT regulators, in part owing to their regulation of EMT-inducing transcription factors.

the importance of EMTs and METs in intratumoural heterogeneity, tumour progression and therapeutic resistance. Finally, we discuss potential approaches to exploit this accumulating knowledge for the improved clinical management of tumours.

Mechanisms of EMT activation

Multiple extracellular signals can initiate an EMT programme, and there is a significant crosstalk among the downstream intracellular signalling pathways and transcription factors that choreograph this complex programme, including multiple positive-feedback loops[3,8] (FIG. 1). This intricate network of interactions leads to increased stability of the acquired mesenchymal cell phenotype that represents the end point of an EMT programme. Recent data in mammary epithelial cells demonstrates that sustained activation of EMT leads to progressive epigenetic alterations in cells, inducing heritable effects that maintain the mesenchymal state even after EMT-initiating signals are no longer present[9]. Hence, under certain conditions, EMTs can yield stable changes in the phenotype and thus lineage identity of cells.

Signalling pathways. The EMT is generally induced in epithelial cells by heterotypical signals, specifically those released by the mesenchymal cells that constitute the stroma of normal and neoplastic tissues. Members of the transforming growth factor-β (TGFβ) family of cytokines are the main and the best-characterized inducers of EMTs occurring during the course of embryonic development, wound healing, fibrotic diseases and cancer pathogenesis[4,10]. Recent data also implicate a role for TGFβ in regulating breast cancer stem cell phenotypes[5,6] and have demonstrated its essential role in maintaining the pluripotency of human embryonic stem cells[11]; the latter may indicate a role of this pathway in inducing stem cell states during all phases of ontogeny.

TGFβ may induce EMTs through multiple distinct signalling mechanisms, including direct phosphorylation by ligand-activated receptors of SMAD transcription factors and by certain cytoplasmic proteins regulating cell polarity and tight junction formation[4,10]. For example, in mammary epithelial cells, the TGFβ type II receptor can directly phosphorylate both SMAD2, SMAD3 and the cell polarity protein PAR6A. Phosphorylation of PAR6A leads to loss of apical–basal polarity and dissolution of existing tight junctions between adjacent epithelial cells[12]. TGFβ also influences the activities of multiple other EMT-inducing signal transduction pathways, including those involving Notch, Wnt and integrin signalling, some of which can act in concert to trigger EMT programmes.

Wnt signalling can lead to EMTs through inhibition of glycogen synthase kinase-3β (GSK3β)-mediated phosphorylation and associated degradation of β-catenin in the cytoplasm. The resulting increased levels of β-catenin enable this molecule to translocate to the nucleus, where it can serve as a subunit of a transcription factor that helps to induce the expression of a large constituency of genes, among them those specifying several EMT-inducing transcription factors[13]. However, β-catenin alone usually does not suffice to induce an EMT. For example, in almost all colorectal carcinomas, the genetic inactivation of *APC* or activation of β-catenin[14] also yield increased intracellular β-catenin pools. However, the majority of these tumours do not display mesenchymal features, suggesting that the activation of this signalling pathway, although necessary in certain cells, may not be sufficient to trigger expression of the EMT-inducing transcription factors that orchestrate this programme (TABLE 1). Multiple studies described differences between the mutational and epigenetic inactivation of epithelial cadherin (E-cadherin) in human breast carcinomas[15,16], although the nuclear accumulation of β-catenin is rarely observed in breast tumours. Further support for the importance of this signalling pathway comes from observations showing that Wnt signalling is increased in colorectal cancers through the silencing of genes specifying key Wnt antagonists: SOX17 (REF. 17), SFRPs[18,19] and DKK1 (REF. 20).

As mentioned, Notch signalling also has a role in the regulation of EMTs occurring during both embryogenesis and tumorigenesis[21]. The complexity of Notch signalling derives from the involvement of multiple receptors, ligands and downstream mediators. Moreover, the outcome of Notch activation is cell type-specific and can be either oncogenic or tumour suppressive[21]. Notch can also induce an EMT by activating the nuclear factor-κB (NF-κB) pathway[22] or by modulating the activity of TGFβ signalling. Hedgehog signalling has also been implicated in EMT and cancer metastasis[21]. Thus, it appears that signalling pathways involved in the regulation of stem cell function and niche–stem cell interactions can play some part in triggering EMT programmes, potentially connected with the role of these programmes in establishing and maintaining stem cell-like characteristics.

Numerous receptor tyrosine kinases (RTKs) have also been found to have crucial roles in embryonic processes that involve EMT programmes, including branching morphogenesis and cardiac valve formation[1–4]. Several of these RTKs are mutated and constitutively active in diverse cancer types. Other

changes of cell surface proteins may also contribute to the triggering of the EMT: E-cadherin expression is lost in many tumours through genetic or epigenetic mechanisms[9]. This often results in the liberation of β-catenin, which is normally sequestered by the cytoplasmic tail of E-cadherin; the resulting liberated β-catenin molecules may then migrate to the nucleus and induce expression of EMT-inducing transcription factors, as discussed above. These two mechanisms — constitutive RTK activation and E-cadherin loss — can lead in many cancer cells to the stabilization of the mesenchymal state, making its expression independent of continuous EMT-inducing heterotypical signalling emanating from the tumour microenvironment.

Hypoxia. Hypoxia is one of the physiological mechanisms that can induce EMTs in tumours through multiple distinct mechanisms, including upregulation of hypoxia-inducible factor-1α (HIF1α), hepatocyte growth factor (HGF), SNAI1 and TWIST1, activation of the Notch or NF-κB pathways, and induction of DNA hypomethylation[23]. Low (3%) O_2 levels have been shown to induce EMTs in multiple human cancer cell lines by inhibiting the activity of GSK3β[24], thereby sparing β-catenin from phosphorylation and subsequent destruction. Accordingly, hypoxic cells have been observed to become more invasive and to display activated Wnt–β-catenin signalling with resulting induction of the EMT-inducing transcription factor SNAI1 (REF. 24). In another study, the activation of Notch signalling was required for hypoxia-induced EMT[25]. Hypoxia may also activate self-reinforcing positive-feedback loops that help to stabilize the mesenchymal state. For example, activation of SNAI1 causes repression of E-cadherin transcription, which leads to the liberation of cytoplasmic β-catenin, which can then activate and further stabilize the expression of EMT-inducing transcription factors in the nucleus.

Other types of extracellular signals may also provoke EMTs. For example, in one study, an EMT was induced in murine mammary epithelial cells in response to the ectopic expression of matrix metalloproteinase 3 (MMP3, also known as stromelysin 1), which derived from increased reactive oxygen species production mediated by a RAC1 splice variant, RAC1B[26].

Epithelial cell–stromal cell interactions as regulators of EMT and MET. As indicated above, the factors inciting EMTs in carcinomas are often components of heterotypical signalling pathways that originate in the tumour-associated stroma from the cells creating the tumour microenvironment. Some of the first reports suggesting a role of stromal cell–epithelial cell interactions in triggering EMT programmes described the increased expression of multiple EMT markers in carcinoma cells at the tumour–stroma interface[27,28]. This was demonstrated in multiple different cancer types both in human tumours and in animal models of tumour pathogenesis[29].

Cancer cells may also undergo METs owing to influences originating in their microenvironment. This was demonstrated by the upregulation of E-cadherin expression and the acquisition of differentiated epithelial cell features when prostate cancer cells were co-cultured with normal hepatocytes[30]. This return to an epithelial state involved, among other things, the formation of cell–cell interactions between normal hepatocytes and the cancer cells, ostensibly mediated by homotypical E-cadherin bridges formed between them. An unresolved issue is whether diffusible factors, specifically growth factors and cytokines of the types mentioned here, are ever involved in actively inducing an MET. An alternative default mechanism is equally if not more plausible: in the absence of signals that actively promote the induction and continued expression of an EMT, many normal and neoplastic cells will revert to the epithelial state as a consequence of a transcriptional default mechanism[31]. This might explain, for example, the METs that seem to occur during the growth of carcinoma metastases[32]. Thus, in the absence of the EMT-inducing signals received from the 'activated' stroma that are present in primary tumours, metastatic cancer cells (which initially experience normal stroma when entering into sites of dissemination) may simply fall back to an epithelial state through an MET[33,34].

Genetic and epigenetic control

Several genes involved in regulating EMT programmes have been shown to be altered in tumours owing to genetic and epigenetic events (BOX 2). As mentioned above, stable loss of E-cadherin can occur through several alternative mechanisms. Mutations in *CDH1*, which encodes E-cadherin, have been identified as a cause of hereditary diffuse gastric cancer[35], and a subset of these patients also have increased susceptibility to develop lobular breast carcinomas[36]. Correlating with this, somatic genetic inactivation of *CDH1* frequently occurs in lobular breast carcinomas[37], but this does not inevitably lead to an EMT, in contrast to the epigenetic silencing of E-cadherin expression discussed above.

Box 1 | EMT and MET in embryonic development

The role of epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) in embryonic development has been summarized in several excellent review articles[2,4]. EMT and MET have important roles in several developmental processes. The earliest EMT event in embryonic development is the formation of mesenchymal cells and mesoderm during gastrulation[2,4]. The formation of placenta, somites, heart valves, neural crest, urogenital tract and secondary palate, and branching morphogenesis of multiple different organs all involve EMT and MET[2,4]. Interactions between transforming growth factor-β, Notch, Wnt, and receptor tyrosine kinase signalling pathways orchestrate these events and these same signalling pathways have a role in the EMT induced during tumour development. The same regulatory mechanisms that convert epithelial cells to migratory mesenchymal cells that are crucial for the formation of organs during embryonic development become abnormally activated in cancer and contribute to invasion and metastasis. Despite their similarities, developmental and cancer-associated EMT and MET processes have some important differences as well, particularly the potentially irreversible nature of these events in certain forms of cancer due to somatic mutations.

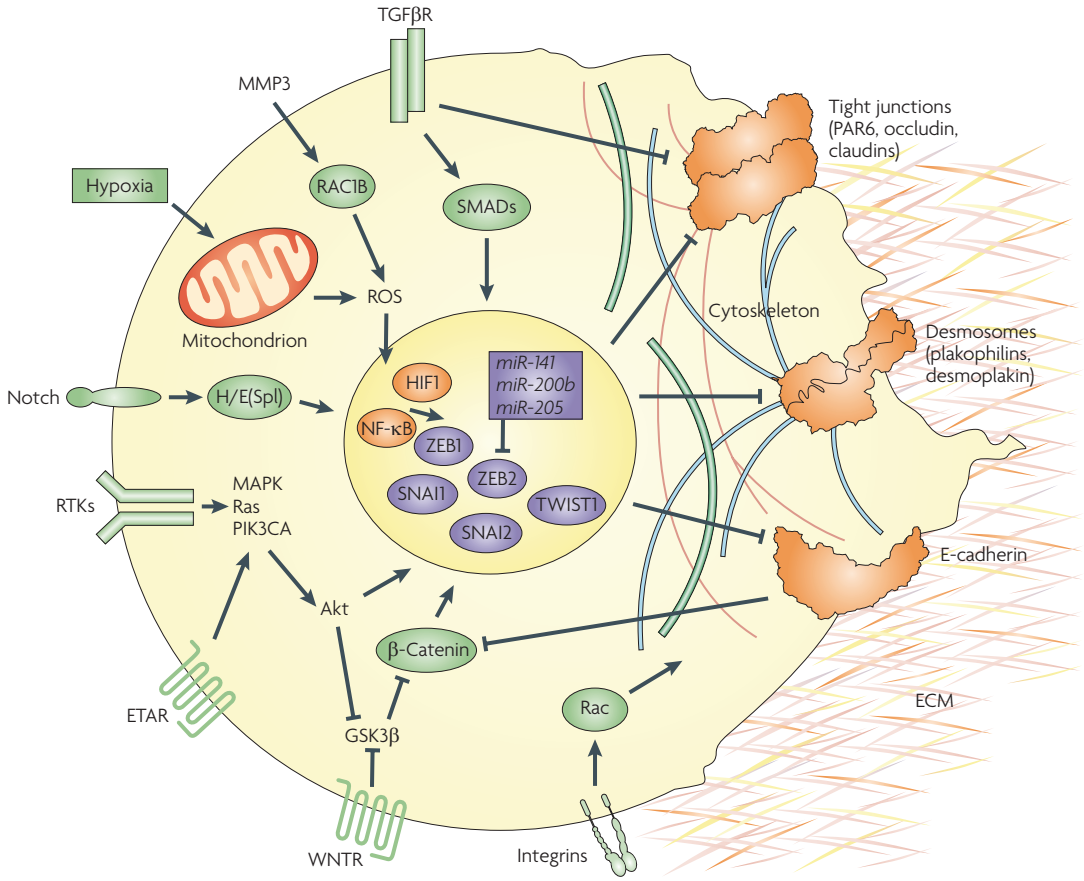

Figure 1 | **A simplified overview of signalling networks regulating epithelial–mesenchymal transitions (EMTs).** Selected signalling pathways and some of their downstream effects and interactions are depicted. Receptor tyrosine kinases (RTKs), transforming growth factor-β (TGFβ), Notch, endothelin A receptor (ETAR), integrins, Wnt, hypoxia and matrix metalloproteinases (MMPs) can induce EMTs through multiple different signalling pathways, and the relative importance of each of these may depend on the particular cellular context. EMTs and mesenchymal–epithelial transitions (METs) are associated with dramatic changes in the cytoskeleton and extracellular matrix (ECM) composition and attachment that act together to alter cell morphology. EMT-inducing signals can lead to the disruption of tight junctions and desmosomes through protein phosphorylation (for example PAR6A phosphorylation by TGFβ signalling[12]) or by repressing protein levels (for example ZEB1 represses plakophilin 3 (REF. 83)). EMT also results in the dramatic reorganization of the ECM as many EMT-inducing factors upregulate the expression of ECM proteins (such as fibronectin and collagens), proteases (such as MMPs) and other remodelling enzymes (such as lysyl oxidase). Hypoxia, RAC1B activation and activation of certain kinase pathways (such as Akt) may lead to increased mitochondrial production of reactive oxygen species (ROS) that elicit pleiotropic effects, including activation of hypoxia-inducible factor 1α (HIF1α) and nuclear factor-κB (NF-κB) (orange circles), signalling and inactivation of glycogen synthase kinase-3β (GSK3β). Besides the interaction among the various signalling pathways, there is also extensive crosstalk among the EMT-inducing transcription factors (purple circles) and the microRNAs (miRNAs) regulating them. E-cadherin, epithelial cadherin; H/E(Spl), hairy and enhancer of split; WNTR, Wnt receptor.

Operating through an alternative mechanism, the hypomethylation of genes specifying transcription factors that programme stem cell phenotypes may lead to EMTs and, in cancer cells, correlate with poorly differentiated cell phenotypes and increased risk of distant metastasis[33]. For example, a recent report described analysis of the comprehensive DNA methylation profiles of stem cell-like $CD44^+CD24^-$ and more differentiated epithelial $CD44^-CD24^+$ cells isolated from either normal or neoplastic breast tissues[33]. This report described several transcription factors implicated in the induction of both EMT and stem cell functions as being hypomethylated and highly expressed in $CD44^+CD24^-$ cells compared with their $CD44^-CD24^+$ counterparts. The ectopic expression of one of these, forkhead box protein C1 (FOXC1), in either MCF-12A or MDCK cells led to a complete EMT, as indicated by decreased E-cadherin, increased vimentin expression, and increased motility and invasion in cell culture. Correlating with the presumed association of relative hypomethylation with stem cell characteristics, treatment of MCF-7 breast cancer cells with 5-aza-cytidine, a DNA methyltransferase inhibitor, increased their invasiveness, tumorigenicity and metastatic capacities concomitant with the upregulation of pro-invasive EMT-associated genes[38,39]. As an aside, these observations raise concerns about the use of

Table 1 | **Expression and clinical relevance of selected EMT-associated genes in primary human tumours**

Gene	Cancer type	Tumour stage	Association with clinico-pathological features	Refs
miR-200 and *miR-205* family	Serous papillary ovarian cancer	FIGO stage III–IV	ND	46
miR-200 and *miR-205* family	Breast cancer	ND	ND	44
miR-335 gene signature	Breast cancer	Primary invasive tumours	Decreased metastasis-free survival	50
miR-10b	Breast cancer	Primary invasive tumours	Presence of metastasis	49
EMT markers	Breast cancer	Primary invasive tumours	Basal-like subtype	58
FOXC2	Breast cancer	Primary invasive tumours	Basal-like subtype	59
SNAI1	Breast cancer	Primary invasive tumours	Poor prognosis	83,85, 86
SNAI2	Breast cancer	Primary invasive tumours	Poor prognosis	83,85, 86
TWIST1	Breast cancer	Primary invasive tumours	Poor prognosis	86
ZEB2	Breast and ovarian cancer	Tumours of different stages	Poor prognosis	87
ZEB1	Uterine cancer	Primary invasive tumours	Aggressive tumour characteristics	83,85

EMT, epithelial–mesenchymal transition; FOXC1, forkhead box protein C1; ND, not determined.

DNA methyltransferase inhibitors for the treatment of breast cancer, as the resulting potential induction of an EMT may increase tumour cell dissemination.

Transcriptional control. Individual members of a group of six to eight transcription factors (FIG. 1; TABLE 1) have been demonstrated to be capable of orchestrating EMT programmes during embryonic development and in cancer[8,40]. These include direct transcriptional repressors of E-cadherin expression — SNAI1, SLUG (also known as SNAI2), SIP1 (also known as ZEB2) and E47 (also known as E2α) — and others, such as TWIST1, FOXC2, FOXC1, GSC and ZEB1, that act less directly on E-cadherin. Emerging data suggest extensive cross-talk among these transcription factors, allowing them to form a signalling network that is responsible for establishing and maintaining mesenchymal cell phenotypes[8,40]. Furthermore, some of these transcription factors, including TWIST1, play a part in overcoming cellular senescence[41] and in generating tumorigenic cancer stem cells[5]. As mentioned earlier, EMT-inducing transcription factors also confer stem cell characteristics on epithelial cells (FIG. 1). This notion is reinforced by the actions of the receptor KIT, an important agent for maintaining the stem cell state in the haematopoietic system, which induces SNAI2 expression, as demonstrated by genetic data in mice[42] and in humans[43].

Non-coding RNAs as regulators of EMT and metastasis. Non-coding RNAs are increasingly recognized as important players regulating gene expression and protein levels (BOX 3). Several recent studies ascribe a role in activating the EMT programme to the *miR-200* family (*miR-200a*, *miR-200b*, *miR-200c*, *miR-141* and *miR-429*) of microRNAs and to *miR-205* (REFS 44–46); a similar role has been associated with a natural anti-sense transcript transcribed from the *ZEB2* locus[47]. In all of these cases, regulation of the EMT is apparently coupled to repression of E-cadherin expression[44,45,48].

The involvement of the *miR-200* family and *miR-205* in regulating EMT was discovered by two independent studies using different experimental strategies[44–46]. In one study, patterns of miRNA expression in various cancer cell lines were correlated with epithelial and mesenchymal characteristics, as defined by E-cadherin (*CDH1*) and vimentin (*VIM*) mRNA levels[46]. Expression levels of *miR-205* and of members of the *miR-200* family were found to vary inversely with vimentin mRNA expression. Subsequent work showed that the targets of these miRNAs include both the ZEB1 and ZEB2 EMT-inducing transcription factors that function as transcriptional repressors of E-cadherin expression. These findings were extended by demonstrations that the expression of these miRNAs correlated positively with *CDH1* and negatively with *VIM* in primary human serous papillary carcinomas of the ovary.

Another report has described the downregulation of both *miR-205* and *miR-200* family members in Madin–Darby canine kidney (MDCK) cells following the induction of EMT by either TGFβ or the tyrosine phosphatase PEZ[44]. Once again, ZEB1 and ZEB2 were predicted to be targets of these miRNAs. Furthermore, downregulation of these miRNAs was sufficient to induce EMT and ectopic expression led to an MET.

A novel mode of regulation of *ZEB2* mRNA levels was discovered by others during SNAI1-induced EMT in human colorectal cancer cell lines[47]. Specifically, the expression of ZEB2 was seen to increase following SNAI1-induced EMT owing to increased mRNA levels, without any noticeable effect on its transcription. Using a combination of approaches, Beltran and

Box 2 | **Epigenetic regulatory mechanisms**

Epigenetic regulatory programmes involve DNA methylation, chromatin (histone) modification and non-coding RNAs. Each of these mechanisms has been shown to have a role in regulating stem cell function and differentiation, and tumorigenesis. For example, DNA methylation has been demonstrated to play an important part in silencing gene expression, imprinting and X chromosome inactivation[71–73]. In addition, DNA methylation was found to be responsible for controlling the cell type-specific expression of certain genes[74].

Inherited defects in DNA methylation and imprinting result in developmental defects and increase the risk of tumorigenesis[71–73]. Recent data also implicate DNA methylation and chromatin changes as initiating events in neoplasia preceding the occurrence of genetic alterations[75–77]. DNA methylation and chromatin modification are interrelated processes and non-coding RNAs may link the two processes[79]. In the past few years the number and type of known histone modifications have increased dramatically, and a large set of enzymes that play a role in mediating these processes has been identified[79]. The potential role for non-coding RNAs in establishing DNA methylation histone modification patterns in mammalian cells is just beginning to be uncovered.

colleagues determined that the expression of ZEB2 is regulated by a natural antisense transcript that prevents the splicing of a large intron in the 5′ untranslated region (UTR) that contains an internal ribosomal entry site. This 5′ UTR also contains a sequence that decreases translation efficiency by inhibiting ribosome scanning, thereby leading to low ZEB2 protein levels in epithelial cells. However, during activation of an EMT, levels of this antisense transcript are increased and its binding to the 5′ UTR inhibits splicing, leading in turn to the retention of the internal ribosomal entry site sequence. This increases translation efficiency and thereby leads to higher ZEB2 and thus lower E-cadherin protein levels.

Through their regulation of EMT and MET, non-coding RNAs are also involved in regulating invasion and metastasis. For example, analyses of the expression of candidate miRNAs in metastatic and non-metastatic breast cancer cell lines revealed that *miR-10b* is a miRNA associated with mesenchymal features and invasive properties[49]. Subsequently, *miR-10b* was found to be induced by TWIST1: its expression increased cell invasion and metastasis by inhibiting the translation of *HOXD10* and upregulating RHOC protein levels. Importantly, the expression of *miR-10b* was higher in primary invasive breast carcinomas in patients with metastasis.

A contrasting role has been associated with *miR-335*, which was identified as a suppressor of invasion and metastasis from array-based profiling of metastatic and non-metastatic derivatives of the MDA-MB-231 breast cancer cell line[50]. *miR-335* appears to regulate metastasis by modulating the expression of the transcription factor SOX4 and the extracellular matrix protein tenascin C. Tavazoie and colleagues also defined a *miR-335* gene signature that is composed of six genes (*COL1A1*, *MERTK*, *PLCB1*, *PTPRN2*, *TNC* and *SOX4*), the expression of which was suppressed by *miR-335* and was high in metastatic cells. This *miR-335* gene signature was associated with decreased metastasis-free survival in breast cancer patients, suggesting a role for *miR-335* in the regulation of metastatic progression.

Improved approaches for the characterization of non-coding RNAs from primary human tissue samples and better understanding of their function will probably lead to the identification of additional non-coding RNAs with crucial roles in EMT and MET. As additional miRNAs become associated with various aspects of cell physiology, it becomes apparent that miRNAs will soon take their place, together with proteins, as integral components of all the regulatory circuits operating within cells.

Mesenchymal–epithelial transition

Recent studies suggest that primary tumours displaying a gene expression signature characteristic of EMT are more likely to be associated with eventual distant metastasis and shorter periods of distant metastasis-free survival[33,34,51,52]. An apparent contradiction of this association between EMT and metastasis comes from repeated observations that distant metastases derived from primary carcinomas are largely composed of cancer cells showing an epithelial phenotype closely resembling that of the cancer cells in the primary tumour[33,34]. If cancer cells must pass through an EMT in order to disseminate, why do resulting metastases closely resemble, at the histopathological level, the primary carcinomas from which they have arisen?

In fact, this discrepancy can be rationalized by the recognition that, following metastatic spread and colonization, an MET often converts the disseminated mesenchymal cancer cells back to a more differentiated, epithelial cell state[32] (FIG. 2). Correlating with this, several studies have demonstrated that the DNA methylation status of the *CDH1* promoter varies at different stages of the metastatic process[53,54]. In primary breast cancers, the tumour cells that undergo transient hypermethylation and silencing of *CDH1* expression are more invasive and metastatic, but subsequently

Box 3 | **Small non-coding RNAs**

The recently identified small non-coding RNAs are a new class of gene expression regulators that are thought to act at the post-transcriptional level, although they may also play a part in establishing epigenetic programmes[80,81]. In the past few years microRNAs (miRNAs) and Piwi-interacting RNAs (piRNAs) have been recognized as essential regulators of stem-cell function, differentiation and embryonic development[80,81]. Furthermore, miRNAs have been shown to act as oncogenes and tumour suppressor genes[82]. miRNAs are processed through multiple steps into short (19-25 base pair) single-stranded RNAs by specialized RNase III enzymes, then incorporated into miRNA-induced silencing complexes (miRISCs) that also contain multiple proteins. Individual miRNA species can bind to multiple mRNA targets and either induce their degradation or prevent translation, and in some cases miRNAs have also been shown to influence transcription by still-undefined mechanisms. piRNAs have been implicated as regulators of transposon mobility in the germ line, but their involvement in other still-unidentified cellular processes in somatic cells cannot be excluded.

E-cadherin expression is re-induced in metastases, a change that is accompanied by the demethylation of the *CDH1* promoter[53,54]. Unfortunately the clonal identities of the cancer cells have not been followed in these studies. This leaves open the possibility that primary cancer cells with an epithelial DNA methylation status are selected from the outset to disseminate to distant tissue sites, where they can dominate the phenotype of resulting macroscopic metastases. Accordingly, the model of sequential EMT and MET has yet to be rigorously tested.

Clinical importance of EMT and MET

The EMT has been implicated in two of the most important processes responsible for cancer-related mortality: progression to distant metastatic disease and acquisition of therapeutic resistance. Both of these processes may be linked, in turn, to a third: the generation by EMTs of cancer cells with stem cell-like characteristics. Two independent groups have demonstrated that, in mammary epithelial cells, expression of the EMT-inducing transcription factors TWIST1 or SNAI1 (REF. 5), or treatment with TGFβ increases the number of stem cells, as defined by their cell surface antigenic profiles, gene expression patterns, ability to form mammospheres in culture and ductal outgrowths in xenotransplant assays[5,6]. Independently of these observations, one group studying EMT analysed the expression of EMT-associated genes in $CD44^+CD24^-$ cells isolated from normal human breast tissue and from primary human breast carcinomas and established that most of the EMT-inducing transcription factors (*TWIST1*, *FOXC2*, *SNAI1*, *ZEB2* (also known as *SIP1*) and *TWIST2*), as well as vimentin and fibronectin, were expressed at far higher levels in $CD44^+CD24^-$ stem cell-like cells than in more differentiated epithelial $CD44^-CD24^+$ cells[5]. These data correlate with prior observations describing the enrichment of invasion-, metastasis- and angiogenesis-associated genes in $CD44^+CD24^-$ breast cancer cells[34] and the increased invasive and metastatic ability of these cells in experimental models[52]. Furthermore, breast cancer cells disseminated in the circulation and bone marrow have been found to be enriched for the $CD44^+CD24^-$ antigen phenotype[55–57].

High frequency of $CD44^+CD24^-$ stem cell-like cancer cells and the expression of EMT-associated genes have also been related to the basal-like subtype of human breast cancer[58,59]. Immunohistochemical analysis of 479 invasive breast carcinomas have demonstrated high expression of EMT-induced markers (vimentin, α-smooth muscle actin, neural cadherin (N-cadherin) and cadherin 11), SPARC, laminin and fascin, and low expression of E-cadherin in basal-like breast tumours compared with other subtypes of breast cancer; these tumours have an especially poor prognosis owing to their increased ability to form distant metastases in visceral organs[60,61]. Others analysed the expression of the EMT-inducing transcription factor FOXC2 in 117 primary invasive breast carcinomas and found a strong association between nuclear staining of FOXC2 and the basal-like subtype[59]. Correlating with these results and the induction of stem cell-like cancer cells by EMT, basal-like breast tumours are enriched for $CD44^+CD24^-$ cancer cells based on dual immunohistochemical analysis[62].

Highlighting the importance of EMT in therapeutic resistance, cancer cells with stem cell characteristics have been found to be enriched in the residual tumours remaining after standard chemotherapeutic treatments[63,64]. For example, in patients with breast cancer undergoing neoadjuvant therapy, there was a significant increase of $CD44^+CD24^-$ cells expressing EMT-associated genes in post-treatment biopsy samples following standard anthracycline–taxane chemotherapy, but not after treatment of $ERBB2^+$ tumours with lapatinib, a dual epidermal growth factor receptor (EGFR) and ERBB2 inhibitor[64]. In certain tumours, including lung and colorectal carcinomas, cells undergoing EMT demonstrated decreased sensitivity to EGFR kinase inhibitors, potentially bypassing their dependence on this pathway by activation of its downstream targets, PI3K and Akt[65].

EMT and acquisition of cancer stem cell features have also been associated with increased resistance to apoptosis[63,66]. EpH-4 and NMuMG murine mammary epithelial cell lines became resistant to ultraviolet-induced apoptosis following EMT induced by TGFβ treatment[66]. Similarly, in breast cancer cell lines, down-regulating the expression of the *let-7* miRNA increased tumour metastatic ability and therapeutic resistance, accompanied by the acquisition of stem cell characteristics and EMT-associated gene expression profiles[63].

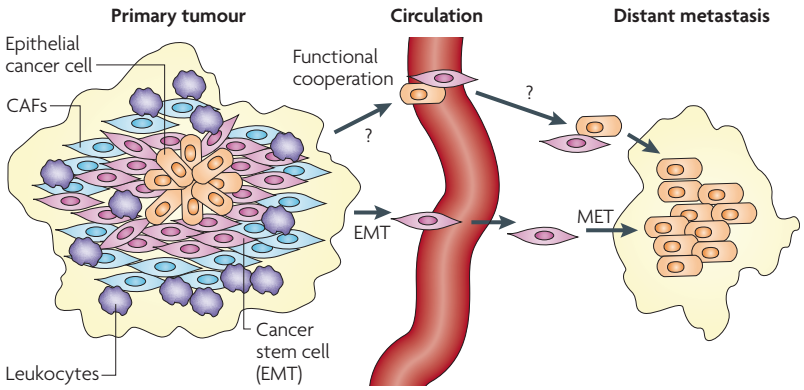

Figure 2 | **Transitions between epithelial and mesenchymal states during carcinoma progression.** In the primary tumour, epithelial–mesenchymal transitions (EMTs) and mesenchymal–epithelial transitions (METs) contribute to intratumoural heterogeneity that can influence therapeutic responses and the ability to metastasize. Interactions with stromal cells, including leukocytes and cancer-associated fibroblasts (CAFs), may induce EMTs and may also preferentially promote the growth and survival of cancer cells with mesenchymal phenotype (including cancer stem cells). Cancer stem cells are more likely to metastasize and are more frequently detected in the circulation and in micrometastases. However, macroscopic distant metastases are more frequently composed of more differentiated epithelial cancer cells. This can be explained by the reversal of EMT through MET after micrometastases grow, due to local selective pressure for the outgrowth of cancer cells with more epithelial features or to the absence of EMT-inducing signals at sites of dissemination. However, the possibility that functional cooperation between mesenchymal and more differentiated epithelial cancer cells operates during metastatic spread cannot be excluded.

Co-culture of tumour epithelial cells with stromal fibroblasts[67] or in hypoxic conditions[68] is also associated with increased therapeutic resistance, potentially owing to their ability to undergo an EMT in response to signals released by the co-cultured fibroblasts.

Conclusions

Increasing evidence suggests that EMT and MET are central regulators of cellular plasticity in carcinomas and have important roles in therapeutic resistance, tumour recurrence and metastatic progression. The contributions of these programmes and their regulators to other types of tumours, specifically neuroectodermal, mesenchymal and haematopoietic neoplasias, remain to be shown. Owing to the clinical importance of the EMT-induced processes, inhibition of EMT is an attractive therapeutic approach that could have significant effect on disease outcome. However, the complexity of the signalling networks that regulate induction of EMTs and the reversibility of the acquired mesenchymal phenotype pose significant challenges. In addition, it remains unclear which tumours should be treated and at what stage of progression. If systemic dissemination occurs at early stages of tumour development, as suggested by recent studies[69], then at the time of diagnosis it may already be too late to successfully target EMT-inducing events. However, if the hypothesis of secondary tumour cell seeding from already established metastases[70] proves to be correct, then targeting further dissemination in cancer patients may still be productive therapeutically.

It is also unclear which signalling pathways should be inhibited in order to most effectively block the initiation of EMT and, at the same time, cause minimal toxicity in normal tissues. The close similarity between the EMT and normal stem cell programmes raises particular concerns in this regard. The reversibility of the EMT and MET programmes provokes further questions about the durability of any initially elicited clinical responses. Finally, a crucial question addresses how the efficacy of an anti-EMT therapy under development can be predicted without waiting years (or even decades in the case of breast cancer) to register differences in the incidence of distant metastases.

These various factors reveal that, despite the large amount of data accumulated on the molecular mechanisms underlying EMT and MET and the undoubted importance of these processes in cancer, a number of fundamental questions require resolution before this knowledge can be translated into truly useful clinical practice. Judging by the pace of ongoing research in this area, it is likely that many of these questions will indeed be answered in the not-too-distant future.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/
5-aza-cytidine | lapatinib
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β-catenin | cadherin 11 | DKK1 | E2α | E-cadherin | ERBB2 | ETAR | fascin | FOXC1 | FOXC2 | GSC | GSK3β | HGF | HIF1α | KIT | N-cadherin | PAR6A | PEZ | SMAD2 | SMAD3 | SNAI1 | SNAI2 | SOX17 | SPARC | TGFβ type II receptor | TWIST1 | vimentin | ZEB1 | ZEB2

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