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Review Article

Epithelial–mesenchymal transition in cancer development and its clinical significance

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The epithelial–mesenchymal transition (EMT) plays a critical role in embryonic development. EMT is also involved in cancer progression and metastasis and it is probable that a common molecular mechanism is shared by these processes. Cancer cells undergoing EMT can acquire invasive properties and enter the surrounding stroma, resulting in the creation of a favorable microenvironment for cancer progression and metastasis. Furthermore, the acquisition of EMT features has been associated with chemoresistance which could give rise to recurrence and metastasis after standard chemotherapeutic treatment. Thus, EMT could be closely involved in carcinogenesis, invasion, metastasis, recurrence, and chemoresistance. Research into EMT and its role in cancer pathogenesis has progressed rapidly and it is now hypothesized that novel concepts such as cancer stem cells and microRNA could be involved in EMT. However, the involvement of EMT varies greatly among cancer types, and much remains to be learned. In this review, we present recent findings regarding the involvement of EMT in cancer progression and metastasis and provide a perspective from clinical and translational viewpoints. (*Cancer Sci* 2010; 101: 293–299)

Development of distant metastases is the final stage of solid cancer progression and is responsible for the majority of cancer-related deaths.[1] Distant metastasis alone or with concurrent locoregional recurrence accounts for nearly 80% of all first relapses in women with breast cancer.[2] While clinically of great importance, the biology of metastasis remains unsolved. The process of tumor metastasis consists of multiple steps, all of which are required to achieve tumor spreading.[3,4] First, cancer cells escape from the primary tumor site. Next, cancer cells invade the tumor stroma and enter the blood circulation directly or the lymphatic system via intravasation. Most circulating cancer cells undergo apoptosis due to anoikis conditions.[5] If cancer cells survive in circulation they may reach more suitable sites by attaching to endothelial cells and extravasating from the circulation into the surrounding tissues. Finally, distal colonization requires that cancer cells invade and grow in the new environment.

Recently, the concept of the epithelial–mesenchymal transition (EMT), as developed in the field of embryology, has been extended to cancer progression and metastasis.[6,7] *In vitro* and experimental animal model data now support the role of EMT in metastasis, concepts supported by analyses of clinical samples. Indeed, the biology of EMT has been clarified in tumor samples through use of EMT-associated markers, such as mesenchymal-specific markers (i.e. vimentin and fibronectin),[8,9] epithelial specific markers (i.e. E-cadherin and cytokeratin),[10,11] and transcription factors (i.e. SNAIL and SLUG).[12]

Most recently, several intriguing studies have described the novel mechanism underlying EMT activation. In the current study, we will discuss the role of small non-coding RNA (microRNA) in regulating EMT-related genes.[13–15] Furthermore, Mani *et al.* disclosed that EMT could generate breast cancer cells with stem cell-like characteristics.[16] Here, we update and discuss recent progress in studies of EMT. These new data improve our understanding of the mechanisms of cancer progression and metastasis as well as therapy resistance. This new information may lead to development of novel clinical targets and improve the clinical management of cancer patients.

Involvement of EMT in Cancer Progression

In the 1980s, Greenburg and Hey first analyzed EMT-associated changes in cell phenotype and mesenchymal states in adult and embryonic epithelia.[17] EMT and the inverse process of mesenchymal–epithelial transition (MET) are major embryological mechanisms for tissue remodeling, as in gastrulation and segment formation.[18] The process of EMT consists of multiple steps.[19,20] First, cell–cell adhesion disintegrates with the loss of epithelial markers such as E-cadherin and the gain of mesenchymal markers such as vimentin. Next, there is a loss of basoapical polarization and the acquisition of front-rear polarization. Then, the cytoskeleton undergoes remodeling, with changes in cortical actin and actin stress fibers. Finally, cell-matrix adhesion is altered, with activation of proteolytic enzymes such as matrix metalloproteases. Note that the process of metastasis in epithelial cancer also consists of multiple steps.[3,4] That is, cells detach from the primary tumor and invade the surrounding tumor stroma. They subsequently enter into the circulation and reach new metastatic sites. Therefore, the process of EMT during cancer progression and metastasis closely resembles that observed in embryologic development. Accordingly, molecular analyses based on EMT in embryology have been applied to cancer progression.

In the 1990s, accumulating evidence indicated that EMT was associated with cancer progression.[7] Indeed, these transformations may be associated with EMT-related signal pathways during development.[7,21] However, Boyer *et al.* stated that EMT during development depends on additional activities of distinct and specific signaling molecules which are highly controlled spatially and temporally, and which do not occur under normal circumstances. On the other hand, EMT in cancer progression could be due to autonomous oncogenic activation of signaling molecules without additional stimulation.[22] Therefore,

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comparisons of EMT signaling pathways in embryological development and cancer progression may make it possible to identify novel pathways specific to cancer progression and to suggest new therapeutic strategies in cancer therapy.[23]

The Molecular Mechanism of EMT in Cancer Progression

Multiple complex signaling systems are required for induction of EMT because epithelial cells undergoing EMT must undergo both functional and morphologic changes. Studies of the crosstalk among the intracellular signal networks could help us to understand the mechanisms regulating EMT. Here, we discuss the regulation of representative molecules, E-cadherin, a major EMT inducer, transforming growth factor-β (TGF-β) signal pathways, and microRNA regulation reported in recent studies (Fig. 1).

E-cadherin regulation. One of the characteristic findings in EMT is the loss of cell–cell adhesion with diminished expression of E-cadherin. E-cadherin, a calcium-dependent transmembrane glycoprotein expressed in most epithelial tissues, constructs a tight junction which connects adjacent cells. The loss of E-cadherin can lead to tumor progression, metastasis, and poorer prognosis in various human carcinomas.[10,11,24,25] Genetic or epigenetic alterations cause a functional loss of E-cadherin. For instance, mutations in E-cadherin are found in diffuse gastric cancer[26] and lobular breast carcinoma.[27] In addition, hypermethylation of the E-cadherin promoter region is found in various human carcinomas, resulting in frequent loss of E-cadherin expression.[28,29] Interestingly, Graff *et al.* proposed that the degree of methylation of the E-cadherin promoter region during metastatic progression is unstable and heterogeneous.[28] This finding suggests that the loss of E-cadherin by methylation in a primary lesion may drive metastatic progression, indicating that EMT is involved in cancer metastasis. Besides genetic or epigenetic control, E-cadherin is regulated by various signal networks, such as TGF-β signaling and transcription factors as discussed in more detail below.

TGF-β signaling. Miettinen *et al.* first revealed that TGF-β induced EMT in normal mammary epithelial cells.[30] In fact, TGF-β is an important inducer of EMT in cancer progression. However, TGF-β is well known to induce multiple responses in cancer progression.[31] For example, loss of the TGF-β signaling pathway results in the progression of cancer because TGF-β is a strong growth inhibitor.[32] Indeed, Hahn *et al.* reported that mutations in TGF-β and Smad4 give rise to pancreatic cancer[33] and colorectal cancer.[34] On the other hand, TGF-β can protect against apoptosis, and promote angiogenesis and immune suppression.[35] TGF-β induces EMT through multiple signal pathways, including direct phosphorylation of Smad 2 and Smad 3. As shown in Figure 1, TGF-β also activates other EMT-related signal pathways, including integrin, Notch, and Wnt signal pathways, all of which trigger EMT programs.

Transcription factors. Transcriptional repressors of E-cadherin such as zinc finger proteins (ZEB1, ZEB2), bHLH protein (Twist), and the snail family of zinc finger proteins (Snail, Slug) are associated with EMT.[36–40] As shown in Figure 1, various signal pathways such as TGF-β,[20] the Wnt cascade, and PI3K/AKT (phosphatidylinositol 3′ kinase–serine/threonine kinase) axis are connected with these transcriptional repressors of E-cadherin.[41] Recent studies have demonstrated that transcriptional repressors of E-cadherin are regulated by microRNAs as described below. Several transcriptional factors such as Snail, Slug, and Twist are useful markers to predict prognosis in various human carcinomas (Table 1). Peinado *et al.* proposed that E-cadherin repressors might participate in the process of EMT as follows. First, Snail and ZEB2 would initiate down-regulation of E-cadherin. Then, Slug and ZEB1 would maintain repression of E-cadherin.[42] However, the effect of E-cadherin repressors on mesenchymal markers such as vimentin and N-cadherin remains unsolved.

Regulation of EMT by microRNA. Recent studies of small non-coding RNAs are shedding light on the regulation of gene expression and proteins in metastasis. It was shown that miR-10b overexpression is associated with invasiveness and metastatic potential.[43] miR-10b is overexpressed in metastatic breast cancer, and up-regulated by EMT transcription factor Twist. Recent independent studies revealed that the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) and miR-205 play critical roles in regulating EMT, targeting the E-cadherin repressors ZEB1 and ZEB2.[13,15] Gibbons *et al.* found that metastasis-prone tumor cells established from

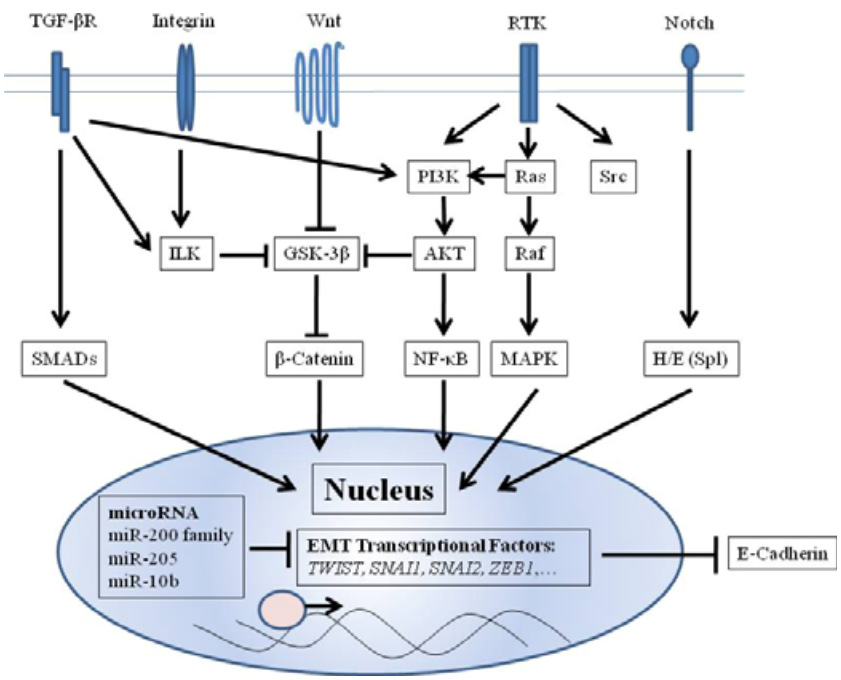

Fig. 1. Depiction of signal pathways regulating the epithelial–mesenchymal transition (EMT). Selected signal pathways regulating E-cadherin are schematized. Transforming growth factor (TGF)-β signals toward the SMAD pathway or the PI3K/AKT axis. Wnt ligands block β-catenin degradation. Excess β-catenin enters the nucleus and upregulates *SLUG* and *SNAIL* transcription. In integrin signaling, overexpression of ILK leads to nuclear translocation of β-catenin. Signals via RTK lead to EMT through the Ras-Raf-MAPK pathway or the PI3K/AKT pathway. AKT, serine/threonine kinase; GSK-3β, glycogen synthase kinase-3β; H/E (Spl), Hairy and enhancer of split; ILK, integrin-linked kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3′ kinase; RTK, receptor tyrosine kinase; TGF-βR, transforming growth factor-β receptor.

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Table 1. Epithelial–mesenchymal transition (EMT)-associated markers in clinical samples predict patient prognosis

EMT-associated gene	Characteristics	Cancer types	Reference (author)
Epithelial marker			
E-cadherin	Type I cell–cell adhesion glycoprotein	Breast cancer	Gould Rothberg and Bracken[25]
		Gastric cancer	Chan *et al.*[24]
		Colorectal cancer	Doridi *et al.*[84]
Claudin-1	Tight junctions restrict lateral diffusion of lipids and membrane proteins	Lung cancer	Chao *et al.*[85]
		Renal cell carcinoma	Fritzsche *et al.*[86]
		Ovarian carcinoma	Kleinberg *et al.*[87]
Mesenchymal marker			
Vimentin	Intermediate filaments represent a third class of cytoskeletal elements	Breast cancer	Thomas *et al.*[88]
		Lung cancer	Al-Saad *et al.*[89]
		Gastric cancer	Utsunomiya *et al.*[90]
N-cadherin	Type I cell–cell adhesion glycoprotein	Esophageal cancer	Yoshinaga *et al.*[91]
		Lung cancer	Nakashima *et al.*[92]
		Urothelial tumor	Lascombe *et al.*[93]
Fibronectin	High-molecular weight extracellular matrix glycoprotein	Bladder tumor	Mutlu *et al.*[94]
		Colorectal cancer	Inufusa *et al.*[95]
		Ovarian carcinoma	Franke *et al.*[96]
Transcription factor			
Snail	Zinc finger transcriptional repressor	Adenocortical carcinoma	Waldmann *et al.*[97]
		Esophageal cancer	Natsugoe *et al.*[98]
		Hepatocellular carcinoma	Miyoshi *et al.*[99]
Slug	Zinc finger transcriptional repressor	Lung cancer	Shih *et al.*[100]
		Colorectal cancer	Shioiri *et al.*[101]
		Esophageal cancer	Uchikado *et al.*[102]
Twist	Basic helix-loop-helix transcription factors	Cervical cancer	Shibata *et al.*[103]
		Ovarian carcinoma	Hosono *et al.*[104]
		Breast cancer	Martin *et al.*[105]

metastatic lung adenocarcinoma (with evidence of mutant K-ras and p53) could transit reversibly between epithelial and mesenchymal states, a property that was regulated by the miR-200 family.[44] Furthermore, two recent independent studies showed that members of the miR-200 family can induce the EMT process and regulate the sensitivity to epidermal growth factor receptor (EGFR) in bladder cancer cells and to gemcitabine in pancreatic cancer cells.[45,46] As for regulating TGF-β, microRNAs related to TGF-β signaling such as miR-155 and miR-29a have been identified in breast cancer tissues.[47,48] It is important to identify microRNAs involved in EMT to elucidate up-stream regulators of various known signal pathways.

Microenvironment and EMT

The tumor microenvironment is composed of the extracellular matrix (ECM), cancer-associated fibroblasts, myofibroblasts, immune cells, and soluble factors required for cancer progression and metastasis. Interaction among cancer cells in the tumor microenvironment can induce EMT by auto- and/or paracrine secretion of mediators such as growth factors, cytokines, and ECM proteins.[21] Media conditioned by cultures of cancer-associated fibroblast induce EMT in breast cancer cells.[49] In a comparison of the central areas of primary colorectal cancer and corresponding metastases, nuclear β-catenin was found in dedifferentiated mesenchyme-like tumor cells at the invasive front and it was localized to the membrane and cytoplasm.[50] This study suggested that the tumor microenvironment may induce or maintain EMT (Fig. 2). For instance, cancer-associated fibroblasts may be supplied from cancer cells undergoing EMT.[51] Similarly, oral squamous cancer cells can directly induce a myofibroblastic phenotype via secretion of TGF-β. TGF-β signaling by stromal myofibroblast can induce secretion of hepatocyte growth factor (HGF) which promotes cancer cell proliferation and invasion.[52]

Drug Resistance and EMT

Cells undergoing EMT become invasive and develop resistance to anticancer agents (Fig. 2). In fact, EMT can be induced by anticancer agents, and stress conditions such as exposure to radiation and hypoxic conditions.[53,54] Up-regulation of *TWIST* was associated with cellular resistance to paclitaxel in human nasopharyngeal, bladder, ovarian, and prostate cancers.[55] In colorectal cancer, stable oxaliplatin-resistant cells established by chronic exposure to oxaliplatin can acquire the ability to migrate and invade with phenotypic changes resembling EMT (spindle-cell shape, loss of polarity, intercellular separation, and pseudopodia formation).[56] In pancreatic and ovarian cancer, stable cell lines resistant to gemcitabine and paclitaxel established by continuous exposure can undergo EMT with increased expression of Snail and Twist, EMT-regulatory transcription factors.[57,58]

Various types of molecularly targeted agents have been developed and used against many carcinomas with or without combination of traditional anticancer agents, leading to improved clinical outcome and survival rate.[59,60] However, EMT reportedly confers resistance to these targeted agents. For example, lung cancer cell lines having undergone EMT, expressing vimentin and/or fibronectin, were insensitive to the growth inhibitory effects of EGFR kinase inhibition (erotinib) *in vitro* and in xenografts[61] as well as other EGFR inhibitors such as gefitinib and cetuximab.[62,63] We have often encountered patients who have suffered relapses after drug treatment, even when the tumors were initially highly sensitive. Thus, EMT can lead to resistance to multiple drugs and permit rapid progression of the tumor. These clinical findings may be attributed to the inherent characteristics of EMT. Clarifying the correlation between EMT and drug resistance may help clinicians select an optimal anticancer drug treatment.

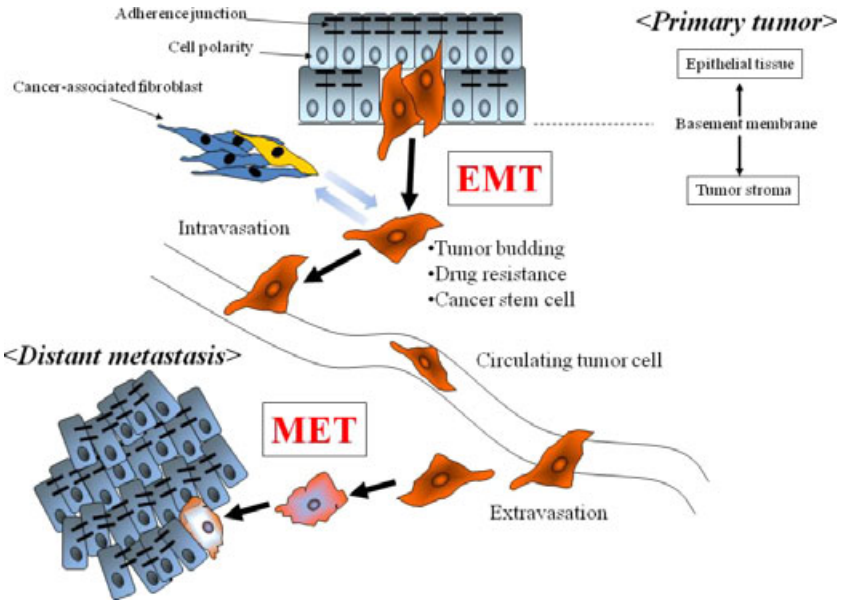

Fig. 2. The epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) are involved in cancer metastasis. Cancer cells undergoing EMT in a primary tumor disseminate through the fragmented basement membrane and acquire the characteristics of drug resistance and cancer stem cells. They can be recognized in tumor buds in histological specimens. EMT cells invade into tumor stroma and enter the circulation, allowing transport to distant organs. At metastatic sites, solitary cancer cells form the new metastatic focus through MET.

Cancer Stem Cells and EMT

Cancer researchers have recently found a minor fraction of cells (cancer stem cells [CSC]) with the ability to self-renew and give rise to differentiated tumor cells. CSC have been identified in breast, colon, and pancreatic cancer.[64–66] CSC as well as cells undergoing EMT are considered to be more resistant to toxic injuries and chemoradiation therapy than differentiated daughter cells.[67,68] Furthermore, cancer cells under hypoxic conditions acquire the properties of CSC.[69,70] Even though evidence indicates a relationship between EMT and cancer cells with the traits of stemness,[71] CSC are rare in whole tumor tissues.[68,72] However, it remains controversial among pathologists whether CSC as well as cells undergoing EMT exist in human cancer tissues.[73] Intriguingly, Mani *et al.* initially disclosed that immortalized human mammary epithelial cells (HMLEs) undergoing EMT are CSC-like as characterized by their $CD44^{high}/CD24^{low}$ phenotype.[16] These investigators induced EMT in HMLEs by ectopic expression of Twist or Snail, known inducers of EMT. The cells undergoing EMT acquired a fibroblastoid mesenchymal appearance. Furthermore, Mani *et al.* observed down-regulation of epithelial markers such as E-cadherin and up-regulation of mesenchymal markers such as N-cadherin, vimentin, and fibronectin. They also noted a $CD44^{high}/CD24^{low}$ expression pattern associated with human breast CSCs. Furthermore, they revealed that the cells undergoing EMT had the properties of CSC, including self-renewal and the capacity to form mammospheres. These findings suggest that EMT may play a role in the development of CSC and properties of invasiveness, metastasis, recurrence, and chemoresistance (Fig. 2).

Clinical Significance of EMT

EMT-associated markers in clinical samples and their effects on prognosis are summarized in Table 1. Most EMT-associated markers have been identified in histological specimens. However, the existence of EMT cells in clinical specimens has been challenged.[74] In response, Voulgari *et al.* suggested that the controversy between experimental and clinical studies is due to the 'spatial' and 'temporal' heterogeneity of EMT (Fig. 3).[19] Cells undergoing EMT may gain metastatic potential but may constitute only a small proportion of the total population of tumor cells. Tumor budding is commonly observed in clinical practice, and it consists of a single cancer cell or small cell cluster at the invasive front of tumor tissues. Indeed, cancer cells in tumor buds have down-regulated E-cadherin[75] and have characteristics of CSC.[76] Therefore, identification of cancer cells undergoing EMT in clinical specimens is difficult for pathologists.

The temporal heterogeneity of EMT (and the reverse, MET) is readily explained. MET is observed *in vitro* following addition of bone morphogenetic protein 7 (BMP7), removal of an EMT-inducer such as TGF-β, and establishment of hypoxic conditions.[54,77] A similar process may occur at metastatic sites which require cancer cells to recover the expression of E-cadherin for cell adhesion. The phenotypes of metastatic specimens are often compared with primary specimens to confirm the diagnosis by hematoxylin–eosin staining. The presence of the same cancer cell characteristics or phenotypes in both primary and metastatic lesions can provide the diagnosis of cancer metastasis. Therefore, the occurrence of MET could make it difficult to prove that EMT, a transient phenomenon that involves only a minority of cells, has occurred in human cancer specimens. However, EMT-associated genes obviously are useful as predictive biomarkers (Table 1). Clinical verification of EMT will require advanced techniques such as *in vivo* imaging.

Treatments Targeting EMT

As shown in Figure 1, EMT-related pathways provide targets for therapy. For instance, inhibition of integrin-linked kinase (ILK) increases the sensitivity of mesenchymal cells to EGFR-target therapy in hepatocellular carcinoma.[63] In *in vitro* studies, Src kinase inhibitors effectively inhibit the growth of cells undergoing EMT.[78] Furthermore, the inhibition of hedgehog signaling can prevent pancreatic cancer cells from acquiring tumor-initiating property and undergoing EMT.[79,80]

RNA interference and microRNA are new technologies in drug development. For instance, silencing of Snail by shRNA induced MET and reduced *in vivo* tumor growth.[81] As for microRNA, Krutzfeldt *et al.* disclosed that specific silencers of endogenous miRNAs, antagomirs, are powerful tools to silence specific miRNAs *in vivo*.[82] Therefore, microRNAs associated with EMT such as the miR-10b and miR-200 family could be exploited as therapeutic strategies in the future.

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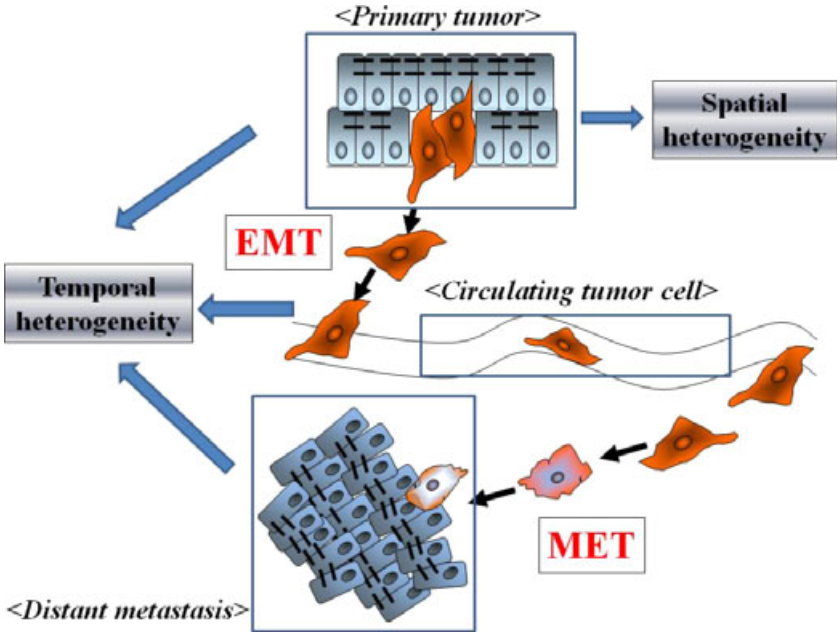

Fig. 3. Spatial and temporal heterogeneity of the epithelial–mesenchymal transition (EMT). Cancer cells undergoing EMT are expected to be only a small proportion of primary tumor tissues. EMT cells transported to metastatic sites are expected to undergo and mesenchymal–epithelial transition (MET). Therefore, the spatial and temporal heterogeneity of EMT/MET severely restricts the ability of pathologists to detect cancer cells undergoing EMT in histological sections.

Furthermore, the tumor microenvironment, which contributes to the maintenance of EMT, could be targeted. A small-interfering RNA targeted at TGF-β reportedly reduces metastasis *in vivo*,[83] and this observation could be applied to TGF-β secreted by tumor stroma. Note that reducing EMT could also lessen the occurrence of anticancer drug resistance and thereby improve the efficacy of conventional therapy. To eradicate cancer cells effectively and cause minimal toxicity to normal cells, further studies are required to define the molecular differences between EMT in embryological development and that in cancer progression.

Perspectives

During the past few decades, an increasing number of studies have shown that EMT is associated with cancer progression, metastasis, and drug resistance. Furthermore, improved understanding of microRNAs and cancer stem cells will clarify the processes underlying EMT. Current understanding of traditional signal pathways coupled with these new concepts could accelerate progress in cancer research. However, the multimodal nature of these complex pathways presents formidable challenges to researchers attempting to inhibit the onset of EMT. Finally, the clinical evidence supporting the role of EMT in cancer progression is still relatively weak. Thus, better methods for EMT detection in patient samples are needed.

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