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Clinical significance of epithelial-mesenchymal transition

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Abstract

The concept of epithelial-mesenchymal transition (EMT), a process where cells change their epithelial towards a mesenchymal phenotype, has gained overwhelming attention especially in the cancer research community. Thousands of scientific reports investigated changes in gene, mRNA and protein expression compatible with EMT and their possible correlation with tumor invasion, metastatic spread or patient prognosis; however, up to now, a proof of clinical significance of the concept is still missing. This review, with a main focus on the role of EMT in tumors, will summarize the basic molecular events underlying EMT including the signaling pathways capable of its induction as well as changes in EMT-associated protein expression and will very briefly touch the role of microRNAs in EMT. We then outline protein markers that are used most frequently for the assessment of EMT in research and diagnostic evaluation of tumor specimens and depict the link between EMT, a cancer stem cell (CSC) phenotype and resistance to conventional antineoplastic therapies. Furthermore, we evaluate a possible correlation between EMT marker expression and patient prognosis as well as current therapeutic concepts targeting the EMT process to slow down or prevent metastatic spread of malignant tumors.

Keywords: Epithelial-mesenchymal transition; Invasion; Metastasis; Prognosis; Therapy

Introduction

Epithelial-mesenchymal transition (EMT) is a central element of embryonic development, wound healing and tumor cell migration, and has thus obtained much attention by the research community since Greenburg and Hay firstly described a mesenchymal-like transformation of epithelial cells when suspended in collagen gels [1]. Basically, the term describes a process in which cells lose epithelial and gain mesenchymal characteristics; this is accompanied by a loss of cell-cell cohesiveness, leading to enhanced migratory capacity [2]. Multiple genes as well as proteins that seem to play a central role in EMT have so far been identified and are either up- or down-regulated during the process, thus serving as possible markers in the assessment of EMT. Since it seems to be a key element in wound healing and tumor cell migration, there is also great interest in EMT as a pharmaceutical target; recent publications even proposed vaccination against

drivers of EMT as an immunotherapeutic approach against tumor progression [3].

However, since many studies on EMT are based on *in vitro* results and not all findings could be confirmed *in vivo*, the clinical significance of the concept remains unclear [4]. This review lays the main focus on EMT in tumor cells and aims at recapitulating what is known about the molecular basis of EMT. Furthermore, we will summarize current markers of EMT that are in clinical and/or diagnostic use and, finally, evaluate EMT from a translational point of view and in the context of clinical feasibility.

Review
The molecular basis of EMT

Basically, EMT stands for a loss of epithelial and a gain of mesenchymal cellular characteristics that enhance migration and invasion by the cell [5]. This process includes loss of cell cohesiveness as well as fundamental reorganization of the cytoskeleton inducing a switch from apical-basal to front-rear polarity, and may furthermore be associated with the acquisition of invasive properties through the secretion of lytic proteases as well as
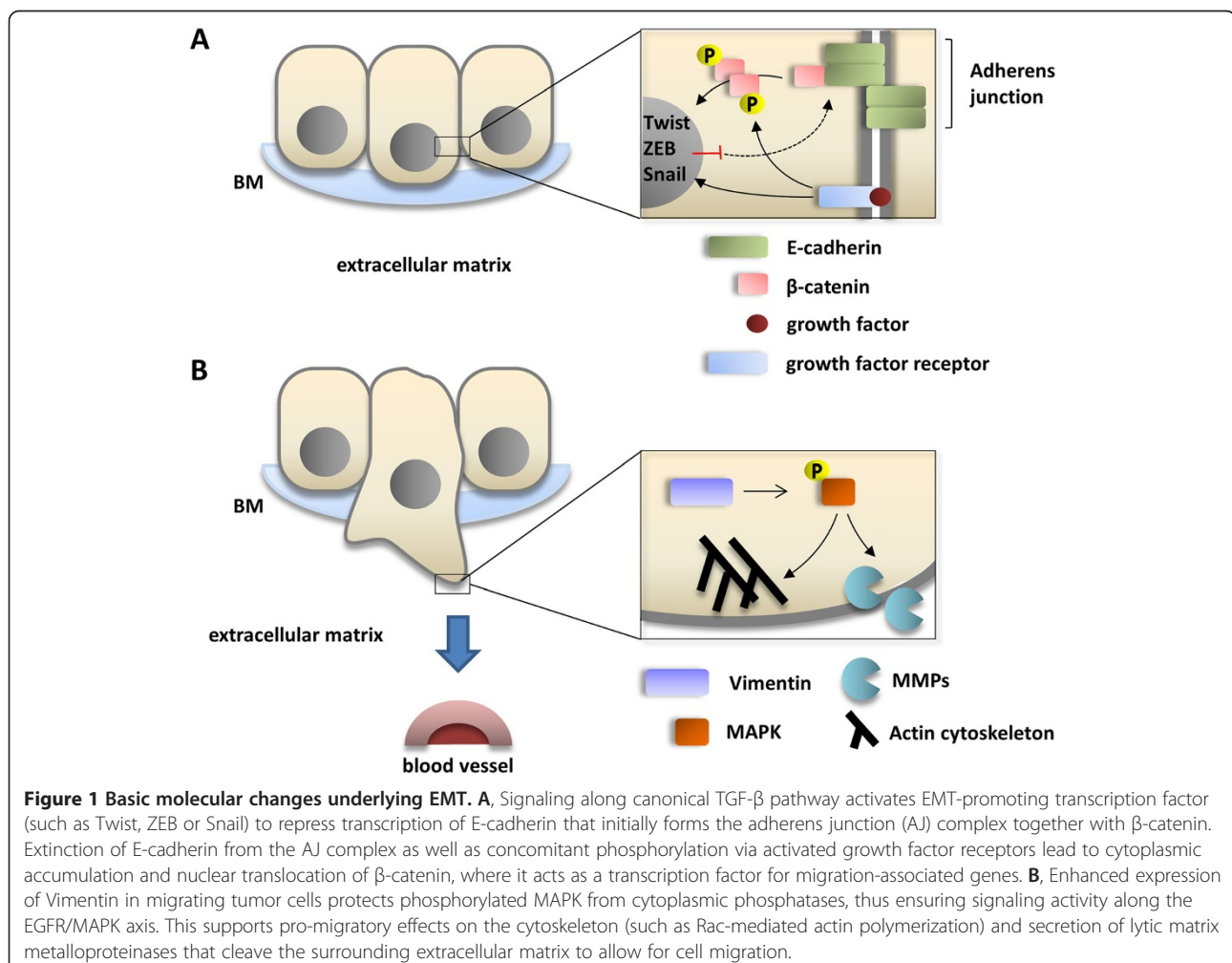
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resistance to senescence and apoptosis [6]. EMT is under tight control of multiple regulatory pathways; first and foremost, transforming growth factor β (TGF-β) signaling activity is enhanced in many physiological and pathological conditions in which EMT is observed, such as organogenesis, inflammation and tumor invasion [7,8]. In canonical TGF-β signaling, binding of TGF-β to its cell surface receptors (type I-III) activates complex formation of Smad family transcription factors, which translocate to the nucleus and cooperate with transcription factors from the Snail and Twist family, so-called "EMT master genes" [9,10]. Non-Smad signaling molecules downstream TFG-β and supportive of EMT include activated Rho-like GTPases, Phosphatidylinositol-3-kinase (PI3K) and mitogen-associated protein kinase (MAPK; the various signaling pathways mediating TGF-β signaling in EMT are excellently reviewed in [6]). Taken together, these effectors mediate transcriptional repression of genes that are involved in cell polarity and cell-cell adhesion, such as RhoA and E-cadherin (Figure 1A) [11,12]. The latter is mediated by the recruitment of histone deacetylases (HDACs) and other repressors to E-box elements in the E-cadherin promoter, leading to chromatin condensation and transcriptional repression [13]. At the same time, the expression of N-cadherin, another member of the cadherin family that allows for enhanced adhesion between mesenchymal cells, is upregulated; this balanced change in cadherin expression has thus been designated "cadherin switch" and is regarded a hallmark of EMT [14]. Not only the expression, but also specific membraneous targeting of E-cadherin is repressed in EMT via loss of the epithelial-specific intermediate filament keratin; therefore, loss of keratin immunostaining is widely regarded as a marker for ongoing EMT [15,16]. Further mechanisms that lead to degradation of cell-cell junctions include a repression of claudin and occludin expression, while zonula occludens 1 (ZO-1) is subsequently lost in a post-transcriptional manner [17-19]. This repression is maintained throughout further progression of EMT [20]. Since protein complexes (such as partitioning defective – PAR) that define the apical compartment of the cell are normally associated with intercellular junctions, degradation

Figure 1 Basic molecular changes underlying EMT. A, Signaling along canonical TGF-β pathway activates EMT-promoting transcription factor (such as Twist, ZEB or Snail) to repress transcription of E-cadherin that initially forms the adherens junction (AJ) complex together with β-catenin. Extinction of E-cadherin from the AJ complex as well as concomitant phosphorylation via activated growth factor receptors lead to cytoplasmic accumulation and nuclear translocation of β-catenin, where it acts as a transcription factor for migration-associated genes. **B**, Enhanced expression of Vimentin in migrating tumor cells protects phosphorylated MAPK from cytoplasmic phosphatases, thus ensuring signaling activity along the EGFR/MAPK axis. This supports pro-migratory effects on the cytoskeleton (such as Rac-mediated actin polymerization) and secretion of lytic matrix metalloproteinases that cleave the surrounding extracellular matrix to allow for cell migration.

of the junctions also weakens the apical-basal polarity cellular phenotype [6]. Moreover, the TGF-β-facilitated signaling along the MAPK axis exerts pro-proliferative and anti-apoptotic effects on the cell, while Ras/MAPK activity alone – without TGF-β induction - has also been linked to enhanced EMT [21-23]. After losing cohesiveness due to degradation of cell-cell junction complexes, mesenchymal-like tumor cells are able to invade through the basement membrane into underlying tissue by the secretion of lytic enzymes such as matrix metalloproteinases (MMPs) and MAPK-mediated reorganization of the actin cytoskeleton which is enhanced by the expression of Vimentin (Figure 1B) [24]. In detail, migration and invasion of moving cells is facilitated by specialized cellular protrusions, such as filopodia, lamellipodia and invadopodia. While filopodia, consisting of actin filaments arranged in a parallel fashion, seem to sense changes to the cellular microenvironment and act as a "guide" through the surrounding matrix, lamellipodia are built upon a branched actin network and allow for actin-myosin interactions as a prerequisite for cellular movement [25,26]. Both filopodia and lamellipodia have been linked to an EMT-like phenotype in migrating tumor cells [27,28]. Invadopodia are closely related to lamellipodia in a sense that they also consist of a branched network of actin filaments, but have the ability to degrade the extracellular matrix (ECM) through the secretion of lytic proteases, such as MMP-1, MMP-7 and MMP-9 (Figure 1B) [26]. Invadopodia formation has been linked to activity of the EMT transcription factor Twist1 cancer, and own results showed high expression of invadopodia-associated proteins, such as Cortactin and Abelson interactor 1 (Abi1), in a colorectal carcinoma cell line with an EMT-like phenotype shown by loss of E-cadherin [29,30]. Accordingly, TGF-β signaling activates small GTPases that enhance local reorganization of the actin cytoskeleton as a prerequisite for lamellipodia and filopodia formation, such as Rho, Rac and Cdc42 [31]. Vimentin, which is frequently upregulated in cells with an EMT-like phenotype, is then required for the further maturation of invadopodia [32]. Besides clearing the way for migrating tumor cells, MMPs that are released during tumor cell invasion are themselves further fueling the EMT process; the same effect is achieved via liberated TGF-β from the ECM [33-35]. In a mouse model of gastric cancer, it could be shown that EMT cooperates with MMP activity to gain access to lymph vessels and to spread distant metastases [36]. Accordingly, blood and lymph vessel infiltration by triple-negative breast cancer cells is associated with the expression of EMT transcription factor Zeb1 in surrounding stroma [37]. Alterations in MMP expression are linked to changes in the integrin repertoire with downregulation of some (epithelial) and upregulation of other integrins that facilitate interaction with extracellular matrix components such as collagen [6].

Targeting transmembrane proteins - like E-cadherin - or increasing the levels of intracellular reactive oxygen species via enhanced activation of Rac1b are further mechanisms of MMP-induced EMT [38,39].

Upon arrival at the site of metastasis, it seems a prerequisite for metastatic colonization that tumor cells undergo a partial reversal of the EMT, the so-called "mesenchymal-epithelial transition" (MET) [40,41]. During that process, tumor cells regain the expression of epithelial markers, such as E-cadherin, while the expression of EMT-associated transcription factors, such as Twist1, is repressed [41]. Thus, EMT can be seen as a reversible and transient process that enables epithelial tumor cells to gain access to the vasculature, allowing for the formation of distant metastasis.

Besides TGF-β, other signaling pathways have also been implied in the activation of EMT; for example, hypoxia-inducible factor (HIF) contributes to EMT in tissue fibrosis and cancer cell invasion by modulating the activity of pro-EMT transcription factors Notch and β-catenin [42-44]. HIF1α induces Twist and Snail expression in endothelial as well as ovarian carcinoma cells [45-47]. Additionally, activation of several receptor tyrosine kinases (RTKs) may result in induction of EMT; in these scenarios, growth factor binding to RTKs as well as activating mutations in oncogenes downstream of the receptors leads to enhanced signaling along the Ras/ MAPK or Akt/mTOR axis, resulting in upregulation of Snail expression [6]. Finally, it has been shown that enhanced wnt signaling activity as well as an upregulation of chemokine receptors (such as CXCR-1) also support the process of EMT [48,49]. Here, wnt signaling leads to an inhibition of glycogen synthase kinase 3β (GSK3β)-mediated phosphorylation of β-catenin; the resulting decrease in proteosomal degradation and cytoplasmic accumulation of β-catenin supports its translocation to the nucleus, where it acts as a transcriptional co-activator of EMT-associated gene expression [50].

In the recent years, the role of small, non-coding RNAs in the EMT process has also been further elucidated. Methylation-depedent expression changes in levels of miR-200c and miR-141, for example, regulate invasion and metastasis in colorectal cancer via altered miR-200c target gene expression; miR-375 is downregulated in tamoxifen-resistant breast cancer cells with EMT-like properties, and its reexpression partly reverses EMT [51,52]. Other miRNAs that have been discussed to play a central role in EMT are, among others, miR-1, 9, 24, 29b, 30a, 31, 124, 155, 192/215 and 661 (reviewed in [6]). Their mechanisms of action include post-transcriptional regulation of "EMT master genes" or of genes defining the epithelial or mesenchymal phenotype of the cell (such as E-/N-cadherin or vimentin). However, a thorough review of the role of miRNAs in EMT

and their clinical significance would lie beyond the scope of this text, where we would like focus on the role of well-characterized proteins in EMT.

Tissue markers of EMT

Unlike the various mechanisms that are known to initiate or repress EMT, the observed hallmarks of established or ongoing EMT are quite consistent. As previously mentioned, loss or degradation of proteins associated with epithelial homeostasis, cell polarity and cell adhesion, such as E-cadherin, RhoA or Plakophilin 2 is frequently observed in EMT (Figure 1A); some proteins that play key roles in cell-cell adhesion when attached to the membrane, such as β-catenin, are redistributed to the cytoplasm [11,12,17]. Moreover, cells undergoing EMT show decreased expression of epithelial cytokeratin filaments, such as keratins 8 and 18 [53]. On the other hand, the intermediate filament protein Vimentin is frequently overexpressed and contributes to cell migration as well as invasion-associated gene expression by stabilizing the phosphorylated state of MAPK and is thus regarded as a stable marker of EMT; moreover, its presence is a prerequisite for the maturation of invadopodia which are indispensable for cell invasion [32,54,55]. Dysregulated expression of transcription factors, such as Notch1, Slug, Snail, Twist or Zeb1 has been described in invasive tumors displaying EMT; these

markers are therefore designated as "EMT master genes". Table 1 provides an overview over selected dysregulated protein markers that have been and still are frequently used in the assessment of EMT.

EMT, tumor invasion and metastasis

The highest clinical significance of the EMT process is linked to its role in tumor cell invasion and metastasis. In a transgenic mouse model of pancreatic beta-cell carcinogenesis, the switch from noninvasive adenoma to invasive carcinoma is associated with a loss of E-cadherin expression [82]; moreover, it has been shown that loss of membraneous β-catenin is associated with tumor cell budding, a morphologic hallmark of invasive tumor phenotype and tumor aggressivity in colorectal cancer tissue specimens [83-85]. In samples from 49 breast cancer patients, the single-cell infiltration pattern that is observed in some lobular carcinomas has been linked to protein truncation mutations in the *CDH1* gene encoding for E-cadherin [86], and hypoxia-induced upregulation of Slug and Snail is associated with increased breast cancer cell migration and invasion *in vitro* [77]. Accordingly, expression of Vimentin can be found in many aggressive breast cancer cell lines [87]. As mentioned above, to allow for tumor cell invasion into the vasculature as a prerequisite for metastatic seeding, EMT cooperates with

Table 1 Frequently used protein markers for epithelial-mesenchymal transition (EMT)

Marker	Original function	Tissue	Reference
Downregulated in EMT			
α-catenin	Cell adhesion molecule	Lung	[56]
β-catenin (membrane)[1]	Cell adhesion molecule	Colon, Pancreas (NET)	[57,58]
Claudin	Cell adhesion molecule	Esophagus, Breast	[59,60]
Cytokeratins	Cytoskeletal filament	Lung, Esophagus	[16,61,62]
E-cadherin	Cell adhesion molecule	Colon, Breast, Lung, Ovary, Esophagus, Prostate, Cervix	[16,61,63-70]
Occludin	Cell adhesion molecule	Ovary	[18,71]
Upregulated in EMT			
Brachyury	Transcription factor	Pancreas, Breast, Lung	[72]
β-catenin (cytoplasm/ nucleus)[1]	Transcription factor	Breast, Cervix	[73]
EGFR	Tyrosine kinase receptor	Cervix	[70]
N-cadherin	Cell adhesion molecule	Ovary, Prostate	[68,74]
Notch-1	Transcription factor	Prostate	[75]
p16[INK4a]	Cell cycle regulator	Colon, Urothelium	[23,76]
Slug	Transcription factor	Breast, Ovary	[11,77]
Snail	Transcription factor	Breast, Cervix, Ovary	[11,70,77]
TTF-1	Transcription factor	Lung	[61]
Twist	Transcription factor	Breast, Stomach	[78,79]
Vimentin	Cytoskeletal filament	Breast, Esophagus, Cervix	[16,55,62,70]
ZEB1	Transcription factor	Colon, Breast, Ovary	[51,68,80,81]

[1]Membraneous depletion, but cytoplasmic accumulation/nuclear translocation.
NET, neuroendocrine tumor; EGFR, epidermal growth factor receptor; TTF-1, thyroid transcription factor-1; ZEB1, Zinc finger E-box-binding homeobox 1.

invadopodia formation and MMP activity [36,37]; circulating tumor cells (CTCs) obtained from peripheral blood of breast cancer patients frequently show an EMT-like phenotype [88,89]. In human and murine malignant melanoma cells, metastatic dissemination is enhanced and accelerated via Snail-induced EMT [90], and bone metastases of human prostate carcinomas show significant overexpression of Notch-1 compared to the primary tumors [75]. In lung carcinoma surgical specimens, tumor dedifferentiation as well as lymphogenous metastasis are also associated with reduced E-cadherin expression [91].

However, as mentioned above, some authors also reported reexpression of epithelial markers, such as E-cadherin, along with loss of EMT-associated transcription factors in established metastases [41]. This apparent reversal of EMT, often referred to as mesenchymal-epithelial transition (MET), has been described for metastases of colorectal carcinoma, non-small cell lung cancer and transitional cell carcinoma [92-94]. There is an ongoing debate regarding the extent to which these findings reflect a basic mechanism in the establishment of metastases or if they are restricted to certain tumor entities or reflect distinct circumjacent conditions [4,41]. There are also critical voices that doubt the role of EMT in invasion at all, since in most histopathologic specimens, many tumors invade and metastasize by cohesive and multicellular rather than single-cell migration, and histopathologists rarely see abundant mesenchymal-like tumor cells in routine surgical specimens [4,95,96]. This apparent contradiction might in part be explained by regarding EMT as a transient state of a small proportion of migrating tumor cells, with only single tumor cells or small clusters of cells obtaining the ideal dynamic configuration for different stages of invasion and metastasis; this reasonable compromise has been referred to as "spatial and temporal heterogeneity of EMT" by Voulgari et al. (Figure 1) [97,98].

Notably, there is another controversy regarding the point whether the EMT program is associated with enhanced or attenuated proliferative activity of the cell. While under normal circumstances TGF-β signaling exerts an anti-proliferative and pro-apoptotic effect, there is experimental evidence that tumor cells having undergone EMT do in fact show enhanced proliferation and resistance to apoptosis [99,100]. This apparent contradiction might also be explained by a possible heterogeneity in the course and the extent of EMT, with specialized cell populations exerting different roles during invasion and metastasis; this is in line with findings that highly metastatic breast cancer cells in fact show strong activity of the TGF-β signaling pathway [101]. It has also been proposed that the two oppositional endpoints of TGF-β signaling might be distinguished by loss of Smad4 in tumor tissue, which promotes TGF-β-mediated tumorigenesis, while in

parallel abolishing its tumor-suppressive functions [102]. Additionally, as described above, signaling along various non-TGF-β-dependent pathways might be capable of overcoming the original anti-tumorigenic effect of TGF-β in the course of an "unfriendly takeover" of central TGF-β signaling nodes and target genes; concurrent PI3K/AKT signaling, for example, thwarts the pro-apoptotic effect of TGF-β, thus selectively allowing for the pro-metastatic effects of the pathway to occur [13,49,50,77].

EMT, cancer stem cells and therapy resistance

Concerning the role of EMT in antitumoral therapies, it has been shown that an EMT-like cellular phenotype in both surgical specimens and cell lines is associated with increased resistance to most conventional approaches, such as chemotherapy [103-105], radiotherapy [106] or hormone withdrawal [107,108]. The observed changes in gene expression during EMT show striking similarity to a rather dedifferentiated state of the cell; in immortalized mammary epithelial cells, induction of EMT not only leads to the gain of a mesenchymal phenotype, but also induces the expression of certain stem cell markers ($CD44^+/CD24^-$) [109]. This generation of breast cancer cells with both cancer stem cell and mesenchymal-like characteristics has again been shown to be dependent on an activation Ras/MAPK signaling, and the link between EMT and cancer cell "stemness" is supported by the fact that genes associated with angiogenesis, invasion and metastasis are overexpressed in stem cell- like $CD44^+/CD24^-$ breast cancer cells; notably, after chemotherapy for breast cancer, residual tumor cells frequently display a stem cell-like phenotype and increased mammosphere formation efficiency [101,110,111]. The sensitivity of non-small cell lung cancer cells to EGFR kinase inhibition depends on their respective EMT phenotype, with mesenchymal-like cells (that express Vimentin or Fibronectin) being less sensitive to EGFR inhibition [112]. In the NSCLC model, it has also been shown that this resistance might be mediated via EGFR-independent MEK-Erk pathway activation and PDGFR, FGFR and TGF-β receptor acquisition in mesenchymal-like tumor cells [113]. An EMT-like gene expression profile in lung cancer cell lines is in fact associated with increased resistance to both EGFR and PI3K/Akt pathway inhibitors, a finding that could even be confirmed in a small patient cohort [114]. Thus, the mechanisms of resistance to antineoplastic therapies might be due to stem-cell like properties of tumor cells that have undergone EMT, allowing for self-renewing of a proportion of cells within the tumor based on the activation of central signaling pathways that are common to both processes, such as TGF-β, wnt, Notch and Hedgehog [13]. Associations between EMT-like properties and a stem-cell like cellular phenotype have not only been described in carcinoma of the breast and in NSCLC, but also in

urinary bladder, head and neck, pancreas, and colorectal carcinoma; here, increased resistance to anti-epithelial growth factor receptor (EGFR)-directed therapy is also associated with an EMT-like phenotype of the tumor cells [115].

EMT and patient prognosis

Since the metastatic spread of malignant tumors accounts for the majority of cancer-specific deaths [116-118], possible correlations between EMT markers and patient prognosis have been intensely studied in multiple tumor entities. However, there is still controversy regarding the impact of the EMT concept on the actual situation in human malignancies [119]. Therefore, much effort has been put into linking the expression of EMT markers to data on patient survival. In colon cancer, the upregulation of genes involved in EMT/matrix remodeling defines a molecularly distinct subtype with very unfavorable prognosis; downregulation of E-cadherin in patient samples, on the other hand, seems to be associated with high TNM stages and distant metastasis [120,121]. Accordingly, basal-like, triple-negative breast cancers that show upregulation of Vimentin have a poor prognosis [54,87]. In a meta-analysis of 1107 breast cancer samples, Tobin et al. showed reduced recurrence free survival in tumors displaying increased gene expression of EMT markers *SNAI2*, *TWIST1* and *VIM,* and decreased levels of *CDH1* (encoding for E-cadherin) [122]. In contrast, only recently Lee et al. were unable to confirm an impact of the tissue expression of EMT markers on disease-free survival or overall survival in breast cancer patients [123]. In prostate cancer, expression levels of EMT markers Twist and Vimentin - as assessed by immunohistochemistry in radical prostatectomy specimens - are independent predictors for biochemical recurrence as defined by a resurgence in serum prostate-specific antigen (PSA) levels following surgery [124]. Additionally, loss of membraneous E-cadherin staining seems to be associated with increased Gleason score, advanced clinical stage, and poor prognosis in prostate cancer [125]. In tissue samples from 354 primary tumors and 30 metastases of endometrial carcinomas, Tanaka et al. reported that EMT status (E-cadherin-negative/ Snail-positive immunostaining) correlated with histological type, FIGO stage, myometrial invasion and positive peritoneal cytology while it was inversely associated with both progression-free survival (HR = 0.443) and overall survival (HR = 0.366) [126].

Taken together, numerous studies in a variety of tumor entities show statistical correlations between patient prognosis and alterations of various markers compatible with EMT. However, it may be difficult to yield reliable prognostic information for an individual patient from the expression pattern of EMT markers in surgical specimens; this is in part due to high variability of marker expression patterns in different tumor areas in a heterogeneous sample [127,128]. Moreover, artificial induction of EMT *in vitro* (under certain cell culture conditions) as well as *in vivo* (in surgical specimens subjected to ischemia) has been shown [129,130]. Another key problem is the lack of a standardized diagnostic definition of which gene or which extent of expression changes is sufficient to determine EMT; in many reports, expression changes of one or two genes are already referred to as EMT or "partial EMT", thus impairing the comparability of studies [4,131]. Furthermore, as has already been discussed above, it is still unclear whether the gene expression changes observed in EMT reflect "passenger mutations" caused by genetic instability during tumor dedifferentiation rather than a real mesenchymal transdifferentiation state of the cell [4]. From this point of view, the expression of EMT markers simply represents a more primitive differentiation state of the cancer cell that is associated with oncogenic activation of a variety of signaling molecules [132].

EMT as a potential target for antineoplastic therapies

Since the population of stem cell-like tumor cells will always bear considerable resistance to conventional therapies and since the hallmarks of EMT have been identified in a significant proportion of these cells as described above, efforts have been made to develop antineoplastic therapies that directly target EMT. The aim of most therapeutic approaches is to block or slow down invasion and metastasis in tumors or, in benign conditions associated with EMT, impede fibrotic organ remodeling [96,133]. Table 2 shows some current therapeutic approaches that are aiming at EMT, most of them targeting kinase signaling pathways upstream EMT master gene expression. In mouse hepatocytes that have undergone EMT, it has been shown that inhibition of STAT3 signaling, for example, reduces EMT-like changes; in renal tubular epithelium, ALK receptor activation via recombinant BMP-7 acts antagonistically to TGF-β and leads to reexpression of E-cadherin [134-136]. Inhibition of kinase signaling downstream FGFR3, ILK, Ras/MAPK or PI3K/AKT downregulates tumor formation, EMT master gene expression and invasive potential in colorectal, lung and pancreatic carcinoma cells *in vitro*, while in some models, re-expression of E-cadherin could be shown upon treatment with kinase inhibitors [137-140]. In a mouse model of hepatocellular carcinoma, transformation with kinase-inactivated integrin-linked kinase (KI-ILK) partially restored the sensitivity to anti-EGFR treatment [141]. Accordingly, our own group showed that Ras-driven EMT is attenuated via Sorafenib-mediated inhibition of Urokinase plasminogen activator (uPA) expression in RT112 urothelial carcinoma cells [23].

Table 2 Therapeutic approaches targeting EMT in benign and malignant processes

Organ/entity	Target	Approach	Mechanism	Effect	Ref.
Liver (Hepatocytes)	STAT3	Sorafenib	Inhibition of STAT3 phosphorylation	TGF-β signaling ↓, Apoptosis ↓, Fibrosis ↓	[134]
Kidney (Tubular epithelium)	ALK3/6 receptors Smad5	Recombinant BMP-7	Antagonistic ALK receptor activation/Smad1 signaling	E-cadherin ↑	[111, [136]
Colorectal cancer	FGFR4	siRNA Knockdown	Reduction of Src and MEK1/2-ERK1/2 signaling	Tumor formation ↓,	[137]
		Targeting antibodies		Cell growth ↓	
		PD173074, TKI-25		Angiogenesis ↓	
Hepatocellular carcinoma	ILK	Kinase-inactivated ILK (S343A)	Reduction of Akt signaling	Sensitivity to anti-EGFR therapy ↑	[141]
Lung adenocarcinoma	HAT/HDAC	Sorafenib	HAT expression↑	Changes in histone acetylation and transcriptional repression of EMT-related genes	[138]
			HDAC expression↓, possibly via inhibition of Ras/Raf/MAPK and ErbB signaling		
	Brachyury	Vaccination (Brachyury-specific T cells)	T-cell mediated cytotoxicity	Lysis of Brachyury-positive tumor cells	[3]
	Axl RTK	SGI-7079	Inhibition of Axl phosphorylation	Growth of mesenchymal NSCLC xenograft tumors ↓	[114]
Breast cancer	LYN kinase	Dasatinib	Inhibition of LYN kinase activity	Invasion ↓	[142]
	EMT master gene expression	Metformin	Transcriptional repression	Twist1 ↓, ZEB1 ↓, Slug ↓, TGF-β 1–3 ↓, MMP-3, MMP-9 ↓, E-cadherin ↑	[143]
Urothelial carcinoma *in situ* (UCIS)	Urokinase plasminogen activator (uPA) expression	Sorafenib	Inhibition of Ras/MAPK signaling	uPA ↓, E-cadherin ↑	[23]
Pancreatic cancer	Gli1, Ptch (Hedgehog target genes)	Cyclopamine, IPI-269609	Inhibition of Hedgehog signaling	Snail ↓, E-cadherin ↑, Metastasis ↓	[139,140]
	EMT master gene expression	Resveratrol	Transcriptional repression	Slug ↓, Snail ↓, ZEB1↓, Migration/Invasion ↓	[144]
	Axl RTK	siRNA Knockdown	Inhibition of MAPK and PI3K/AKT kinase signaling	GTP-bound Rho/Rac↓, Slug ↓, Snail ↓, Twist ↓, MMP-9 ↓, Migration/Invasion ↓	[145]

STAT3, Signal transducer and activator of transcription 3; TGF-β, transforming growth factor β; ALK3, activin-like kinase 3; BMP-7, bone morphogenetic protein 7, FGFR4, fibroblast growth factor receptor 4; Src, sarcoma kinase; MEK, mitogen-associated protein kinase kinase; ERK, extracellular signal-related kinase; siRNA, small interfering RNA; ILK, integrin-linked kinase; AKT, protein kinase B; HAT, histone acetyltransferase; HDAC, histone deacetylase; EGFR, epidermal growth factor receptor; RTK, receptor tyrosine kinase; NSCLC, non-small cell lung cancer; LYN, Lck/Yes-related novel protein tyrosine kinase; MMP, matrix metalloproteinase; uPA, urokinase plasminogen activator; ZEB1, Zinc finger E-box-binding homeobox 1; PI3K, phosphatidylinositol-3-kinase.

From the knowledge of the diverse kinase-dependent signaling pathways that are activated during EMT, it is not surprising that the application of multi-kinase inhibitors such as Sorafenib is capable of reversing the process to a certain extent. Up to now, concepts that are directly targeting EMT master genes or their effectors are rare. Interesting new approaches include the previously mentioned vaccination against Brachyury-positive tumor cells and the transcriptional repression of EMT master gene expression by the anti-diabetic drug Metformin (Table 2) [3,72,143]. Resveratol, a dietary polyphenol, downregulated expression of EMT master genes Zeb1, Snail and Slug and impaired CSC self-renewal capacity, tumor growth and invasion in a mouse model of pancreatic ductal adenocarcinoma [144]. However, despite the abundance of literature on effectors of EMT, there is a lack of studies that show a solid effect of a specific compound in an *in vivo* system additionally to cell culture data, and to our best knowledge, a study that rescued the EMT phenotype after application of a certain compound - for example by overexpressing an EMT-inducing transcription factor - has so far not been conducted. Therefore, most of the data on drugs targeting EMT has to be regarded as preliminary, and further research is needed to identify valuable pharmacologic targets during the induction or progression of the EMT process.

Conclusions

Taken together, the concept of EMT is a valuable model for the morphologic and molecular changes observed in tumor cell invasion as well as tissue fibrosis. However, it is still unclear whether or to which extent cells in fact do undergo a complete conversion of cell type or show only transient changes in cellular morphology and protein expression patterns that are supportive of a migratory phenotype. Despite the controversies dealing with the definition and extent of EMT, the association between an EMT-like cellular phenotype - as shown by changes in marker protein expression - and tumor aggressivity has been well-proven in a variety of malignancies. In recent years, first promising results have been reported concerning a possible use of the EMT process as a pharmacological target, especially with multi-kinase inhibitors such as Sorafenib. However, since most of these results are actually derived from *in vitro* data and definite proof of druggable EMT *in vivo* is still missing, the clinical utility of these approaches remains to be elucidated in future studies.

Abbreviations

AJ: Adherens junction; CXCR-1: CXC motif chemokine receptor 1/Interleukin-8-receptor alpha; ECM: Extracellular matrix; EMT: Epithelial-mesenchymal transition; FGFR: Fibroblast growth factor receptor; FIGO: Fédération internationale de Gynécologie et d'Obstétrique; GSK3β: Glycogen synthase kinase 3β; HDAC: Histone deacetylase; HIF: Hypoxia-inducible factor; MAPK: Mitogen-associated protein kinase; MEK: Mitogen-associated protein kinase; MET: Mesenchymal-epithelial transition; MMP: Matrix metalloproteinase; NSCLC: Non-small cell lung cancer; PAR: Partitioning defective; PDGFR: Platelet-derived growth factor receptor; PI3K: Phosphatidylinositol-3-kinase; RTK: Receptor tyrosine kinase; TGF-β: Transforming growth factor β; TNM: Tumor/Nodes/Metastasis (clinical classification system for tumor spread); uPA: Urokinase plasminogen activator; ZO-1: Zonula occludens 1.

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