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EDITORIAL

Epithelial-mesenchymal, mesenchymal-epithelial, and endothelial-mesenchymal transitions in malignant tumors: An update

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Abstract

Epithelial-to-mesenchymal transition (EMT) represents conversion of an epithelial cell in an elongated cell with mesenchymal phenotype, which can occur in physiologic and pathologic processes such as embryogenesis (type 1 EMT), wound healing and/or fibrosis (type 2 EMT) and malignant tumors (type 3 EMT). The proliferation rate, metastasizing and recurrence capacity, as also the individualized response at chemotherapics, in both epithelial and mesenchymal malignant tumors is known to be influenced by reversible switch between EMT and mesenchymal-to-epithelial transition (MET). Although much research work has already been done in these fields, the specific molecular pathways of EMT, relating to the tumor type and tumor localization, are yet to be elucidated. In this paper, based on the literature and personal experience of the authors, an update in the field of EMT *vs* MET in epithelial and mesenchymal tumors is presented. The authors tried to present the latest data about the particularities of these processes, and also of the so-called endothelial-to-mesenchymal transition, based on tumor location. The EMT-angiogenesis link is discussed as a possible valuable parameter for clinical follow-up and targeted therapeutic oncologic management. The paper begins with presentation of the basic aspects of EMT, its classification and assessment possibilities, and concludes with prognostic and therapeutic perspectives. The particularities of EMT and MET in gastric and colorectal carcinomas, pancreatic cancer, hepatocellular and cholangiocarcinomas, and lung, breast and prostate cancers, respectively in sarcomas and gastrointestinal stromal tumors are presented in detail.

Key words: Gastrointestinal stromal tumor; Carcinoma; Gastrointestinal cancer; Hepatic cancer; Sarcoma

Core tip: This review, based on the personal experience of gastrointestinal pathologists, which correlates with literature data, is intended to provide an up-date in the field of epithelial-mesenchymal transition and mesenchymal-epithelial transition in epithelial and mesenchymal-malignant tumors, respectively. The molecular mechanism of these processes and their possible role in tumor progression, metastasis and therapy are presented in detail.

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INTRODUCTION

Because of paucity of data on biological behavior of epithelial and mesenchymal malignant tumors, the process of metastasis is still so poorly understood that it is difficult to block it therapeutically. In recent years, one of the newest described mechanisms that seems to contribute to migratory and invasive properties of tumor cells is the epithelial-mesenchymal transition (EMT)[1-3]. Till now, it is known to be implicated in carcinomas and in some mesenchymal tumors, such as sarcomas and gastrointestinal stromal tumors (GIST)[1-21]. However, the molecular mechanisms underlying EMT and distant metastasis remain somewhat unclear.

In this review we intend to present the main characteristics of EMT in a logical manner, beginning with the basic molecular mechanisms and possibilities of its quantification on histological slides. Then, the particularities of EMT in several carcinomas and sarcomas are explored and a future perspective synthesized.

BASIC INFORMATION ABOUT EMT

Definition of EMT

EMT represents conversion of an epithelial cell in an elongated cell with mesenchymal phenotype, which can occur in physiologic and pathologic processes[22]. Being influenced by several biochemical mechanisms, EMT involves mainly loss of cell polarity, dissolution of cell membrane, disintegration of cytokeratin filaments and desmosomes, and migration of the newly formed cells that have a mesenchymal phenotype[23,24]. Besides increase in migratory capacity, cell resistance to apoptosis also increases with associated cytoskeletal disorders and excessive deposits of extracellular matrix components[23-25].

Classification of EMT

Based on the pathogenetic role, three types of EMT are described. Type 1 EMT, involved in embryogenesis and organ development, mainly designed to generate the primary mesenchyme[23,26]. The first report on type 1 EMT in vertebrata embryogenesis was published in 1995[22].

Type 2 EMT is involved in non-neoplastic processes and genesis, and recruitment of fibroblasts in chronic inflammation-related tissue repair, wound healing, tissue regeneration and fibrosis[23-27].

Type 3 EMT represents cytoskeleton disorders that result from dissolution of cell-cell adhesion, cell scattering and loss of apical-basal cell polarity in malignant tumors[1,2,23,28].

EMT and endothelial-to-mesenchymal transition in non-neoplastic lesions

Type 1 EMT plays important roles in embryo implantation, embryogenesis and organ development[23,26]. At the same time, the reverse mesenchymal-to-epithelial transition (MET) is necessary to generate a secondary epithelium of kidney and other organs[23,24]. The secondary epithelia can remain in this status or can be re-differentiated into several structures such as fibroblasts, connective tissue, astrocytes, adipocytes chondrocytes, osteoblasts, and muscle cells[23,24]. The EMT-to-MET switch is mediated by several genes such as paired box2 (*Pax2*), Wilms tumor1 (*Wt1)*, and *Bmp7*[23]. In the embryonic heart, during formation of the atrioventricular/endocardial cushion, EMT is induced during somitogenesis by the HGF (hepatocyte growth factor) and its c-Met/Crk proteins, secreted by mesenchymal cells[23-25]. EMT is mandatory for the formation of epicardium-derived progenitor cells and their migration in the sub-epicardial space and it is mediated by several factors, such as transforming growth factor-β 1 (TGF-β1) and myocardin[29].

Type 2 EMT was described not only in physiologic repairing processes but also in renal, pulmonary, and hepatic fibrosis and even in pathogenesis of fistulae in Crohn's disease[23-27]. In renal fibrosis, about 35% of fibroblasts originate from tubular epithelial cells, which are detached and mobilized through a damaged basement membrane, and they are responsible for specific lesions such as chronic glomerulonephritis, diabetic nephropathy, lupus nephritis, and Alport syndrome[24]. The other fibroblasts are recruited from bone marrow (15%) and renal stroma (50%)[24]. In cardiac fibrosis, TGF-β1-mediated genesis of fibroblast from damaged vessels-endothelial cells, known as endothelial-to-mesenchymal transition (EndMT), was suggested as the predominant event, as it is involved in cardiac embryogenesis too[25]. In renal fibrosis, it is observed that the EMT-related fibroblasts are SMA-positive myofibroblasts; it is suggested that a combined EMT-EndMT is the key event of any fibrosis[24].

Because TGF-β action and EndMT are experimentally

inhibited and E-cadherin expression is restored in epithelial cells by bone morphogenetic protein 7 (BMP-7), it is supposed that systemic administration of this protein may delay progression of cardiac fibrosis and prevent chronic rejection of transplanted heart[25], besides delaying renal failure[24]. Rapamycin may also ameliorate the amount of fibrotic tissue by blocking the mTOR signaling[30].

EMT in malignant tumors (type 3 EMT)

In malignant tumors, EMT-related invasion and migration of tumor cells in blood flow is associated with poor prognosis, high metastatic rate, and low disease-free survival time[1-21]. In cancer cell lines, EMT is considered when the cells gradually change from epithelial to migratory elongated spindle-like mesenchymal cells with fibroblastic morphology; expression of epithelial markers decreases as tumor cells progressively display the mesenchymal markers[1,2]. Gaining mesenchymal signature facilitates detachment of tumor cells, accompanied by proteolytic digestion of basement membrane, vascular invasion (intravasation), and migration of circulating tumor cells to distant sites[2,3,24].

As not much is known about the cytoskeleton of detached and circulating tumor cells, it is supposed that EMT produces detyrosination of some proteins, such as α-tubulin (storage of Glu-tubulin)[28]. This process, a vimentin-dependent one, induces formation of tubulin microtentacles (microtubules-based membrane protrusions), which are distinct from the well-known actin-based prolongations (lamelipodia, filopodia) and confer on the circulating tumor cells with mesenchymal phenotype the capacity of attachment at the endothelial layers and further extravasation at distant sites[28]. Moreover, Glu-tubulin increases the intravascular life of tumor cells, from 3-5 min to about 16 h, favoring cell extravasation[28,31].

At the same time, in distant metastatic tissue, a reverse MET can be acquired (EMT-MET switching) according to the metastatic microenvironment; regaining of epithelial features allows re-proliferation of tumor cells in these secondary-formed tumor clusters in lung and liver metastases, but not in bone metastatic tissue[2,3,15,18]. It is also worth mentioning that, in primary tumors, in parallel with EMT, the transformed tumor cells acquire a stem cell pattern or activate multidrug-resistant stem cells, and the converted mesenchymal cells that did not migrate in the blood flow are responsible for local recurrence, probably due to a reverse MET in the primary tumor, besides metastatic sites[18,32].

EMT MARKER PROTEINS

Literature describes several markers, which are involved in EMT. During EMT, E-cadherin expression is reduced to near-zero level, E-cadherin-to-N-cadherin (Neural cadherin) switch is installed and other EMT-related markers [vimentin, fibronectin, smooth muscle actin (SMA), desmin, Sox, SNAIL1/SNAIL, SNAIL2/SLUG, Axl, zinc finger E-box-binding homeobox 1 (ZEB1), Notch-1, v-ets erythroblastosis virus E26 oncogene homologue 1 (ETS1), fms-related tyrosine kinase 1 (FLT1), V-set and immunoglobulin domain-containing protein 1 (VSIG), stromelysin-3, Twist, FOXC2, HOXB7, ACTA2, platelet derived growth factor (PDGF), *etc.*] are overexpressed[4,11,32], predominantly at the invasive front[23]. Other epithelial cell-cell adhesion molecules such as claudins (types 3, 4, and 7), α-catenin, γ-catenin, occludin, desmoplakin and plakoglobin are also down-regulated in cells with mesenchymal signature[5,11,32]. Immunohistochemically, E-cadherin and catenins mark the cell membrane; N-cadherin, vimentin, and PDGF present a predominant cytoplasmic expression; Notch-1 and ZEB1 mark the nuclei, whereas SNAIL, SLUG, and Twist mark the cytoplasm, as well as the nuclei of the tumor cells.

It is important to note that some of the transformed cells present only mesenchymal phenotype, whereas other tumor cells display a dual epithelial-mesenchymal expression[32], also known as an amphicrine pattern[33]. As regards the stem cells with malignant potential, it is still unclear if they are identical to the cells with mesenchymal signature, or they represent two distinct cell types; they are marked by both mesenchymal markers and stem cell markers such as CD44 and ALDH (aldehyde dehydrogenase)[34].

SLUG is a zinc-finger transcription inhibitory protein, a member of the SNAIL family (SNAIL 1 and 2) that is primarily described in the neural crest and embryonic mesodermal cells; it also plays roles in type 1 and type 3 EMT[32-36]. SNAIL 1/SNAIL is a transcriptional hypoxia-activated protein that interacts with Wnt- and also with serine/threonine kinase receptor signalling pathways; the tumor cells' invasiveness and resistance to apoptosis are influenced by its overexpression in both tumor cells and stromal fibroblast[32,37]. SNAIL 2/SLUG is involved in cancer progression and suppresses E-cadherin, desmoplakin, and keratin-18 expression, but the exact mechanism is not known; it is supposed to interfere with the Wnt/GSK3β/β-Trcp1 pathway[4].

Vimentin is a mesenchymal marker that can be acquired during EMT but its expression is low and most difficult to be quantified, as compared with other EMT markers that indicate a mesenchymal-like phenotype[1].

The receptor tyrosine kinase Axl is an EMT marker whose mRNA expression is strongly correlated with vimentin. It is involved in EMT of breast, and pancreatic and lung cancers and is expressed more in mesenchymal than in epithelial lines[6-8].

Dismantling of epithelial cell membrane and remodeling of extracellular matrix are also influenced by matrix metalloproteinases (MMP-2, MMP-3, and MMP-9) that act as proteolytic enzymes and may link the EMT with increased invasion and metastasis, and shortened survival time in almost all types of human

cancer[23,24,38].

VSIG, also known as radioiodinated cell surface A33 antigen or glycoprotein A34, is a single-pass 387 amino acid membrane protein involved in cell-cell adhesion[39].

Cathepsin family (*e.g.,* Cathepsin Z located at 20q13.3, Cathepsin X, *etc*.) is also considered to be involved in EMT and to contribute to tumor metastasis through down-regulation of E-cadherin, α-catenin, and β-catenin, and up-regulation of vimentin, fibronectin, MMP-2, MMP-3, and MMP-9[38].

MOLECULAR FACTORS INVOLVED IN EMT

In the last few years, several research works were carried out to understand the mechanism of EMT in both carcinomas and mesenchymal tumors, but data are elusive.

As regards DNA, the next-generation sequencing-based methods proved that the genome-wide DNA methylation reprogramming is not involved in EMT; the progressive EMT and genetic disorders are rather related to altered histone modifications[1,9] and epigenetic mechanisms[11]. Recently, it is suggested that a 76-gene EMT-signature is involved in the metastatic process and that it influences the therapeutical answer of tumor cells[7], but the results are still confusing.

E-cadherin's transcriptional down-regulation is one of the key factors of EMT but other factors, such TGF-β also play important roles in these processes[1,9]. It is worth noting that there are no deletions or mutations of *E-cadherin* gene, but only epigenetic down-regulation or transcriptional silencing which allows its further re-expression in primary or metastatic tumors[18]. E-cadherin is codified by the *CDH1* gene; it interferes with other EMT-related genes, such as vimentin, fibronectin 1 *(FN1), CDH2* (which codifies the protein N-cadherin), *ZEB1* (target of SNAIL), *ZEB2/SIP1*, *K-ras, integrin, Notch,* and *Axl* (a tyrosine kinase inhibitor)[7]. The cancer cell lines with epithelial phenotype are characterized by overexpression of other genes such as *claudins 4* and *7, MUC1, RAB25, SPINT2,* and *ERBB2*[7]. However, some of these molecular markers (SNAIL 1/2, ZEB 1/2, E47) are direct inhibitors of the transcription of *E-cadherin* gene, whereas Twist, Goosecoid, FoxC2, and E2.2 are indirect E-cadherin inhibitors[11,16,17]. At the same time, SNAIL 1/2, ZEB1/2, and Twist are activated by TGF-β, a cytokine, secreted by mesenchymal stromal and inflammatory cells, whereas N-cadherin is activated by Twist[32,40]. If we take into account that TGF-β also induces renal EMT, besides mobility of endothelial cells, followed by EndMT and subsequent renal and cardiac fibrosis[24,25], and regulation of matrix accumulation[32], then we can suppose that stromal fibrosis is generated by an interaction between EMT and EndMT. Moreover, TGF-β is a tumor suppressor in early-stage carcinomas but induces tumor cells' proliferation, migration, and metastases, in advanced stages[32].

Epidermal growth factor (EGF) is a mitogenic factor involved in tumor proliferation and aggressiveness, through its receptor, EGFR. Although *EGFR* and *K-ras* genes' status are used as indicators for targeted therapy with anti-EGFR drugs of several tumors, such as pulmonary and colorectal cancers, the origin of this factor or its prognostic role is not firmly proved. As regards the role of this pathway in EMT, it seems that the Ras-activated/SNAIL/SLUG pathway interacts with FoxC2 and the phosphatidylinositol 3'-kinase (PI3K)/Akt/mTOR axis, at least in the case of colorectal cancer[40].

One of the newest factors found as pivotal regulators of EMT and as negative regulators of E-cadherin are the post-transcriptional gene regulators microRNAs (miRs): miR-21, miR-26b, miR-29c, miR-31, miR-124, miR-212, and the five members of the miR-200 family (miR-200a, 200b, 200c, 141, and 429) with their most prominent gene targets *ZEB1* and *ZEB2* (also known as *SIP1* and *SFHX1B*)[2,10,11,17,18,21,40]. Their overexpression is believed to inhibit EMT in human carcinoma cells and to decrease tumor cell proliferation and migration in the blood flow[2,10,11,17,18,21]. EMT-related interaction of miR-200 family with *p53* gene is also assumed[19].

Their elective affinities for specific types of cancer cell lines are described below.

EMT, ENDMT AND ANGIOGENESIS

The data available on the link between EMT and angiogenesis is so scattered that it does not elucidate if the link is any possible important therapeutic implications. For example, the MMP family members (MMP-2, MMP-3, and MMP-9), which are released by fibroblasts and macrophages, are known to influence both EMT and angiogenesis[23,38], though not proved to mediate the interaction among these two processes. At the same time, EMT, activated *via* SNAIL/Twist, is responsible for the attachment of tumor cells to the activated endothelial cells *via* α-tubulin detyronisation[28]. The transcription factor Twist, a target gene of SNAIL, is reportedly implicated in embryogenesis (EMT type 1), but its hypoxia-activated over-expression is also proved in several human carcinomas[41]. However, its role in carcinogenesis and metastasis is not well defined. In experimental models, Twist inhibition does not decrease tumor cell proliferation rate, but reduces circulating tumor cells significantly[28]. In cancer tissues its expression is increased at the invasion front[41]. Based on these observations, it is supposed that Twist increases direct invasion of tumor cells in the surrounding tissues, and cell penetration inside the endothelial layer, but does not influence tumor growth[28,41]. As a therapeutic target, its inhibition may decrease the rate of metastasis.

Because the endothelium of pre-existing mature vessels, involved in angiogenesis of both epithelial

and mesenchymal tumors, is activated by CD105 (endoglin)[42,43], new studies are necessary to prove if the Twist-attached endothelial cells are also CD105 positive or other mechanism is involved in this attachment.

Most recently, a possible TGF-β1 mediated-EndMT of intratumor endothelial cells is reported[44]. It relates to the cases in which the intratumor endothelial cells lose the cell-cell junction and immunoexpression of CD31 and gain positivity for mesenchymal markers such as α-SMA (smooth-muscle actin)[44]. It is important to note that the endothelial cell-specific miR-302c is proved to inhibit this EndMT, but decreases the tumor cell motility[44]. In previous researches, our team observed inconstant positivity of Kaposi sarcoma cells[42] and gastric tumor cells for the endothelial marker CD105 (Gurzu *et al*[42], personal communication), which could indicate even a possible epithelial-to-endothelial transition or an incidental positivity of epithelial cells with mesenchymal signature.

A relationship between inflammation, angiogenesis and EMT is suggested by COX-2-mediated EMT, which is stimulated by TGF-β through a PGE2-dependent mechanisms[23,35], and also by an MMP-related signaling[23].

Other arguments that favor a link between angiogenesis and EMT-mediated metastasis are that, on the one hand, the endothelial cell-secreted factors, such as EGF, increase cell mobility and inhibit apoptosis, whereas, on the other hand, the EGF-related EMT is more prominent in the perivascular area, but the intraluminal tumor cells exhibit an epithelial phenotype[45]. It is important to prove that the involved vessels present CD105-positive activated endothelium. EGF seems to also induce a stem-like phenotype of EMT-transformed tumor cells (in both intra- and perivascular tumor cells) and SNAIL positivity through PI3k-Akt pathway, whereas down-regulation of EGF in endothelial cells decreases tumor growth rate and the immunoexpression of stem cell markers[45]. The data suggests that antiangiogenic therapy should be based on inhibition of tumor cells from secreting pro-angiogenic substances such as vascular endothelial growth factor-A (VEGF-A), and of the activated endothelial cells to synthesize pro-angiogenic/pro-stem/pro-EMT substances such EGF[45].

EMT IN GASTROINTESTINAL CARCINOMAS

EMT in gastric carcinoma

Fewer than 50% of GC cells express E-cadherin, and SLUG cytoplasmic positivity is reported in about 30% of the cases[36]. Most of the E-cadherin negative cases are hereditary[46] or acquired diffuse/poorly-cohesive gastric carcinomas (GCs)[47]. The 5-year survival rate is strongly related to E-cadherin expression, which is 88.6% in positive cases and 63.5% in cases with loss of E-cadherin expression[36]. As regards SLUG immunoexpression, the 5-year survival rate is about 78% in negative cases and only 54.3% in SLUG positive-GCs[36].

Independent of the histologic type, E-cadherin negative/SLUG positive-GCs are associated with higher risk for lymph node and distant metastases, as compared to the E-cadherin positive/SLUG negative-GCs[36]. The reverse correlation of E-cadherin/SLUG is observed in only 75.5% of GCs, while the other cases present double positivity for E-cadherin and SLUG[36]. In E-cadherin positive-GCs, association of SLUG positivity induces a lower survival rate, as compared to SLUG negative cases (92% *vs* 46.7%)[36].

In Chinese patients, the cell-cell adhesion molecule VSIG is also reduced in GC samples, as compared with paired gastric mucosa, whose decreased levels are proved with PCR, western-blot and IHC examinations[38]. Using IHC methods, total loss of VSIG was found in more than 50% of the cases, especially in GCs with distant metastases, and decreased levels in 46% of them, which are strongly correlated with the overall survival rate and disease-free survival[38].

Cathepsin X expression was detected in *Helicobacter pylori*-infected normal gastric mucosa, which was 3-12 fold up-regulated in 68% of metastatic GCs with EMT, especially in the cases with an intestinal type architecture[38,48].

Twist is especially overexpressed in diffuse type GCs and is correlated with N-cadherin expression; both of them are down-regulated in intestinal type-GC, as compared to paired normal gastric mucosa[49]. N-cadherin is expressed in chief and parietal cells of the normal mucosa. Based on these observations, it was suggested that E-cadherin-to-N-cadherin/Twist switch is specific for carcinogenesis of diffuse type GC, whereas intestinal-type GC is more related to TGF-β-dependent ZEB2 (SIP1) up-regulation/SNAIL weak down-regulation/E-cadherin loss[49]. At the same time, release of TGF-β, which activates the EMT-inductors SMAD 2/3 and PI3K/Akt signaling, is caused by extreme hypoxia[50].

Among the miR-200 family members, miR-141 is reported to have been down-regulated in about 80% of primary GCs, as compared with matched normal gastric mucosa and also in several human gastric cancer cell lines[11,18]. Its overexpression inhibits proliferation of GC cells, but decreased level of miR-141 favors occurrence of distant metastases[18].

As regards the therapeutic approaches, EMT may induce in GCs a Wnt/β-catenin signaling-dependent resistance to adriamycin, which can be reverted by therapy with the proton pump inhibitor pantoprazole[51].

EMT in colorectal carcinoma

In advanced-stage colorectal carcinomas (CRCs), a predominant-epithelial phenotype was reported from the center of primary tumor, and mesenchymal features in the cells from the invasion front[11,20]. As regards SLUG expression, its positivity induced in CRC cells an EMT-related aggressive behavior, but no relation could be proved between E-cadherin and SLUG expression[36,52]. Aggressivity was also induced

by SLUG overexpression in fibroblasts of the tumor stroma[37].

Increased E-cadherin and decreased vimentin-expression were noted in liver metastases, as compared with the primary tumor[11]. This immunophenotype, which indicates an EMT in primary tumor, predominantly in the invasive areas, and a reverse MET in hepatic metastases, seems to be related to altered functions of miRNAs such as miR-21[40], miR-29c[2], miR-31[40], miR-212[10], and some members of miR-200 family[11].

The miR-21 and miR-31 are particularly considered related to the TGF-β-pathway, because of their being targeted by the T lymphoma and metastasis gene 1 (*TLAM1*); their down-regulation increases CRC cells motility and invasion[40].

The miR-29c is down-regulated in primary CRC tumor cells with high invasive potential and re-expressed in hepatic metastases[2]. In CRCs lines, miR-29c decreased the proliferation, migration and metastatic potential of tumor cells, negatively regulated the Wnt/β-catenin signaling pathway and inhibited EMT *via* PI3K/AKT and GSK-3β/β-catenin signaling[2,40]. Immunohistochemical examinations showed increased nuclear β-catenin expression, loss of E-cadherin, up-regulation of T-cell factor/lymphoid enhancing factor transcriptional activity, and increased cell migration and invasiveness in miR-29c knockdown CRC cells, whereas overexpression of miR-29c induced a nuclear-to-cytoplasmic shift of β-catenin[2]. In matched liver metastastases foci, elevated levels of miR-29c induced MET[2]. In CRC, the two target genes of miR-29c and EMT-MET switch are the protein tyrosine phosphatase type IVA 1 and guanine nucleotide binding protein alpha13[2].

Overexpression of miR-212 seems to be responsible for inhibition of CRC cells proliferation; besides, by delaying their migration in blood flow, it becomes protective for both hepatic and lung metastases[10]. On the other hand, low level of miR-212 associates with overexpression of manganese superoxide dismutase, which mediates the EMT of CRC cell lines and induces a more aggressive tumor phenotype and a shorter survival rate of patients diagnosed with CRC[10].

In metastatic CRC of miR-200 family members, the miR-141 and miR-200c, mapped to chromosome 12, were found to be overexpressed in liver metastatic tissue, as compared with primary tumor cells[11]. Owing to gradually decreasing expression of miR-200c in tumor cells, as compared to adjacent normal colonic mucosa, and also in the tumor invasion front, it was found that this molecular factor, which is re-expressed in liver metastases, induces cell proliferation but suppresses cell invasion and migration[11]. On the other hand, high level of miR-200c/141 promoter region in CRC cell lines is associated with epithelial signature, resulting in up-regulation of E-cadherin, down-regulation of vimentin expression, and lower risk for distant metastases[11].

The other two members of miR-200 family, miR-200b and miR-429, which are mapped to chromosome 1, do not influence the EMT in CRC cell lines; miR-200b, but not miR-429, is down-regulated in liver metastatic tissue, as compared with primary tumor cells[11]. However, the most significant member of miR-200 family, involved in EMT and metastatic process of CRC, is miR-200c, which exerts negative regulation of its gene targets *ZEB1, ETS1* and *FLT1*[11]. Although the miR-200 family interacts with *p53* gene in other cancer cell lines[19], no correlation between mi-R200c and p53 was found in CRC[11].

Based on the above-mentioned expression of mi-RNAs, the most recent studies suggest that these molecular factors may serve as potential diagnostic markers and targets for therapeutic strategies in patients with metastatic CRC[2,10,11]. Further *in vivo* studies are necessary to confirm this supposition and to emphasize the relationship between mi-RNAs and other genes such as *p53* and mismatch repair (*MMR*) genes.

As regards the circulated CRC cells, it was proved that, inside the blood vessels, disruption of actin filaments increased cell adhesion, whereas destabilizing cytoskeletal microtubules prevented cell attachment and tumor intravasation at the junctions located between endothelial cells[28,53].

EMT IN PANCREATIC CANCER

In pancreatic cancer cells, EMT is induced by several molecular factors, such as the receptor tyrosine kinase *AXL*[7,8], loss of FOXA1/A2 expression[17], and dysregulation of miR-200 family[17], but this event is not so frequent as the other carcinomas are[17,54]. Moreover, loss of E-cadherin expression, a result of *E-cadherin* gene methylation, is correlated with histological grade of differentiation, which is noticed in about one quarter of well-differentiated ductal adenocarcinomas, half of the poorly-differentiated ones and few or none of the non-cohesive (undifferentiated and signet ring cell carcinomas) cases[17,55]. In chemoresistant well/moderately-differentiated pancreatic adenocarcinomas, E-cadherin loss and a membrane-to-cytoplasm shift of β-catenin expression pattern were noticed in the non-cohesive foci[17,55]. N-cadherin does not mark the normal acinar, ductal, and pancreatic islets; it is expressed in more than 50% of pancreatic carcinomas and is more intense in infiltrating cells from the invasive tumor front, independent of the tumor stage[56]. As regards the other IHC markers that indicate a mesenchymal-like phenotype of the epithelial tumor cells, SLUG was reportedly expressed in half percent of the cases, while SNAIL 1 marked 75% of pancreatic carcinomas. SNAIL 1 presents predominant positivity in the ductal cells of the tumor center and in undifferentiated carcinomas, as compared with the more cohesive carcinomas, whereas SLUG is more intense in the invasive front[56]. Moreover, 50% of the pancreatic carcinoma cells present double positivity for SNAIL and E-cadherin[56]. Twist is negative in 97% of pancreatic carcinomas and its expression is

10-fold higher after exposure of pancreatic cell lines to hypoxia for 48 h[56].

Hypomethylation and overexpression in pancreatic carcinomas cells and elevated serum levels of miR-200a and miR-200b are associated with retention of E-cadherin expression, as also with epigenetic silencing through methylation of *ZEB2 (SIP1)* gene[11,17]. As SIP1 expression is absent in most of the pancreatic cancers, it seems that E-cadherin dysregulation is rather related more to miR-200a and miR-200b than to *SIP1* gene[17]. The serum levels of these miR-factors were noticed to be elevated in patients with chronic pancreatitis, as compared with healthy controls[17]; they can be used as a screening parameter to identify patients with chronic pancreatitis and high risk for ductal adenocarcinoma.

EMT IN HEPATIC CANCER

EMT in hepatocellular carcinoma

The EMT-angiogenesis-stem cell-like phenotype crosstalk is supposed as a key factor of hepatocellular carcinoma (HCC), based on the fact that the number of tumor-associated macrophages, which secrete TGF-β and are involved in angiogenesis, is directly correlated with the number of activated stem cells, as well with EMT-dependent tumor cells invasiveness[57]. Just as other carcinomas do, TGF-β induces EMT through E-cadherin down-regulation and up-regulation of several factors, including twist, N-cadherin, and SNAIL[58].

Cathepsin Z was also reported to be up-regulated and involved in EMT and metastasis of about 43% of HCCs; its increased level was correlated with high serum level of α-fetoprotein and also with poorer 5-year and 3-year survivals[38].

Down-regulation of miR-124 and miR-26b was reported in HCC cells, in cases with a more aggressive behavior, whereas their overexpression suppressed tumor cell proliferation, inhibited EMT and prevented cytoskeletal disorders through blocking formation of stress fibres, filopodia and lamellipodia[21,58]. In HCC, the two target oncogenes, negatively regulated by miR-124 are ROCK2 and EZH2[21].

As regards the therapy, the advanced-stage HCC is currently treated with the multityrosine kinase and angiogenesis inhibitor, sorafenib. Because MET is also involved in metastatic behavior of HCC, the new MET-inhibitor, Tivantinib (ARQ 197), is being tested in the ongoing trials, as a second-line therapy of HCC[59].

EMT in cholangiocarcinoma

Based on the scanty data available, it is believed that the EMT in cholangiocarcinomas is regulated mainly by the transcription factors ZEB2 and S100A4, which are modulated by TNF-α (tumor necrosis factor)[60]. TNF-α also stimulates TGF-β activity[60] which in turn activates other EMT-inducers, such Twist, N-cadherin, and vimentin[61]. Just as other carcinomas do, EMT induces a high rate of metastasis and a short overall survival rate[60], and thus help in blocking TGF-β by BMP-7[61].

EMT IN LUNG CANCER

Lung cancer is one of the tumors that is known for rapid progression accompanied by a large number of genetic and epigenetic changes[1,7]. EMT is identified in about 23% of non-small cell lung cancer (NSCLC) lines and in one third of the patients with metastatic lung cancer. Based on the histology of the cell lines, the squamous pattern associates with a mesenchymal phenotype in about 50% of lines; adenocarcinomas are characterized mostly by epithelial phenotype, and the other patterns (neuroendocrine, large cell carcinoma) by only mesenchymal signatures[7]. Although it was experimentally proved that DNA methylation is not involved in EMT of lung cancer cell lines, after transition, the expression of some DNA-related enzymes, such as methyltransferases 1, 3a, and 3b, and ten eleven translocation (TET1), were significantly altered and some histone methylation was changed[1].

In A549 lung cancer cell lines, during TGF-β-dependent EMT, miRNA relative expression of E-cadherin was progressively lost and the most significant overexpression was attributed to N-cadherin, followed by SNAIL 1 and vimentin[1]. Maximum level of N-cadherin was observed after 96 h of incubation, with significantly increasing levels from 24 to 96 h[1]. SNAIL 1 becomes significantly expressed after 4-12 h of incubation, followed by progressive overexpression in the next 24-96 h; its level is quite low compared to that of N-cadherin[1]. The overexpression of vimentin does not differ significantly at 12, 24, and 96 h. Moreover, its level is maintained at a constant value, which is similar to the levels of SNAIL 1 and N-cadherin, quantified at 10-12 h of incubation[1]. In NSCLCs, SNAIL 1 and vimentin's expressions are reversely correlated with the miR-30a level[62].

In contrast, another experimental study proved that 76 genes were involved in EMT, the main ones being *CDH1* and *vimentin,* followed by several EMT transcription factors such as *FN1, MMP2* (matrix metalloprotease-2), and *ZEB1*; the gene *CDH2* was identified inconstantly and was not included in the EMT molecular signature[7]. In mesenchymal cells from NSCLC lines, *K-ras* mutation, loss of *STK11 (LKB1)*, and *SMARCA4* mutations/deletions are more frequent than in epithelial lines; the last ones rather show CDKN2A and CDKN2B loss[7].

In lung cancer cell lines, EMT also induces down-regulation of some histones methylation, such as DNMT1, DNMT3a, DNMT3b, H3K4me3, H3K9me2, TET2, and TET3, and up-regulation of TET1, and H3K36me3[1].

As regards the prognosis, retention of the epithelial phenotype, proved by E-cadherin positivity in the tumor cells, was associated with longer time to progression

and a longer survival, independent of the mutation status of the *EGFR* (epidermal growth factor receptor) gene[7,12]. *EGFR* wild type/*K-ras*-wild type molecular profile seems to indicate a less aggressive NSCLC[7].

EMT as the therapeutic target in lung cancer

Patients with *EGFR*-mutated NSCLCs showed significant improved outcome after having been introduced to anti-EGFR drugs, such as erlotinib (Tarceva)[12] and gefitinib[13]. However, the *EGFR* wild type and *K-ras* mutated NSCLCs are resistant to EGFR inhibitors, and the data about the effect of these targeted drugs on *EGFR* wild type/*K-ras*-wild type patients is scanty[7].

In NSCLC cell lines, EMT was proved to induce resistance to erlotinib, whereas resistance to Akt/mTOR and PI3K/Akt1 pathway inhibitors was seen in both EGFR wild-type and mutated tumors with mesenchymal signature[7]. However, the mesenchymally-transformed cells are not pan-resistant; they are sensitive to sorafenib and the commonly used chemotherapics such as cisplatin, gemcitabine, vinorelbine, pemetrexed, docetaxel, paclitaxel, and platinum-doublets[7].

On the one hand, retention of the epithelial phenotype (high E-cadherin and low vimentin/fibronectin) is associated with erlotinib sensitivity, even in wild-type *EGFR* and wild-type *K-ras* NSCLCs[7,14]. It was then suggested that E-cadherin's diffuse positivity could be used as an indicator of erlotinib/gefitinib sensitivity, especially in wild-type cases[12]. On the other hand, in culture cell lines with mesenchymal signatures, which are insensitive to anti-EGFR drug gefitinib, restoration of the epithelial phenotype could induce re-sensitivity to this drug[7,13].

The level of the receptor tyrosine kinase Axl increases in mesenchymal NSCLC lines, because the cells, having a dose-dependent synergistic effect with erlotinib, are sensitive to the Axl inhibitor SGI-7079; the maximum synergy level was obtained at high concentrations for both erlotinib and SGI-7079[7].

Based on the foregoing data, a 76-gene EMT signature (including the genes *CDH1, CDH2, vimentin,* and *FN1*) was experimentally designed to predict 8-wk disease control in NSCLC-patients treated with erlotinib, independent of their molecular profile[7]. However, the exact underlying biological mechanism is not fully understood and hence deserves further investigation.

EMT IN BREAST CANCER

EMT in breast[4] is dependent on reducing mi-RNA expression of E-cadherin, which is related to *CDH1* promoter methylation, but not to mutational inactivation[9]. Just as the other carcinomas are, E-cadherin is re-expressed in hepatic metastatic tissues[11], and local recurrence may be dependent on the EMT-MET switch in the primary tumor[32].

Claudins (types 1, 3, 4, and 7) were described to be down-regulated in certain types of breast cancer, such as fibromatosis-like metaplastic carcinoma, a low-grade *EGFR* (exons 18, 19, 20, and 21) wild-type basal-like spindle cell carcinoma with predominant HER-2 and hormone receptors negativity, diffuse positivity for basal keratins and focal vimentin and E-cadherin expression[5]. Fibroblastic-like phenotype of breast carcinoma cells (with negative or low E-cadherin expression), intense angiogenesis (indicated by overexpression of VEGF-A) and chromosome instability can also be induced by twist, *via* Wnt/β-catenin[32,63].

SNAIL 1, known to induce invasiveness in several human cancers, seems to be a hypoxia-protective factor for breast tumor cells, *via* β-catenin activation, which regulates expression of HIF-1 (hypoxia-inducible factor 1)-dependent genes, besides being an inductor of hormone-resistance[32,63]. Based on these facts, it is suggested that SNAIL 1 may be used as an indicator of response of breast cancer to antiangiogenic therapy[64].

Twist expression was proved to be up-regulated in tandem with tubulin detyrosination, in both invasive ductal[28] and lobular-type adenocarcinomas[41], at the invasion front[28,41]. This interaction, which is mainly twist-dependent, seems to promote penetration of tumor cells through the endothelial cell layer, endothelial engagement and, probably, consecutive angiogenesis that favors metastasis[28].

EMT is also induced by the receptor tyrosine kinase *AXL*[6,7], Pyk2 and TGF-β *via* SNAIL 1/2 and ZEB 1/2[32], and it seems to have particular pathways in triple negative breast cancers[32].

As regards miRNA, the miR-200 family-mostly miR-200a/b/c, miR-141, and miR-429-is considered to influence EMT of breast cancer cells with inhibition of the E-cadherin repressors ZEB 1/2[32,65]. An interaction between *p53* gene and miR-200c is also defined in breast cancer cell lines[19]. The miR-21s also influences the EMT phenomenon *via* tumor suppressor gene PTEN and AKT/ERK1/2 axis[32,66]. Besides down-regulation of cyclin-dependent kinase (CDK8), β-catenin targeted-miR-26b is the other reported negative prognostic factor in breast cancer[58,67].

EMT is reported to be involved in breast cancer resistance to tamoxifen; the most down-regulated factor is the methaderin-targeted miR-375[68], whereas miR-519a is up-regulated in tamoxifen-resistant breast cancer cell lines with mesenchymal-like signature[69]. Re-expression of miR-375 and inhibition of miR-519a might serve as potential therapeutic approaches for estrogen receptor-positive breast carcinomas[68,69]. It was also experimentally proved that some contraceptive pills, such as centchroman, may be used to inhibit EMT and to play a dose-dependent antiapoptotic and antiproliferative role in human breast cancer cells, *via* down-regulation of HER2/ERK1/2/MMP-9 signaling[70].

EMT IN PROSTATIC CANCER

Prostatic carcinomas have a predilection for bone metastases, the metastatic cascade being also related

to EMT that is linked with stem cell signature of the prostatic carcinoma cells[15]. Although vimentin and ZEB 1 contribute to EMT, the most involved marker that plays a role in bone metastasis seems to be Notch-1[15].

In liver metastases, prostate carcinoma cells are bound to hepatocytes in an E-cadherin-dependent manner[18]. In primary tumor tissues, EMT is characterized by activation of EGFR and subsequent down-regulation of E-cadherin, whereas, in liver metastases, down-regulation of EGFR signaling induces re-expression of E-cadherin and cell-cell adhesion[18]. In bone metastatic tissue, E-cadherin remains down-regulated and Notch-1 is up-regulated[15].

EMT AND MET IN MESENCHYMAL TUMORS

EMT and MET in sarcomas

Inhibiting MET is one of the therapeutic goals currently being tested in human sarcomas. However, because of the aberrant expression of the EMT markers noticed in many mesenchymal tumors, most of the researchers believe that EMT is involved in grade of recurrence, metastasis rate and overall survival rate[71-75]. To our knowledge, it is more about MET than EMT, but the molecular aspects are neither well explored nor well defined.

In chondrosarcomas, CXCR4 and survivin were tested as candidates for molecule-targeted therapy, and they were found expressed in more than 80% of chondrosarcomas. Besides inducing SNAIL and N-cadherin up-regulation, they are directly correlated with recurrence rate, *via* MEK/ERK and PI3K/AKT signaling[73].

In osteosarcoma cell lines, HIF-1α-mediated hypoxia induces increased level of E-cadherin and decreased vimentin expression. This aberrant pattern is associated with a higher proliferation rate and increased invasivity of the osteosarcoma cells that can be inhibited by resveratrol[74], which has an antiangiogenic effect due to increased nitric oxide production in endothelial cells[76].

In neuroblastomas, several EMT pathways are up-regulated; EMT induces not only aggressiveness but also chemoresistance to doxorubicin[75].

EMT and MET in gastrointestinal stromal tumors

Although they are mesenchymal tumors, one third of GIST cells are proved to express not only the mesenchymal markers such as vimentin and SNAIL, but also as being positive for the cell-cell adhesion molecule E-cadherin and AE1/AE3 keratin[71,72]. Moreover, SNAIL-positive/E-cadherin negative GISTs, independent of vimentin expression, presented a higher risk for distant metastases[71] and keratin/E-cadherin positive-cases were predominantly vimentin/N-cadherin negative[72]. Based on these facts, it is supposed that both EMT and the reverse MET processes are involved in GISTs metastasis.

The post-transcriptional factor miR-137, which is targeted by Twist1, enhances the epithelial cell morphology and it serves as an apoptosis inductor, decreasing GIST cell motility[72]. In conclusion, miR-137 induced-EMT seems to indicate a lower risk for distant metastases and hence may be used as a potential therapeutic tool.

SUMMARY AND FUTURE PERSPECTIVES

Although the literature provides several new insights into EMT of tumor cells, supplementary molecular exploration is necessary for therapeutical reducing the rate of metastasis. The literature review shows that several factors are indeed involved in EMT, most of them having prognostic and/or predictive value. However, the features relating specifically to organ-related carcinomas need further elucidation, because most papers dealing with this subject contain no specific and valuable data.

If EMT is indeed involved in the resistance of tumor cells to specific drugs, such as anti-EGFR substances, tamoxifen, and classic chemotherapics, identification of transition pathways could be of immense help in evolving an appropriate targeted therapy for both carcinomas and malignant mesenchymal tumors, including GISTs.

Additional studies of complex molecular profiling are necessary to elucidate the particularities of EMT in terms of histological type and localization of the tumor and angiogenesis-EMT interaction. Complex studies should also take into account not only cases with E-cadherin loss, but also the particularities of those cases, which show focally decreased expression and aberrant immunohistochemical pattern. We believe that, in the near future, the particularities of EMT will be a very useful parameter for proper clinical follow-up, individualized therapy and a more refined molecular classification of malignant tumors.

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