Topic: Reviews of Recent Advances in Research and Treatment for Gastroenterological Malignancies

Epithelial-mesenchymal transition in gastroenterological cancer

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ABSTRACT

Epithelial-mesenchymal transition (EMT) was first reported as an essential process in embryonic cells and later showed that cancer cells, regardless of the context, exhibited a similar phenomenon that was crucial for tumor progression. Epithelial cells lose their adhesive characteristic capacity which is necessary for their functions but gain a mesenchymal phenotype. This change from epithelial to the mesenchymal phenotype of cancer cells makes it difficult to understand the mechanism underlying cancer biology and tumor progression. A number of transcription factors involved in tumor cell EMT and microRNA-regulated EMT have been reported. This review discussed recent findings and new players in EMT in gastrointestinal cancers. Since the molecular mechanisms of tumor progression are sometimes context-dependent, the recent findings of EMT have been reviewed in a context-dependent manner.

Key words: Epithelial-mesenchymal transition, gastrointestinal cancer, microRNA, transcription factor

Introduction

Epithielial-mesenchymal transition (EMT) is a well-known phenotype and essential for tumor invasion and metastasis.[1-3] The phenotype change in EMT is drastic, so the theory has fascinated many investigators, and several mechanisms have been reported to date. However, the number of factors essential for EMT is increasing; thus, it is challenging to integrate those factors to understand their networking. In this review, we briefly updated the recent EMT findings in a context-dependent manner, because the mechanisms underlying a disease substantially depend on the original function of the affected organ. Theoretically, the concept of EMT explains various cancer characteristics including tumor cell invasion, metastasis, chemo resistance and stem cell phenotype; therefore, it has considerable clinical significance. Thus, this review explores both the molecular mechanism of EMT and its clinical significance.

Although many EMT players, such as transcription factors and microRNAs (miRNAs) have been introduced so far such as transcription factors and miRNAs, their roles are to some extent-dependent on the context. Therefore, we discussed the role of each molecule in a context-dependent manner to clarify the specific role of each player.

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Esophageal Cancer

Esophageal cancer (EC) has two distinct histological subtypes, that is, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC).[4] The former commonly occurs in Asia, whereas the latter is common in the United States and Western countries. Transforming growth factor-β1 (TGF-β1) was reported to induce EMT in EAC via the mothers against decapentaplegic homolog (SMAD) 4 pathway and this signaling was inhibited by bone morphogenetic protein 7, another member of the TGF-β1 superfamily.[5] Using immortalized esophageal keratinocyte, TGF-β1 was shown to regulate mitochondrial superoxide dismutase 2 (SOD2) which possesses antioxidant activity, to convert $CD44_{low}$ to $CD44_{high}$ cells. Expression of SOD2 was transcriptionally regulated by NF-κB and zinc finger E-box binding homeobox 2 (ZEB2), but not ZEB1.[6] In the same cells, it was also reported that TGF-β1-mediated EMT required *p53* mutation accompanied by up-regulation of ZEB1 and the loss of epithelial growth factor receptor (EGFR)-dependent

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senescence program.[7] Epithelial cell adhesion molecule (EpCAM), a well-known marker for circulating tumor cells in many solid tumors, is down-regulated in TGF-β1-mediated EMT. However, EpCAM expression in disseminated tumor cells (DTCs) was associated with lymph node metastasis and decreased overall survival of patients with EC. The conflicting evidence that DTCs need the process of EMT but express epithelial cell marker EpCAM is supported by the result that high expression of EpCAM promoted tumor outgrowth after xenotransplantation of esophageal carcinoma cells, suggesting that EpCAM expression changes dynamically over the course during cancer progression.[8]

A notable EMT inducer that has recently been reported is interleukin-23 (IL-23). IL-23 is mainly produced by Th17 cells that infiltrate in the tumor microenvironment and contributes to EMT via activation of the Wnt/β-catenin pathway in esophageal squamous carcinoma.[9] Eukaryotic initiation factor 5A2 (eIF5A2) was first isolated as an oncoprotein and was later found to be involved in EMT. Increased expression of eIF5A2 induced ESCC metastasis and angiogenesis via the hypoxia inducible factor-1 signaling pathway in esophageal squamous cell lines.[10] The clinical investigation revealed Snail overexpression in 40% of patients with SCC tissue samples, which was associated with vascular invasion, advanced clinical stage and the EMT phenotype.[11]

Gastric Cancer

Distinct carcinogenetic pathways have been reported for intestinal and diffuse type gastric carcinoma, but EMT has been mainly discussed for the latter phenotype.[12] The link between EMT and gastric adenocarcinoma could be partly because of the *H. pylori* cytotoxin-associated gene A (CagA) oncoprotein, which is responsible for the "hummingbird" phenotype *in vitro*, which mimics EMT.[13] CagA overexpression in gastric cancer (GC) cells up-regulated the expression of mesenchymal markers and CD44, which is a cancer stem cell marker in GC.[14] CagA overexpressing cancer cells also showed high tumorigenic ability *in vivo*. Immunohistochemical analysis of samples from individuals with *H. pylori* infection confirmed high CD44 expression and expression of different mesenchymal markers.[15] Tissue microarray analysis of samples from 385 GC patients revealed three miRNAs (miR-200c, miR-200b and miR-125b) to be significantly associated with survival. Functional experiments in a mouse model demonstrated that miR-200b suppressed ZEB1 and E-cadherin and inhibited cell migration and tumor growth.[16] *In vitro* analysis revealed that overexpression of miR-200b also down-regulated ZEB2 expression, which in turn significantly reduced cellular proliferation, migration and invasion in GC cells.[17] miR-7, which is down-regulated in highly metastatic GC cell lines, was found to be involved in metastasis by regulating its direct target, insulin-like growth factor-1 receptor. Overexpression of miR-7 was able to suppress Snail expression, increase E-cadherin expression and partially reverse EMT.[18] Several other EMT inducers have been reported recently. For example, erythropoietin-producing hepatocellular (Eph) A2 overexpression resulted in up-regulation of the EMT markers N-cadherin and Snail, and the Wnt/β-catenin targets TCF4, Cyclin-D1 and c-Myc. In contrast, Eph A2 silence by short hairpin RNA had the opposite effect.[19] SALL4, a zinc-finger transcriptional factor for embryonic stem cell's self-renewal and pluripotency, has been suggested to be involved in tumorigenesis. SALL4 overexpression induced EMT with increased expression of Twist1 and N-cadherin, and decreased expression of E-cadherin.[20] Telomerase activation through induction of human telomerase reverse transcriptase (hTERT) induced malignant transformation by stabilizing telomeres. hTERT overexpression could promote EMT and stemness of GC cells. TGF-β1 and β-catenin-mediated EMT was abolished by depletion of hTERT.[21] In the gastric epithelium, the runt domain transcription factor RUNX3 functions as a key mediator of the TGF-β pathway. Loss of RUNX3 in gastric epithelial cells results in EMT and production of tumorigenic stem cell-like subpopulation expressing gastric stem cell marker Lgr5. Loss of both RUNX3 and *p53* caused gastric epithelial cells to be sensitized to TGF-β-induced EMT, during which the resultant induction of Lgr5 is enhanced by aberrantly activated Wnt pathway.[22]

Colorectal Cancer

EMT is critical in transdifferentiation of polarized epithelial cells to an invasive mesenchymal phenotype. The function of EMT transcription factors in colorectal cancer (CRC) has been reported. Snail, an activator of EMT, was expressed at high levels in CRC colonospheres. Overexpression of Snail in CRC cells induced colonosphere-forming property and cell dedifferentiation. Blocking IL-8 expression or activity disrupted the Snail-induced stem cell-like features of colonospheres.[23] Snail directly induced zinc finger protein 281 (ZNF281) transcription and repressed miR-34a/b/c, thereby protection of ZNF281 mRNA from direct down-regulation by miR-34. Furthermore, *p53* activation resulted in miR-34a-dependent repression of ZNF81.[24] Syngeneic Twist1-positive colon carcinoma cells (CT26) that invaded tissues surrounding tumors demonstrated the mesenchymal phenotype.[25] Genotype also affected the mechanism of EMT. TGF-β1 induced changes in cell morphology, gene expression, motility and invasion consistent with EMT in microsatellite stable colon cancer cells, whereas cells exhibited

IL-6-dependent activation of signal transducer and activator of transcription 3 (STAT3), a conserved and direct target of miR-34a.[26] Stimulation of EMT results in the nuclear translocation of pyruvate kinase M2 (PKM2) in colon cancer cells. EMT stimulation causes direct interaction of PKM2 in the nucleus with TGF-β-induced factor homeobox 2, a transcriptional cofactor repressor of TGF-β signaling.[27] The roles of miRNA in EMT in CRC have been reported. For example, liver metastatic tissues showed higher expression of miR-200c than that of the primary tumor, and miR-200c overexpression was significantly associated with hypomethylation of the miR-200c promoter.[28] Overexpression of miR-212 inhibited CRC cell migration and invasion *in vitro* and intrahepatic and pulmonary metastasis *in vivo*. Manganese SOD (MnSOD) was identified as a direct target of miR-212, and an inverse correlation has been observed between the level of miR-212 and MnSOD protein in colorectal tumor samples. MnSOD was required for down-regulation of epithelial markers and up-regulation of mesenchymal markers in CRC cells.[29]

Hepatocellular Carcinoma

TGF-β is a major microenvironmental factor to affect hepatocellular carcinoma (HCC) dedifferentiation, inducing EMT and acquisition of metastatic phenotypes. Transcriptomic analysis on human HCC tissue samples revealed that TGF-β signaling was activated in a subpopulation of HCC, called Wnt-TGF-β subclass.[30,31] Sequential transcriptome analysis suggested that TGF-β signaling was a late event accompanied with extensive gene alterations.[32] TGF-β has been shown to induce hepatocyte nuclear factor-4α (HNF-4α) post-translational modifications that correlate with the early loss of the ability of HNF-4α to bind to target gene promoters via glycogen synthase kinase-3β (GSK-3β) kinase during EMT.[33] The receptor tyrosine kinase Axl binds to 14-3-3ζ as a result of phosphorylation of the linker region of SMAD3 at Ser213, which causes the up-regulation of TGF-β target genes such as PAI1, MMP9 and Snail.[34] The function of EMT transcription factors have been updated recently. Accumulative data on non-coding RNA have revealed a novel mechanism of EMT in HCC. For example, miR-200c was down-regulated in HCC with bile duct tumor thrombus, which occurred in 30 out of 1,240 patients, and regulated ZEB1 expression as well as an invasive phenotype.[35] The miR216a/217 cluster induced EMT and its direct targets, phosphatase and tensin homolog and SMAD7 were identified.[36] miR-331-3p-mediated inhibition of PH domain and leucine-rich repeat protein phosphatase resulted in stimulation of protein kinase B (AKT) and subsequent EMT.[37] miR-424-5p reversed resistance to anoikis, blocked EMT progression and inhibited its direct target ICAT/CTNNBIP1, a novel β-catenin-interacting protein.[38] A non-coding antisense transcript, ZEB1-antisense1 (ZEB1-AS1), promoted EMT and metastasis in HCC. The zeb1-as1 promoter was hypomethylated in human HCC samples and resulted in tumor specific up-regulation of ZEB1-AS1.[39] lncRNA-AL589182.3 (ENST00000493038), which can be activated by TGF-β, up-regulated ZEB1 and ZEB2 through competitively binding to the miR-200 family and induced tumor cell EMT and invasion.[40] Interestingly, hepatitis C virus (HCV) has also been found to contribute to EMT. HCV core protein down-regulated secreted frizzled-related protein 1 (SFRP1) expression by inducing hypermethylation of the SFRP1 promoter.[41] A previous transgenic mouse study demonstrated that overexpression of HCV core protein in HCC cells increased active TGF-β levels in culture supernatants and induced SMAD2/3 phosphorylation. HCC cells expressing HCV core protein could activate stellate cells in co-culture and this activation was TGF-β-dependent.[42] CD44s, a known cancer stem cell marker in many malignancies, mediated TGF-β-induced EMT, and regulated mesenchymal phenotype in HCC.[43,44]

Cholangiocarcinoma

Since this disease is not common, clinical and basic research on human cholangiocarcinoma (CCA) samples is limited. CCA is one of the solid cancers that have no effective molecular targeted therapy to date. Gemcitabine plus platinum is the only chemotherapeutic drug that to some extent inhibits CCA progression.[45] Several EMT-related molecules are also known to play pivotal roles in CCA. Inactivation of miR-200c is reported to induce the expression of mesenchymal markers and NCAM1, a known hepatic stem/progenitor cell marker.[46] STAT3-driven expression of small proline-rich protein 2a suppressed the interaction of miR-200c/141 with ZEB1.[47] Although the efficacy of the EGFR tyrosine kinase inhibitors, erlotinib and cetuximab, has not been confirmed in CCA treatment,[48] activation of the EGF-EGFR axis is known to abolish gefitinib-mediated EMT progression.[49] ANXA8 was found to be involved in EGF-forkhead box protein O signaling-mediated EMT progression.[50] The sonic hedgehog ligand is highly expressed in human CCA, and treatment with the hedgehog inhibitors, cyclopamine and 5E1, suppressed cell proliferation, migration and invasion by down-regulating the target genes hepatoblastoma 1 and 2. Furthermore, these inhibitors have been shown to attenuate EMT.[51] In addition to the above-mentioned molecules, some unique molecules have also been linked to EMT recently in CCA, which include 4 histamines (H1-H4) and their receptor (HR). Loss of H3HR expression or overexpression of H4HR has been shown to significantly decrease CCA proliferation and disrupt EMT progression.[52]

Pancreatic Cancer

Pancreatic cancer is one of the worst solid cancers in terms of prognosis and treatment outcome, because there is no promising molecular target identified to

date. EMT was first reported in this malignancy two decades ago, and the major functional interactions of the EMT-transcription factors have also been reported. The genomic landscape of pancreatic cancer has been partially unveiled.[53] However, the role of each key molecule involved in EMT remains to be elucidated, an effective therapeutic molecular target is yet to be identified for pancreatic cancer. The epigenetic analysis revealed that the Class I histone deacetylase inhibitor mocetinostat suppresses ZEB1 and induces miR-203 re-expression, thus, leading to the repression of stemness properties and drug resistance.[54] TGF-β1 was highly up-regulated in pancreatic cancer.[55] TGF-β1 has been shown to induce EMT, SMAD2/3 phosphorylation, restoration of retinoblastoma 1 expression and SMAD-dependent up-regulation of Wnt7b in KRC cell line. In *in vivo* orthotopic models, inhibition of TGF-β1 signaling suppressed those effects, resulting in tumor regression and decrease in metastasis.[56] The calcium-/calcineurin-responsive nuclear factor of activated T cells, a transcription factor expressed during inflammation, drives EMT in a sex determining region-box 2-dependent manner and loss of *p53* induced EMT, and acquisition of cancer stem cell-like properties by down-regulating miR-200c.[57] Ataxia telangiectasia Group D complementing gene, which is highly expressed in pancreatic cancer,[58] up-regulated CD44 in mouse and human PanIN lesions via activation of β-catenin signaling. This in turn results in the induction of EMT phenotype and expression of ZEB1 and Snail1.[59]

Perspectives

Increasing evidence supports the role of EMT in cancer progression, metastasis and drug resistance. Recent studies of EMT transcription factors and microRNAs are shown in Tables 1 and 2 respectively. In a tumorigenic mouse model, it was shown that EMT precedes pancreatic tumor formation.[60] However, whether EMT occurs in the early stage or late stage of tumor formation remains to be confirmed. The mesenchymal phenotype is essential for tumor cell migration and invasion. The epithelial phenotype might be required for cancer cells to spread to other organs. Cancer cells tend to acquire both phenotypes under specific conditions, and the functional aspect of each phenotype regarding chemoresistance remains elusive.[61] EMT has been categorized into three types: developmental (Type I), fibrosis and wound healing (Type II), and cancer (Type III). Of these, Type III EMT is the least well understood.[62] If Type III EMT can be classified further into subgroups based on the molecular mechanisms, it would be possible to develop personalized cancer therapeutic approaches based on the specific EMT stage.

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Table 1: EMT TFs in gastroenterological malignancies

TFs	EC	GC	CRC	HCC	PDCA	CCA
ZEB1/2		[16,17]	[63]	[64]	[54]	
Twist1			[25]	[65]		
Snail	[11]		[23,24,66]	[65]		
SHP-1				[67]		
SMAD3/4	[5]				[56]	
FoxC1				[68]		
FoxC2		[69]				
FoxM1					[70,71]	
FoxQ1				[72]		
NFATc1					[57]	

EC: Esophageal carcinoma; GC: Gastric cancer; CRC: Colorectal cancer; HCC: Hepatocellular carcinoma; PDCA: Pancreatic ductal adenocarcinoma; CCA: Cholangiocarcinoma; SMAD: Mothers against decapentaplegic homolog; ZEB1/2: Zinc finger E-box binding 1/2; TFs: Transcription factors; EMT: Epithelial-mesenchymal transition; NFATc1: Nuclear factor of activated T cells; SHP-1: Small heterodimer partner-1

Table 2: MicroRNA in gastroenterological malignancies

MicroRNA	Targets	EC	GC	CRC	HCC	PDCA	CCA
miR7	IGF1R		[18]				
miR9	CDH1	[73]					
miR34a/b/c	ZNF281			[24]			
miR125b	SMAD2/4						
miR130b	IRF1				[74]		
miR146a	Numb			[66]			
miR148a	Snail				[75]		
miR200b/c	ZEB1, NCAM1		[16,17]	[28]	[35]	[76]	[46]
miR212	MnSOD			[29]			
miR216a/217	PTEN/ SMAD7				[36]		
miR223	Fbw7					[77]	
miR331-3p	PHLPP				[37]		
miR338-3p	ZEB2		[78]				
miR424-5p	ICAT/ CTNNBIP1				[38]		
miR655	ZEB1	[79]					
lncRNA	ZEB1/2				[40]		

EC: Esophageal carcinoma; GC: Gastric cancer; CRC: Colorectal cancer; HCC: Hepatocellular carcinoma; PDCA: Pancreatic ductal adenocarcinoma; CCA: Cholangiocarcinoma; PHLPP: PH domain and leucine-rich repeat protein phosphatase; SMAD: Mothers against decapentaplegic homolog; PTEN: Phosphatase and tensin homolog; IGF1R: Insulin-like growth factor-1 receptor; ZNF281: Zinc finger protein 281; IRF1: Interferon regulatory factor 1; ZEB: Zinc finger E-box binding; NCAM1: Neural cell adhesion molecule; MnSOD: Manganese superoxide dismutase

Conflicts of interest

There are no conflicts of interest.

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