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TOPIC HIGHLIGHT

2016 Gastric Cancer: Global view

Emerging molecular basis of hematogenous metastasis in gastric cancer

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Abstract

Lymphatic metastasis is commonly observed in gastric cancer (GC), but hematogenous metastasis is more likely responsible for the cancer-related mortality. Since Stephen Paget first introduced the "seed and soil hypothesis" a century ago, growing evidence recognizes that numerous essential secreted factors and signaling pathway effectors participate in the pre-metastatic niche formation and distant organ metastasis. The cross-talk between GC cells and surrounding microenvironment may consist of a series of interrelated steps, including epithelial mesenchymal transition, intravasation into blood vessels, circulating tumor cell translocation, and secondary organ metastasis. Secreted factors including vascular endothelial growth factor (VEGF), matrix metalloproteinases and cancer-derived extracellular vesicles, especially exosomes, are essential in formation of premetastatic niche. Circulating tumor cells and microRNAs represent as "metastatic intermediates" between primary tumors and sites of dissemination. Many biomarkers have been identified as novel metastatic markers and prognostic effectors. In addition, molecular therapy has been designed to target biomarkers such as growth factors (human epidermal growth factor receptor 2, VEGF) and chemokines, although they have not clearly proven to be effective in inhibiting GC metastasis in clinical trials. In this review, we will systematically discuss the emerging molecules and their microenvironment in hematogenous metastasis of GC, which may help us to find new therapeutic strategies in the future.

Key words: Gastric cancer; Metastasis; Molecular mechanism

Core tip: The premetastatic niche is a novel predictor of cancer metastasis. The following steps including local invasion, intravasation into vessel lumen, survival in the circulation and extravasation also contribute to gastric cancer progression through a variety of mechanisms.

This review provides an overview of the complex interaction between tumors and their microenvironment in hematogenous metastasis cascade.

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INTRODUCTION

Gastric cancer (GC) is the second leading cause of cancer-related death in the world[1]. Many GC patients are diagnosed at advanced stage with metastasis, and miss the possibility for curative resection. It has been documented that patients with advanced stage GC have a poor prognosis with a five-year survival rate less than 15%[2]. Thus, metastasis is demonstrated to be an essential event in the prognosis of GC. Successful hematogenous metastasis cascade depends on intrinsic factors of the tumor cells and their subsequent communication with the surrounding microenvironment[3]. During metastatic progression, tumor cells possess continuous inter-dependent strategies[4]. First, tumor cells form a microenvironment, escape from primary site through surrounding extracellular matrix (ECM) and intravasate into the lumina of blood vessels. Translocation system is then formed and circulating tumor cells (CTCs) survive in the circulation and arrest at the secondary organs. Subsequently, tumor cells survive with the microenvironment of distant tissues, thereby micrometastasis and metastatic colonization emerge. In this review, we will discuss the emerging molecular mechanism that regulates hematogenous metastasis in GC.

TUMOR MICROENVIRONMENT FORMATION AS A "PRE-METASTATIC NICHE"

Stephen Paget has introduced the "seed and soil hypothesis" for a century, but emerging evidence recognizes numerous essential secreted factors and cancer-derived extracellular vesicles (EVs) as potential new effectors in pre-metastatic niche formation and metastasis.

Secreted factors

Multiple chemokines and growth factors secreted by cancer cells and their associated stromal cells are involved in recruitment of bone marrow-derived hematopoietic progenitor cells (HPCs) to support the new vessel formation and tumor metastatic microenvironment[5]. Vascular endothelial growth factor (VEGF) is initially identified as an endothelial cell-specific mitogen, which can embed endothelial cells (ECs), stimulate the formation of new blood vessels and induce angiogenesis[6]. VEGF-A, VEGF-B and placental growth factor (PIGF) binding to VEGFR-1 are considered to be key regulators of blood vessel growth, while VEGF-C and VEGF-D binding to VEGFR-2 regulate lymphatic angiogenesis[7]. High expression of VEGF was observed in patients with GC and significantly linked to the microvessel density (MVD) and the presence of vascular invasion[8,9]. Furthermore, VEGF correlated with tumor size, poor TNM stage and overall survival and appears to be a significant prognostic factor for hematogenous metastasis of GC[10]. However, a phase Ⅲ randomized trial has demonstrated that anti-VEGF-A monoclonal antibody, bevacizumab, failed to improve survival in advanced GC patients[11]. The expression of other angiogenic factors such as platelet-derived growth factor (PDGF-B) was seen with a higher MVD score in 50% of diffuse-type gastric adenocarcinoma cases[12]. Examination of human tissues from relapsed GC patients demonstrated higher VEGFR-1 (FLT-1) expression[13]. Circulating VEGFR-1$^+$ HPCs and VEGFR-2$^+$ ECs may precede the arrival of tumor cells, then VEGFR1$^+$ cells could maintain the expression of primitive cell surface markers, including CD34, CD11b, c-kit, and Sca-1 in the pre-metastatic niche[14]. In a smaller phase Ⅰ study, there was no significant difference in median overall survival time between the HLA-A*2402-positive and -negative groups that were vaccinated with URLC10 and VEGFR1 peptides[15]. Recently, the phase Ⅲ trial REGARD[16] has demonstrated that ramucirumab, a fully human IgG1 anti-VEGFR-2 monoclonal antibody, significantly improved median overall survival compared to the placebo group, suggesting VEGFR-2 signaling inhibition as an important therapeutic modality in advanced GC.

Fibroblasts and matrix metalloproteinases (MMPs) probably also contribute to pre-metastatic niche formation by secretion of pro-angiogenic and ECM-remodeling factors[17]. The interaction of VLA-4 (integrin $\alpha4\beta1$) with its ligand, fibronectin, is essential for tumor cell migration. VCAM-1, the counter receptor of VLA-4, is significantly increased in serum of GC patients. Concentration of soluble VCAM-1 is positively associated with invasion depth and the presence of distant metastases[18]. MMPs, zinc-dependent endopeptidases capable of degrading ECM proteins, can drive the loss of the basement membrane. The activation of MMPs and urokinase-type plasminogen activator (uPA) is also required for expression of transcription factor Snail-1 which finally inhibits E-cadherin[19]. Recent studies have confirmed that elevated expression of MMP-2, MMP-7, MMP-9 and MT1-MMP in gastrointestinal cancers increases their ability to metastasize[9,20-22]. In particular, MT-MMP immunolocalized in 61% of GC cases is implicated in vascular invasion of the tumor

cells through the activation of proMMP-2[23]. Following exposure to *Helicobacter pylori* (*H. pylori*) strain 60190, the expression of MMP-7 is upregulated, which is dependent on gastrin[24]. Meta-analyses showed that overexpression of MMPs (such as MMP-2 and MMP-9) is a poor prognostic factor of GC[25,26]. Moreover, human epidermal growth factor receptor 2 (HER2) knockdown resulted in the downregulation of the expression of MMP-9, which abrogates the invasion promoted by HER2 signaling in GC[27]. Clinical trials on MMP inhibitors (MMPIs) in advanced stage cancer patients have yielded inconsistent outcomes[28], however, marimastat as a broad-spectrum MMPI was illustrated to increase overall survival in patients with advanced GC in a phase Ⅲ randomized trial[29].

Despite that, the release of other soluble secreted factors including lysyl oxidase, FGF, SDF-1[30], tumor necrosis factor (TNF)-α[31] and transforming growth factor (TGF)-β have been shown to play critical roles in the formation of the pre-metastatic niche.

Cancer-derived EVs

Recent research advances provide provocative insights into EVs, which have implications regarding the steps of the tumor pre-metastatic niche formation. According to their origin, EVs can be organized into several categories, including exosomes, tumor-derived microvesicles (TMVs), and large oncosomes. We will focus on the mechanisms that exosomes mediate metastasis by conditioning both the bone marrow and the premetastatic niche.

Exosomes are small, membrane-bound vesicles (40-100 nm) that transfer RNAs and proteins from one cell (site of origin) to distant locations. Several studies have indicated that hypoxia promotes the secretion of exosomes with enhanced angiogenic and metastatic potential in different tumor types[32]. The release mechanisms of exosomes involve the Rab family of small GTPases in vesicle trafficking and micro-vesicle budding pathway[33]. As expected, exosomes derived from tumor cells promote metastasis by conditioning both the bone marrow and the pre-metastatic niche[34]. Peinado *et al*[17] proposed a model for tumor-derived exosomes as a systemic factor increasing metastatic behavior through educating bone marrow derived cells. Recently, it has been suggested that GC-derived exosomes could promote human umbilical cord-derived MSCs migration[35]. After injecting fluorescently labeled exosomes from metastatic melanoma cells into mice, exosomes were observed to exit the circulation to localize in metastatic sites: the lung, bone marrow, liver and spleen[36]. However, little is known about whether and how GC-derived exosomes could promote distant organ metastasis. Interestingly, microRNAs (miRNAs) detected in human serum and saliva are mostly concentrated in exosomes[37]. Recent evidence has found that AZ-P7a, a metastatic GC cell line, released let-7 miRNAs *via* exosomes into the extracellular environment to maintain the oncogenesis[38]. The enrichment of let-7 miRNA family in the exosomes from AZ-P7a cells may reflect metastasis in GC. Later on, Melo *et al*[39] revealed that miRNA biogenesis in exosomes inhibited the expression of their respective mRNA targets, such as phosphatase and tensin homolog (PTEN) and the transcription factor homeobox D10 (HOXD10), indicating the contribution to breast cancer progression.

Cancer stem cells or cancer initiating cells

Accumulating evidence suggested that tumor cell subpopulation in the primary tumor mass termed Cancer stem cells (CSCs) or cancer initiating cells (CICs) has the potential to successfully form metastasis in a distant organ[40]. Since Takaishi *et al*[41] identified CD44 as a gastric CSC (GCSC) marker, isolation and culture of GCSCs have become a novel model for GC research. Interestingly, chronic *H. pylori* infection leads to an expansion of the compartment of gastric epithelial stem cells. It has been confirmed that CD44+ cells induced by *H. pylori*/CagA exhibited the hummingbird phenotype and stimulated the expression of mesenchymal markers[42]. Initial evidence identified the high expression of CD44 and CD133 in precancerous lesions, malignantly transforming tissues and drug-resistant GC tissues[43]. Some other molecules including TR3, Musashi-1, ALDH and Nanog have been proposed as novel metastatic markers on GCSCs, bringing great opportunities for therapy of GC patients[41].

LOCAL INVASION AND INTRAVASATION

After a pre-metastatic niche is established, metastatic carcinoma cells invade locally through surrounding ECM, which is termed as "local invasion". Subsequently, tumor cells escape from the primary site, and disseminate *via* the hematogenous circulation into the vessel lumen, which is termed as "intravasation". In the process of epithelial mesenchymal transition (EMT), tumor cells lose their epithelial cell feature and cell polarity, which makes cell permeability increase. Then the loss of polarity and alteration of the actin cytoskeleton disrupt the cell-cell adhesion and cell-ECM adhesion, mediating the concomitant formation of membrane protrusions required for invasive growth[44].

E-cadherin encoded by the *CDH1* gene is essential in the activation of transcriptional regulators which disassemble intercellular junctions. A recent meta-analysis showed that[45] downregulation of E-cadherin is frequently observed in patients with diffuse type GC. The downregulation of E-cadherin results in the loss of connection of the E-cadherin-dependent cytoplasmic cell-adhesion complex and dysregulation of cellular signaling pathways including Wnt signaling, Rho GTPases, and epidermal growth factor receptor (EGFR)[46]. β-catenin acting as a transcription cofactor with T cell factor/lymphoid enhancer factor (TCF/LEF)

in Wnt signaling pathway is also down-regulated in 47% of GC cases[47]. Importantly, abnormal expression of E-cadherin and β-catenin was correlated with advanced tumor stage and lower 5-year survival rate of GC patients[45,48]. Evidence suggests that Rac1, RhoA and RhoC were overexpressed in metastases GC tissues and related to higher TNM stage[49,50]. Moreover, siRNA mediated RhoC knockdown inhibits the migration and invasion of GC cells[50]. Recent findings demonstrate that RhoA mutations induced by E-cadherin are quite common in diffuse GC[51,52].

Another signaling pathway involved in EMT is TGF-β, which enhances tumor invasion and metastasis by stimulating angiogenesis and cell motility[53]. During TGF-β-induced EMT, not only the production of potent angiogenic inhibitor such as thrombospondin 1 is induced, but also transmembrane claudins, occludins and scaffold proteins are down-regulated, leading to the degradation of tight junctions[54]. Inactivation of TGF-β signaling components including TGF-β receptors, Smad2 and Smad4 consists in almost all types of GC[53]. Several studies have showed the immunoreactivity for TGF-β in gastric carcinomas, with positive rates ranging from 22.8% to 73.8%[55]. Decreased SMAD4 expression and increased SMAD7 expression have been found in GC, ensuring the inactivation of TGF-β-R1 and TGF-β-R2. Treatment with recombinant TGF-β1 or cagE-positive *H. pylori* significantly altered EMT-related marker and enhanced the ability of cancer cells to migrate[56]. Moreover, TGF-β is also known to regulate invasion and metastasis of GC cells *via* ERK, MAPK, BMP, and JNK signal pathways[57]. Significant upregulation of serum concentrations of TGF-β1 in GC patients is correlated with tumor mass and metastasis[58]. In summary, these studies suggest that activation of TGF-β signaling can regulate its associated target genes, enhancing distant metastasis in GC.

SURVIVAL IN THE CIRCULATION AND EXTRAVASATION

After tumor cells intravasate into the circulation, they disseminate widely through the venous and arterial vessels and finally migrate to a limited subset of target organs. Evidence indicates that CTCs and miRNAs represent as "metastatic intermediates" between primary tumors and sites of dissemination in a variety of cancers[4].

CTCs

Since CTCs were first discovered in the nineteenth century, there have been numerous studies on the detection and isolation of CTCs in peripheral blood of patients with various carcinomas[59], including gastrointestinal cancers[60]. In spite of the low count of CTCs in peripheral blood, the advances of molecular and cytological techniques enable us to detect or characterize CTCs[59,61,62]. To date, several clinical studies have demonstrated that CTCs can be a potential prognostic marker or be used to monitor the recurrence of cancers[61,63]. Numerous studies increasingly attach our attention to CTCs because of their significant utilities in the diagnosis, therapy or prognosis of various cancer types, including GC.

CTCs, as the metastatic intermediate, connect primary tumors and metastatic tumors[4,64]. Although these complex processes of translocation of tumor cells have not been fully uncovered, tumor cell shedding from primary tumors and then invading into vessels are regarded as the initiation, in which EMT plays vital roles[65,66]. Once in the blood vessels, CTCs are confronted with several natural obstacles, such as shear forces, the attack of immune cells and anoikis, which result in only 0.01 percent of CTCs alive and impede the metastatic process[67,68]. The phenomenon that CTCs in the hematogenous circulation are arrested by vessels is mainly explained by two hypotheses. One is about physical trapping of CTCs, with large diameters of 20-30 μm, in which carcinoma cells could be arrested by capillaries with the luminal diameter of 8 μm around[4], whereas the other hypothesis is that CTCs favor the trapping of particular tissues through adhesive interactions[69].

A recent meta-analysis indicated that CTCs could be a predictor of the survival of GC patients and the patients with detectable CTCs had shortened recurrence-free survival[61]. It has been reported that the positive rate of CTCs was high in advanced GC patients and indicated a poor prognosis[70]. A study also showed the tumorigenicity of CTCs from advanced GC patients[63].

miRNAs

MiRNAs play critical mechanistic roles in angiogenesis and cancer metastasis. The levels of several individual miRNAs are dysregulated in GC. A study identified that diffuse histologic type, tumor invasion and progressive stage were associated with low expression of miR-200b in patients with GC[71]. miR-200b suppresses expression of ZEB2 EMT-inducing transcription factors and enhances expression of E-cadherin[71]. We have reported that miR-141 and miR-375 were significantly downregulated in GC[73,74]. Snail-regulated miR-375 inhibited the migration and invasion of GC cells partially by targeting JAK2 oncogene[74]. Liu *et al*[75] reported that miR-10b promoted invasion of gastric cells by activating RhoC-AKT signaling through targeting HOXD10. In addition, miRNA signaling is converged with several crucial molecular signaling pathways. miR-21 up-regulated in GC tissues has been shown to target RECK[76], PTEN[77] and PDCD4[78] tumor suppressor genes which inhibit tumor metastasis and angiogenesis *via* modulating MMPs and PI3K/Akt pathway.

Table 1 Biomarkers identified as mediators of metastasis in gastric cancer

Biomarkers	Target gene(s)	Ref.
Growth factors		
VEGF	*CD34, CD11b, c-kit,* and *Sca-1*	[14]
PDGF-B		[13]
EGF	*EGFR*	[46]
Chemokines		
SDF-1	*CXCR4*	[30]
TNF-α	*TNFR I/II*	[31]
TGF-β	*TSP1, SMAD4*	[55,56]
EVs		
Exosomes	*HOXD10* and *PTEN*	[39]
ECM Proteases		
E-cadherin		[45]
Rho GTPases: Rac1, RhoA and RhoC		[49,50]
MMP-2, MMP-7, MMP-9 and MT1-MMP		[20-22]
VLA-4	*VCAM-1*	[18]
MicroRNAs		
miR-200b	*ZEB1* and *ZEB2*	[71]
miR-141	*ZEB1*	[72]
miR-375	*JAK-2*	[73,74]
miR-10b	*HOXD10*	[75]
miR-21	*RECK, PTEN* and *PDCD4*	[76-78]

SDF: Stromal derived factor; TNF: Tumor necrosis factor; TGF: Transforming growth factor; VLA: Very late antigen; PDGF-B: Platelet-derived growth factor; EGF: Epidermal growth factor; VEGF: Vascular endothelial growth factor; EVs: Extracellular vesicles.

CONCLUSION

During the past decades, studies have indicated numerous molecular biomarkers involved in progression of the GC metastatic cascade (Table 1). There are complex processes during hematogenous metastasis in GC. In early stage, tumor cells arrive in distant sites termed as "premetastatic niche". Secreted factors and cancer-derived EVs, especially exosomes, are essential in formation of premetastatic niche. Then these cells arrest in organs, adhere to vessel wall and invade locally through surrounding ECM. During metastatic progression, CTCs and miRNAs represent as metastatic mediators between primary tumors and sites of dissemination. We shall dwell deeper into the mechanisms of GC stem cell self-expansion, as well as crosstalk between GC-derived EVs and CTCs with surrounding microenvironment. Identification of potential molecular targets of GC metastasis will be of great importance for cancer therapy, which is a challenging issue in ameliorating the morbidity and mortality burden of GC.

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