

Cancer-Stromal Cell Interaction and Tumor Angiogenesis in Gastric Cancer

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Abstract Recent studies in molecular and cellular biology have shown that tumor growth and metastasis are not determined by cancer cells alone but also by a variety of stromal cells. The stroma constitutes a large part of most solid tumors, and cancer-stromal cell interaction contributes functionally to tumor growth and metastasis. Angiogenesis is the result of an imbalance between positive and negative angiogenic factors released by tumor and host cells into the microenvironment of the neoplastic tissue. In gastric cancer, tumor cells and stromal cells produce various angiogenic factors, including vascular endothelial growth factor, interleukin-8, and platelet-derived endothelial cell growth factor. The microenvironment in the gastric mucosa may also influence the angiogenic phenotype of gastric cancer. *Helicobacter pylori* infection increases expression of several angiogenic factors by tumor cells. Activated fibroblasts and macrophages in tumor stroma also play an important role in angiogenesis and tumor progression. We review the current understanding of cancer-stromal cell interaction as it pertains to tumor angiogenesis in gastric cancer.

Keywords Gastric cancer · Angiogenesis · Tumor-stromal cell interaction · Carcinoma-associated fibroblast (CAF) · Tumor-associated fibroblast (TAM) · Mesenchymal stem cell (MSC)

Abbreviations

CAF Carcinoma-associated fibroblast
TAM Tumor-associated fibroblast

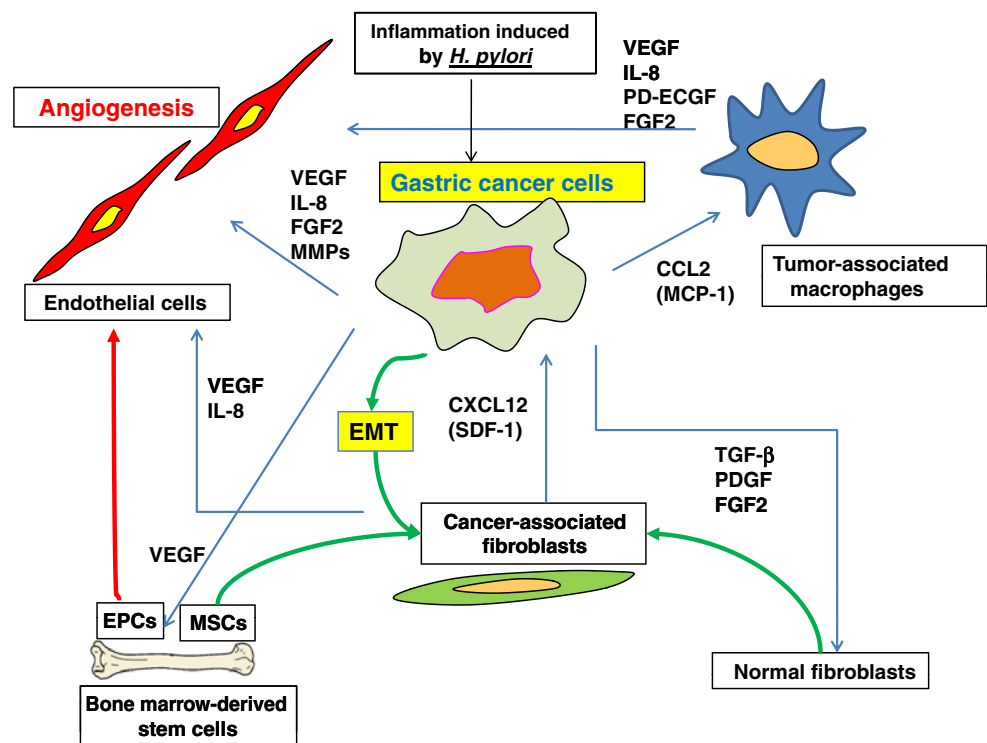
MSC	Mesenchymal stem cell
VEGF	Vascular endothelial growth factor
IL	Interleukin
FGF	Fibroblast growth factor
PD-ECGF	Platelet-derived endothelial cell growth factor
MVD	Microvessel density
<i>H. pylori</i>	<i>Helicobacter pylori</i>
COX	Cyclooxygenase
CagA	Cytotoxin-associated gene A
NF-κB	Nuclear factor-κB
MMP	Matrix metalloproteinase
MCP	Monocyte chemoattractant protein
TGF	Transforming growth factor
FAP	Fibroblast activation protein
EMT	Epithelial-to-mesenchymal transition

Introduction

Cancer tissue consists of both tumor cells and stromal cells, all of which are surrounded by extracellular matrix. Tumor growth is determined not only by tumor cells themselves but also by stromal cells. Recent studies have shown that interactions between tumor and stromal cells create a unique microenvironment that is essential for tumor growth and metastasis [1, 2]. Tumor stroma contains several types of cells including activated fibroblasts (myofibroblasts), endothelial cells, and inflammatory cells including macrophages (Fig. 1). It has become clear that activated fibroblasts in cancer stroma are prominent modifiers of tumor progression. As such, they are called carcinoma-associated fibroblasts (CAFs) [3]. Angiogenesis, which is necessary for tumor progression, is also influenced by the organ microenvironment. Stromal reaction (desmoplasia) is

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Fig. 1 Interaction between gastric cancer cells and stromal cells influences angiogenesis through various angiogenic factors and cytokines. *EMT* epithelial-to-mesenchymal transition; *MSCs* mesenchymal stem cells; *EPCs* endothelial progenitor cells



observed in gastrointestinal cancers but not in non-invasive neoplasms [4]. The generation of tumor stroma is triggered by tumor cells and induces the ingrowth of new blood vessels and mesenchymal cells from the adjacent normal tissue [5]. However, recent studies revealed that bone marrow-derived stem cells are integrated into tumor stroma and differentiate into myofibroblasts and vascular endothelial cells [6, 7].

Gastric cancer is one of the most frequent malignancies in the world [8]. Previous studies have indicated that a large number of genetic and epigenetic alterations in oncogenes and tumor suppressor genes as well as genetic instability determine the multi-step process of gastric carcinogenesis [8, 9]. In addition, the molecular events that characterize gastric cancer differ, depending on the histologic type, whether intestinal (well-differentiated) or diffuse (poorly differentiated) gastric cancer [9]. Gastric cancer cells express a wide array of growth factors, angiogenic factors, and cytokines that act via autocrine, paracrine, and juxtacrine mechanisms in the tumor microenvironment [8]. Herein, we discuss the importance of the organ-specific microenvironment and cancer-stromal cell interaction in the growth and angiogenesis of human gastric cancer.

Angiogenic Factors in Human Gastric Cancer

Weidner et al. [10] first reported a direct correlation between the incidence of metastasis and the number and density of blood vessels in invasive breast cancers. Similar

studies have confirmed this correlation in gastrointestinal cancers [11–14]. Induction of angiogenesis is mediated by various molecules released by both tumor and host cells [15, 16]. Several growth factors that regulate angiogenesis have been identified. Gastric cancer cells produce various angiogenic factors, including vascular endothelial growth factor (VEGF) [13], interleukin (IL)-8 [17], fibroblast growth factor (FGF)-2 [18], and platelet-derived endothelial cell growth factor (PD-ECGF) [19].

Among the various angiogenic factors, VEGF (now termed VEGF-A) is considered one of the strongest promoters of angiogenesis in gastrointestinal tumors [20]. VEGF-A is released by cancer cells, but fibroblasts and inflammatory cells in tumor stroma are also sources of host-derived VEGF-A [21]. VEGF-A, also known as vascular permeability factor, is a secreted protein that plays a pivotal role in hyperpermeability of the vessels in addition to angiogenesis [22]. Several studies have shown a correlation between VEGF-A expression and microvessel density (MVD) in human gastric cancer [13]. The prognosis of patients with VEGF-A-positive tumors is poorer than that of patients with VEGF-A-negative tumors [12, 14].

For gastric cancer, prognosis is dependent on both the histologic type and the disease stage [23]. The intestinal-type gastric cancer tends to metastasize to the liver by hematogenous dissemination. In contrast, the diffuse-type gastric cancer is more invasive; peritoneal dissemination is predominant. We have found that the angiogenic phenotype differs between intestinal-type and diffuse-type gastric cancers [13]. Intestinal-type gastric cancer is more depen-

dent on angiogenesis than is the diffuse-type. Intestinal-type but not diffuse-type tumors express high levels of VEGF-A, levels of that correlate significantly with vessel counts [13]. In contrast, FGF2 expression is higher in diffuse-type tumors, especially scirrhus-type cancer [18]. These findings suggest that VEGF-A promotes angiogenesis and progression of human gastric cancers, especially those of the intestinal type.

IL-8 is a multi-functional cytokine that can stimulate division of endothelial cells. IL-8 can induce migration of some tumor cells [24] and has been implicated in the induction of angiogenesis in such diverse diseases as psoriasis and rheumatoid arthritis and in some malignant diseases. IL-8 is a known angiogenic factor for human lung cancer [25, 26] and is also produced by melanomas [27] and bladder [28] and prostate [29] cancers. In human gastric cancer, most tumor tissues express IL-8 at levels higher than those in the corresponding normal mucosa [17]. The level of IL-8 mRNA in neoplasms correlates strongly with vascularization, suggesting that IL-8 in tumor tissue regulates neovascularization [17]. Furthermore, gastric cancer cells transfected with the IL-8 gene were shown to produce rapidly growing, highly vascular neoplasms at the orthotopic site (gastric wall) in nude mice [30].

PD-ECGF, an endothelial cell mitogen initially purified to homogeneity from human platelets, has chemotactic activity for endothelial cells in vitro and is angiogenic in vivo [31]. PD-ECGF was shown to be identical to thymidine phosphorylase, an enzyme involved in pyrimidine nucleoside metabolism [32]. PD-ECGF expression is elevated in several types of solid tumor including colon cancer [33–35]. PD-ECGF is expressed at high levels in vascular tumors that express low levels of VEGF-A [35]. In these colon cancers, the major source of PD-ECGF is the infiltrating macrophages. A positive association between PD-ECGF expression and MVD has also been reported for human gastric cancer [19, 36]. In human gastric cancer, PD-ECGF is expressed more frequently in infiltrating cells than in tumor epithelium [19]. There is an association between PD-ECGF expression by infiltrating cells, VEGF-A expression by tumor epithelium, and vessel counts in intestinal-type gastric cancer but not in diffuse-type gastric cancer [19].

Lymphangiogenic Factors in Human Gastric Cancer

VEGF-C and VEGF-D are ligands for VEGF receptor (VEGFR)-3 and VEGFR-2. VEGFR-3 is a tyrosine kinase receptor that is expressed predominantly in the endothelium of lymphatic vessels [37]. Alitalo et al. [38] described VEGF-C as a lymphangiogenic factor that can selectively induce hyperplasia of the lymphatic vasculature. Other growth factors are reported to be lymphangiogenic, such as

FGF-2 [39], platelet-derived growth factor (PDGF)-BB [40], and angiopoietin-2 [41]. VEGF-C/VEGFR-3 signaling is a key primary proliferation pathway for lymphatic vessels, whereas angiopoietin-2 is important in later remodeling stages [41]. Although the importance of FGF-2, PDGF-BB, and angiopoietin-2 for lymphatic metastasis of gastric cancer is unknown, a significant correlation between lymph node metastasis and VEGF-C expression has been reported in human gastric cancer [42, 43]. VEGF-C immunoreactivity was associated with lymphatic invasion, lymph node metastasis, and increased MVD, however, there was no association between VEGF-D immunoreactivity and clinicopathologic features in submucosally invasive gastric cancer [44]. These results suggest that VEGF-C is a dominant regulator of lymphangiogenesis in early-stage human gastric cancer.

Helicobacter pylori (*H. pylori*) Stimulates Angiogenesis in Gastric Cancer

The most significant advance in understanding the pathogenesis of gastric cancer is recognition of the role of *H. pylori* in gastric carcinogenesis. *H. pylori* infection is thought to contribute significantly to the pathogenesis of atrophic gastritis and intestinal metaplasia. Epidemiologic studies have indicated that infection with *H. pylori* is a risk factor for gastric cancer, and the WHO/IARC classified this bacterium as a definite biologic carcinogen in 1994 [45]. In addition, *H. pylori* inoculation into the stomach of Mongolian gerbils was shown to be associated with the occurrence of chronic gastritis, intestinal metaplasia, and adenocarcinoma [46, 47]. There are several virulence-associated *H. pylori* genes, including *cagA*, *vacA*, *iceA*, and *babA* [8]. The *cagA*-positive strains are associated with a higher grade of gastritis and higher risk of gastric cancer than are the *cagA*-negative strains [48]. The *cagA* gene is located within the *cag* pathogenicity island (PAI). The *cagPAI* encodes components of a type IV secretion system, by which the *cagA* gene product, CagA, is delivered into gastric epithelial cells. CagA binds Src homology 2 (SH2) domain-containing tyrosine phosphatase SHP-2 and activates its phosphatase activity. A recent study showed that *H. pylori* penetrates normal, metaplastic, and neoplastic epithelium to cause a strong immune-inflammatory response and promote gastric carcinogenesis [49].

In addition to its deregulation of SHP-2 by CagA, *H. pylori* is a potent activator of nuclear factor- κ B (NF- κ B) in gastric epithelial cells. Activation of NF- κ B by *H. pylori* infection induces a variety of cytokines, angiogenic factors, MMPs, and adhesion molecules [50, 51]. We reported previously that *H. pylori*-infected gastric cancer patients have greater tumor vascularity than that of gastric cancer

patients after *H. pylori* eradication [52], suggesting that *H. pylori* infection influences angiogenesis in gastric cancer. Some studies suggested that the *cagA*-positive strain of *H. pylori* plays an important role in tissue remodeling, angiogenesis, cancer invasion and metastasis [53–55]. Crabtree et al. [53] reported that *H. pylori* infection induces IL-8 production by gastric epithelium. We found that coculture of gastric cancer cells with *H. pylori* induces expression of mRNAs encoding IL-8, VEGF-A, angiogenin, urokinase-type plasminogen activator, and MMP-9 by gastric cancer cells [54]. Wu et al. [55] also reported that *H. pylori* influences expression of VEGF-A and MMP-9 and promotes gastric cell invasion via COX-2- and NF- κ B-mediated pathways. Cox-2 inhibition decreases expression of angiogenic factors and MMP activity [56].

Role of Tumor-Associated Macrophages (TAMs) in Angiogenesis

Macrophages belonging to the mononuclear phagocyte system exhibit functions in endocytosis and cytotoxicity and secrete more than 100 biologically relevant substances [57]. Macrophages recruited to tumor stroma are called TAMs. The role of TAMs in tumor progression is complicated and wide ranging. Although activated macrophages may have anti-tumor activity, tumor cells have been reported to escape the anti-tumor activity of TAMs [58]. Indeed, removal of macrophages by genetic mutation reduced tumor progression and metastasis [59]. TAMs are recruited from circulating monocytes into tissues in response to chemoattractants, and they interact with tumor cells to make cancer stroma. One important characteristic of macrophages is the potential for angiogenic activity. Activated macrophages produce various factors that induce angiogenesis in wound repair [60], in chronic inflammatory diseases such as rheumatoid arthritis [61] and psoriasis [62], and in atherosclerotic plaques [63]. We previously reported that macrophage infiltration into tumor tissue correlates significantly with tumor vascularity in human esophageal and gastric cancers [64, 65]. Ishigami et al. [66] also found a direct association between the degree of TAM infiltration and depth of tumor invasion, nodal status, and clinical stage in gastric cancer. Macrophage recruitment is mediated by a variety of chemoattractants, including monocyte chemoattractant protein-1 (MCP-1/CCL2), macrophage inflammatory protein 1 α (MIP-1 α /CCL3), regulated upon activation, normal T cell expressed and secreted (RANTES/CCL5). Of these CC chemokines, MCP-1 is one of the most potent [67]. We found that MCP-1 produced by tumor cells is associated significantly with macrophage infiltration and malignant behavior, such as angiogenesis, tumor invasion, and lymphatic infiltration [64, 65]. Trans-

fection of the MCP-1 gene into gastric cancer cells causes strong infiltration of macrophages into tumors and enhanced tumorigenicity and metastatic potential in a mouse orthotopic implantation model [68]. Because activated macrophages produce VEGF-A, IL-8, FGF2, and PD-ECGF, MCP-1 expressed by gastric cancer cells plays a role in angiogenesis via recruitment and activation of macrophages.

Cyclooxygenase 2 (COX-2) expressed by cancer and stromal cells has been shown to contribute to tumor angiogenesis [69]. The expression levels of COX-2, in both tumor and stromal cells, correlated well with VEGF levels and MVD in gastric cancer tissue [70]. The CD40 ligand (CD40L)/CD40 interaction has been shown to induce COX-2 expression in macrophages, fibroblasts, and endothelial cells [71, 72]. Furthermore, MCP-1 and CD40L were shown to have a synergistic effect on COX-2 expression and subsequent VEGF production by macrophages in gastric cancer [72].

CAFs Promotes Tumor Growth via Angiogenesis

It has been reported that normal fibroblasts inhibit progression of cancer. Overexpression of transforming growth factor (TGF)- β and hepatocyte growth factor in fibroblasts resulted in tumorigenic outgrowth of breast epithelium, which was inhibited by normal fibroblasts [73]. The inhibition of TGF- β signaling in fibroblasts resulted in prostate intraepithelial neoplasia, indicating that normal fibroblasts suppress carcinogenesis [74]. However, numerous studies provided evidence that CAFs indeed promote the growth of tumors [75, 76]. CAFs show gene expression profiles that are distinct from those of normal fibroblasts [77], and they acquire a modified phenotype, similar to fibroblasts associated with wound healing. Although the mechanisms that regulate activation of fibroblasts and their accumulation in tumors are not fully understood, PDGF, TGF- β , and FGF2 are known to be partly involved in this process [75, 76, 78]. Many human tumors secrete PDGF ligands and PDGF receptors are expressed by various stromal cell populations such as CAFs and pericytes [78–80]. Inhibition of paracrine PDGF signaling reduces stromal reaction and disrupt pericyte support, resulted in destabilizing tumor vasculature and inhibiting angiogenesis [81–83]. In mouse model of cervical cancer, inhibition of stromal PDGFR by imatinib suppressed the expression of FGF-2 (a proangiogenic factor) and FGF-7 (a growth factor for epithelial cell) by CAFs [82]. Recently, we found that PDGF receptor is highly expressed by CAFs, pericytes, and lymphatic endothelial cells in human gastric cancer and targeting PDGF receptor by imatinib inhibited tumor growth and metastasis in the orthotopic gastric cancer model (Sumida et al., unpublished data). Fibroblast activa-

tion protein (FAP) is expressed by activated fibroblasts during wound healing and within tumor stroma. An antibody to human FAP, sibtrotuzumab, is now under clinical study in patients with colorectal cancer and non-small cell lung cancer [84]. CAFs might serve as novel therapeutic targets in cancer.

Bone Marrow-derived Mesenchymal Stem Cells (MSCs) as a Source of CAFs

Although CAFs have been implicated in important aspects of solid tumor biology including tumor growth, angiogenesis, and metastasis, sources of CAFs have not been well defined [85]. There are some candidates for the origins of CAFs, such as fibroblasts residing in local tissues [76], periadventitial cells including pericytes and vascular smooth muscle cells [86], endothelial cells [87], or bone marrow-derived cells including various stem cells [6]. Worthley et al. [88] recently reported that bone marrow-derived cells can differentiate CAFs in human gastric cancer that developed in female recipients of male allogeneic stem cell transplantation. Major types of stem cells in the bone marrow are hematopoietic stem cells and MSCs. MSCs can be defined according to their ability to self-renew and differentiate into tissues of mesodermal origin, including bone, cartilage, and adipose and connective tissues [89]. MSCs were reported to migrate to sites of tissue injury, sites of inflammation, as well as to stroma in solid tumor, where they interact with tumor cells [90]. Several studies have implicated molecules such as CXCL12 (SDF-1)/CXCR4, CCL2 (MCP-1)/CCR2, and PDGF in the tumor-homing ability of MSCs [91–93]. In an *in vitro* experiment, MSCs exposed to tumor-conditioned medium over a prolonged period of time assumed a CAF-like myofibroblastic phenotype, which promotes tumor cell growth both *in vitro* and *in vivo* [94]. Banerjee et al. constructed an *in vitro* model for the tumor-stroma interaction by culturing MSCs exposed to tumor-conditioned medium (representing CAFs), tumor cells, and differentiated HL60/U937 cells as surrogates for TAM, and found that the presence of CAFs and TAMs promotes growth of tumor cells. Indeed, CAFs and TAMs actively participate in altering the growth activity and drug resistance of tumors *in vivo* [95, 96]. Guo et al. [97] recently constructed a gastric cancer mouse model (Gan mice) by simultaneous activation of prostaglandin E2 and Wnt signaling in the gastric mucosa. MVD increased significantly, and the expression of VEGF-A was predominantly induced in the stromal cells of gastric tumors in the Gan mice. Moreover, they showed by bone marrow transplantation experiments that a subset of gastric myofibroblasts is derived from bone marrow. We examined the

role of MSCs in the tumor microenvironment using orthotopic nude mice models of gastric and colon cancers. Systemically administered MSCs possessed the ability to migrate to the orthotopic tumor site, where they differentiate into CAFs. Tumor cells mixed with MSCs and implanted orthotopically resulted in a greater tumor volume and lower survival rate than did implantation of tumor cells alone (Shinagawa et al., unpublished data).

Epithelial-to-Mesenchymal Transition (EMT) as a Source of CAFs

EMT is conversion of epithelial cells to migratory fibroblastoid cells [98]. Cancer cells undergoing EMT lose epithelial polarity, acquire a spindle-shaped morphology, and develop invasive and migratory ability. In various cancers, EMT is associated with poor histologic differentiation and tumor progression [99, 100]. Several authors have described the expression patterns of EMT-related genes such as E-cadherin, β -catenin, S100A4, Snail, Slug, Twist, and SIP1 in gastric cancer [101–103]. Down-regulation of E-cadherin expression and high-level of S100A4 expression are associated with peritoneal dissemination, serosal invasion, an infiltrating growth pattern, and a poor prognosis [103]. Up-regulation of Slug, SIP1, and Snail is associated with E-cadherin down-regulation in gastric cancer [101, 102]. Using a tissue array method, Kim et al. [104] examined expression of EMT-related protein in the gastric cancer tissues of 598 patients and found that loss of epithelial proteins and acquisition of mesenchymal proteins are associated with poorly differentiated histology and a poor outcome.

Recent studies revealed that there are genetic alterations that occur at similar frequencies in both cancer cells and surrounding stromal cells [105, 106]. In addition, a non-random X-chromosome-inactivation pattern was found in both stromal fibroblasts and cancer cells in a breast cancer model [107]. Thus, cancer cells and a subset of CAFs might be of the same origin. EMT of cancer cells may also account for CAFs that are present in tumors.

Future Perspectives

It has been believed that endothelial cells in tumor vessels are genetically stable and that these cells will not become drug resistant in response to antivascular therapy. However, recent studies showed that endothelial cells in certain tumor vessels are aneuploid and express neoplastic markers [108]. Similarly, it has become clear that CAFs in certain tumors have somatic gene alterations. We should elucidate whether genetic and/or epigenetic alterations exist in CAFs and endothelial cells in tumor vessels.

The different cell types populating the tumor stroma, i.e., CAFs, CAMs, endothelial cells, and pericytes, can help to create an environment permissive of tumor growth, angiogenesis, and invasion. The evidences presented in this Review indicate that stromal cells might serve as novel therapeutic targets in cancer. Targeted therapy that prevents the stromal support of tumor progression might provide a complementary approach to conventional treatments that target the cancer cells themselves. Because most solid tumors have reactive stroma, targeting of stromal cells may have broad clinical implications as a therapeutic strategy. Further understanding the cellular and molecular mechanisms that regulate cancer-stromal cell interaction and inhibition of stromal cell activation may facilitate development of an effective anti-tumor therapy.

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