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Tumor microenvironment in gastric cancers

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Abstract

The tumor microenvironment favors the growth and expansion of cancer cells. Many cell types are involved in the tumor microenvironment such as inflammatory cells, fibroblasts, nerves, and vascular endothelial cells. These stromal cells contribute to tumor growth by releasing various molecules to either directly activate the growth signaling in cancer cells or remodel surrounding areas. This review introduces recent advances in findings on the interactions within the tumor microenvironment such as in cancer-associated fibroblasts (CAFs), immune cells, and endothelial cells, in particular those established in mouse gastric cancer models. In mice, myofibroblasts in the gastric stroma secrete R-spondin and support normal gastric stem cells. Most CAFs promote tumor growth in a paracrine manner, but CAF population appears to be heterogeneous in terms of their function and origin, and include both tumor-promoting and tumor-restraining populations. Among immune cell populations, tumor-associated macrophages, including M1 and M2 macrophages, and myeloid-derived suppressor cells (MDSCs), are reported to directly or indirectly promote gastric tumorigenesis by secreting soluble factors or modulating immune responses. Endothelial cells or blood vessels not only fuel tumors with nutrients, but also interact with cancer stem cells and immune cells by secreting chemokines or cytokines, and act as a cancer niche. Understanding these interactions within the tumor microenvironment would contribute to unraveling new therapeutic targets.

KEYWORDS
CAFs, endothelial cells, gastric cancer, gastrin, TAMs

1 | INTRODUCTION

Cancer development is accompanied by a prominent desmoplastic reaction involving various stromal cell types and immune cells in the local microenvironment. Immune cells are primarily recruited from the bone marrow, although some are expanded from local resident immune cells. The origin of other stromal cells, such as fibroblasts, endothelial cells, and nerves, remains controversial. However, it is likely that most of these cell types are expanded or transformed from normal cells in the original organ, while a few may originate from bone marrow-derived cells.[1-3] All these components constitute a tumor microenvironment that favors the growth and expansion of cancer cells. Cells in the tumor microenvironment fuel and stimulate other cell types in a paracrine fashion, creating an environment that allows tumor cells to escape from host immune surveillance and become more resistant to cancer therapy. Thus, a detailed and precise understanding of interactions within the tumor microenvironment is critical to establishing new cancer therapies. In this review, we focus on the role of tumor-associated cells in gastric cancers. We give particular consideration to findings validated in mouse models.

2 | CANCER-ASSOCIATED FIBROBLASTS

Within the tumor microenvironment, cancer-associated fibroblasts (CAFs) are a dominant stromal component and contribute in many ways to tumor progression. In earlier studies using preclinical models, CAFs were identified as large spindle-like stromal cells in solid tumors with abundant connective tissue (as in pancreatic cancer).[1] Normal stroma in non-neoplastic tissues contains a small number of fibroblasts. CAFs differ in being characterized by abundant expression of α-smooth muscle actin (α-SMA), a marker of activated fibroblasts.[3]

Genomic analyses have identified genetic or epigenetic alterations in human and mouse CAFs, compared with non-cancer fibroblasts, in several cancer types.[4-6] Tumor cells, as well as other stromal components such as immune cells, probably contribute to these gene modifications in activated CAFs through tumor-stroma interaction. Activated CAFs can produce abundant soluble molecules, including basic fibroblast growth factor (bFGF), members of the vascular endothelial growth factor (VEGF) family, platelet-derived growth factor (PDGF), ligands of epidermal growth factor receptor (EGFR), interleukins, and TGF-β. These molecules, in turn, regulate tumor growth and inflammatory responses via direct cell-to-cell contact or in a paracrine manner. Although many products secreted from CAFs were once thought to be tumor-promoting, recent studies have revealed that CAFs may also have an inhibitory effect on tumor progression.[7,8] Emerging analyses at the single-cell level have clearly demonstrated that CAFs are largely heterogeneous, and consist of multiple molecular subsets with different effects on tumor progression.[9]

Gastric cancers, in particular undifferentiated gastric cancers, often exhibit excessive fibrosis with massive infiltration of CAFs. A study using a mouse model of inflammation-associated gastric cancer demonstrated that mouse CAFs promote gastric cancer cell growth and progression via secretion of IL-6, CXCL12, Wnt5a, Gremlin-1, etc.[10] To be more specific, EGF, FGF, IL-6, CXCL12, and PDGF can directly promote tumor cell proliferation,[10-18] EGF, PDGF, IL-1β, IL-6, IL-8, and PGE2 can promote tumor invasion and metastasis,[10,11,17] and TGF-β, IL-6, FGF, TNF-α, and IL-1β can promote epithelial-mesenchymal transition (EMT)[10,11,17,19] (Figure 1). Our group has previously reported for a chemically induced mouse model of gastric cancer that CAFs are the primary source of IL-6 and that knockout of IL-6 inhibits gastric tumor growth.[18] IL-6 production and secretion from CAFs are regulated in part by an autocrine loop mediated by IL-1 signaling or miR-149-dependent transcriptional modulation. IL-6 activates STAT signaling in tumor cells, leading to the promotion of tumor growth and metastasis.[15-17] In addition, TGF-β and Lumican/FAK signaling may play a role in the crosstalk between gastric cancers and CAFs.[11,19] Furthermore, CXCL12 produced by myofibroblasts and endothelial cells promotes gastric carcinogenesis through the CXCL12/CXCR4 pathway.[20] Therefore, targeting CAF-mediated crosstalk would be beneficial in the treatment of gastric cancers (Table 1).

Given the heterogeneous nature of CAFs, it is likely that CAFs originate from various cells. Numerous publications have

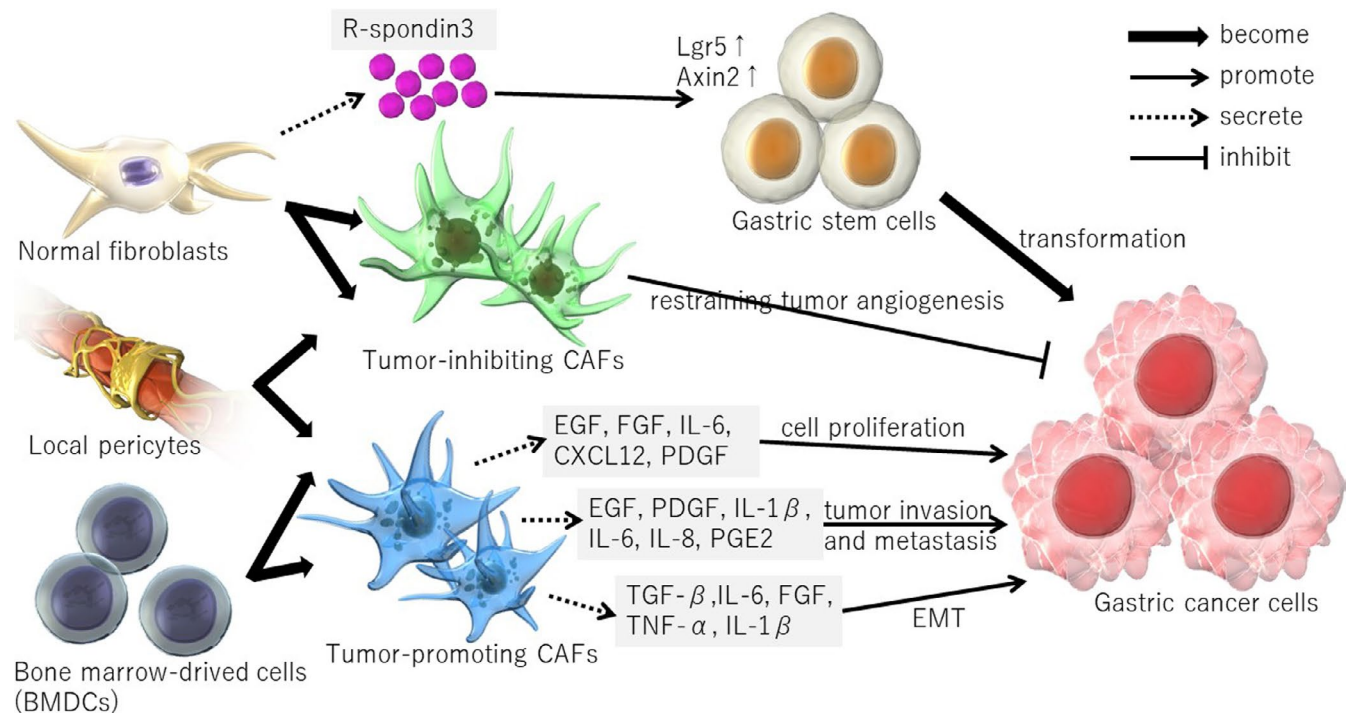**FIGURE 1** The interplay between cancer-associated fibroblasts (CAFs) and gastric cancer. CAFs regulate tumor growth and inflammatory responses by secreting various molecules. Although most CAFs have been thought to promote tumor growth, a subset of CAFs may also have an inhibitory effect on tumor progression. Normal fibroblasts, a likely origin of CAFs, support gastric (cancer) stem cells via R-spondin3 secretion

TABLE 1 Function of each soluble molecules in gastric cancer microenvironment

Soluble molecules	Secreting cells	Target cells	Function	References
IL-1	CAFs, macrophages	Immune cells	Induce the recruitment and activation of inflammatory cells	(13,43,44)
	CAFs	Cancer cells	Promote tumor invasion and metastasis and EMT	(10,13)
	M1 macrophages	MDSCs	Activate and recruit MDSCs	(27-29,31)
	Macrophages	parietal cells	Propagate the inflammatory response and inhibit gastric acid secretion	(46)
	Local mucosa		Induce stepwise spontaneous gastric inflammation, metaplasia, dysplasia, and carcinoma	(14,119)
IL-4	TH2 cells, basophils,ILC2	M2 macrophages	M2 polarization of macrophages	(14,26,32,49)
IL-6	CAFs	Cancer cells	Promote tumor cell proliferation, tumor invasion and metastasis, and EMT	(10,14-18)
	CAFs	Immune cells	Induce inflammation	(13)
	Macrophages		Induce the recruitment and activation of inflammatory cells	(43,44)
IL-8	CAFs	Cancer cells	Promote tumor invasion and metastasis	(17,119)
	CAFs	Immune cells	Induce inflammation	(13,119)
	M1 macrophages	MDSCs	Activate and recruit MDSCs	(27-29)
	macrophages		Induce the recruitment and activation of inflammatory cells	(44)
IL-10	M2 macrophages, other inflammatory cell types, mucosal cells	Unclear		(31,35,46)
	regulatory macrophages (Mregs)		Counter-regulate the M1 response	(49)
IL-11	Parietal cells	gp130	Activate JAK-STAT signaling pathways, induce atrophic gastritis	(14)
IL-12	M1 macrophage	TH1 cells	Amplify a type 1 immune response	(26)
IL-13	TH2 cells, basophils, ILC2	M2 macrophages	M2 polarization of macrophages	(32)
IL-17	Th17		Induce gastritis and oxyntic atrophy	(14)
IL-22	CAFs	Cancer cells	Promote gastric cancer cell invasion	(14)
IL-33	gastric mucosal cells	ILC2, TH2	Activate ILC2 and Th2 immunocytes, and thus initiate a Th2 cytokine response	(33)
IFN-γ	Th1 cells		Suppresses inflammation and induces gastritis and oxyntic atrophy	(14)
	M1 macrophages			(31)
			Angiostatic activities and chemoattracts anti-tumoral lymphocytes	(119)
TNF-α	CAFs	Immune cells	Induce inflammation	(13)
	CAFs	Cancer cells	Promote EMT	(10)
	M1 macrophages	MDSCs	Activate and recruit MDSCs	(27-29,31)
	Macrophages, other inflammatory cell types, mucosal cells		Induce the recruitment and activation of inflammatory cells	(44,46)

TABLE 1 (Continued)

Soluble molecules	Secreting cells	Target cells	Function	References
	macrophages	Epithelial cells	Promote Wnt signaling, contributes to gastric tumorigenesis	(120)
Wnt5a	MSC-derived CAF	Cancer cells	Promote tumor growth	(10)
PGE2	CAFs	Cancer cells	Promote tumor invasion and metastasis	(13)
	Gastric mucosal cells	Macrophages	Recruit macrophage to gastric tumors, and promote the Wnt/β-catenin signaling activity, which thus contributes to gastric cancer	(47,120)
COX-2		Cancer cells	promote tumor growth by inhibiting apoptosis, promote cell proliferation and stimulating angiogenesis within cancer cells	(119)
NOS	Macrophages	Epithelial cells	Causes methylation of genes associated with tumor suppression	(31)
	MDSCs	T cells	Suppress host immunity	(55,58)
ROS	MDSCs	T cells	Suppress host immunity	(55,58)
Arg-1	MDSCs	T cells	Suppress host immunity	(55,58)
PD-L1	MDSCs	T cells	Suppress host immunity	(55,58)
MMPs	CAFs	Cancer cells	Inhibit tumor growth, invasion, and metastasis	(13)
	CAFs	Inflammatory cells	Participant in reconstruction of the tumor microenvironment	(13)
	Epithelial cells	M1 macrophage	Suppress M1 macrophage polarization, exerts a restrictive role on *H. pylori*-induced gastric injury and the development of premalignant lesions	(50)
TGF-β	CAFs	Cancer cells	Promote EMT	(10,11,13,19)
	Cancer cells	Fibroblasts and cancer cells	Stimulate collagen synthesis in both fibroblasts and cancer cells, which leads to diffuse fibrosis in the case scirrhous GC	(119)
	M2 macrophage	Unclear		(35
EGF	CAFs	Cancer cells	Promote tumor cell proliferation and tumor invasion and metastasis	(13,17)
HGF	CAFs	Cancer cells	Affect the proliferation and migration of cancer cells	(11,13)
VEGF	CAFs	Cancer cells	Affect the proliferation and migration of cancer cells	(11,13)
FGF	CAFs	Cancer cells	Promote tumor cell proliferation and EMT	(11)
PDGF	CAFs	Cancer cells	Promote tumor cell proliferation and tumor invasion and metastasis	(11,13)
CXCR4	endothelial cells	Cancer cells	Aggressive tumor behavior, such as tumor invasion, metastasis, and poor differentiation	(119)
CXCL9	M1 macrophage	TH1 cells	Amplify a type 1 immune responses	(26)
CXCL10	M1 macrophage	TH1 cells	Amplify a type 1 immune responses	(26)
CXCL12	CAFs	Cancer cells	Promote tumor cell proliferation	(10,12,20)
STAT-3	Cancer cells	Dendritic cells	Increase the capacity of tumors to evade the immune system by inhibiting the maturation of dendritic cells, thereby suppressing the immune response	(119)
Gremlin-1	MSC-derived CAF	Cancer cells	Promote tumor growth	(10)
R-spondin3	Myofibroblasts	Gastric stem cells	Lead to an increase in proliferation of Wnt-responsive Axin2$^+$Lgr5$^-$ stem cells and finally gastric gland hyperplasia	(21,22)

shown that CAFs originate from local normal fibroblasts, bone marrow mesenchymal stem cells (MSCs), or local pericytes.[2] Recent studies have shown that normal gastric myofibroblasts secrete R-spondin3 and contribute to stem cell activity, at least in the gastric antrum.[21] Antral glands are maintained by long-lived stem cells residing at the gland base, some of which are characterized by the expression of Lgr5 or Axin2. During mucosal inflammation and regeneration, R-spondin3-expressing myofibroblasts expand and promote proliferation and stem cell function in Axin2^{+} stem cells.[22] Therefore, normal myofibroblasts act as an important component of the gastric stem cell niche, and likely give rise to CAFs that activate stem cells in cancer tissues via R-spondin signaling (Figure 1). Studies using bone marrow transplantation in mice have shown that MSCs display a remarkable feature called tumor-specific tropism, in which they actively migrate to tumor sites.[10,23] In fact, in a mouse model of gastric cancer, at least 20% of CAFs were estimated to arise from bone marrow-derived MSCs.[10] Notably, the presence of bone marrow-derived cells in the tumor mesenchyme was confirmed in human gastric adenocarcinomas and rectal adenomas in patients who developed tumors following bone marrow transplantation.[24] In addition to bone marrow-derived MSCs, a local MSC-like cell type expressing Gremlin-1 has been identified in the gastrointestinal mucosa.[25] Whether CAFs are indeed derived from Gremlin-1[+] local MSCs remains uncertain. However, lineage tracing studies in mice will elucidate more details of the cellular origin of CAFs in future.

3 | IMMUNE CELLS

3.1 | Macrophages

In the tumor microenvironment, macrophages or monocytes are known as tumor-associated macrophages (TAMs) and are among the most abundant immune cells. The degree of TAM infiltration in tumor tissues is positively correlated with poor prognosis in various cancers. It is thought that TAMs promote cancer progression by secreting various factors, including inflammatory cytokines, growth factors, and proteolytic enzymes. In addition, TAMs interact with other stromal components and often suppress the host immune response, resulting in immune escape and the subsequent growth of tumor cells.

Macrophages are classified into 2 main types: M1 and M2. M1 macrophages have a pro-inflammatory role and produce various cytokines (Figure 2). M1 macrophages, through their expression of cytokines and chemokines such as IL-12, CXCL9, and CXCL10, drive the polarization and recruitment of TH1 cells, thereby amplifying a type 1 response.[26] In addition, other previous reports have suggested that M1 macrophages secrete IL-1β, TNF-α, and IL-8, to activate and recruit MDSCs.[27-30] Furthermore, activated macrophages are known to produce nitric oxide synthase (NOS) and/or reactive oxygen species (ROS) that causes epigenetic changes in the gastric epithelial cells.[31]

Conversely, M2 polarization of macrophages are driven by TH2 cells, basophils, and type 2 innate lymphoid cells (ILC2s) through
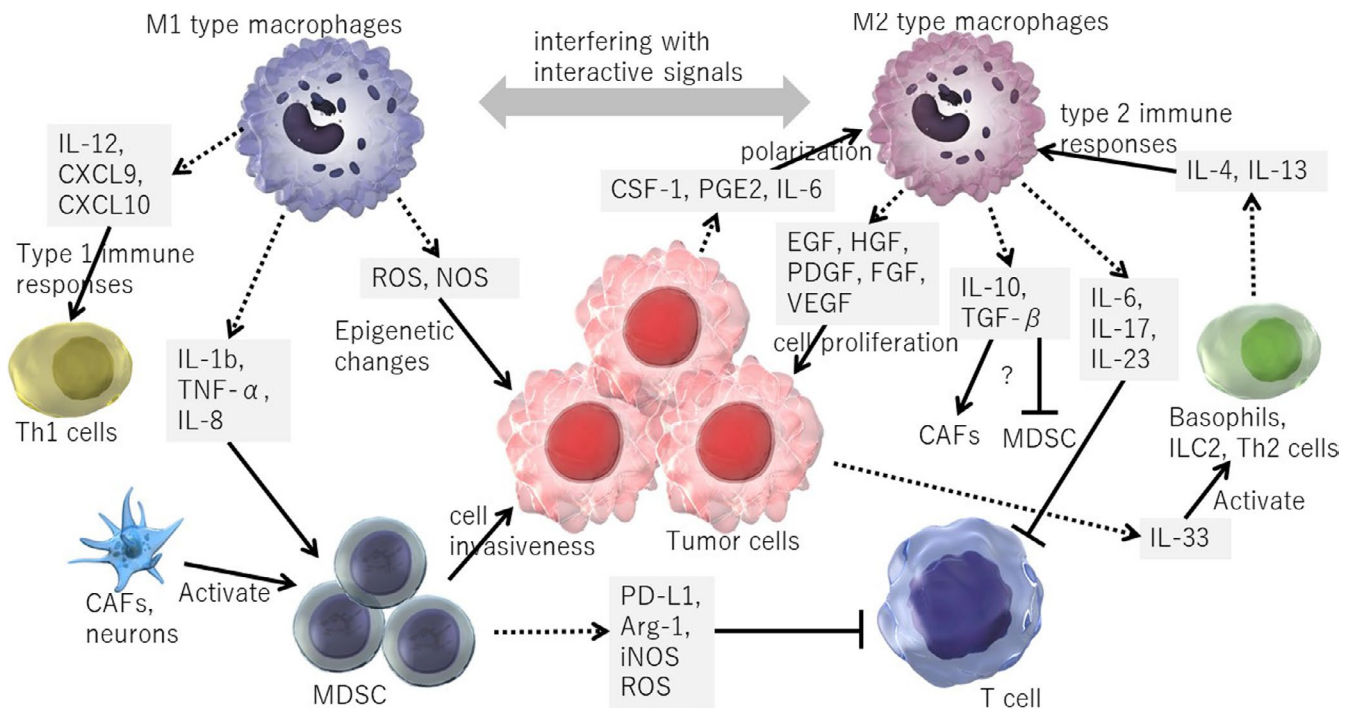

FIGURE 2 The interplay between tumor-associated macrophages (TAMs) and gastric cancer. TAMs and tumor cells stimulate each other by secreting several cytokines and growth factors. M1 and M2 macrophages can be reprogrammed into each other, leading the immunological shift within the tumor microenvironment. Myeloid-derived suppressor cells (MDSCs) suppress host immunity by inhibiting T-cell responses, and have pro-tumorigenic effects

their secretion of IL-4, IL-13, or IL-33[26,32] (Figure 2). IL-33 is also expressed by a subset of epithelial cells and could be released upon epithelial injury.[33,34] In tumor tissues, the majority of macrophages are polarized to M2. A previous study using peritoneal macrophage showed that M2 macrophages produce anti-inflammatory cytokines, including TGF-β and IL-10.[35] Nevertheless, the cellular targets of molecules secreted by M2 macrophages remain largely unclear. Presumably, TGF-β is likely to contribute to tumor progression by activating CAFs and promoting fibrosis in gastric cancer models.[10,36] IL-10 may trigger activation of the T-cell–inhibitory receptor PD-L1 on TAMs, which favors the inhibition of tumor-specific T-cell immunity,[26] therefore contributing to the general suppression of anti-tumor activities in the tumor environment. TAMs can also promote angiogenesis and contribute to remodeling of the tumor microenvironment.

There is a direct crosstalk between tumor cells and TAMs. Tumor cells secrete several cytokines and growth factors that induce M2 polarization in TAMs, such as colony stimulating factor 1 (CSF-1), PGE2, and IL-6. TAMs, in turn, can directly stimulate tumor cell growth by secreting EGF, HGF, PDGF, FGF, and VEGF[37,38] (Figure 2). M2 macrophages can be reprogrammed into M1 macrophages by interfering with these interactive signals, leading to the shift in immune microenvironment.[39,40]

In mouse models of gastritis and gastric cancers, there is a marked infiltration of macrophages. These macrophages are recruited by epithelium-derived chemokines and cytokines.[41-46] They produce pro-inflammatory cytokines such as TNF-α, and stimulate tumor growth, in part through Wnt activation.[45,47] Indeed, depletion of macrophages in these mouse models suppresses epithelial proliferation and tumorigenesis.[44,46,47] In this context, the polarization of macrophages is altered by factors that include MMP-7, IL-33, and EGF signaling.[33,48,49] M1 and M2 macrophages appear to make distinct contributions to the crosstalk between macrophages and epithelial cells.[46,50] Macrophages, in addition to directly stimulating epithelial growth, probably interact with other stromal components and contribute to angiogenesis or tumor immunity.[51] Future studies are needed to clarify the precise role of macrophages in gastric tumors.

3.2 | Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) comprise a population of myeloid progenitor and immature myeloid cells, and are regulatory immune cells associated with sites of chronic inflammation and cancer that suppress CD8^{+} T-cell function by their expression of PD-L1 and CTLA-4. Therefore, targeted in vivo depletion of the granulocytic MDSC subset is sufficient to induce the intratumoral accumulation of endogenous CD8^{+} T cells and tumor cell apoptosis in certain cancer models.[52,53] Treatment with immune checkpoint inhibitors blocks the suppressive effects of MDSCs on CD8^{+} T cells, causing tumor regression in various mouse models of cancers.[54] MDSCs are also endowed with the ability to suppress host immunity

through Arg-1, iNOS, and ROS, like macrophages. It was reported that MDSCs also have direct pro-tumorigenic effects.[55]

MDSCs are characterized by strong dual expression of CD11b and Gr-1. Increased myeloid CD11b^{+}Gr-1^{+} cells are particularly associated with inflammation-associated cancers. In the context of inflammation and cancer, the spleen becomes an important source of myeloid cells in animal models and human patients.[56,57] In mouse models of gastrointestinal (GI) and pancreatic cancers, MDSCs are activated and recruited to local tissues by sustained expression of inflammatory cytokines such as IL-1β, TNF-α, DKK-1, and IL-8[27-29] (Figure 2). Chemokine signaling, modulated by CXCR4 or CXCR2 receptors, also mediates the activity of MDSCs.[53,58,59] Other tumor microenvironmental components, such as CAFs or neurons, also contribute to differentiation and activation of MDSCs.[10,58] While MDSCs might have somewhat beneficial effects on regulating insulin tolerance in diabetes patients and maternal-fetal tolerance in pregnancy, a large body of cancer research works has suggested that MDSCs promote tumor progression in numerous cancer models.[60] Therefore, targeting MDSCs might be a novel approach for stimulating host immunity against tumor cells and subsequent tumor regression.

3.3 | Lymphocytes

Tumor-infiltrating lymphocytes (TILs) consist of T cells, B cells, and natural killer (NK) cells, and T-cell-mediated adaptive immunity is considered to play a major role in anti-tumor immunity. The subsets of T cells are represented by CD8^{+} cytotoxic T cells, CD4^{+} T helper cells, FOXP3^{+} regulatory T cells, memory T cells, and NK cells. These lymphocytes can infiltrate stroma and tumor cells to modulate the host immune response against tumor cells, while upregulated PD-L1 or CTLA-4 expression suppresses these anti-tumor immune responses in certain tumor microenvironment.

Nevertheless, evidence to the role of lymphocytes in mouse gastric cancer models remains quite limited.[61] Mohammed and colleagues demonstrated the number of infiltrating lymphocytes in the Tff1-knockout gastric tissues that develop chronic inflammation and gastric neoplasms at the antropyloric gastric regions. In this context, loss of TFF1 causes decreased IL-17 expression in CD8^{+} T cells, which resulted in loss of cytotoxic function by T-cell–mediated tumor immunity.[62] O'Reilly and colleagues showed that NF-κB1 deficiency resulted in aberrant JAK-STAT signaling, which dysregulated the expression of effectors of inflammation, antigen presentation, and immune checkpoints. They also demonstrated that gastric PD-L1 upregulation was driven by aberrant STAT1 activation, and this provides a functional link between loss of NF-κB1, aberrant STAT1 activation, increased PD-L1 expression, and GC development.[63]

In human GCs, high densities of T lymphocytes in tumor tissues are associated with favorable survival, while PD-L1 expression correlates with shortened overall survival.[64] There are also T regulatory cells (Tregs) as a component of TIL, which are able to inhibit the immune response mediated by CD4^{+} and CD8^{+} T cells, by suppressing

T-cell proliferation, antigen presentation, and cytokine production.[65] Recent genome sequencing studies have revealed that high levels of PD-L1 expression and favorable response to immune checkpoint inhibitors are associated with Epstein-Barr virus (EBV) and microsatellite instability (MSI) tumor molecular subtypes of GC.[66] However, mouse models that recapitulate such GC subtypes in human have not been well established. To understand the precise mechanism in TIL-mediated tumor immunity in GCs, generation and analysis of new mouse models with high PD-L1/CTLA-1 expression would be needed in the future.

4 | ENDOTHELIAL CELLS

Angiogenesis is a process by which cancers supply the tumor microenvironment with nutrients and oxygen, thereby contributing to tumor growth.[67] Multiple drugs that target angiogenesis-related molecules, such as VEGF, are available in clinics and are effective in many cancers. Endothelial and vascular blood vessel cells have a function beyond supplying nutrition to tumor tissues; they also act as cancer niche cells and create a cancer-promoting environment.[68] For example, endothelial cells can stimulate and activate colorectal cancer stem cells in a paracrine or juxtacrine fashion by mediating Notch and Akt signaling.[69,70] Similarly, lymphatic endothelial cells can activate metastatic signatures[71,72] and immunoregulatory function[73] in gastric cancers via secretion of soluble factors, suggesting a paracrine tumor-promoting function of endothelial cell lineage in gastric cancers.

Our group had previously identified a unique perivascular niche that supports normal and preneoplastic stem cells in the mouse stomach.[20,74,75] Long-lived gastric stem cells exist at the proliferative zone of each gastric gland, which is called the isthmus, and maintain the mucosal homeostasis. Stem cells are supported by their local stem cell niches, which consist of various stromal, immune, and even epithelial cell types.[76,77] Given that previous studies have suggested that inflammatory CXCL12/CXCR4 signaling has an important role in gastritis and gastric cancer,[10,12,78] we examined the expression pattern of CXCL12 and its receptor CXCR4 in the stomach using fluorescent reporter mice. Interestingly, each molecule is expressed in specific cell types. CXCL12 is expressed in stromal cells, in close proximity to isthmus stem cells. Immunofluorescence and FACS analysis has confirmed that these gastric CXCL12$^+$ cells are indeed a subset of CD31$^+$ endothelial cells.[20,74] In contrast, CXCR4 expression was also observed in cells adjacent to CXCL12$^+$ endothelium, including type-2 innate lymphoid cells (ILC2s) and isthmus stem/progenitor cells (Figure 3).

In models of mouse gastritis and gastric cancer, isthmus stem cells are increased and become more proliferative with the expansion of CXCL12$^+$ endothelial cells and CXCR4$^+$ ILC2s. Gastric cancer development in mice is inhibited by genetic deletion of CXCL12, pharmacological inhibition of CXCR4, and ablation of ILC2s, indicating that the CXCL12/CXCR4-dependent stem cell niche has a significant contribution to gastric carcinogenesis.[20,74] Mechanistically, gastric ILC2s express high levels of Wnt5a, one of the non-canonical Wnt ligands (Figure 3). They also activate RhoA signaling in gastric stem cells, the change of which is often observed in human gastric cancers.[79-81] These findings again highlight the importance of the endothelial perivascular niche in regulating tissue stem cells and carcinogenesis in multiple organs.[82-85]

Furthermore, it has been suggested that the perivascular network is important in the metastasis of various cancers, including gastric cancer.[71,86] A potential mechanism of metastatic promotion by vascular endothelial cells is the activation of tumor cells by locally invaded vessels or endothelial cells, which induces EMT and allows the tumor cells to enter systemic circulation more efficiently. In addition, circulating tumor cells or tumor-derived soluble factors may change vascular formation and other features of distant organs in a way that creates a favorable microenvironment for cancer cells. This microenvironment is known as the pre-metastatic niche. Indeed, CXCL12/CXCR4 signaling contributes to the development of the pre-metastatic niche and disruption of CXCL12/CXCR4 signaling is effective in inhibiting metastasis.[83,87-89]

Recent scRNA-seq studies have determined that tumor endothelial cells are heterogeneous at a molecular level, and that the various subtypes contribute to angiogenesis and tumor progression and
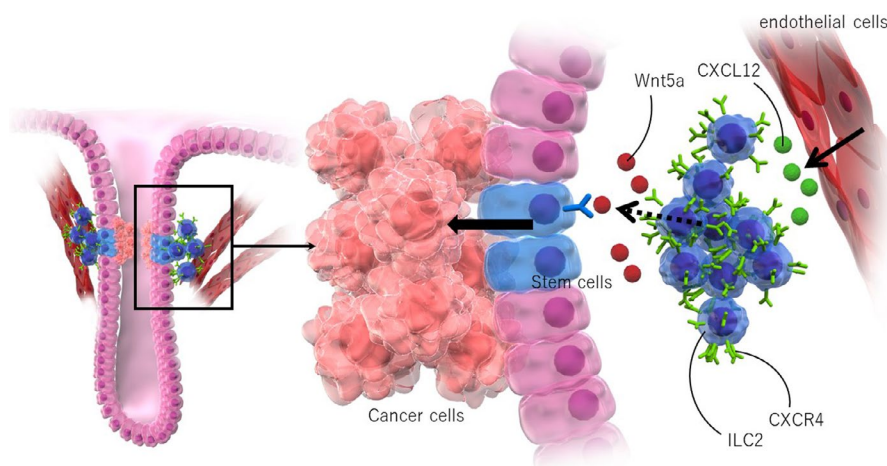

FIGURE 3 Endothelial cancer niches promote gastric cancer progression. CXCL12 is expressed in stromal endothelial cells, in close proximity to gastric isthmus stem cells, while CXCR4$^+$ type 2 innate lymphoid cells (ILC2s) are recruited by CXCL12$^+$ endothelial cells and enriched in this region. Isthmus stem cells become more proliferative with the expansion of CXCL12$^+$ endothelial cells and CXCR4$^+$ ILC2s

respond to targeting therapies in different ways.[90-92] Detailed subtyping and a deeper understanding of this heterogeneity would require an extensive functional validation study, but would be helpful in establishing new therapeutic strategies beyond VEGF targeting. Disrupting the interaction between compromised tumor endothelial cell types and cancer cells may be a promising approach to controlling the tumor microenvironment.

5 | GASTRIN

The peptide hormone gastrin is produced by G cells in the gastric antrum. It has been suggested that gastrin, in addition to having a physiological role in regulating acid secretion, may help to promote gastric cell proliferation and carcinogenesis. More details are provided in a separate review.[93] Here, we briefly review the role of amidated gastrin in stem cell regulation and cancer development.

Transgenic mice with hypergastrinemia (INS-GAS mice) show increased epithelial proliferation and develop metaplasia and cancer in the proximal stomach, suggesting that gastrin has a proliferative effect in this part of the stomach.[94] The development of gastric tumors in INS-GAS mice is significantly inhibited by an antagonist of CCK2R, a gastrin receptor, indicating that proliferation and carcinogenesis occur through the gastrin/CCK2R pathway.[95] Nevertheless, gastrin knockout (GAS-KO) mice, in which gastric tumor development is, in theory, inhibited, often develop spontaneous gastric tumors, particularly in the distal section of the stomach.[96] In fact, studies using hypergastrinemic INS-GAS mice and GAS-KO mice demonstrate that gastrin has different effects on proximal and distal gastric carcinogenesis; gastrin promotes proximal tumor development but inhibits distal tumor development[97,98] (Figure 4).

In situ hybridization imaging and lineage tracing studies in mice have revealed the specific site of CCK2R expression in murine stomachs.[99] In the proximal stomach, CCK2R is strongly expressed in mature parietal and enterochromaffin-like (ECL) cells, consistent with its role in acid secretion. In addition, a subset of proliferating progenitors in the neck zone of the gastric corpus glands also express CCK2R. These cells can expand and supply daughter cells in response to hypergastrinemia, which is likely to have been through activation of the ERK pathway in progenitor cells.[100,101] Therefore, the tumor-promoting effects of gastrin in the proximal stomach probably occur via stimulation of CCK2R-expressing proliferating progenitors in the isthmus-neck region. Given that the serum gastrin level is usually elevated in patients with long-term proton pump inhibitor (PPI) use, increased gastric cancer risk in such patients might be affected by activation of the gastrin/CCK2R pathway.[102]

Conversely, CCK2R has been found to be expressed in rare cells residing near the base of the antral and cardiac glands.[99,103] These basal CCK2R+ cells generally do not overlap with Lgr5+ basal stem cells, but show robust proliferation and act as long-lived stem cells.[104] CCK2R+ stem cells normally divide asymmetrically and supply daughter cells within the gland. Interestingly, gastrin suppresses the stem cell activity of CCK2R+ cells. In gastrin-deficient conditions, CCK2R+ stem cells
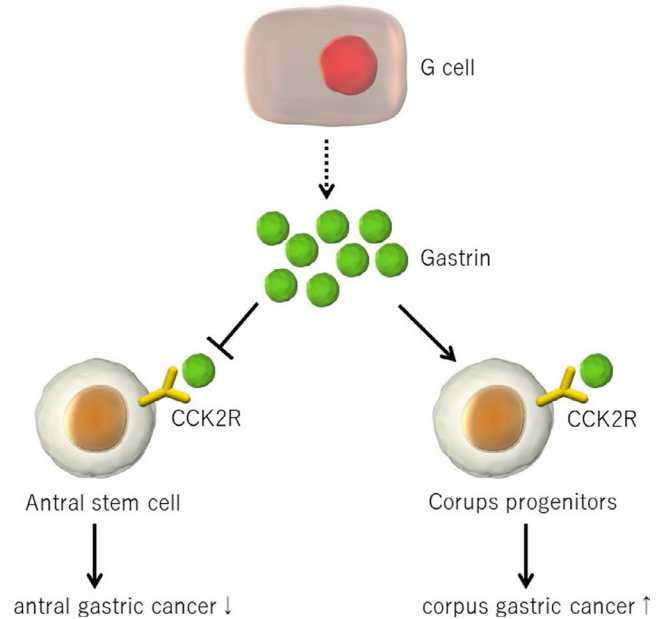

FIGURE 4 Gastrin's effects on the development of stomach cancer. Gastrin receptor Cckbr is expressed in gastric stem and progenitors in both the proximal and distal stomach. While gastrin promotes proliferation of Cckbr+ cells in the proximal stomach, gastrin/Cck2r suppresses proliferation and stem cell function in the distal stomach

divide more rigorously and more symmetrically. This increase in symmetric stem cell division results in robust accumulation of gene mutations during carcinogenesis,[105] which could account for the promotion of antral carcinogenesis by gastrin deficiency. Thus, these data support the idea that the proximal and distal portions of the stomach are really 2 distinct organs, based, in large part, on differences in the regulatory role of gastrin, and highlight the importance of the gastrin-dependent hormonal niche within the gastric tumor microenvironment.

6 | CONCLUSION

The tumor microenvironment is rather complex and very different from the microenvironment of normal tissue. Inflammation appears to be a key initial step in the creation of the tumor microenvironment, and is associated with the recruitment of various immune cells and the activation of epithelial stem or immediate progenitors, which are the main origin of gastrointestinal cancers.[76,104,106-108] Recruitment, growth, or differentiation of CAFs and blood vessels is caused by numerous interactions between tumor cells and other stromal components. Recent studies have suggested that nerves and neurotransmitters also play a critical role within the tumor microenvironment for many cancers, including gastric cancers.[109-115] Some tumor stromal components may be anti-tumorigenic,[7,58,116] but tumor cells and other stromal cells appear to acquire the ability to escape this anti-tumorigenic host defense. Understanding the precise biology of the tumor microenvironment is of critical importance. There is a limitation in currently available mouse gastric cancer models, as most of them

do not develop invasive or metastatic cancer but show modest meta-plastic or dysplastic changes that have been used as an alternative endpoint.[117] However, it remains uncertain whether such a lesion is a true precursor of gastric cancers.[106,108,118] Therefore, generation of invasive gastric cancer models coincident with a proper tumor micro-environment in mice would be encouraged. Targeting pro-tumorigenic stroma and activating an anti-tumorigenic microenvironment could be promising new therapeutic approaches in cancer treatment.

DISCLOSURES

The authors disclose no conflicts.

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