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Review

Regulation of lymphangiogenesis in human gastric cancer

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ABSTRACT

Tumor lymphangiogenesis is regulated by lymphangiogenic factors released by tumor cells and stromal cells into the microenvironment of the neoplastic tissue. Of known lymphangiogenic factors, the best-characterized are vascular endothelial growth factor (VEGF)-C and -D, which bind to VEGF receptor-3 on lymphatic endothelial cells. In gastric cancer, tumor cells produce both VEGF-C and -D. Expression of VEGF-C, but not VEGF-D, correlates directly with lymph node metastasis of early-stage gastric cancer. Some gastric cancer cells express VEGF receptor-3 as well as VEGF-C and -D ligands, and VEGF-C and -D can stimulate growth and invasion and inhibit apoptosis of gastric cancer cells in an autocrine manner. We found recently that platelet-derived growth factor (PDGF) receptor-β is expressed in tumor-associated lymphatic endothelial cells and that cancer cells produce PDGF-BB, which also acts as a lymphangiogenic factor for gastric cancer. We review the current understanding of molecular mechanisms involved in lymphangiogenesis and lymph node metastasis of human gastric cancer.

KEYWORDS: lymphangiogenesis, gastric cancer, PDGF, VEGF

INTRODUCTION

Gastric cancer is the world's fourth most common malignancy and the second leading cause of cancer deaths [1]. In Asian countries such as Korea and China, gastric cancer is the leading cause of cancer death. Conventional therapies for gastric cancer with metastasis include surgery and chemotherapy, but the prognosis for advanced-stage disease remains poor. Novel therapeutic strategies are needed, but their development depends on understanding cancer biology, especially at the molecular level. A large number of genetic and epigenetic alterations in oncogenes and tumor suppressor genes as well as genetic instability drive the multi-step process of gastric carcinogenesis [2]. In addition, the molecular events that characterize gastric cancer differ, depending on the histologic type, whether intestinal- or diffuse-type gastric cancer [2]. Metastases of gastric cancer are commonly found in the lymphatic system. The precise mechanisms by which cancer cells detach from the primary site, invade into lymphatic vessels and regrow within the sentinel lymph nodes are not well clarified.

Both angiogenesis and lymphangiogenesis are essential for tumor growth and metastasis. Increased vascularity enhances the growth of primary neoplasms by supplying nutrients and oxygen; it also provides an avenue for hematogenous metastasis [3, 4]. Lymph node metastasis is one of the major negative prognostic factors in most cancers including gastric cancer [5]. Lymphatic metastasis is believed to occur by invasion of pre-existing lymphatic vessels or by formation of new lymphatic vessels at the tumor periphery in a process known as tumor-induced lymphangiogenesis [6]. Previous studies by us and by others have shown that the number of lymphatic vessels in the

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tumor periphery correlates directly with lymphatic metastasis in cases of gastric cancer [7, 8]. Although induction of tumor angiogenesis is known to be a complex process that involves the interplay of a variety of tumor-derived growth factors [3, 4], how tumors induce lymphangiogenesis is poorly understood. Herein, we discuss the importance of lymphangiogenesis in the growth and metastasis of human gastric cancer.

Role of VEGF family members in lymphangiogenesis

Gastric cancer cells produce various angiogenic and lymphangiogenic factors. Of these, members of the vascular endothelial growth factor (VEGF) are considered some of the strongest promoters of vasculogenesis, angiogenesis and lymphangiogenesis of gastrointestinal tumors [9]. The VEGF family includes VEGF-A, -B, -C, -D, -E and -F and placental growth factor (PlGF) [10]. VEGFs bind to receptor tyrosine kinases VEGF receptor (VEGFR)-1, VEGFR-2 and VEGFR-3, which are specifically expressed in vascular and lymphatic endothelial cells. The VEGF-A/VEGFR-2 signaling axis plays a pivotal role in angiogenesis [11]. Several groups of investigators have reported correlation between VEGF-A expression levels and microvessel density (MVD) of human gastric cancer [12-14]. VEGF-A-positive tumors have been shown to lead to a poorer prognosis than do VEGF-A-negative tumors [15-17].

Recent studies have suggested that VEGF-A can act as a direct or indirect lymphangiogenic factor [18, 19]; however, the main regulators of lymphangiogenesis are VEGF-C and VEGF-D [20]. These two factors are secreted glycoproteins that are structurally similar, sharing areas of homology. They are specific ligands for VEGFR-3, a receptor tyrosine kinase that is expressed predominantly in the endothelium of lymphatic vessels [21]. Both cytokines are subject to proteolytic processing, which allows them to bind to VEGFR-2 expressed on vascular endothelial cells.

Skobe *et al*. [22] described VEGF-C as a lymphangiogenic factor that can selectively induce hyperplasia of the lymphatic vasculature. VEGF-C and VEGF-D have mitogenic effects on vascular and lymphatic endothelial cells and promote survival of lymphatic endothelial cells via VEGFR-3. The lymphangiogenic ability of VEGF-C and VEGF-D was confirmed *in vivo* in various mouse models [20]. Yanai *et al*. [23] reported that transfection of VEGF-C gene into AZ521 human gastric carcinoma cells increased the percentage of mice with lymph node metastasis and the number of lymphatic vessels in a mouse orthotopic implantation model. Upon examination of clinical specimens of human gastric cancer, significant correlation was found between lymph node metastasis and VEGF-C expression [7, 8]. VEGF-C is expressed by tumor cells, and the expression level is highest at the invading edge of early-stage gastric cancer (confined to the mucosa or submucosa) [7]. There are fewer reports regarding VEGF-D, and it remains controversial whether VEGF-D promotes lymphangiogenesis in human tissues. Juttner *et al*. [24] analyzed expression and prognostic significance of VEGF-D along with VEGF-C and VEGFR-3 in 91 gastric adenocarcinomas. They found that the presence of VEGF-D or VEGF-C correlated with lymphatic metastases and decreased survival. Shida *et al*. [25] examined expression of VEGF-C and VEGF-D at the mRNA level by means of RT-PCR in 50 gastric cancer specimens. Both mRNA levels correlated significantly with lymphatic invasion, but only VEGF-C expression was related to lymph node metastasis. We examined expression of VEGF-C and VEGF-D by immunohistochemistry in 140 archival surgical specimens of submucosally invasive gastric cancer [26]. VEGF-C immunoreactivity was associated with lymphatic invasion, lymph node metastasis, and increased MVD. However, there was no association between VEGF-D immunoreactivity and any clinicopathologic feature. We also quantified mRNA levels of VEGF-C, VEGF-D and VEGFR-3 in biopsy specimens of human gastric cancer by real-time RT-PCR and analyzed correlation between these levels and lymph node metastasis [27]. With this method, the mRNA levels of factors produced by both tumor cells and stromal cells are evaluated in limited amounts of tissue. Expression of VEGF-C and VEGFR-3 (but not of VEGF-D) was significantly greater in patients with lymph node metastasis than in those without such metastasis, suggesting that VEGF-C is a dominant regulator of lymph-angiogenesis in human gastric cancer.

VEGF-C and VEGF-D as autocrine factors

VEGFRs are expressed by a variety of cancer cell lines. VEGF-A and VEGFR-1/2 are co-expressed in a number of cancers, including breast [28], prostate [29], colon [30], and pancreas [31, 32] cancers, suggesting that VEGF-A has a direct effect on tumor cells via an autocrine mechanism. VEGFR-3 is also found in several types of malignant cells, although the significance of such expression remains unclear. We recently studied expression and function of VEGFR-3 in gastric cancer cells [33, 34]. VEGFR-3-specific immunoreactivity was detected on tumor cells from 17 of 36 human gastric carcinoma specimens. *In vitro* treatment of gastric cancer cell line KKLS, which expresses VEGFR-3, with its ligands, VEGF-C and -D, stimulated cell proliferation and increased expression of mRNAs encoding cyclin D1, Bcl-2, PlGF and autocrine motility factor [33, 34]. VEGF-C/D transfection into KKLS cells resulted in stimulation of angiogenesis, lymphangiogenesis and cell proliferation and in inhibition of apoptosis. Thus, VEGF-C/D may participate in the progression of human gastric carcinoma via autocrine and paracrine mechanisms (Figure 1).

Role of platelet-derived growth factor (PDGF)-BB in lymphangiogenesis

Although the best-characterized lymphangiogenic factors are VEGF-C and -D [20], it is unlikely that these are the sole factors regulating processes noted above. Various lymphangiogenic factors produced by tumor cells, endothelial cells and stromal cells have recently been identified. These include VEGF-A [35], PDGF-BB [36], fibroblast growth factor (FGF)-2 [37], hepatocyte growth factor (HGF) [38] and members of the angiopoietin (Ang) family [39]. FGF-2 promotes lymphatic vessel growth in the mouse cornea, but this effect is believed to occur indirectly via induction of VEGF-C expression and activation of VEGFR-3 signaling [37]. Ang-2 is crucial for establishing the lymphatic vasculature. VEGF-C/VEGFR-3 signaling is a key primary proliferation pathway for lymphatic vessels, whereas Ang-2 is important in later remodeling stages [40]. So far, the importance of FGF-2, HGF and Ang-2 for lymphatic metastasis of human gastric cancer is unknown.

Interesting preclinical studies have indicated that PDGFs and PDGF receptors (PDGFRs) not only

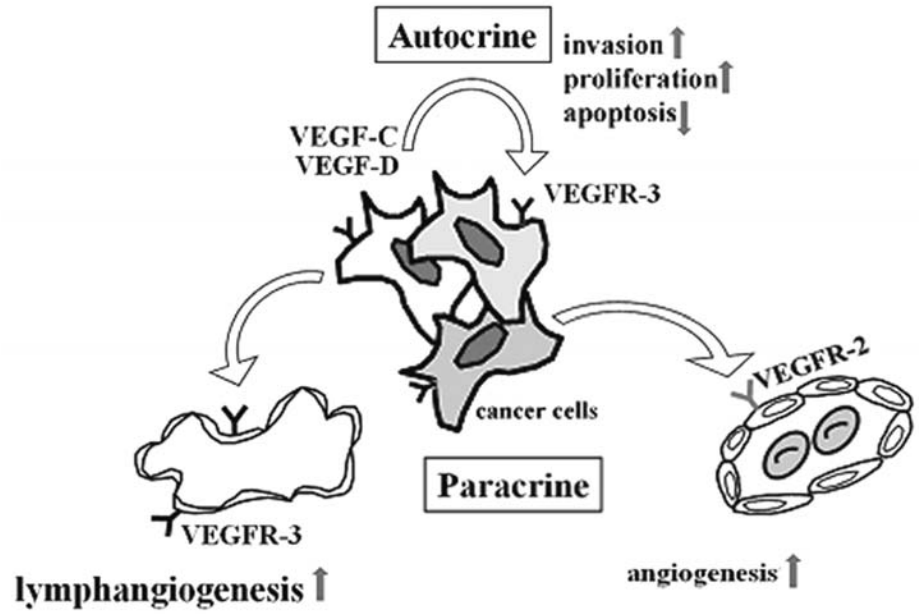

Figure 1. Human gastric carcinoma cell lines express VEGF-C, VEGF-D and VEGFR-3. VEGF-C and -D may be involved in the progression of human gastric carcinoma, acting via autocrine and paracrine mechanisms.

promote hemangiogenesis and direct tumor cell growth but are important players in lymph-angiogenesis [36, 41] (Figure 2). PDGFs have been found to induce tumor growth by directly stimulating growth of certain types of tumor cells [42], to stimulate angiogenesis [43], to recruit pericytes [44] and to control the interstitial fluid pressure in stroma, influencing transvascular transport of chemotherapeutic agents in a paracrine manner [45]. Recently, Cao *et al*. [36] showed that members of the PDGF family can induce lymphatic vessel growth in the mouse cornea and that expression of PDGF-B in murine fibrosarcoma cells induces tumor lymphangiogenesis, leading to enhanced lymph node metastasis. Correlation between PDGF-B expression levels and lymph node metastasis has been reported in non-small-cell lung [46] and esophageal squamous cell carcinomas [47].

We examined expression profiles of PDGF-B and PDGFR-β in human gastric carcinoma [48]. Expression of PDGF-B and PDGFR-β in the tumors was significantly higher in patients with lymph node metastasis than in those without and was also significantly higher in diffuse-type carcinoma than in intestinal-type carcinoma [48]. Immunohistochemistry revealed that tumor cells expressed PDGF-B, but PDGFR-β was expressed predominantly by stromal cells, such as lymphatic endothelial cells, pericytes and carcinoma-associated fibroblasts. PDGFR-β is preferentially expressed by activated, proliferating lymphatic endothelium but not by quiescent lymphatic vessels in normal tissue [48].

Anti-lymphangiogenic therapy

Although the efficacy of anti-angiogenic therapy has been studied extensively, the concept of

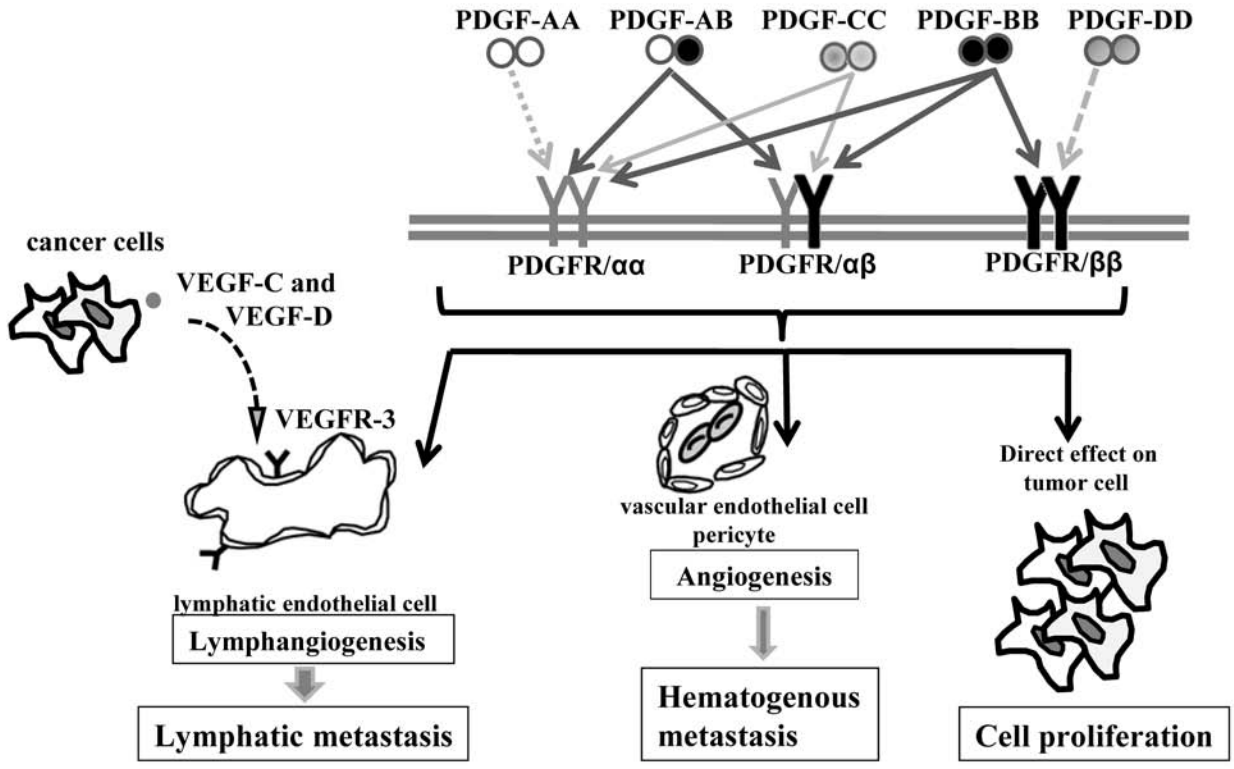

Figure 2. VEGF-C or VEGF-D and VEGFR-3 signaling system is a key regulator of tumor lymphangiogenesis. Members of the PDGF family are expressed at high levels in many malignant tissues. Activation of PDGF receptors is known to lead to stimulation of cell growth and angiogenesis. Cao demonstrated recently that PDGFs play a direct role in promoting lymphangiogenesis and metastatic spread to lymph nodes [36].

targeting lymphangiogenesis to obtain a therapeutic advantage in cancer is a recent one. The lymphatic network plays an important role in cancer metastasis, allowing tumor spread to draining lymph nodes. Thus, targeting the induction of tumor lymphangiogenesis and functional alteration of existing lymphatic vessels may help eliminate a route for lymphatic metastasis.

In mouse experiments, a neutralizing antibody to VEGFR-3 was shown to completely block tumor lymphangiogenesis with no effect on preexisting vessels [49], suggesting that anti-lymphangiogenic therapy could be used safely in adults. Soluble VEGFR-3 fusion proteins as well as antibodies targeted to VEGFR-3, VEGF-C and VEGF-D are currently being developed. However, there is a possibility of increasing interstitial fluid pressure in the tumors by destroying lymphatic vessels, thereby impairing the delivery of other anti-cancer drugs.

A number of small molecule inhibitors that inhibit VEGFR signaling are available [50]. Many of them also inhibit the activity of other related receptor-type tyrosine kinases such as PDGFRs and c-KIT, due to similarity in the kinase site. We investigated the effects of imatinib, PDGFR-β tyrosine kinase inhibitor, on lymphatic vessels and cancer-associated fibroblasts in tumors grown up from implantation of human gastric cancer cells into the stomachs of nude mice. The tumors treated with imatinib showed reduced areas of lymphatic vessels and stromal reaction in comparison to those in untreated tumors [48, 51]. Treatment with imatinib alone did not inhibit lymph node metastasis [51]; however, imatinib combined with cytotoxic chemotherapeutic drugs did inhibit lymph node metastasis [51]. Therefore, for inhibiting lymph node metastasis, reduction of the lymphatic vessel area seems to be insufficient.

CONCLUSIONS

VEGF-C and PDGF-B expressed by tumor cells and stromal cells are associated with lymph-angiogenesis and lymphatic metastasis of gastric cancer. Thus, blockage of the factors that induce lymphangiogenesis may be a reasonable approach to prevention and treatment of lymphatic metastasis. Interfering with the growth of lymphatic endothelial cells via several different signaling pathways should enhance efficacy of the anti-metastatic treatments. Further knowledge of the cellular and molecular mechanisms that regulate lymph-angiogenesis of tumors may facilitate development of effective anti-lymphangiogenic therapies.

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