

The role of tumour microenvironment in gastric cancer angiogenesis

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Abstract

Gastric cancer is one of the most common cancers in the world. More than 95% of gastric cancers are adenocarcinomas originating from the glandular epithelium of the stomach lining. Unfortunately, a large number of patients are diagnosed when the tumour is at unresectable stage. Therefore, it is very important to understand the mechanisms involved in gastric cancer pathogenesis. One of them is angiogenesis, which means the formation of new blood vessels from pre-existing vasculature. This process is dependent on interactions between the tumour and surrounding stromal cells which create the tumour microenvironment. Moreover, both tumour and stromal cells release a wide array of angiogenic factors that have an influence on endothelial cell recruitment and thus affect the process of angiogenesis. In this paper we discuss the role of tumour microenvironment in gastric cancer angiogenesis.

Introduction

Gastric cancer is one of the most common cancers in the world, which seriously affects the patients' quality of life. More than 95% of gastric cancers are adenocarcinomas originating from the glandular epithelium of the stomach lining. There are two major histological types of this disease: intestinal and diffuse. The intestinal type is determined by cohesive neoplastic cells, which form gland-like tubular structures, and it is strongly connected with dietary and environmental risk factors such as *Helicobacter pylori* infection. The sequence of histological changes leading to intestinal type gastric cancer include gradual transition through chronic gastritis, gastric atrophy, intestinal metaplasia, and dysplasia. According to Lauren's classification, diffuse type gastric cancer is characterised by poorly differentiated cells and no glandular structures. In this case, a major risk factor is also *H. pylori* infection [1, 2].

In Western countries, a large number of gastric cancer patients are diagnosed when the tumour is at an unresectable stage. Currently, the only solution for these patients is systemic chemotherapy with the aim of maintaining quality of life and prolonging survival. Un-

fortunately, survival of patients with advanced gastric cancer treated with palliative chemotherapy remains low [3]. Therefore, it is very important to understand the mechanisms involved in gastric cancer pathogenesis. It is thought that one of the most important mechanisms is angiogenesis, which means the formation of new capillaries from pre-existing vasculature [4]. This is because tumour vasculature enables malignant cells to escape from the primary site and establish distant metastasis elsewhere [4]. It is not surprising then that targeting tumours with therapy based on angiogenesis remains a highly pivotal area of study. In this context, it should also be mentioned that during vascular network formation a very significant role is played by interactions between the tumour and the surrounding stromal cells, which creates a unique tumour microenvironment. Moreover, both tumour and stromal cells release a wide array of angiogenic factors that influence endothelial cell recruitment and thus affect the process of angiogenesis [5].

In this paper we will discuss the role of the tumour microenvironment, especially cancer associated fibroblasts (CAFs) and tumour associated macrophages (TAMs) in gastric cancer angiogenesis.

The role of angiogenic factors in gastric cancer pathogenesis

Numerous reports indicate that angiogenesis in tumour tissue is under the control of various factors released both by tumour and stromal cells. In the case of gastrointestinal tumours, the most significant angiogenic factors are: vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), interleukin 8 (IL-8), and platelet-derived endothelial growth factor (PD-ECGF) [6]. Among these, VEGF is thought to be one of the most prominent determinants of angiogenesis in gastric cancer. It has been reported that a high concentration of VEGF may induce aggressive tumour growth and metastasis. Therefore, patients with VEGF positive tumours have poorer prognosis than patients with VEGF negative tumours [7]. In this context, the study of Schimanski *et al.* is very interesting; they evaluated the impact of VEGF-A, -B, -C, and -D on tumour dissemination and survival in gastric cancer patients. Their studies have showed that VEGF-D expression was significantly correlated with distinct metastatic disease but not with patient survival [8]. In turn, the study of Han *et al.* demonstrated that patients with positive staining for VEGF-C showed significantly less favourable survival rates compared with patients who had negative staining for VEGF-C. The survival rates were also significantly lower in patients who had positive staining for VEGF receptor-3 (VEGFR-3) compared with those who did not show VEGFR-3 expression. So it can be said that patients with positive expression for both VEGF-C and VEGFR-3 exhibit the most unfavourable prognosis [9].

The next growth factor involved in tumour blood vessel formation is FGF. In the context of angiogenesis, the two most extensively studied FGFs are acidic fibroblast growth factor (aFGF or FGF-1) and basic fibroblast growth factor (bFGF or FGF-2). A growing body of evidence indicates that FGF can act together with VEGF to amplify tumour angiogenesis [10]. Therefore, targeting the FGF and VEGF pathways synergistically may be more efficient in suppressing tumour growth and angiogenesis than targeting either factor alone. It has been reported that in gastric cancer patients FGF is overexpressed [11]. A positive correlation was demonstrated between the highest expression of FGF and tumour invasiveness and lymph-node metastases. It is worth noting that expression of FGF-2 in gastric cancer may also predict recurrence following resection [11, 12].

The cytokine that may stimulate division of endothelial cells and then promote angiogenesis is IL-8. The study of Kuai *et al.* demonstrated that overexpression of IL-8 in gastric cancer cell lines MKN-45 is probably responsible for increased cell adhesion, migration,

and invasion. It is thought that IL-8 expression is also connected with resistance to oxaliplatin. In contrast, inhibition of IL-8 expression with small interfering RNA decreased adhesion, migration, invasion, and oxaliplatin resistance in KATO-III gastric cells. These results suggest that IL-8 may act as a target in gastric cancer therapy [13]. It should be noted that the level of IL-8 mRNA in neoplasms is significantly correlated with vascularisation. Therefore, it may suggest that IL-8 regulates neovascularisation in cancer tissue [14]. Moreover, gastric cancer cells transfected with the IL-8 gene have been shown to produce highly vascular neoplasms in the gastric wall in nude mice [15]. In the case of colon cancer IL-8 may not only provide a proliferative advantage but may also promote the metastatic potential [16].

The next known endothelial cell mitogen is PD-ECGF, which has been demonstrated to have chemotactic activity for endothelial cells *in vitro* and angiogenic activity *in vivo*. For example, in pancreatic cancer patients the expression of PD-ECGF was correlated with poor prognosis, but there was no significant association between the expression of PD-ECGF and clinicopathological features, except for tumour histology [17]. In the case of gastric cancer, expression of PD-ECGF is detected in infiltrating cells and in tumour epithelium. Moreover, in intestinal type of gastric cancer there is a clear correlation between PD-ECGF and VEGF-A expression and vessel counts [18].

Cancer-associated fibroblasts and their role in gastric cancer angiogenesis

As it was mentioned above tumour tissue consists of tumour and stromal cells. Among stromal cells the most significant role in cancer development and progression is played by CAFs. In contrast to normal, non-activated fibroblasts, CAFs possess an activated phenotype and can be detected by their expression of fibroblast-specific protein 1 (GSP1), vimentin, desmin, and α -smooth muscle actin (α -SMA). It is thought that CAF presence in cancer tissue is associated with development of high-grade malignancies and poor prognoses [19]. The studies of Orimo and Weinberg have shown that stromal fibroblasts extracted from invasive breast tumours are more competent to promote the growth of mammary carcinoma cells and to enhance tumour angiogenesis than are comparable cells derived from outside non-pathological tissues. In contrast, normal fibroblasts have a role in maintaining epithelial homeostasis by suppressing proliferation and oncogenic potential of adjacent epithelial cells [20, 21]. A study with the use of immunofluorescence microscopy demonstrated that the frequency of myofibroblasts

in the CAF group from the tumoural gastric wall named CaF-29 was greater than in the group of normal fibroblasts. Moreover, transforming growth factor- β (TGF- β) significantly increased the α -SMA expression in CAFs. Conditioned medium from human gastric cancer cell lines from scirrhous gastric cancer upregulated the α -SMA expression in CAFs. Interestingly, cells from non-scirrhous gastric cancer like MKN-45 or MKN-74 did not show this effect [22].

It has been reported that CAFs may differentiate from resident local fibroblasts. Although the mechanism regulating activation of fibroblasts and their accumulation in tumour tissue has not been established, it is thought that this process is mediated by many cytokines and growth factors, including VEGF, bFGF, and PD-ECGF [6]. In turn, data from animal models and studies on human breast cancer suggest that a very significant CAF source is represented by bone marrow-derived cells which, when exposed to tumour-conditioned medium, assume a CAF-like phenotype, including characteristic markers such as α -SMA or tenascin-c. Guo *et al.* constructed a gastric cancer mouse model (Gan mice) by simultaneous activation of prostaglandin E2 and Wnt signalling in the gastric mucosa. During this experiment, expression of VEGF-A was observed in the stromal cells. Moreover, increased microvessel density (MVD) was been demonstrated as well. Of interest, they showed, by bone marrow transplantation experiments, that a subset of gastric myofibroblasts is derived from bone marrow [23]. A third proposed source of CAFs origin is epithelial-to-mesenchymal transition (EMT). During EMT cancer cells lose epithelial polarity, gain a spindle-shaped morphology and develop invasive and migratory phenotype [6]. Kim *et al.* analysed the expression of EMT-related factors in 598 gastric cancer patients and they found that loss of epithelial proteins and acquisition of proteins connected with mesenchymal tissue tend to correlate with poorly differentiated histology and poor prognosis [24].

Inflammatory responses and gastric cancer angiogenesis

It has been demonstrated that approximately 15–20% of cancers are initiated by inflammatory responses. In cancer pathogenesis, two particular macrophage phenotypes have been shown: classically activated macrophages (M1) and alternatively activated macrophages (M2). It should be mentioned that macrophages localised within the tumour stroma are named TAMs. These cells are recruited from circulating monocytes in response to chemoattractants including monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 α (MIP-1 α) [25]. The study by Wu *et al.* revealed

that TAMs may promote angiogenesis and lymphangiogenesis in gastric cancer patients, probably by the way of VEGF enhancement. There is also a positive correlation between TAM count and MVD [26]. Moreover, in gastric cancer tissue a high number of TAMs is prominently correlated with lymph node metastasis, intestinal type of tumour and Fas ligand (FasL) expression. These results may suggest that TAMs 'cooperate' with tumour-derived FasL and serve as a barrier against infiltration of CD8⁺T cells into the cancer nest. It should be mentioned that expression of FasL in CD8⁺T cells and in natural killer cells (NK) plays a very significant role in Fas-mediated tumour killing [27].

As was mentioned above, macrophage recruitment is mediated by a wide array of chemoattractants. One of the most significant chemoattractants is MCP-1, which is produced by tumour cells. Kuroda *et al.* demonstrated that transfection of the MCP-1 gene into gastric cancer cells is responsible for strong infiltration of macrophages into tumour tissue and enhanced metastatic potential in the mouse orthotopic implantation model [28]. The notion that MCP-1 plays a significant role in cancer pathogenesis is strongly supported by the study of Futagami *et al.*, who examined the expression of MCP-1, its receptor CCR2, and expression of CD40 ligand (CD40L) in cancer tissue. Their reports indicate that in gastric cancer tissue there was a significant correlation between microvessel density and CD40L, MCP-1, and CCR2 expression [29]. It is worth noting that CD40L, a member of the tumour necrosis factor superfamily, is primarily expressed by activated T cells and platelets. In contrast, CD40 a receptor of the tumour necrosis factor superfamily is expressed by numerous cell types including monocytes and endothelial cells. Therefore, it may play a pivotal role in tumour angiogenesis [29]. Cyclooxygenase-2 (COX-2) has also been shown to contribute to tumour angiogenesis. In this study, Futagami *et al.* revealed that COX-2 expression levels were significantly higher in CD40L-stimulated macrophages and was correlated with increased VEGF production. The addition of MCP-1 to CD40L-stimulated macrophages had a synergistic effect on COX-2 expression and VEGF production, which may have an influence on tumour angiogenesis [30].

In summary, the tumour microenvironment plays a very significant role in gastric cancer angiogenesis. Moreover, stromal cells including CAFs and TAMs might serve as a novel, promising target in cancer therapy. Therefore, understanding the molecular mechanisms regulating interactions among cancer and stromal cells will be a significant step in clinical oncology and gastroenterology because it may facilitate development of a novel anti-cancer therapy.

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