The role of cancer-associated fibroblasts in the stemness of gastric cancer stem cells

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Gastric cancer remains one of the leading causes of global cancer mortality worldwide. In recent years, several studies revealed that gastric cancer contain cancer stem cells (CSCs), a unique subpopulation in tumors, which display stem cell properties: the ability to sustain self-renewal and supply cancer cells. CSCs properties are modulated by their microenvironment, called CSC niche. Recent studies revealed that the interactions between CSC and niche play an important role for the progression of gastric cancer. This brief review presents the current development in gastric cancer stem cell study, which will be useful to find novel therapeutic strategies for gastric cancer.

Keywords: Gastric cancer; Cancer stem cells; Tumor microenvironment; Cancer-associated fibroblasts


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Introduction

Gastric cancer (GC) is one of the most common cancers worldwide[1]. Regardless of development in traditional treatments including surgery, radiotherapy, chemotherapy, targeted therapy, and immune therapy, the overall survival rate in advanced GC patients is very low, which is mainly attributed to tumor relapse and metastasis[2, 3]. Recent studies propose that metastasis and recurrence are associated with the presence of cancer stem cells (CSCs), also called tumor-initiating cells[4, 5].

Normal stem cells possess two unique characteristics: self-renewal potency, which supplies an adequate number of cells to maintain the organ’s function, and pluripotency, which allows mature cells to compose a specific organ[6]. Stem cells tend to be found in specific areas of an organ where a special microenvironment called niche maintains stem cells functions. Cancer is regarded as a disease of stem cells, which forms the basis of CSC theory[7]. In 1937, Furth and Kahn indicated this theory that a single leukemic cell is capable of transmitting systemic disease in mice[8]. This was first published paper about CSCs. However it has taken a long time for the concept of CSCs to become well recognized. In 1997, CSCs were first identified and isolated in acute myeloid leukemia (AML) by Bonnet and Dick with surface markers CD34 and CD38[9].

Al-Hajj et al. revealed that the first solid CSCs were
identified in breast cancer in 2003 using two surface markers CD44+/CD24low[10]. Since then, CSCs have been identified in various types of solid tumors[10-12]. Various potential biomarker combinations have been published to detect CSCs from malignancies[13, 14]. Gastric cancer stem cells (GCSCs) have also recently been reported in various studies using GC cell lines and primary GC tissues[15].

Solid tumors consist of cancer cells and various types of stromal cells, fibroblasts, endothelial cells, and hematopoietic cells. Tumor progression has been recognized as the product of crosstalk between the cancer cells and its surrounding tissue or tumor stroma[16]. Also, fibroblasts are associated with tumor growth and progression in various carcinomas. Interactions between cancer cells and their microenvironment can have considerable impact on tumor characteristics. Furthermore, it has been reported that niche stromal cells have a critical role in the characteristics of CSCs[6]. Recently, the mechanism between GCSCs and their niche in human GC have gradually been revealed. A deeply understanding this mechanism is important, which has become a critical focus of basic research that could yield potential novel therapeutic tools to eradicate GC. In this brief review, we present the current evidence concerning interaction between GCSCs and their niche.

**Gastric Cancer Stem Cells**

Recently, accumulating studies promote a common recognition of the accurate definition for CSCs proposed by the American Association for Cancer Research workshop in 2006[17]. Many reports showed the presence of CSCs in many solid tumors, including breast[10], brain[11, 18], prostate[19], liver[20, 21], pancreas[22-24], colon[25-28], lung[29], renal[30], and head and neck cancers[31] and melanoma[32, 33].

There are some useful methods to identify and isolate CSCs, such as flowcymetoric analysis using surface markers and side population fraction. In 2009, Takaishi et al.[25] first defined CD44 as a potential cell surface marker of GCSCs in several GC cell lines. They found that CD44+ fraction has a sphere-forming ability and makes xenograft tumors in immunodeficient mice. Zhang et al.[26] showed a combination of the cell surface markers CD44 and CD24 in GC cell lines and primary GC tissues from five patients, suggesting that CD44+/CD24+ expression may act as a putative GCSC marker because high tumorigenicity in immunodeficient mice. Chen et al.[27] isolated CSCs from human gastric cancer tissues and the peripheral blood of gastric cancer patients using CD44 and CD54 surface markers, and suggested that CD44+/CD54+ cells possess a higher ability to initiate tumors in vivo and re-establish the cellular hierarchy of tumors from single-cell implantation. Han et al.[30] identified the epithelial cell adhesion molecule (EpCAM) and CD44 as putative markers. Katsuno et al.[35] demonstrated that aldehyde dehydrogenase1 (ALDH1) is a candidate marker for gastric cancer stem cells.

In other studies, CD90[36], CD71[37], and CD133, ABCB1 and ABCG2[38], were identified as GCSC markers using human GC tissues or GC cell lines. Recently, Nagano et al.[39] indicated that CD44 variant isoform (CD44v) is also one of the cell surface markers of GCSCs. However, many of these published markers are not completely specific to CSCs, or have yet to facilitate isolation of CSCs[40-42].

In addition to the surface markers, many studies demonstrated the presence of CSCs in side population (SP) cells isolated from human cancer tissues and cell lines. The SP fraction was detected by flow cytometry, using DNA-binding dye Hoechst 33342. SP cells highly express several subtypes of the ATP-binding cassette (ABC) transporter family that is responsible for the extrusion of both Hoechst and some drugs[43].

Since identification of the SP phenotype as a hematopoietic stem cell marker in 1996, the SP cells have emerged as a promising method to identify stem cell in a variety of tissues[44]. SP cells have been isolated in various tumors including glioma, medulloblastoma, hepatocarcinoma, breast, prostate thyroid, colon, and gastric cancers[45-47].

SP cells display a capacity for self-renewal and higher tumorigenicity in vivo and in vitro than that of non-SP cells, raising the hypothesis that the SP subset may represent a universal CSC population. In gastric cancer, several reports indicated that SP population has stem cell properties. Haraguchi et al.[48] isolated SP cells from many human GC cells lines and found that the cell lines. Fukuda et al.[49] reported FACS sorted-SP cells from gastric cancer cell lines (MKN-45, KATOIII, MKN74, MKN28, and MKN1) and human GC tissues are more tumorigenic and with greater capability of xenograft formation in immunodeficient mice, and possess a higher potential for peritoneal metastasis with the upregulated mRNA expression levels of integrins and CD44. Also, Ehata et al.[52] characterized the SP cells within diffuse-type gastric cancer cell line, OCUM-2M, OCUM-2D, and OCUM-2MD3, produce both SP cells and non-SP cells, have greater capability of xenograft formation in immunodeficient mice, and possess a higher potential for peritoneal metastasis with the upregulated mRNA expression levels of integrins and CD44. Also, Ehata et al.[52] characterized the SP cells within diffuse-type gastric cancer cell line, OCUM-2M, OCUM-2D and OCUM-
2MD3, have higher chemoresistance than that unsorted cells.\textsuperscript{[53]} Recently, we characterized the SP cells from diffuse-type gastric cancer cell lines, OCUM-12, OCUM-2MD3. They displayed higher ability of sphere formation, tumorigenicity, and mRNA expression level of CSC markers, \textit{ABCG2}, \textit{ALDH1}, \textit{CD44}, \textit{NANOG}, and \textit{OCT3/4}.\textsuperscript{[54]}

These reports may offer a useful method to identify GCSCs by SP assay, and provide a new target for cancer therapy. However, the use of the SP fraction to identify GCSCs remains controversial.\textsuperscript{[25]} Burkert et al.\textsuperscript{[55]} revealed that SP cells and non-SP cells isolated from GC cell lines do not differ with respect to the number of stem-cell-like cells, and concluded that the SP fraction of GC is not sufficient to CSCs. Furthermore, Zhang et al.\textsuperscript{[56]} showed that SP cells from BGC-823 display same tumorigenicity as non-SP cells. These results suggested that not all SP cells might contain CSCs in GC cell lines. Putative CSCs analyzed by potential markers and SP expression may contain not only a uniform stem cell population, but also early progenitor cells. These results suggest that combination between SP assay and surface marker may be useful method to identify and isolate CSC population precisely.

**Cancer Stem Cells Microenvironment**

Tumor microenvironment is composed of diverse cells and molecules, such as mesenchymal stromal cells, secreted molecules, and extracellular matrix.\textsuperscript{[57]} The tumor stroma consists of, including fibroblasts, macrophages, a vascular network, and extracellular matrix. Tumor progression has been recognized as the product of interaction between the cancer cells and tumor stroma.\textsuperscript{[16]} Fibroblasts in the tumor microenvironment are termed cancer-associated fibroblasts (CAFs) which are a subpopulation of the tumor-stroma environment that in many cases determines tumor outcome.\textsuperscript{[58]}

Cancer cells themselves may alter their adjacent stroma to form a supportive environment for tumor progression. Cancer cells modify and activate their microenvironment by secreting growth factors and proteins, while stromal cells also affect cancer cells by secreting soluble factors such as growth factors or cytokines.\textsuperscript{[59]} These interactions can act in an autocrine and a paracrine manner. CAFs have been reported to promote various types of tumor via secretion of growth factors and cytokines.\textsuperscript{[60, 61]} Gastric fibroblasts secrete several growth factors, such as transforming growth factor-\(\beta\) (TGF-\(\beta\)), fibroblast growth factor2 (FGF2), FGF7, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and hepatocyte growth factor (HGF), which affected the proliferation and migration of GC cells.\textsuperscript{[62]}

Fuyuhiro et al. revealed that TGF-\(\beta\) from GC cells upregulates the number of myofibroblasts in CAF.\textsuperscript{[63]} The number of myofibroblasts of gastric fibroblasts was upregulated by TGF-\(\beta\) secreted from cancer cells. Furthermore, the number of myofibroblasts was more increased by TGF-\(\beta\) in CAFs compared with normal fibroblasts. TGF-\(\beta\) produced by tumor cells may contribute to maintaining the myofibroblastic phenotype and might play an important role in the malignant phenotype in the tumor microenvironment.\textsuperscript{[63]} Otherwise, Fuyuhiro et al. also reported that cancer-associated orthotopic myofibroblasts stimulate the motility of GC cells with TGF-\(\beta\).\textsuperscript{[64]} Fibroblast cells increased the migration and invasion ability of GC cells. These motility-stimulating activities of CAFs were downregulated by Smad2 siRNA treatment and anti-TGF-\(\beta\) neutralizing antibody.

Webber et al. reported that some cancer-derived exosomes could affect on fibroblasts.\textsuperscript{[65]Sigma} Some cancer-derived exosomes could trigger elevated \(\alpha\)-SMA expression and other changes consistent with the process of fibroblast differentiation into myofibroblasts. It has been reported that the cancer cell-derived TGB-\(\beta\) modulates myofibroblast differentiation in colon cancer,\textsuperscript{[66]} breast cancer,\textsuperscript{[67]} and squamous cancer.\textsuperscript{[68]}

Current evidence suggests that the characteristics of tissue stem cells are controlled by the surrounding microenvironment, called as ‘stem cell niche’. In the stomach, the niche surrounding stem cells contributes to the maintenance of these cells, regulation of cell numbers, and directing differentiation.\textsuperscript{[69]} Tissue stem cells in the stomach are surrounded by myofibroblasts that act as a niche and secrete different types of soluble factors, including bone morphogenetic proteins (BMP), TGF-\(\beta\)-1, Wnt ligands, SDF-1, and matrix metalloproteinases (MMPs)\textsuperscript{[70, 71]}.

Like normal stem cells, CSCs have the capacity for self-renewal and multipotency. Reported evidence suggested that the behavior of stem cells and CSCs is controlled by a tissue-specific niche microenvironment.\textsuperscript{[57, 72-81]} CSCs relay on a similar niche, called the ‘CSC niche’, which regulates their proliferation and differentiation.\textsuperscript{[78, 82-84]} There are many components in niche, which are involved in tumor growth, including extracellular matrix, stromal cells, vascular and endothelial molecules, secreted modifier proteins, growth factors, myofibroblasts, and hypoxia. Furthermore, the niche also protects CSCs from various toxic drugs, which may contribute to their chemoresistance.\textsuperscript{[85, 86]} Recently, various studies have provided remarkable evidence of the regulation of CSCs by the niche, based on similar stem-cell-like properties.\textsuperscript{[57]} Therefore, CSC properties within a tumor may depend on the microenvironment niche.

Normal stem cells, such as embryonic stem cells and induced pluripotent stem cells require niche fibroblasts as
feeder cells to supply stemness factors. Recently, it has been reported that niche stromal cells have a critical role in the characteristics of CSCs[6]. We revealed that CAFs significantly increased the SP population, the number of spheroid colonies, and the expression level of CSC markers in scirrhous GC cell lines. This stimulating activity was decreased by TGF-β inhibitors, but not FGF receptor inhibitor and cMet inhibitor, which suggested that CAFs might sustain the stemness of scirrhous gastric cancer cells by TGF-β signaling.

TGF-β was reported to maintain the stemness of glioblastoma[87, 88] and leukemia[89]. Ikushima et al. and Penuelas et al. demonstrated that TGF-β signaling increased glioma-initiating cells. Naka et al. demonstrated that TGF-β-FOXO signaling maintains leukemia-initiating cells in chronic myeloid leukemia. In contrast, Ehata et al.[52] reported that TGF-β decreased the SP cells and the sphere formation ability within scirrhous gastric cancer cells, due to decrease expression of ABCG2 transporters. Moreover, Katsumo et al.[35] also confirmed that TGF-β decreased the number of ALDH1+ CSCs in diffuse-type gastric cancer cells, and downregulated ALDH1 and REG4 expression, which were consistent with the inverse correlation between ALDH expression and Smad3 phosphorylation.

TGF-β probably functions as a tumor suppressor before the initiation of cancer, and during the early stages of carcinogenesis. In contrast, during the advanced stages of cancer, TGF-β signaling promotes cancer progression and metastasis[90]. This TGF-β functional change from tumor suppressor to promoter has been well recognized[91, 92]. These controversial results might be the dual function of TGF-β on the progression of cancer cells. Understanding and control of the dual role of TGF-β would contribute the cancer therapy.

A novel therapeutic strategy

Various types of conventional anticancer therapies, including chemotherapy, radiation, and immunotherapy, have been used in clinical practice, however, the tumors frequently relapsed possibly because of the presence of quiescent CSCs[93]. The quiescent CSCs tend to escape from anti-proliferative chemotherapeutic agents, thereby contributing to tumor recurrence, metastasis, drug resistance, and poor clinical outcomes[17, 94].

Recent focus of cancer research has shifted to targeted therapies of CSCs rather than cytotoxic therapies that indiscriminately kill tumor cells[40, 98]. Furthermore, many lines of evidence presented in this brief review indicate that the niche of CSCs plays a critical role in maintaining the properties of CSCs. Targeting the unique molecules in the tumor microenvironment and their signaling interactions may be a promising therapeutic strategy, and may provide a complementary approach to conventional therapies targeting the malignant cells themselves[59]. The crosstalk between CSCs and their microenvironment is important for the regulation of tumor behavior. Impairing this interaction and interfering with the establishment of a supportive niche by anti-stroma therapy will promote eradication of CSCs and provide a more effective therapy for tumor treatment[96].

Tranilast (N-(3, 4-dimethoxyphenyl) anthranilic acid), a drug used clinically for the treatment of excessive proliferation of fibroblasts. Tranilast decreases gastric carcinoma growth through its effect in blocking the growth-interactions between fibroblasts and scirrhous gastric cancer cells[97]. The combination therapy with tranilast and cisplatin decreases the xenografted tumor size, fibrosis, and mitosis, and increases apoptosis[98]. Tranilast might be a promising new drug to inhibit the interaction between fibroblasts and scirrhous gastric cancer cells[99].

Brabletz et al demonstrated that CSCs have the potential to metastasize and recurrence, and these traits have been widely considered to be the consequence of dysregulation of stem cell control[100]. Nishii et al showed that SCs like SP cells have higher ability of peritoneal dissemination of GC. We reported that TGF-β from CAFs might sustain the stemness of GCSCs. TGF-β signaling might be a promising target for cancer therapy against metastasis and recurrence of CSCs. Kawajiri et al indicated that the administration of TGF-β receptor inhibitor, A-77, improves the prognosis of the mice with peritoneal dissemination[101]. A-77 administration reduces the fibrosis and causes the medullary formation of cancer cells in vivo. The invasiveness of scirrhous gastric cancer cells is significantly decreased in a co-culture with fibroblasts, and A-77 significantly decreases the invasion ability of scirrhous gastric cancer cells. A-77 is thus considered to be useful inhibiting the peritoneal dissemination of scirrhous gastric carcinoma. Shinto et al. demonstrated that TGF-β receptor inhibitor, Ki26894, significantly suppressed the growth interactions between diffuse-type gastric cancer cells and fibroblast cells in vitro[102]. In vivo, treatment with a combination of Ki26894 and S1, which is a 5-fluorouracil analog, showed a synergistic anticancer effect on the tumor size, number of the metastatic lymph nodes[103]. GCs inhibiting lymphatic involvements by TGF-β receptor inhibitor might have high sensitivity for anticancer drug[104]. These results suggested that the metastatic ability of CSCs might be decreased by TGF-β receptor inhibitor, A-77 and Ki26894. TGF-β seems to stimulate tumor progression in the tumor-stroma interaction, while the specific role of TGF-β in tumor progression is still controversial[105]. Additional studies are necessary to investigate the effect of TGF-β receptor inhibitors on cancer progression and CSC regulation, to use them for a new cancer therapy.
Conclusions

Accumulative evidence supports the existence of CSCs that have ability to generate tumors are resistant to chemotherapy, and can produce more differentiated non-tumorigenic cells within gastric tumors. CAFs have been proven to play an important role in the maintenance or regulate of CSCs. Therefore, the understanding of the interaction between CSCs and CAF may lead to the development of promising therapy for GC.

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Conflict of interests

There are not any financial or other interests with regard to the submitted manuscript that might be construed as a conflict of interest.

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