

The Gastric Cancer Stem Cells and Resistance for Chemotherapy Therapy

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ABSTRACT

Gastric cancer remains a major global health threat, and most patients with advanced stage disease require chemotherapy. Resistance to therapy is a major obstacle in the management of gastric cancer, which may be due to cancer stem cells that are defined as “cancer cells within a tumor that possess the capacity for self-renewal and that can cause the heterogeneous lineage of cancer cells that constitute the tumor.” Gastric cancer stem cells exhibit characteristic biomarkers, signaling pathways, and crosstalk networks with tumor microenvironment. Targeting of these characteristics, which play important roles in cancer stem cells resistance, may provide new therapeutic modalities for gastric cancer.

Keywords: Cancer stem cell; Gastric cancer; Side population; Stem cell marker; Chemoresistance; Microenvironment; Molecular target.

INTRODUCTION

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 comprise a specific organ [1]. It has recently been demonstrated that cancer originates from a small subpopulation of cells known as cancer stem cells (CSCs) that possess the abilities of self-renewal and tumorigenesis [2,3]. CSCs retain the capacity to produce a hierarchy of phenotypically diverse progeny [4]. This theory was first proposed by Furth and Kahn [5] in 1937, and CSCs were first identified and isolated by Bonnet and Dick [6] in 1997. In 2006, the American Association for Cancer Research workshop created a consensus definition of CSC as

“cells within a tumor that possess the capacity for self-renewal and that can cause the heterogeneous lineage of cancer cells that constitute the tumor” [7]. Recent accumulating data support the hypothesis that CSCs may exist in several solid tumors, including gastric cancer (GC) [8]. In addition to their self-renewal capacity, CSCs have the potential to metastasize and recurrence [4,9]. Various biomarkers and signaling have been utilized to detect and characterize CSCs, including those in human GC [8,10-12]. CSCs have been demonstrated to be preferentially spared by traditional cancer therapies because standard chemotherapy and radiation therapy target the differentiated tumor cell bulk, which results in cancer recurrence [12,13]. The identification of the CSC component of a tumor may open a new therapeutic perspective on the basis of selective

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targeting of this small population of cells. In this chapter, the characteristic properties of gastric cancer stem cells (GCSCs) are reviewed with regard to surface markers and self-renewal signaling.

Gastric Cancer and Gastric Cancer Stem Cells

Gastric cancer remains one of the most common cancers worldwide and represents a major global health threat. Traditionally, the clonal evolution model has been used to explain GC growth: GC cells result from multiple mutations over time resulting in a population of continually diversifying cells. In contrast, the CSC theory suggests that only CSCs can self-renew and promote tumor growth [14]. Because gastric carcinoma manifests a histological heterogeneity [15], multipotent CSCs may explain this heterogeneity evident in gastric tumors [16]. Although investigation of the origin of GCSCs is ongoing, numerous recent studies suggest that gastric stem or progenitor cells or bone marrow-derived cells (BMDCs) are candidates for GCSC [17-19]. Houghton et al. reported that BMDCs migrate to and repopulate the gastric mucosa during infections, and over time, contribute to metaplasia, dysplasia, and gastric carcinogenesis [17]. A recent study by Varon et al. provided compelling evidence that long-term *Helicobacter pylori* infection induces the recruitment and accumulation of BMDCs in the gastric epithelial mucosa, which then participate in dysplasia and GC development [20].

Although most patients with advanced stage GC require chemotherapy, the development of chemoresistance is a major obstacle in therapy. Because the survival of CSCs is better than that of proliferating progenitor cells or differentiated tumor cells on the administration of intensive anticancer therapies [21], it may be important to understand CSC drug resistance mechanisms in the development of a promising therapy aimed at reducing chemoresistance. Several signals are known to be associated with the stemness of GCSCs and targeting their cellular pathways, which may play important roles in CSC resistance, may provide new therapeutic modalities for advanced stage GC [22-26].

GCSC Markers

Several candidate GCSC cell surface markers have been reported [8,10-12]. CD44 is a class I transmembrane glycoprotein that acts as a receptor for extracellular matrices such as hyaluronic acid, and it is a known downstream target of the Wnt/ β -catenin pathway [27]. CD44 is associated with cell signaling, migration, and homing and is expressed in lower glandular cells of the gastric antrum. It has multiple isoforms, including CD44H that exhibits high affinity for hyaluronate,

and CD44 splice variants (CD44v) that exhibit metastatic properties. In recent years, CD44 expression correlating with CSC-like characteristics has been used to identify CSC populations in several tumor types, including GC. Chen et al. demonstrated, for the first time, the existence of CD44+ cells within GC tumors that are endowed with stem cell properties and also provided a plausible explanation for the

chemoresistance that is frequently observed in patients with GC [28]. Moreover, Takaishi et al. reported that CD44+ cells have a sphere-forming ability and serially reproduce morphologically and phenotypically heterogeneous diseases of the original GC tissues, thereby demonstrating CD44 to be a potential biomarker of GCSCs [27]. Furthermore, Nishii et al. reported side population (SP) cells with CD44 expression exhibiting high potential for peritoneal metastasis and suggested that CD44 is associated with GCSCs [9]. In addition, Han et al. proved that as few as 500 FACS-sorted epithelial cell adhesion molecule (EpCAM)+/CD44+ cells from human GC tissues are capable of forming xenograft tumors in immunodeficient mice and proposed EpCAM and CD44 as putative GCSC markers [29]. Chen et al. reported that GCSCs isolated from human tumor tissues and peripheral blood carried CD44 and CD54 surface markers [28]. Ishimoto et al. recently reported that GCSC-like cells expressing CD44v revealed an enhanced capacity for reduced glutathione (GSH) synthesis and defense against reactive oxygen species (ROS) [30]. On the other hand, Rocco et al. reported that CD44+ and CD133+ cells neither expressed stem-like properties nor exhibited tumor-initiating properties [31].

CD90 is a glycosylphosphatidylinositol glycoprotein anchored in the plasma membrane and is involved in signal transduction; in addition, it may mediate adhesion between thymocytes and thymicstroma. Jiang et al. identified a CSC population in gastric primary tumors, characterized by their CD90 phenotype, and in a cell population with the CD90 phenotype enriched in sphere-cultured cells from human gastric primary tumors, suggesting CD90 as a potential GCSC marker [32]. Notably, CD90+ cells have self-renewal properties and the ability to establish a tumor hierarchy from single-cell implantation; furthermore, CD90 expression closely correlates with the *in vivo* tumorigenicity of gastric primary tumor models.

CD24 is a glycoprotein expressed at the surface of most B lymphocytes and differentiating neuroblasts. Zhang et al. suggested that the CD44+CD24+ subpopulation of human GC cell lines AGS is composed of GCSCs [33].

CD71 (transferrin receptor) mediates the uptake of transferrin-iron complexes and is highly expressed on the surface of the cells of the erythroid lineage. Ohkuma et al. reported that the CD71- cell fraction was present in both the G1/G0 cell cycle phase and the invasive fronts of cancer foci, indicating high tumorigenicity, multipotency, and invasiveness [34]; they suggested that CD71- is useful in detecting CSCs in human gastric adenocarcinoma.

CD133, a pentaspan transmembrane glycoprotein, was initially considered to be a marker of hematopoietic stem cells. Smith et al. recently demonstrated that a moderate to high percentage of GC samples have CD133 expression with moderate to strong membranous and apical expression [35]. Although CD133 is closely associated with CSCs in various tumors, its significance in GCSCs remains unclear [31].

ALDH1 (aldehyde dehydrogenase 1) is a ubiquitous aldehyde dehydrogenase family of enzymes that catalyzes the oxidation of aromatic aldehydes to carboxyl acids. Katsuno et al. identified

ALDH1 as an additional marker of GCSCs [36]; ALDH1+ cells from a human GC cell line revealed higher tumorigenic potential *in vitro* and *in vivo* compared with that of ALDH1- cells and were capable of self-renewal and generating heterogeneous cell populations. Moreover, transforming growth factor- β (TGF β) therapy reduced the number of ALDH1+ cells and their tumorigenicity via ALDH1 downregulation and regeneration of the expression of islet-derived family member 4 (REG4) [36].

Lgr5 (leucine-rich repeat-containing G protein-coupled receptor5) was identified as a novel stem cell marker of the gastrointestinal tract, including the gastric gland fundus [37-39]. CD44+, ALDH1+, and CD133+ cells coexisted with Lgr5+ cells in the stem cell zone of adjacent normal gastric mucosa and were also detectable in GC [38]. Barker et al. demonstrated that Lgr5+ cells at the base, rather than the isthmus, of gastric glands in adult transgenic mice continuously gave rise to all antral unit cells under normal homeostatic conditions [39]. Simon et al. reported that an increase in LGR5+ putative stem cells during gastric tumorigenesis may play a role in the development and progression of GC [40].

SP, identified and isolated by the ability to efflux Hoechst 33342 dye, is known as a CSC-rich population [9,41-43]. GC cell lines were found to contain 0.02%-2.2% SP cells [41,44]. Nishii et al. isolated SP cells by using GC cell lines OCUM-2M, OCUM-2D, and OCUM-2MD3 [9]; they confirmed that serially sorted SP subsets from GC cell lines exhibited higher engrafted tumor formation and possessed a higher potential for peritoneal metastasis with upregulated expression levels of the adhesion molecules α 2-, α 5-, β 3-, and β 5- integrins and CD44 compared with those of the non-SP subsets. Moreover, the mRNA expression of CSC markers ALDH1, CD44, NANOG, and OCT3/4 was significantly increased in SP cells, which possess properties similar to those of stem cells [9]. Furthermore, similar findings were reported by Fukuda et al., who demonstrated that SP cells from GC cell lines and human GC tissues are more tumorigenic and chemoresistant compared with unsorted cells [41]. These sorted cells remained in an undifferentiated state and revealed a distinct hierarchy in malignancy. Further evidence of the link between GCSCs and the SP phenotype was recently provided in a report by Ehata et al., who demonstrated that SP cells within human diffuse-type GC cells display greater tumorigenicity *in vivo* compared with that in non-SP cells and produce both SP cells and non-SP cells, indicating the self-renewal activity and multipotency of stem cell-like characteristics [45]. Collectively, these observations may offer a novel tool to identify and isolate GCSCs using SP assay, and provide a new insight into novel strategies for GC therapy by targeting CSCs in clinical trials. Schmuck et al. reported that SP cells were smaller and expressed CD133 and MSI-1, which yielded SP and non-SP cells in recultivation experiments [43]. In addition, Zhang et al. reported that SP cells from MKN-45 possess CSC properties and proved that they were gastric cancer stem-like cells. However, SP cells from BGC-823 did not possess CSC properties, proving that not all SP cells contain cancer stem-like cells in GC cell lines [46]. Moreover, Burkert et al. revealed that SP and non-SP cells isolated from four GC cell lines did not differ with regard

to the number of stem cell-like cells [47]. Nevertheless, the utility of SP to identify GCSCs remains controversial [27].

ABC transporters, including ATP-binding cassette subfamily B member 1 (ABCB1/MDR1) and ATP-binding cassette subfamily G member 2 (ABCG2), can confer multidrug resistance to cancer cells. The expression of these transporters is correlated with the response to therapy and survival [48]. SP cells are determined by their differential potential to efflux the fluorescence dye Hoechst 33342 via ABC transporters, which are associated with SP chemoresistance properties [9,43,49]. Jiang et al. demonstrated the expression of the CSC markers ABCB1/MDR1 and ABCG2 in human GC tissue samples and cell lines and concluded that the expression of these markers varied in GC with various degrees of differentiation [32].

These putative markers may be useful to identify CSCs and determine the therapeutic molecules. However, many of the published markers are not absolutely specific to stem cells. In addition, different types of CSCs may coexist within one tumor mass. Cancer cells determined by these potential markers may contain not only stem cells but also progenitor cells. Therefore, a single surface marker may not be sufficient to identify and characterize GCSCs, and thus, a combination of these markers is required.

Gastric Cancer Stem Cells Signaling

The dysregulation of several major signal transduction pathways may be involved in gastric tumorigenesis and in the self-renewal of GCSC as stem cell regulators [50]. Sonic hedgehog (Shh) expression levels are the highest in the stem cell region of the gastric unit [51]. The Shh signaling pathway is dysregulated in GC [52,53], and aberrant activation of the Shh pathway positively correlates with poorly differentiated and aggressive GC [54]. In addition, Shh signaling promotes the motility and invasiveness of GC cells through TGF β -mediated activation of the ALK5-Smad3 pathway, indicating its potential role in GC metastasis [55]. The Shh signaling pathway is essential for the maintenance of cancer stem cell-like cells in GC [56]. Evidence supporting the role of Shh signaling as a driving force and intrinsic regulator of GCSCs has been established from several recent studies of human GC cell lines and cancerous tissue samples. CD44+/CD24+ stem-like cells from a GC cell line revealed upregulated mRNA expression in the Shh signaling molecules, Patched 1 (PTCH1), and GLI compared with that in the nontumorigenic CD44-/CD24- subpopulation [33]. The Shh pathway may provide a rational therapeutic approach to targeting GCSCs for GC therapy [56,57].

Wnts are secreted glycoproteins that bind to cell surface receptors to initiate signaling cascades that are a key to the development and maintenance of gastric epithelia. Wnt/ β -catenin signaling primarily involves balancing the ratios of stemness, proliferation, and differentiation [58]. Aberrant activation of Wnt signaling has been shown to regulate the self-renewal of stem cells and trigger a variety of tumors [59,60], including GCSCs [61]. Ishimoto et al. reported that a subpopulation of rare CD44+ stem cell-like slow-cycling cells was consistently present in the gastric glands at the squamo-

columnar junction in normal mouse stomach [62]. By the combined activation of PGE₂, Wnt signaling enhances the expansion of these stem cell-like cells, leading to gastric tumorigenesis [63]. Moreover, soluble Wnt antagonists play a negative role in GC growth and contribute to the maintenance of the stem cell pool in deep gastric glands [64]. The Wnt/ β -catenin signaling pathway may play an important role in maintaining self-renewal and the undifferentiated state of GCSCs [61,65].

TGF β has been reported to maintain the stemness in glioblastoma [66,67] and leukemia [68]. The TGF β superfamily is essential for gut morphogenesis, cellular differentiation, and adult homeostasis [65,69]. Ehata et al. recently reported that TGF β decreased the SP cell numbers within diffuse-type gastric carcinoma cells [45]. Hasegawa et al. recently demonstrated that TGF β signaling significantly increased the expression levels of CSC markers, ALDH1, CD44, Nanog, and Oct3/4, in GCSCs [70].

Embryonic stem cell-expressed Ras (ERas) is a recently identified Ras family oncogene that supports the tumor-like propagation of ES cells [71]. ERas product is a constitutively active Ras protein in the absence of mutation [72]. ERas oncogene is expressed in viviparity phase cells but not in somatic cells because of the epigenetic regulation of the ERas oncogene in the somatic phase. Yashiro et al. reported that ERas activation may be associated with tumorigenesis in gastric carcinoma and may be one of the molecules responsible for cancer stem cell-like characteristics. Moreover, Kubota et al. suggest that ERas is activated in a significant population of GC, where it may play a crucial role in GC cell survival and metastases to the liver via downregulation of E-cadherin [73]. In addition, they reported that ERas induces chemoresistance to CPT-11 via activation of the phosphatidylinositol-3 kinase-protein kinase β mTOR pathway and NF κ B, consequently leading to ABCG2 upregulation [74].

The Notch pathway has been known to developmental biologists for decades; its role in the control of stem cell proliferation has now been demonstrated for several stem cell types including hematopoietic, neural, and mammary [75]. Notch signaling is another key pathway in the self-renewal of stem cells, cell fate determination, and differentiation during developmental and adult cell homeostasis as well as in tumorigenesis [76-79]. Although notch signaling-mediated stem-like properties in GC have not yet been fully defined, abnormal activation of Notch signaling was observed in GC. Approximately 75% of primary GCs expressed the Notch ligand Jag1, with the expression status correlating with cancer aggressiveness and patient survival rate [80].

The stemness factors, Sox2, Oct3/4, Klf4, and Nanog, have been associated with induced pluripotent stem cells [81,82], and few studies have suggested that these factors may play a role in human malignancy [83]. Yupeng et al. reported that Sox2 may promote cell proliferation and tumorigenesis in breast cancer [83]. In addition, Oct3/4 expression has been suggested to be implicated in self-renewal and tumorigenesis via activation of its downstream genes in cancer stem-like cells of cancer cells [84,85]. Other studies have reported that Oct4 expression is

associated with the early stage of pancreatic carcinogenesis [86] and is correlated with lymph node metastasis [87]. In GC, Matsuoka et al. reported that Sox2 and Oct3/4 are independent prognostic factors for patients with GC. Further, Tian et al. reported that Sox2 plays a pivotal role in sustaining stem cell properties [88]. Liu et al. reported that nonadherent spheroid body-forming cells from the GC cell line MKN-45, cultured in a stem cell-conditioned medium, exhibited GCSC characteristics of sustained self-renewal, high proliferation, chemoresistance, and high expression of CSC markers such as Oct3/4, Nanog, Sox2, and CD44, compared with those in the parental cells [89].

GCSC Microenvironment (niche)

Normal stem cells, such as embryonic stem cells and induced pluripotent stem cells, require niche fibroblasts as feeder cells to supply the stemness factors. In the stomach, the niche surrounding stem cells in the isthmus/neck region of mucosa contributes to the maintenance of these stem cells, the regulation of cell numbers, and their differentiation [90]. Gastric stem cells are surrounded by a sheet of subepithelial myofibroblasts that acts as a niche and secretes

different types of growth and differentiation factors [91,92]. It was recently reported that niche stromal cells play a critical role in the characteristics of CSCs [1]. There are several components of the niche that have been suggested to regulate CSC properties, and these components are involved in tumor growth, including extracellular matrix, stromal cells, vascular and endothelial molecules, secreted modifier proteins, growth factors, bone marrow-derived myofibroblasts, and hypoxia. Hasegawa T. et al reported that carcinoma-associated fibroblasts might regulate the stemness of CSCs in gastric cancer by TGF β signaling [70]. Bone marrow-derived myofibroblasts were recently considered as major components of the niche for gastric carcinogenesis and tumor growth [54,93-95]. Moreover, carcinoma-associated fibroblasts originating from bone marrow-derived mesenchymal stem cells create a niche to sustain cancer progression [96,97].

Guo et al. reported that gastric tumor cells activate the stromal fibroblasts (SFs) and become myofibroblasts [93]; they suggested that suppressing the fibroblast activation by inhibiting tumor cell-derived factors would be an effective strategy for chemoprevention in GC. Moreover, Shibata et al. demonstrated that the overexpression of stromal-derived factor 1 (SDF1)/CXCL12, a ligand for CXCR4 (C-X-C chemokine receptor type 4), induces the GC recruitment of BMDCs and the modulation of the progenitor niche [97]. Hepatocyte growth factor secreted by cancer-associated fibroblasts increased the self-renewal of colon CSCs through activation of the Wnt signaling pathway [98]. Chronic gastritis can recruit bone marrow-derived mesenchymal stem cells, and these differentiate into cancer-associated fibroblasts that sustain cancer progression [11,96]. Moreover, several inflammatory cells, including macrophages, can affect the self-renewal of CSCs [98,99]. Uehara et al. demonstrated the relationships between

H. pylori colonization, GC, and DNA damage within Lgr5+ epithelial stem cells in the stomach of patients with GC[100];

they found that Lgr5⁺ cells expanded in the presence of *H. pylori* in the antrum of patients with GC. In addition, Tsugawa et al. used CD44v9-expressing GC cell lines to study the potential of intracellular CagA to avoid autophagy and found a molecular link between *H. pylori*-derived CagA and GC stem-like cells [101]. Chronic inflammation caused by

H. pylori infection plays an important role in transforming resident stem cells into tumor cells.

Hypoxia is another critical aspect of the CSC niche and is involved in the maintenance of self-renewal and the undifferentiated state of the CSC population in various solid tumors [102,103]. Hypoxic conditions may be implicated in the stemness of GCSCs, although the underlying mechanisms remain unknown [104]. Hypoxia-inducible factor (HIF)-1 α down-regulated CD133 expression in cancer cells [105]. Understanding the origin of CSCs and their interaction with niches would be helpful in precise targeting of CSCs.

Resistance of GCSCs to Cancer Therapy

In addition to conventional cancer therapies such as surgery, cytotoxic chemotherapy, and radiation, selective therapies on the basis of cancer biology have become available [106]. The resistance of CSCs to these therapies may be explained by various mechanisms, including characteristic properties of CSCs and their microenvironment, as described above [107]. Conventional chemotherapy and radiation kill differentiated tumor cells en masse, resulting in tumor size reduction; however, tumor relapse occurs because of the presence of residual quiescent CSCs. There is a need to design drugs that specifically target CSCs, including stem cell-targeting drugs, stemness inhibitors, and microenvironment-modulating drugs [108-110]. For targeting GCSCs, several novel strategies have been suggested, including tumor stem cell differentiation induction, targeting GCSC cell surface molecules, targeting the GCSC microenvironment, and inhibiting GCSC self-renewal pathways.

Chemoresistance of GCSCs

Most patients with GC in the advanced-stage disease require chemotherapy, and resistance to therapy is a major obstacle in the management of gastric cancer. One of the critical problems in cancer therapy is the heterogeneity of cancer cells. Anticancer therapies are effective against proliferating progenitor cells or differentiated tumor cells, but quiescent CSCs can survive chemotherapy and produce progenitor cells or differentiated tumor cells [21,111]. Therefore, the development of a therapy against CSC is important in reducing chemoresistance.

CSCs identified as SP cells exhibit chemoresistance related to the ABC transporter expressed in these cells. Two ABC transporters have been identified as capable of effluxing Hoechst 33342 dye and mediating the SP phenotype in both CSCs and normal cells: ABCB1/MDR1 and ABCG2 [9]. Overexpression of efflux pumps by ABC transporters may allow cancer cells that exhibit stem-like properties to escape the cytotoxic effects of anticancer drugs, compromising chemotherapeutic outcomes [112-114]. Axitinib, a multitargeted

tyrosine kinase inhibitor against vascular endothelial growth factor receptor 1 (VEGFR-1), VEGFR-2 and VEGFR-3; platelet derived growth factor receptor (PDGFR); and c-Kit, targeted CSCs to enhance efficacy of chemotherapeutic drugs via inhibiting the drug transport function of ABCG2 [115]. Therefore, selective inhibition of ABC transporters could be beneficial in combination with chemotherapy, particularly in the eradication of multidrug-resistant cancer cells [116-118] (Figure 1).

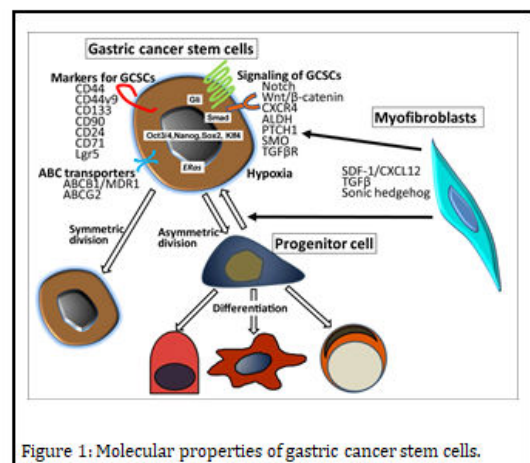


Figure 1: Molecular properties of gastric cancer stem cells.

Tumor cell hierarchy is consistent with a gastric cancer cells population at the hierarchical apex of cancer stem cells. Gastric cancer stem cells (GCSCs) have the capacity to self-renew and to differentiate into various kinds of daughter cells, including progenitor-type cells and more differentiated tumor cells. GCSCs reveal the characteristic biomarkers and stemness-maintaining signaling pathways. In addition, GCSCs reveal crosstalk networks with stromal cells (such as myofibroblasts), which may secrete factors that regulate stemness and cancer cell differentiation in the tumor microenvironment.

CSCs manifest enhanced protection against ROS, rendering them resistant to chemotherapy or radiotherapy. Ishimoto et al. revealed a role for CD44v in the protection of CSCs from high levels of ROS in the tumor microenvironment. CD44v interacts with and stabilizes xCT, a subunit of a glutamate-cystine transporter, and thereby promotes cystine uptake for GSH synthesis. CSCs provide a rationale for CD44v-targeted therapy to impair ROS defenses and sensitize them to conventional chemotherapy [30]. Tamada M. et al. suggest that CD44 ablation enhanced the effect of chemotherapeutic drugs in p53-deficient or hypoxic cancer cells, and that metabolic modulation by CD44 is a potential therapeutic target for glycolytic cancer cells that manifest drug resistance [119]. Park et al. discussed a near-infrared-sensitive molecular imaging probe based on hydrogel complexes that can target the GCSC marker CD44 [120].

The WNT/ β -catenin signaling pathway is required for normal stem and CSC self-renewal in various cell types [121,122]. In addition, this pathway can confer chemoresistance to 5-fluorouracil or doxorubicin in hepatocellular carcinoma [123] and neuroblastoma [124]. Although the mechanisms by which the Wnt pathway mediates chemoresistance are not completely clear, one potential mechanism is through the upregulation of

ABC transporter pumps. ABCG2 expression and chemoresistance to both cisplatin and paclitaxel were reversed by β -catenin siRNA knockdown [125]. Further, the inhibition of the Shh pathway has been demonstrated to sensitize CSCs in a variety of tumors, including GC [56].

Jiang et al. administered trastuzumab (humanized anti-ERBB2 antibody) to gastric tumor cells expressing CD90 and found that this reduced the CD90+ population in tumor size and growth when combined with traditional chemotherapy [126]. Zhi et al. reported the following with regard to the properties of ALDH-high cancer cells when compared with those of ALDH-low cancer cells: ALDH-high cancer cells exhibited high CSC properties because they express higher levels of Sox2, Nanog, and Nestin; they express more floating spheroid bodies and more colony formation; and they exhibit more resistance to traditional chemotherapeutic drugs such as 5-fluorouracil and cisplatin. The Notch signaling pathway has been identified to play an important role in numerous processes during tumor progression and metastases and self-renewal of CSCs [127-129]. Recent evidence suggests that Notch may also contribute to chemoresistance in CSCs through the maintenance of MDR1-expressing CSCs [130]. Nishikawa et al. suggested that ALDH generates chemoresistance in GC cells through Notch1 and Shh signaling [131]. ALDH inhibitors [132] might be promising for CSC targeted therapy. Liu et al. reported that inhibition of Notch1 with shRNA could decrease ABCC1 expression, and improve chemosensitivity in prostate CSCs. Notch1 signaling could transactivate ABCC1, resulting in higher chemoresistance ability of prostate CSCs [133]. Akagi et al. reported the role of myeloid cell leukemia-1 in the apoptosis resistance in CSC-like populations in GC [134]. Zhan et al. demonstrated that the orphan receptor TR3, a regulator of cell proliferation and apoptosis, is increased in gastric tumorsphere cells and suggested that TR3 is essential for CSC maintenance in human GC cells; therefore, TR3 could be used as a new therapeutic target for GC [135]. Xi et al. reported that Lgr5 is associated with chemotherapy resistance in GC, and the inhibition of Lgr5 expression with small interfering RNA increased the sensitivity of GC cells to chemotherapy [136]. Although the abovementioned strategies would be helpful in developing anti-CSC drugs to cure GC, not all pathways/markers may be active in each CSC in the tumor tissues. Therefore, early diagnosis and multiple-target therapy are crucial in the CSC-based therapy of GC and other types of cancer.

Radioresistance of GCSCs

The National Comprehensive Cancer Network (NCCN) guideline on GC therapy includes radiotherapy as a standard therapy for patients at the advanced stage. Radiobiological research over the past decades has provided evidence that both content and intrinsic radiosensitivity of CSCs vary between tumors, thereby affecting their radiocurability. Moreover, the application of cell surface markers to discriminate CSCs and nonstem cells is expected to allow more direct investigations of CSC radioresistance. In glioblastoma, the CD133- high cell fraction was found to have decreased sensitivity to radiation-induced apoptosis [137]. Furthermore, the overexpression of the

Wnt- catenin pathway was demonstrated to enhance the radioresistance of mammary progenitor cells using breast cancer cell lines [138].

Microenvironmental factors may also lead to radioresistance of cancer cells. A majority of experimental and human tumors contain hypoxic cells, and hypoxic tumor cells are more radioresistant than well-oxygenated cells [139], which is supported by experimental and clinical studies demonstrating that this protection may be reduced by hypoxic cell sensitizers or oxygen-enriched breathing gases [140]. Hypoxia can affect stem cell generation and maintenance in tumors through the expression of OCT4 [141] and Myc activity [142] induced by HIF. In addition, both acute and chronic hypoxia increase the radioresistance of GC cells by cell cycle arrest, and reoxygenation decreases the radioresistance of hypoxic cells [139].

New Targets for GCSCs

Targeting the characteristic signaling pathways of CSCs may represent a promising strategy for GC therapy [12]. Since chemotherapy is not able to kill quiescent CSCs, it might be useful to develop a novel drug that can differentiate quiescent CSC into active cells. Inhibitors of signaling pathways that are most likely employed in the maintenance of the self-renewal capacity and the perpetual proliferation of CSCs have emerged as an important novel class of therapeutic agents. Gastrointestinal tumors have been linked to Hh expression, and inhibition of Hh signaling by Hh antagonists such as cyclopamine and robotnikinin may be effective in the management and prevention of such cancers [143,144]. Yan et al. indicated that GCSCs play an important role in tumor angiogenesis and that Notch-1 is one of the mediators involved in these processes. β -Elemene was effective at attenuating angiogenesis by targeting GCSCs, and attenuated tumor angiogenesis by targeting Notch-1 in GCSCs [145,146]. Small molecules that target both the β -catenin-dependent Wnt signaling cascade and the anti-Wnt antibodies are awaiting translation into clinical practice [61,147]. The potency of salinomycin is based on the suppression of the Wnt/ β -catenin signal transduction, which is associated with the GCSC signaling. Wang et al. reported a novel Ad5/35-DKK1-based approach to abrogate the Wnt signaling in CSCs and demonstrated that the GCSC-targeting gene therapy was effective in preclinical experiments [148]. In addition, Zhi et al. reported that ALDH-high cancer cells were highly sensitive to salinomycin compared with ALDH-low cancer cells [149]. Lee et al. suggested that Wnt/ β -catenin signaling maintains self-renewal and tumorigenicity of cancer stem-like cells by activating Oct3/4, and proposed the inhibition of Wnt/ β -catenin signaling as a novel therapeutic strategy for targeting cancer stem-like cells [150]. Nephew et al. showed a preclinical epigenome-targeting evidence that DNA methyltransferase inhibitor, SGI-110, reduced the stem-like properties of ALDH+ cancer cells, including their tumor initiating capacity, resensitized chemoresistance, and decreased tumor progression [151]. Zieker et al. suggested that inhibiting the phosphoglycerate kinase 1 (PGK1), a key metabolic enzyme,

stimulate stem cell differentiation of CD44+GC cells, which may represent a promising avenue of research into overcoming chemoresistance in GC [152].

Several tyrosine kinase signals are known to be associated with the stemness of CSCs; therefore, targeting their cellular pathways, which might have important roles in the resistance of CSCs, may provide new treatment modalities for GC. I have reported that a c-Met inhibitor SU11274 increases the chemosensitivity of GCSCs to the irinotecan by decreasing UGT1A1 which metabolizes irinotecan [9,43,49]. Repression of c-Kit by p53 is mediated by miR-34 and is associated with reduced chemoresistance, migration and stemness markers such as CD44 and Lgr5 [133]. Inhibitors of insulin-like growth factor-1 receptor and its downstream PI3K/Akt/mTOR pathway reduced the ALDH+ breast CSCs [153].

It is possible that multiple interactions of the CSC niche contribute to resistance against conventional therapies. Therapeutic targeting of CSCs along with their niche appears a promising approach for future research in combination with conventional anticancer therapy.

Conclusion: Despite recent aggressive studies of GCSCs, few specific GCSC markers have been identified, and understanding of GCSC signaling mechanisms is poor. Also, current studies suggest that CSC behavior is regulated by the complicated tumor microenvironment. However, on the basis of these observations, the CSC theory offers a novel approach via therapeutic targeting of CSCs, which are assumed to be responsible for tumor growth, recurrence, and chemoresistance. CSC-targeted therapy, such as blocking of CSC signaling pathways, targeting of specific CSC antigens, and controlling crosstalk between CSCs and their microenvironment may lead to the development of novel therapeutic strategies for GC in the future.

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