Chapter 11

Gastric Cancer with Liver Metastasis (GCLM) and the Importance of Dormant Cancer Stem Cells

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Additional information is available at the end of the chapter

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Abstract

Surgical treatment of gastric cancer with liver metastasis (GCLM) is even more interested for oncologists. Liver resection or RFA (radiofrequency ablation) is not commonly indicated in gastric cancer with liver metastasis (GCLM). There is no direct marker defining the degree of biological aggressiveness of the tumor (indicating or contraindicating the surgical treatment), therefore we are left to rely on indirect prognostic factors: 1. cancerous invasion in the gastric wall serosa; 2. the presence of three and more liver metastases; 3. the size of metastasis exceeding 50 mm. Clarification of the nature of biological behavior of gastric cancer is a turning point of this treatment. Small light in explanation of the above problem is cancer stem cells (CSCs) theory. This theory proposes that CSCs serve not only as the basis for the development and progression of tumors, but also as the primary reason for tumor recurrence and metastasis. A better understanding of CSCs' contribution to clinical tumor dormancy and metastasis will provide new therapeutic revenues to eradicate metastatic tumors and significantly reduce the mortality of cancer patients.

Keywords: gastric cancer with liver metastasis (GCLM), radiofrequency ablation (RFA), cancer stem cells (CSCs), metastasis, tumor dormancy, cell dormancy, epithelial-mesenchymal transition (EMT)

1. Introduction

Gastric cancer is the fourth most common cancer worldwide [1]. The stage of gastric cancer at diagnosis determines treatment options and has a strong influence on the length of the patient's survival.

Early diagnosis of earlier stages of the disease with adequate treatment/R0 resection of stomach + D2 lymphadenectomy + suitable perioperative chemotherapy/bring a better outlook [3] (**Figure 1**).

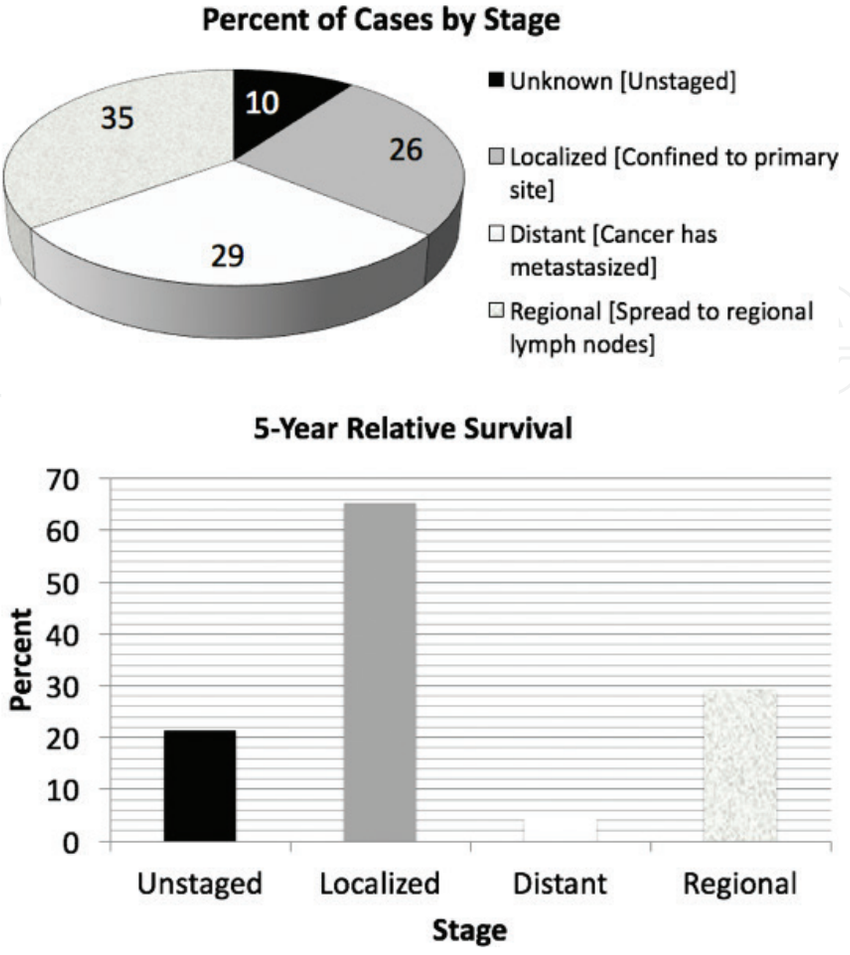

Figure 1. Percentage of cases and 5-year relative survival by stage at diagnosis: gastric cancer. The earlier gastric cancer is diagnosed, the better chance a patient has of surviving 5 years after diagnosis. For gastric cancer, 26.0 and 29.0% of cases are diagnosed at the local and distant stage, respectively. Of note, the stage of disease is unknown at diagnosis in more than one-third of cases. The 5-year survival for localized gastric cancer is 65.4%, compared with 4.5% for distant stomach cancer [2].

Remote metastases as a sign of systemic disease reduce the overall patient survival. The most common site for gastric cancer metastasis is the liver [4].

For the sake of comparison, at present, the liver resection is currently accepted as a treatment for liver metastases of colorectal cancer with referred 5-year survival in 40–56% of patients [5]. Thanks to advances in surgical techniques and perioperative chemotherapy, the indication range keeps expanding.

Compared with colorectal cancer, the gastric cancer represents a more aggressive cancer disease with heterogenic nature [6].

Other metastatic lesions associated with gastric cancer such as peritoneal carcinomatosis or extensive involvement of the regional lymph nodes significantly deteriorates the patient's outcome, contraindicating the surgical treatment.

GCLM is considered a systemic disease with adverse outcome and systemic chemotherapy is indicated as the first line of treatment [7].

Thanks to the effort on the part of some of the surgeons to reverse the adverse outcome in resectable GCLM, who performed resection or RFA surgery on the liver, we were able to collect interesting outcomes—5-year survival of 0–45% of patients [8, 9].

These studies are greatly handicapped by the low number of patients, mostly from a single center [8].

However, over 90% of mortality in cancer patients is described to the subsequent spread of cancer cells to distant tissues [10]. In patients, the threat of tumor can return after chemotherapy and radiation remains terrifying and painfully real.

This phenomenon is described as tumor or cell dormancy. The experimental models have revealed that cancer patients may have hundreds to thousands of disseminated cancer cells in circulation but only a small portion of these cells progresses to form clinically overt metastases [11].

Metastasis is a multistep process. The metastatic cancer cells acquire epithelial-mesenchymal transition (EMT)-like phenotype allowing them to disseminate from the primary tumor into circulation; the early step of metastasis (intravasate, survival, arrest, and extravasation) is a very complicated and complex process [12].

However, only a small subset of these cells (~2%) can initiate growth as micrometastases, and an even smaller fraction of these cells (~0.02%) is able to persist and forms macrometastases [12].

The sub-population of cancer cells has stem-like properties and is capable of initiating tumor, invasive growth, and spread to distant organs [13]. These cancer stem cells (CSCs) have the ability to self-renew, to produce more cancer cells, as well as undergo differentiation to give rise to phenotypically diverse nontumorigenic cancer cells.

2. Gastric cancer with liver metastasis (GCLM) and surgery

Liver resection/RFA is not a frequent treatment modality for gastric cancer with liver metastasis (GCLM).

This is well documented by a Korean study, where in 10% of the 100,000 GCLM patients, only 4% had hepatic surgery. At present, there is no clear consensus supporting liver resection in this type of tumor [14].

In this respect, the study of Kinoshita et al. has become a breakthrough [15]. It describes a 5-year disease-free survival in 30% of carefully selected patients. This confirmed that a small sub-population of patients with GCLM may benefit from liver resection or RFA. The median recurrence-free survival time was 9 months.

Half of the patients had recurrence within 1 year, in spite of R0 resection and careful selection. On the other hand, there was sufficient number of patients with long-term survival. This can be explained by varying tumor sub-populations with differing biological behavior [15, 72].

The question is: Which GCLM patients are suitable for surgical intervention?

At present, there is no direct marker available, defining the degree of biological aggressiveness of the tumor (indicating or contra-indicating the surgical treatment), therefore we are left to rely on indirect prognostic factors—number of liver metastases, size of metastatic lesion [8, 72].

Several studies have attempted to identify the prognostic factors defining adverse outlook for patients and contraindicating surgical intervention.

Among these studies, a multicenter study by Japanese authors stands out [15]. This study defines three adverse prognostic factors:

1. Invasion of serosa by primary tumor
2. Three and more liver metastases
3. Size of liver metastasis exceeding 50 mm.

The study noted a significant difference in survival between patients without a prognostic factor and patients with one of the three prognostic factors. The authors recommend to consider surgical intervention in the presence of any of the three risk factors.

Patients with lower number of risk factors had better 3- and 5-year survival following liver resection [15].

The indication for surgical intervention in GCLM is subject to overall clinical condition of the patient, but liver resection should definitely be contraindicated in the presence of all three adverse prognostic factors (no long-term survival was noted) [15].

Repeated hepatectomy was performed only in 14.4% of patients, which is significantly lower number of hepatectomies compared to patients with colorectal cancer. This is caused by different pathophysiological course of gastric cancer relapse [16].

Hepatic resection is presently considered and justified only in case of solitary relapsing metastasis of GCLM [16].

The role of chemotherapy in GCLM is not clearly defined. Neo-adjuvant chemotherapy is being brought forward that can be used to differentiate responders from nonresponders. Surgical intervention is contraindicated in nonresponders [17].

GCLM patients treated by systemic chemotherapy alone have 1.7% 5-year survival [17].

Several studies assessed the use of RFA in GCLM, recommending it for solitary lesions up to 30 mm in size, located in the periphery of the liver. No clear advantage of RFA compared to surgical resection has been shown [18, 72].

Surgical treatment is not able to provide patients with GCLM a complete cure. Half of the patients had recurrence within 1 year, in spite of R0 resection and careful selection. On the other hand, there was sufficient number of patients with long-term survival. This can be explained by varying tumor sub-populations with differing biological behavior [15].

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The number of studies aimed to clarify the explanation of the process invasive gastric cancer growth, metastasis, and particularly its biological aggressiveness essentially failed.

We do not differentiate the varying degrees of biological aggressiveness of gastric cancer.

A small light in explanation of the above problem is cancer stem cells (CSCs) theory.

This theory proposes that CSCs serve not only as the basis for the development and progression of primary tumors, but also as the primary reason for tumor recurrence and metastasis (theory of minimal residual disease).

Micrometastases involving dormant cancer stem cells are mistaken for small macrometastases. These are distinct disease entities responsible for late recurrence (months, years) with high resistance to current chemotherapy.

The combined use of traditional therapies with targeted CSC-specific agents may target the whole cancer and offer a promising strategy for lasting treatment and even its cure.

3. Gastric cancer stem cells (CSCs) theory

3.1. Minimal residual disease—definition

Minimal residual diseases are remnant tumor cells that are left after treatment and that cannot be detected by conventional clinical studies. These cells can persist in the primary site or as disseminated tumor cells in proliferative and/or dormant phases [19].

A source of minimal residual disease is considered systemic micrometastatic diseases caused by early dissemination of cancer cells from the primary tumor. These cells have the ability of dormancy [17].

In gastric cancer patients, minimal residual disease is defined as micrometastases and isolated tumor cells (ITC). First, micrometastasis is defined as tumor cell clusters between 0.2 and 2.0 mm in the greatest dimension, whereas ITC are defined as single tumor cells or small clusters of tumor cells less than 0.2 mm in size (seventh Tumour-Node-Metastasis Classification classification) [20].

Cancer stem cells (CSCs) are the cornerstone of micrometastases and define their characteristics and behavior. The clinical implications and/or prognostic significance of the micrometastases are still a matter of debate.

3.2. Definition of cancer stem cells (CSCs)

As defined by the American Association for Cancer Research Workshop on Cancer Stem Cells, a cancer stem cell (CSC) is a cell within a tumor that possesses the capacity to self-renew and to give rise to the heterogeneous lineages of cancer cells that comprise the tumor. Because they have an intrinsic ability to propagate tumor cells, CSCs are also referred to as "tumor-initiating cells" or "tumorigenic cells" [21]. The ability of stem cells to self-renew and give rise to multiple cell lineages is termed as "stemness" [22] (**Table 1**).

Self-renewal	CSCs serially transplant through multiple generations
Differentiation	CSCs generate symmetrical and asymmetrical cells
Tumorigenicity	CSCs can propagate tumor cells
Specific surface markers	Allow for separation of CSCs from nonstem cells

Table 1. Characteristics of the cancer stem cells [21].

3.3. Brief history of cancer stem cells (CSCs)

History of cancer stem cells dates back to the nineteenth century. A hypothesis of cancer stem cells (CSCs) that have similar properties to stem cells (SCs) was first described by Rudolf Virchow and Julius Conheim in 1855 [23]. Virchow suggested that cancers arise from the activation of dormant cells present in mature tissue, which are remainders of embryonic cells (perhaps similar to cells now known as stem cells) [23]. Virchow believed that cancer is caused by severe irritation in the tissues, and his theory came to be known as chronic irritation theory. However, Conheim had suspected that the remaining embryonic cells from which cancers form during organogenesis were "lost."

In 1997, Bonnet and Dick described a subpopulation of cells with the presence of a specific surface marker CD34 ($CD34^+$) and the absence of a CD38 marker ($CD38^-$) in patients with acute myeloid leukemia capable of inducing a cancerous disease after transplanting those cells to mice with an altered immunological system—leukemic-initiating cells [24, 25].

CSCs have already been identified in breast, lung, ovarian, prostate, gastric, colorectal cancer, and brain tumors [26].

It is estimated that in these malignancies, CSCs constitute <5% of all tumor cells [26].

3.4. Origin of the gastric cancer stem cells (CSCs)

The origin of gastric cancer stem cells (CSCs) is described as follows:

1. CSCs are derived from progenitor and normal stem cells [27].
2. Dedifferentiated gastric cells, via nuclear factor-kappa-B (NF-κB) modulation of Wnt signaling [27].
3. Bone marrow-derived progenitor cells progressing through metaplasia and dysplasia to cancer (**Figure 2**) [29].

Helicobacter pylori infection triggers inflammation and changes the local gastric microenvironment. This change might affect the differentiation of gastric stem cells and could induce gastric cancer. Helicobacter pylori colonizes and manipulates both progenitor and leucine-rich repeat containing G protein-coupled receptor-5 ($Lgr5^+$) stem cells, which then change gland turnover and cause hyperplasia [28].

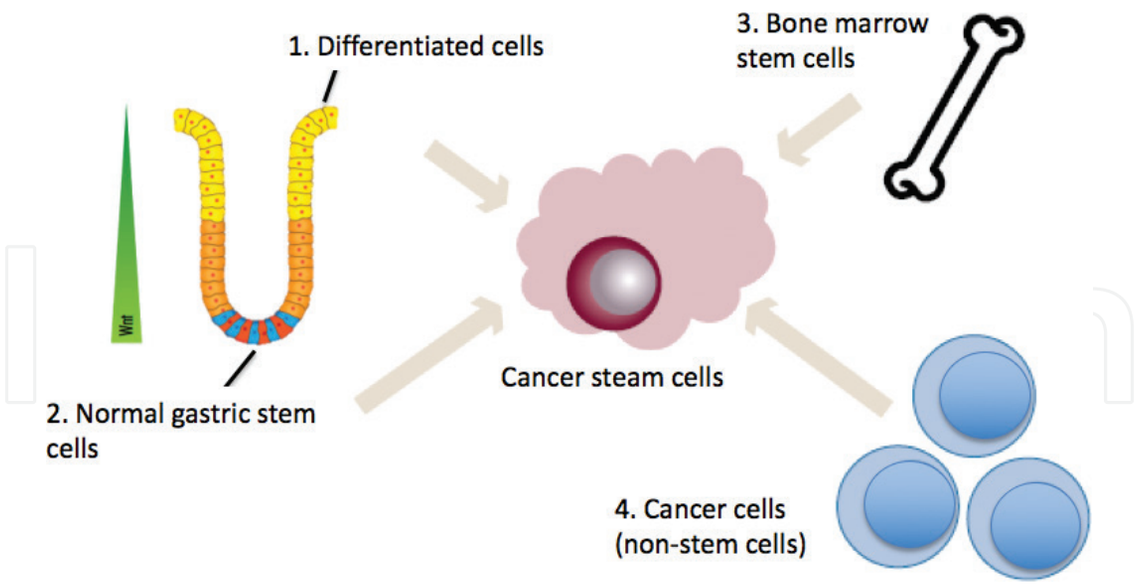

Figure 2. Gastric cancer stem cells formation [28].

Chronic infection with Helicobacter felis caused inflammation and induced the reconstruction of gastric tissue with bone marrow-derived cells, whereas acute inflammation does not lead to bone marrow-derived cell recruitment [29].

3.5. Cancer stem cells (CSCs) properties

Within both primary and metastasized tumors, cell subpopulations can differ on the basis of such factors as morphology, expression of surface antigens, specific alterations of the genome, and patterns of gene expression [30]. Likewise, CSCs are heterogeneous with varying degrees of self-renewal capacity, development potential, and expression of cellular markers. Like normal stem cells, CSCs exist in a hierarchy [31–33]. Their capacity for self-renewal and differentiation places CSCs at the top of a cellular hierarchy from which all other cells within a tumor are derived (**Table 2**) [32].

Using glioma stem cells, research has shown that CSCs can divide symmetrically, producing new CSC progeny, or asymmetrically, producing nonstem cell and stem cell progeny [34]. Intratumoral heterogeneity likely derives from asymmetrical division and differentiation of CSCs [33]. Over time, unrestrained differentiation and proliferation produces the heterogeneous

Characteristic	Normal stem cells	CSCs
Self-renewal	✓	✓✓
Differentiation	✓	✓✓
Plasticity	–	✓
Quiescence	✓✓	✓

Table 2. Characteristics of normal stem cells and cancer stem cells [32].

populations of primary and metastatic tumor cells that contribute to tumor properties, such as recurrence, resistance to therapy, and metastasis [30].

The manifestation of CSCs heterogeneity:

First, different subsets of cancer stem cells express different surface markers. Wright et al. described that breast CSCs could be divided into $CD44^+/CD24^-$ and $CD133^+$ subsets based on differences in surface marker expression [35].

Second, the heterogeneity of CSCs is manifested in the differences of the cell properties. Specifically, some cancer stem cell subsets possess a strong invasive capability, whereas other cancer stem cell subsets are in a quiescent (dormant) state and do not differentiate [36, 37].

Third, the dormant state of cancer stem cells is not permanent. Under the influence of appropriate external or internal stimuli, dormant cancer stem cells may undergo invasive transformation and become invasive cancer stem cells [38]. Therefore, an investigation of the factors that promote quiescent stem cell transformation is of great clinical significance.

3.6. Cancer stem cells (CSCs) dormancy

Many solid tumors undergo an extended period of "dormancy," characterized by the presence of minimal residual disease over many years before overt metastases may eventually arise.

Gastric CSCs consisted of both quiescent gastric CSCs and invasive gastric CSCs (increased metastatic activity). Invasive gastric CSCs are defined as $CD26^+$ $CXCR4^+$ double-positive cells and the $CD26^-$ $CXCR4^-$ double-negative cells as quiescent gastric CSCs based on surface marker expression [39].

In 2007, Aquirre-Ghiso postulated two different states of "cancer dormancy," tumor-cell dormancy, and tumor mass dormancy [19, 40, 41].

Tumor mass dormancy (micrometastasis) occurs when cancer cell proliferation is counterbalanced by apoptosis owing to poor vascularization (angiogenic dormancy) or by an immune response. In this case, the cancer cells are never truly inactive, but rather are incapable of expanding beyond a certain number (**Figure 3**).

Tumor-cell dormancy is defined as the condition in which cancer cells enter the G0 phase of the cell cycle and have low metabolism. This form of dormancy is clinically asymptomatic.

However, this conceptual framework is still under debate. At present, little is known about the factors that might have a role in the "awakening" of dormant tumor cells that leads them into the dynamic phase of macrometastatic formation.

CSCs exist within a microenvironment of surrounding vasculature, stromal cells, immune cells, and secreted factors produced by these cells. These create a niche wherein the CSCs can survive and thrive in order to propagate and differentiate into the cells that make up the tumor mass. In essence, the niche is a regulatory microenvironment that nurtures the stem-cell–like

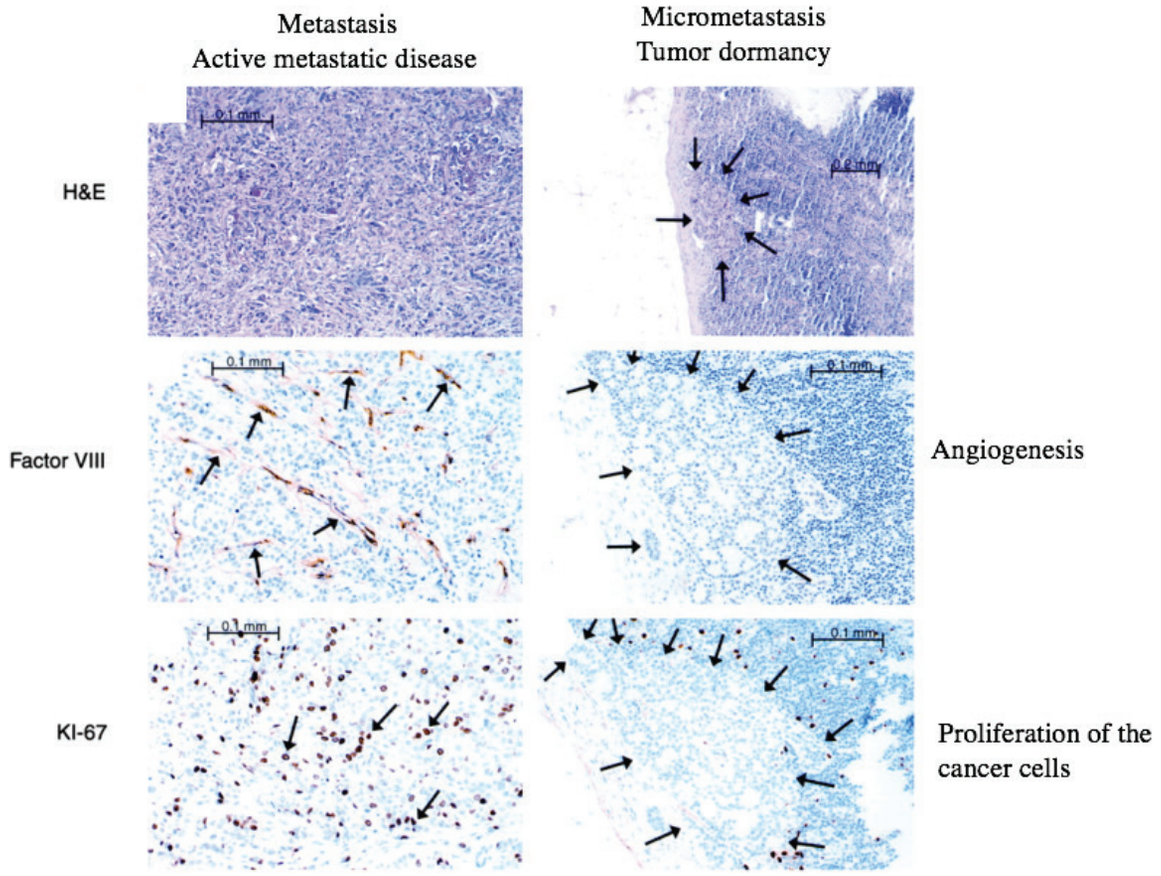

Figure 3. Metastasis vs. micrometastasis. H&E staining of breast cancer lymph node macrometastases (*A*, ×400) and micrometastases (*B*, *arrows*, tumor-lymph node interface, ×200). Immunohistochemical analysis of vascularization of human breast cancer lymph node metastases (*C*, ×400) and micrometastases (*D*, ×400). Tumor vascularization was analyzed by staining with polyclonal antibody against factor VIII, an endothelial-speci c marker. In breast cancer metastases (*C*), there was marked neovascularization (brown stain; arrows, representative blood vessels). In contrast, breast cancer micrometastases (*D*) had a marked decrease in tumor microvessel density. *Arrows*, tumor-lymph node interface. Immunohistochemical analyses of proliferation of breast cancer metastases (*E*, ×400) and micrometastases (*F*, ×400). Tumor proliferation was analyzed by staining with antibody against Ki-67. In breast cancer metastases, there was a much higher rate of proliferation (*E*, red/brown stain; arrows, representative proliferating cells) compared with micrometastases (*F*, *arrows*, tumor-lymph node interface) [71].

characteristics of CSCs so that they can generate or regenerate the tumor bulk and maintain their self-renewing potential. Intracellular and intercellular signals operate within CSC microenvironments and support CSC activities. The internal signals include molecular pathways that regulate stemness, whereas extracellular signals consist of cells designed to anchor CSCs within the microenvironment, and cell receptors and secreted factors that are necessary for maintaining CSCs in their quiescent state [42].

Signaling pathways are key components in all cells. They stimulate a wide variety of cell processes—from cell growth, proliferation, and differentiation to invasion and apoptosis. Well-known internal signals or pathways that function in normal stem cell niches include the Wnt, Notch, Hedgehog (Hh), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways [42]. Several intracellular signaling pathways may be altered in the process of malignant transformation of stem cells. For example:

1. The evolutionarily conserved *Wnt family* of proteins is cysteine-rich, secreted glycoproteins that control tissue homeostasis, and regulate diverse processes during development. Wnt pathway dysregulation has been identified in several hereditary diseases and is associated with gastrointestinal cancers [43].
2. *The Notch pathway* has crucial roles in stem cell control and cell-fate determination. Research has found that a signature of the Notch pathway is found in CSCs identified patients with poorly differentiated lung adenocarcinoma, and was prognostic for poorer overall survival. By inhibiting the Notch pathway, CSCs were prevented from forming tumors when implanted into mice [44].
3. *The Hedgehog (Hh) protein family members* turn on the genes that regulate the cell cycle and determine cell fate. They are also known to be key regulators of carcinogenesis. Hh and downstream factors have been shown to have significant roles in pancreatic cancer, gastric cancer, glioma, and basal cell carcinoma. Inhibition of the Hh pathway in pancreatic cancer depressed the self-renewal of CSCs and impaired their resistance to chemotherapy [45].
4. *The Hippo pathway* and its related mediators Yes/Yap regulate several tumor suppressor genes to control cellular processes such as survival, proliferation, differentiation, apoptosis, and stem or progenitor cell expansion [46]. Dysregulation of the Hippo pathway has been identified in multiple cancers including liver, lung, colorectal, gastric, ovarian, and prostate [46]. Researchers also found that the expression levels of Yes/Yap genes were prognostic for survival in patients receiving certain types of chemotherapy [46].
5. *NANOG* is a transcription factor involved in the self-renewal and maintenance of pluripotency in normal stem cells. Experimental inhibition of NANOG or related transcription factors has been shown to decrease stem-cell–like activities in breast cancer, colorectal cancer, gastric, prostate cancer, and melanoma [47].
6. *The STAT family* of transcriptional factors cooperates with NANOG to transcribe stemness genes that are required for modulating pluripotency [33]. The STATS are upstream signals activated by interleukin-6 (IL-6).

Activated STAT3 has been found in leukemia, squamous cell carcinoma of the head and neck, multiple myeloma, breast cancer, and prostate cancer. Blocking the STAT3 signaling pathway has been shown to inhibit the clonogenic and tumorigenic potential of CSCs in prostate cancer [26]. In addition, it has been shown that blockade of STAT3 activity inhibits both tumor growth and tumor-initiating potential in colon CSCs [48].

Cancer-associated cells in the microenvironment may secrete growth factors and cytokines to support CSCs. Examples of these include cytokines such as stromal cell-derived factor-1, IL-6, and IL-8, all of which function to regulate CSC activity [49].

During dormancy, micrometastases are somehow able to evolve and acquire a full complement of metastasis-colonization functions that they did not express before. It is difficult to envision how this progression could occur in CSCs (section of micrometastasis) that remain in a state of replicative quiescence. Although CSCs in bone marrow look quiescent, the overall CSCs population is not static. Circulating cancer cells can be detected in blood in the apparent

absence of active metastatic disease. If not in the bone marrow, at least in other tissues, micrometastases may be constantly exiting and re-entering a dormant state, and become familiar with the environment, undergoing further selection for colonization traits during the active interludes. Transition between quiescent and proliferative states is a property of adult stem cells that may be hijacked by CSCs [49].

3.7. Epithelial-mesenchymal transition (EMT): the source of cancer stem-like cells

Elizabeth Hay first described an "epithelial-mesenchymal transformation" [50].

The term "transformation" has been replaced with "transition," pointing to reversibility of the process and the fact that it is different from neoplastic transformation [51].

An epithelial-mesenchymal transition (EMT) is defined as the process that allows a polarized epithelial cell, which normally interacts with basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of extracellular matrix (ECM) components [51].

These processes are consistent with the acquisition of a "cancer stem-like cell" phenotype that is also known as "stemness" or cancer stem cell (CSCs) characteristics [52, 53, 37].

EMTs are encountered in three distinct biological settings that carry very different functional consequences:

1. EMTs associated with implantation, embryo formation, and organ development;
2. EMTs associated with wound healing, tissue regeneration, and organ fibrosis;
3. EMTs associated with cancer progression and metastasis.

While the specific signals that delineate the EMTs in the three discrete settings are not yet clear, it is now well accepted that functional distinctions are apparent.

Pathologists have accepted the hypothesis of EMT in carcinogenesis albeit skeptically.

However, increasing evidence have demonstrated that the process of EMT is vitally important in cancer progression and metastasis, where cancer cells acquire a more invasive and metastatic phenotype [54].

Metastatic cancer cells with a mesenchymal phenotype are believed to undergo reverse transition, i.e., mesenchymal-to-epithelial transition (MET) at the site of metastasis to gain the pathology of their corresponding primary tumors [55].

This process is an important step by which metastatic tumor cells grow at the metastatic site.

Epithelial-mesenchymal transition is associated with carcinogenesis, invasion, metastasis, recurrence, and chemoresistance, which have been shown to be tightly linked with the function

of CSCs. However, the direct relationship between CSCs and EMT in terms of molecular mechanisms remains to be elucidated.

3.8. The cancer stem cells phenomenon and the clinical course of the disease

Gastric cancer is usually diagnosed at later stages. This may be because patients often do not exhibit symptoms until their disease has progressed, or their symptoms have been vague and attributed initially to cause other than cancer.

Dormant CSCs (micrometastases) cannot be detected by current imaging examination methods and are overlooked.

When the primary tumor is treated, whether with preoperative chemotherapy and/or chemoradiation followed by surgery, we observe several phenomena. The primary tumor is often resistant to therapy. We know from our experience that the more resistant the primary tumor is, the more metastatic potential it has. In other words, this aggressive biology, which is probably related to the number of CSCs (and evolved species of CSCs) present in the primary tumor or volume of the tumor, dictates metastatic potential. In addition, although it may appear that local treatment has been successful, highly resistant metastatic disease often becomes apparent very quickly.

We distinguish three patterns of response and resistance observed in patients with advanced and metastatic gastric cancer following first-line therapy (docetaxel, cisplatin, and 5-FU).

A first pattern involves patients with gastric cancer, where there is almost a 50% chance they will experience some reduction in tumor volume and improvement in their symptoms for a short time, but, after a few months, the cancer starts to grow. Second-line therapy produces less reduction in tumor volume and for a shorter duration response. Between these patients, it can be seen that the CSCs population is enriched by cancer treatments, making the tumor more resistant.

A second pattern involves patients whose tumors exhibit primary resistance. These patients never experience tumor shrinkage even with the initial treatment option.

A third resistance pattern is one in which the patient has a mixed treatment response. Metastatic lesions in the liver, for example, will become smaller, while those in abdominal lymph nodes increase in size. This phenomenon is intrapatient tumor heterogeneity. Not only can tumors in different organs exhibit different molecular characteristics, but multiple metastases in the same organ can have different somatic profiles.

While an anti-HER2–targeted therapy is showing efficacy, only about 20% of gastric cancers overexpress HER2 [56]. In a review of first-line therapy in patients with metastatic disease, the inclusion of an anti-HER2–targeted agent provided a modest increase in survival to slightly more than 1 year [56]. Patients receiving anti-HER2 therapy develop resistance immediately after treatment. Recently, the mechanism of acquired resistance to the anti-HER2–targeted agent trastuzumab in gastric cancer has been explored. Treatment of gastric cancer cells for 20 weeks with trastuzumab resulted in epithelial-mesenchymal transition (EMT) induction in drug-resistant cells. This EMT induction was characterized by loss of E-cadherin and ZO1, as well as overexpression of claudin-1, vimentin, β-catenin, ZEB1, Slug, and Snail 22. These drug-resistant cells also exhibited an aggressive tumor phenotype, including higher motility, invasion potential, tumor formation

potential, and metastatic capacity [56]. Furthermore, the drug-resistant cells exhibited other CSC properties, including higher sphere-forming capacity and expression of the CSC markers Oct4, CD133, and CD44 [56]. The increase in CSC potential was accompanied by downregulation of the AKT signaling pathway and upregulation of the STAT3 pathway. The STAT3 pathway was activated by Notch-dependent autocrine secretion of interleukin 6 [56].

These are real problems in the clinic, which are difficult to control. In solving this problem, it is necessary to penetrate into the cellular or molecular basis of gastric cancer and speculate whether different clinical outcomes reflect different CSC populations or molecular characteristics.

Gastric cancer is a heterogeneous disease with diverse molecular characteristics. Multiple experimental and clinical investigations have implicated a wide range of germ line and somatic alterations that drive tumor progression [57]. Recently, the Cancer Genome Atlas Research Network analyzed nearly 300 samples of previously untreated gastric and gastroesophageal cancer and grouped them into four major molecular subtypes [58]:

1. The Epstein-Barr Virus (EBV)–positive group, which made up 9% of gastric cancers. This group displays high prevalence of DNA hypermethylation, including promoter methylation of the tumor suppressor CDKN2A (p16INK4A). There is a high incidence of PIK3CA mutations, amplifications of several oncogenes, including ERBB2, and recurrent amplifications of chromosome p9 (leading to overexpression of PD L1/2 and JAK2) [58].
2. The microsatellite instability (MSI) group, which made up 22% of gastric cancers. This group is characterized by enrichment for microsatellite instability (MSI), including hypermethylation at the MLH1 promoter. The MSI subgroup exhibits mutations in many cancer "hotspots," such as PIK3CA, ERBB3, ERBB2, EGFR, and overexpresses mitotic pathway components [58].
3. The genomically stable subgroup, which made up 20% of gastric cancers. This group exhibited mutations in CDH1 and in RHOA, a protein important in cell motility and the STAT3 signaling pathway [58].
4. The high chromosomal instability (CIN) group, which made up about 50% of gastric cancers. This subgroup is concentrated at the gastroesophageal junction. The CIN group exhibited hyperactivation of EGFR and other RAS-driven receptor tyrosine kinases, mutation of the tumor suppressor TP53, and high levels of aneuploidy. Chromosomal instability has been shown to be prevalent in several solid tumors, including those of the head and neck, testes, lung, and liver, as well as in gastric and gastroesophageal cancers. Fewer CINs are seen in melanoma, and even fewer in Wilms' tumors [59].

3.9. Identifying CSCs

Cancer stem cells in solid tumors were first reported in breast cancer ($CD44^+CD24^-$/low fraction) [60].

The first report of gastrointestinal CSCs was in the $CD133^+CD44^+ALDH1^+$ fraction of colorectal cancer [61].

Subsequently, gastrointestinal CSCs have been detected in cancers of esophagus, stomach, liver, and pancreas [62].

To distinguish CSCs from other cancer cells, researchers have developed profiles of unique cellular markers. These profiles allow detection of CSCs within a tumor and enable the separation of CSCs from nonstem cancer cells for ***research purposes***.

Markers and characteristics of the cancer stem cells:

1. surface markers (e.g., CD24, CD26, CD44, CD90, CD133, and CD166) [63].
2. high aldehyde dehydrogenase (ALDH) activity [63].
3. formation of the spheres when cultured in nonadherent conditions [63].
4. high tumorigenic potential when xenografted into immunocompromised mice.

The existence of CSCs in gastric cancer was first revealed by analyzing a panel of gastric cancer cell lines [64, 65]. Cancer stem cells from either gastric cancer cell lines or resected tumors were isolated using cell surface markers, such as CD44 and epithelial cell adhesion molecule (EpCAM) [65]. Moreover, gastric CSCs can even be isolated from the peripheral blood of gastric cancer patients using CD44 and CD54.

Leucine-rich repeat containing G protein-coupled receptor-5 (Lgr5) is a gastric CSC marker and $Lgr5^+$ stem cells in the stomach could be the origin of gastric CSCs [66]. Patients with gastric cancer containing $Lgr5^+$ cells have a short median survival [66].

Stem cells that express villin exist in the pyloric gland and villin + gastric stem cells might be converted to gastric cancer cells [66]. Kruppel-like factor-4 (KLF4) might play a critical role in gastric cancer initiation and progression in villin + gastric stem cells [66].

In addition, ALDH1, CD90, CD71, and CD133 could be candidate markers of gastric CSCs. MicroRNAs might regulate the properties of gastric CSCs by inducing epithelial-mesenchymal transition [67].

It must be noted, however, that no set of markers are exclusive to CSCs, and also that CSC phenotypes vary over time and between individual patients' tumors of the same subtype. These facts have caused researchers to speculate whether different clinical outcomes reflect different CSC populations [63].

3.10. Treatment of the cancer stem cells

Multiple research findings indicate that conventional therapies, which target the rapidly dividing cells in tumors, have limited efficacy or even adverse effects on CSCs [30] and lead to treatment failure, chemoresistance, and recurrence.

Consequently, two types of cancer therapies targeting CSCs have been investigated: first, to induce and/or maintain dormancy of tumor cells, and second, to induce cell death in residual dormant cancer cells by targeting their markers. Consequently, gastric cancer therapies targeting CSCs have been investigated (**Table 3**) [70].

Target molecules/pathways		Target tumors	Therapeutic agents
Surface markers	CD44	Gastric cancer	Sulfasalazine
Signaling pathways	JAK/STAT signaling	Gastric cancer	Napabucasin (BBI-608), fedratinib, pacritinib
Microenvironment	VEGF/VEGF-R	Gastric cancer	Bevacizumab, cediranib, ziv-aflibercept
Epigenetic system	Histone deacetylases	Gastric cancer	Entinostat, vorinostat, mocetinostat, romidepsin, belinostat, panobinostat
	EZH2 inhibitor	Gastric cancer	Tazemetostat (EPZ-6438)
Others	ABC transporters	Gastric cancer	Zosuquidar, tariquidar, laniquidar
	Immune-mediated antitumor effect, insulin resistance	Gastric cancer	Metformin

JAK, Janus-activated kinase; VEGF-R, VEGF receptor; EZH2, enhancer of zeste homolog 2; ABC, ATP-binding cassette.

Table 3. Target molecules and pathways for gastric cancer stem cells.

3.11. The risks of anticancer stem cells (CSCs) therapy

1. Many markers for CSCs are also found on normal stem cells, which is a disadvantage in terms of their use as therapeutic targets. Thus, the best way to eradicate CSCs is to discover the molecules responsible for the specific properties of CSCs, but not of normal cells, such as variants of stem cell surface markers, such as CD44v8–10 in gastric cancer [68].
2. The second challenge is the need to rethink the use of traditional endpoints of tumor regression in clinical trials. Because CSC-targeting agents do not cause tumor regression, investigators must determine how to demonstrate conclusively that these agents provide a benefit. The circulating tumor cells are highly enriched in stem cell markers in patients. Whereas 1–5% of cells are CSCs in primary cancers, studies have shown that closer to 30–50% of circulating tumor cells express stem cell markers [25]. Circulating tumor cells may prove useful as biomarkers for patients in clinical trials. Isolating and measuring circulating tumor cells may be a way to monitor patients and determine the efficacy of potential treatments [69].

4. Conclusion

Surgical treatment is not able to provide patients with GCLM a complete cure.

Advanced gastric cancer is one of the most difficult challenges in clinical practice. Research has shown that CSCs can initiate tumor development and play a significant role in tumor relapse and metastasis. Indeed, evidence is accumulating that treatments, such as chemotherapy and

radiation, can increase the proliferation of CSCs. Investigations are underway into the molecular signaling pathways involved in tumor cell repopulation. The small subpopulation of CSCs in gastric cancer may be a rational treatment target.

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