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Review article

Gastric carcinogenesis and the cancer stem cell hypothesis

Yoshiro Saikawa, Kazumasa Fukuda, Tsunehiro Takahashi, Rieko Nakamura, Hiroya Takeuchi, and Yuko Kitagawa

Department of Surgery, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Abstract

Normal stem cells (NSCs) are reported to exist in most tissues, including the brain, bone marrow, and probably the gastrointestinal tract. In the latter case, they are thought to possess both the self-renewal capacity and asymmetrical division capacity to generate progenitor cells which differentiate into epithelial cells. NSCs in the normal gastric mucosa are thought to be present in the proliferative zone of the neck/isthmus region, and to undergo a complex bipolar migration from the neck/isthmus region either upward or downward, becoming differentiated normal epithelial cells. NSCs in human gastric mucosa are difficult to identify due to the current lack of a useful marker. A precise definition of cancer stem cells (CSCs) is still under discussion. CSCs are generally defined as malignant cells with NSC capacity. However, many studies of CSCs have demonstrated their rapid growth and high metastatic potential, while NSCs are thought to be slow-growing and self-renewing, and to lack functional capacities such as cell migration and attachment. Recent evidence suggests the existence of CSCs in a wide variety of solid tumors. In this review, we will discuss the existence and cell biology of gastric NSCs and CSCs. We will also discuss whether gastric CSCs originate as organ-specific stem cells or as bone marrow-derived cells (BMDCs). Under certain conditions, the local microenvironment may promote the development of gastric cancer. Thus, *Helicobacter pylori* infection and the accompanying chronic inflammatory processes will supply critical initiators inducing cell growth and the tissue repair response, leading to carcinogenesis. This mechanism will be discussed in light of stem cell research. Progress in stem cell research in the gastric field is still limited to experimental animal models. However, recent studies should enhance our understanding of human cancer biology, and provide novel tools for the treatment of incurable gastric cancer.

Key words Gastric cancer · Cancer stem cell

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Etiology of gastric cancer

At the beginning of 2000, gastric cancer was the second most common cancer worldwide. Although the United States now has one of the lowest rates in the world [1], gastric cancer remains common in China, South America, Eastern Europe, Korea, and Japan. Although it is conventionally accepted that diet and nutrition play a critical role in gastric carcinogenesis, the mechanisms that account for the observed geographic and temporal incidence patterns have not yet been established.

A number of factors are known to suppress or promote gastric cancer [2, 3] (Table 1). These include the protective role of nutrition, particularly the intake of fruit and vegetables; the benefits of vitamin C intake; the protective effects of modern food processing and storage, thereby reducing both spoilage and the use of salt-curing, pickling, and nitrates for preservation; *Helicobacter pylori* infection and interactions with dietary factors; natural carcinogens or precursors such as nitrates in food; the production of carcinogens during the grilling or barbecuing of meats; and carcinogen synthesis from dietary precursors in the stomach. Of the two general types of gastric cancer (intestinal type and diffuse type), the intestinal type is more common and more often distal. In contrast, the diffuse type has a poorer prognosis, tends to occur in younger patients, and can occur anywhere in the stomach, but especially in the cardia [2].

Mucosal changes resulting from various kinds of environmental insult can eventually lead to chronic atrophic gastritis, and then to intestinal metaplasia, a precursor to intestinal-type gastric adenocarcinoma [2]. Various host-related, environmental, and infectious components have been implicated in the etiology of gastric adenocarcinoma. Host-related factors that may lead to increased gastric cancer risk include low serum ferritin levels, pernicious anemia, distal gastrectomy for benign peptic ulcer, Barrett's esophagus, adenomatous polyps,

Table 1. Risk factors for gastric cancer

Definite: surveillance suggested	Possible
Familial adenomatous polyposis	Excess alcohol ingestion
Gastric adenomas	Hamartomas
Gastric biopsy revealing high-grade dysplasia	High intake of salted, pickled, or smoked foods
Definite	Low intake of fruits and vegetables
Chronic atrophic gastritis	Ménétrier's disease
Gastric metaplasia or biopsy	Peutz-Jeghers syndrome
Helicobacter pylori infection	Tobacco smoking
Hereditary nonpolyposis colorectal cancer (Lynch II syndrome)	Questionable
Probable	Benign gastric ulcers
History of subtotal gastrectomy (>20 years)	Fundic gland polyps
Pernicious anemia	Hyperplastic polyps
Tobacco smoking (adenocarcinoma of cardia)	

Studies that have attempted to identify the causes of gastric cancer have elucidated a number of factors that appear to be involved

and hereditary nonpolyposis colorectal cancer (HNPCC) [2, 3]. In terms of environmental causes, food refrigeration has probably reduced the dietary exposure to various carcinogens, such as nitrates and nitrites, by reducing the bacterial and fungal contamination of food. Refrigeration has also reduced the consumption of smoked, cured, and salted foods, thereby reducing the incidence of intestinal-type gastric cancer. Fruits and vegetables are known to be protective against gastric cancer carcinogenesis, while a modest increase in risk has been reported for increased meat consumption, with a positive correlation with longer cooking times. Other environmental factors, such as smoking or industrial dust exposure, may also be associated with gastric cancer. Chronic infections of the stomach by *H. pylori* or Epstein-Barr virus have been reported in epidemiological studies, and a relationship between chronic infection and gastric carcinogenesis has been suggested [4].

The Unitarian hypothesis and cell lineages in the gastrointestinal (GI) tract

The most popular concept of tissue regeneration in the GI tract is the Unitarian hypothesis, in which a single stem cell gives rise to all cell lineages in the epithelium (Fig. 1) [5–9]. Holzer's concept that pluripotentiality can be the property of only a group of cells rather than a single cell has led to the proposal of "committed progenitor cells" [10], but ultimately, all cell lineages might originate from a single cell. Only recently, various investigators have obtained definitive evidence for the existence of such committed progenitors in the intestinal epithelium [8–11]. The main evidence for the Unitarian hypothesis was the finding of radiolabeled debris in all cell lineages in the small-intestinal epithelium after a minimum dose of isotope (tritiated thymidine) that killed only basal crypt cells which were phagocytosed by adjacent surviving stem cells [12]. However, reservations have been raised about the proposal of committed progenitor cells [13], including questions about the selective phagocytic ability of crypt base cells. Moreover, conclusions about the endocrine cell lineage were confined to the finding of nonradioactive debris in a single endocrine cell, perhaps scant basis for such a definitive conclusion. However, all cell lineages are present in colonies of intestinal cells regenerating after irradiation [14]. In addition, mouse fundic stomach mucosa, autotransplanted to subcutaneous tissue, is colonized by a layer of simple immature mucosal cells, which gives rise to parietal, mucous neck, and argyrophil cells, with eventual reconstitution of the gastric mucosa [15]. In the acid-secreting gastric mucosa, classical continuous and flash labeling methods with tritiated thymidine have shown that undifferentiated "granule-free" cells located in the isthmus of the gland are self-renewing and give rise to all lineages in the gastric tubule via three committed precursors — the prepit cells, the preparietal cells, and the preneck cells, with the peptic or zymogenic cell as the end-stage differentiated progeny of the neck cells [7]. A recent review by Wright [11] has suggested that the intestinal stem cell has a repertoire which includes all the main cell lineages within the intestinal crypt systems. In addition, the stem cell can derive reparative lineages in appropriate situations, can produce new crypts, and can even give rise to GI carcinomas [10].

Stem cells and normal epithelial cell lineages in the stomach

The epithelial cell lineages of the stomach undergo constant turnover, with complete self-renewal every 2 to 7 days under normal circumstances, while cell turnover is

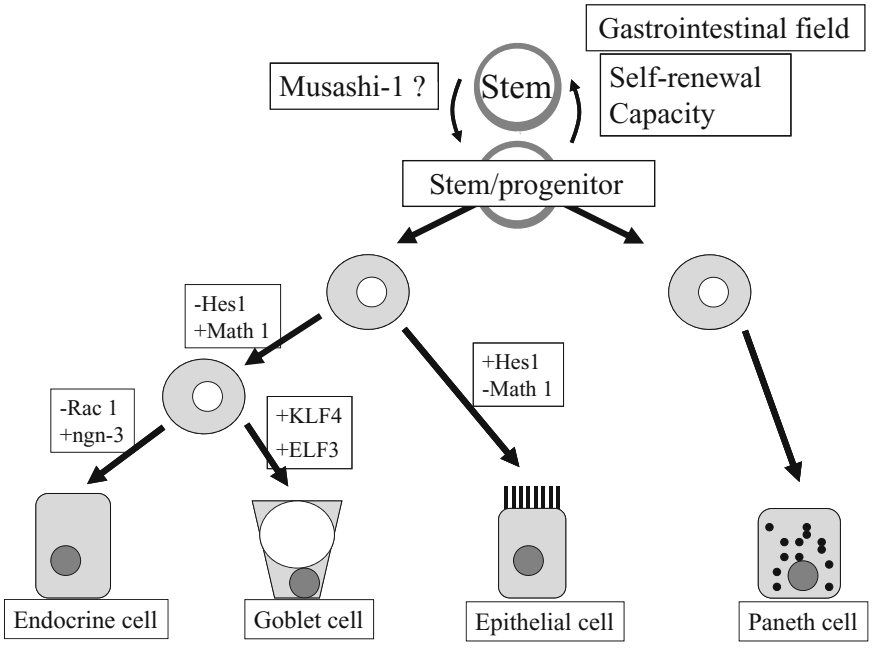

Fig. 1. Theory of normal stem cells in the gastrointestinal (GI) tract. The most popular concept of tissue regeneration in the GI tract is the Unitarian hypothesis, in which a single stem cell gives rise to all cell lineages in the epithelium, and all cell lineages are derived from a stem/progenitor cell in the gastrointestinal epithelium

enhanced by tissue damage [16]. Wright [11] proposed the concept of intestinal stem cells and cell lineages, and the same system can be extended to the gastric mucosa. Thus, it is believed that there is a hierarchical system of proliferating and differentiated cells generated by multipotent stem cells. Those stem cells remain unidentified in a distinct "niche", their highly primitive nature resulting in a lack of any definitive phenotypic or morphological markers. Recent studies of the mouse stomach have demonstrated that three classes of progenitor cells (prepit, preneck, and preparietal cells) are derived from multipotent granule-free cells in the isthmus region [17]. Other studies have demonstrated useful markers for stem cells [18–20]. Several reports suggest that progenitor cells are present in normal human gastric mucosa, and that gastric stem cells are present in the proliferative zone of the neck/isthmus region, giving rise to all the differentiated epithelial cell types [18]. A previous study has demonstrated that Musashi-1 (Msi-1) is a useful marker for mouse intestinal stem cells [19]. Msi-1 has been identified on progenitor cells in gastric mucosa in the neck/isthmus [20] (Fig. 2). In differentiation, cells derived from stem cells undergo a complex bipolar migration from the neck/isthmus region, moving either upward or downward. *Helicobacter pylori* infection modifies the distribution and migration of cells derived from the neck/isthmus region [21].

Table 2. Tumor-initiating cells in several types of solid tumors

Type of tumor	Specific markers
Brain cancer	$CD133^+$ / SP
HNSCC	$CD44^+$
Lung cancer	$CD133^+$ / $Sca\text{-}1^+CD34^+$ / SP
Breast cancer	$CD44^+$ $CD24^{-/low}$ / SP
Gastric cancer	CD44 / SP
Liver cancer	$CD133^+$ / SP
Pancreatic cancer	$CD133^+$ / $CD44^+CD24^+ESA$ / SP
Colon cancer	$CD133^+$ / $EpCAM^{high}CD44^+$ /$\alpha_2\beta_1{}^{hi}$
Prostate cancer	$CD133^+$ / $CD44^+$
Melanoma	$CD20^+$

HNSCC, head and neck squamous cell carcinoma

Definition of cancer stem cells (CSCs)

Interest in gastric cancer stem cells (CSCs) has arisen in the broader context of the CSC hypothesis, which first appeared more than a century ago when European pathologists observed that tumors were composed of a heterogeneous mixture with partially differentiated cell types, similar in many respects to a normal organ [22, 23]. Dick's laboratory group (Lapidot et al. [24] and Bonnet and Dick [25]) first demonstrated the existence of CSCs more than a decade ago, when they proved the CSC hypothesis in human acute myeloid leukemia. The leukemic stem cell, which was defined by the CD34+/CD38– immunophenotype, serially reproduced the disease in nonobese diabetic/severe combined immunodeficiency (SCID) mice. The leukemic CSCs were remarkably long-lived and possessed self-renewal capacity. Thus, the growth of specific tumor cells with defined markers in immunodeficient mice has become the gold standard for identifying CSCs [26] in breast cancer [27], brain cancer [28], prostate cancer [29], melanoma [30], colon cancer [31–33], liver cancer [34],

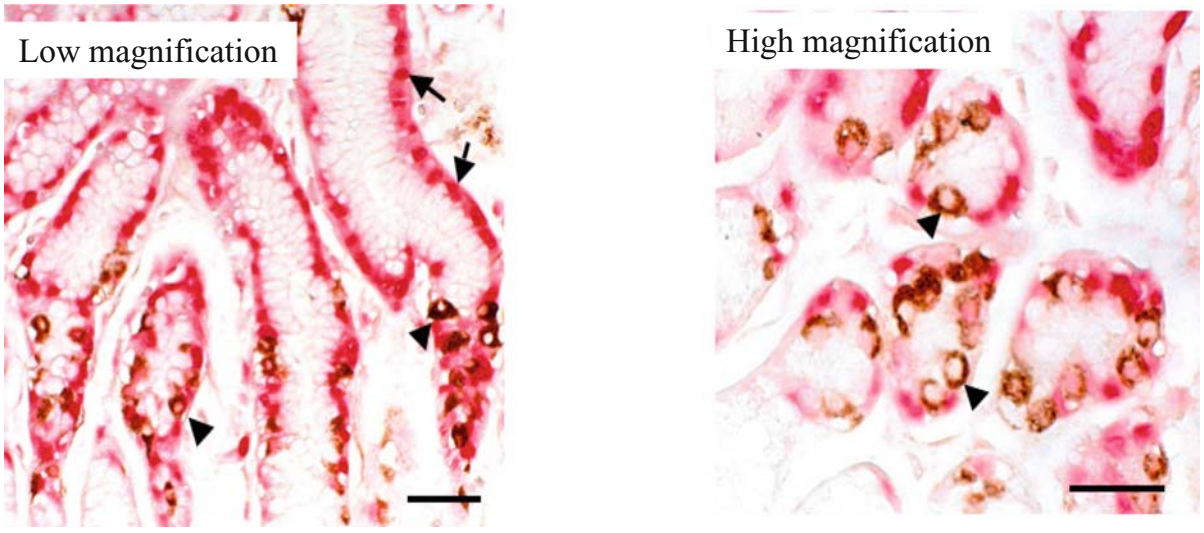

Fig. 2. Stem cells/progenitor cells with Musashi-1 (Msi-1) and proliferating cell nuclear antigen (PCNA) immunohistochemical staining in the adult human antral mucosa. Musashi-1 (Msi-1) is a useful marker of mouse intestinal stem cells. Msi-1 has identified stem/progenitor cells in gastric mucosa; Msi-1+ cells were detected predominantly in the isthmus/neck region (the putative position of stem cells) of the adult antrum on immunohistochemical staining. Msi-1+ cells coexpressed neither PCNA nor Ki 67. Double staining: *brown*, Msi (*arrowheads*); *red*, PCNA (*arrows* in *left panel*) (from reference [20] with permission)

pancreatic cancer [35, 36], and head and neck cancer [37] (Table 2). In such studies, as few as 100 cells of a CSC subpopulation were capable of forming a tumor in immunodeficient mice, far fewer than the number of cells that was required with a non-CSC subpopulation. At a recent American Association for Cancer Research workshop, a working group used the available data to create a consensus definition of CSCs 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" [38]. Accumulating data support the hypothesis that CSCs exist in many solid tumors, though little evidence has been reported for gastric CSCs [21, 39]. Recently, the term "tumor-initiating cells" (TICs) has been adopted in experimental studies instead of "CSCs", indicating the uncertainty associated with the CSC hypothesis (Fig. 3).

Several experimental procedures have been used to identify CSCs or TICs [39–41]. An in vitro method termed "spheroid colony formation," involves growing candidate CSCs in culture dishes specially coated with adhesion molecules in serum-free medium containing only epidermal growth factor and basic fibroblast growth factor. The growth of spherical colonies after a few weeks suggests self-renewal ability consistent with a CSC phenotype. An in vivo method involving the implantation of candidate CSCs under the skin or within organ-specific sites (e.g., orthotopic) of SCID or NOD/SCID/interleukin receptor (ILR)γ null (NOG) mice is generally regarded as the standard method for proving true tumorigenicity and the existence of CSCs (Fig. 4). In a third methodology, flow cytometric isolation of "side population" (SP) cells, first described by Goodell et al. [42], permits the enrichment of cells with stem cell activity and distinctive expression of the ATP-binding cassette (ABC) transporters. SP cells are reportedly enriched in primitive, nondifferentiated cells [43, 44], and recent studies have shown the existence of SP cells in human solid cancers [39, 45–49] (Fig. 5). Many studies have already suggested that the SP fraction is an enriched fraction for TICs.

Using the above-mentioned procedures, investigators have reported the possible existence of CSCs or TICs in solid malignant tumors [40, 41]. Thus, solid cancers can be viewed as differentiated cells, such that only CSCs are able to sustain and propagate the tumors and give rise to invasive lesions and metastases. This new paradigm may enable us to establish novel approaches to cancer therapy targeted against CSCs. Presumably, the eradication of non-CSCs will be somewhat easier [48].

Biological markers of normal stem cells (NSCs) and CSCs (Table 2)

While markers specific for NSCs have been identified in several human tissues, reliable makers for NSCs in

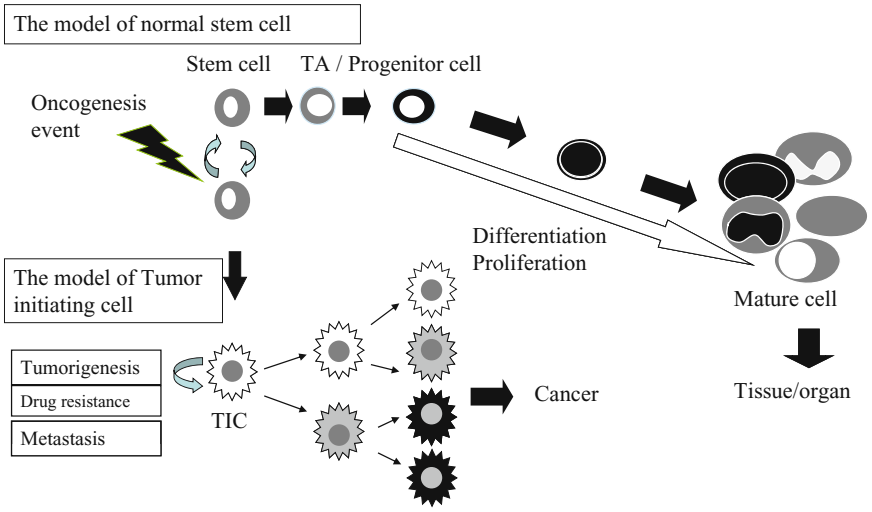

Fig. 3. Hierarchy of tumor-initiating cells (*TIC*s). At a recent American Association for Cancer Research workshop, the latest available data were used to create a consensus definition of cancer stem cells (CSCs) as "cells within a tumor that possess the capacity for self-renewal and that can cause the heterogeneous lineage of cancer cells that constitute the tumor." Tumor-initiating cells (TICs) are now defined rather broadly to take into account uncertainties in the properties of CSCs

the human stomach have not yet been elucidated. As our previous study showed, Msi-1 is a candidate stem cell marker in mouse intestine and human stomach [19, 20], and serves as a marker for progenitor cells in the human stomach. Msi-1+ cells were detected immunohistochemically in the isthmus/neck region (the putative location of stem cells) of the adult antrum. Msi-1+ cells were intermingled with proliferating cell nuclear antigen (PCNA) + cells in the isthmus/neck region of the adult antrum, but did not coexpress PCNA or Ki 67 [20]. Msi-1 expression overlapped partly with the expression of MUC 5 and MUC 6, indicating that Msi-1+ cells retain some features of both foveolar and pyloric gland cell differentiation phenotypes [20], and suggesting that Msi-1 might be a marker of cells with progenitor characteristics before active proliferation in the human antrum.

Takaishi et al. [21] recently identified gastric cancer-initiating cells from a panel of human gastric cancer cell lines, using the cell surface marker CD44. They isolated and analyzed subpopulations of CD44(+) TICs from MKN-45, MKN-74, and NCI-N87 lines, characterizing spheroid colony formation, and tumorigenic ability in the stomach and skin of SCID mice. The study by Takaishi et al. [21] demonstrated that SP cells isolated by flow cytometric sorting did not include TICs. However, our previous study demonstrated that the SP fraction of MKN-45 cells had sphere-forming ability and high tumorigenicity in NOD mice, resistance to anticancer drugs, and an immunophenotype similar to that of stem cells [39]. The inconsistencies between these two studies require further analysis. However, the possibility remains that established gastric cancer cell lines maintain a specific population of TICs or CSCs.

NSCs and CSCs: microenvironmental niches

The microenvironment surrounding stem/progenitor cells in the isthmus/neck region contributes to the maintenance of stem/progenitor cells, the regulation of cell numbers, and the directing of differentiation. Important microenvironmental factors likely include three-dimensional intercellular relationships, vascular/lymphatic factors, cytokines, immune factors, tissue O_2/CO_2 pressure, and stem/progenitor cell dynamics. The microenvironmental stem cell compartment is termed the "niche" and it maintains optimal conditions for cells with stem cell capacity [50–52]. In the GI epithelial stem cell niche, the underlying cells of the mesenchymal lamina propria probably regulate stem cell behavior through secreted basement membrane factors and the paracrine secretion of various growth factors and cytokines, the receptors for which are situated on GI epithelial cells [50]. The detailed properties of the niche in the gastric isthmus/neck region are still under investigation [51].

The mechanism by which the microenvironment of the stem cell niche maintains both stem cell renewal and differentiative capacity is unclear [52–59]. Using GFP-expressing neural stem cells [52] or GFP-expressing bone marrow stem cells (BMSCs) [53], several studies have suggested fusion with embryonic stem (ES) cells was important. However, these phenomena were observed using genetically artificial ES cells in culture, and seemed not to be the same as the in vivo observations of adult stem cell plasticity. These fusion events were too infrequent to account for reported BMSC plasticity in vivo, as only 2–11 hybrid clones were formed per 10^6 BMSCs [53], while 6 weeks after BMSC transplantation, almost 60% of intestinal

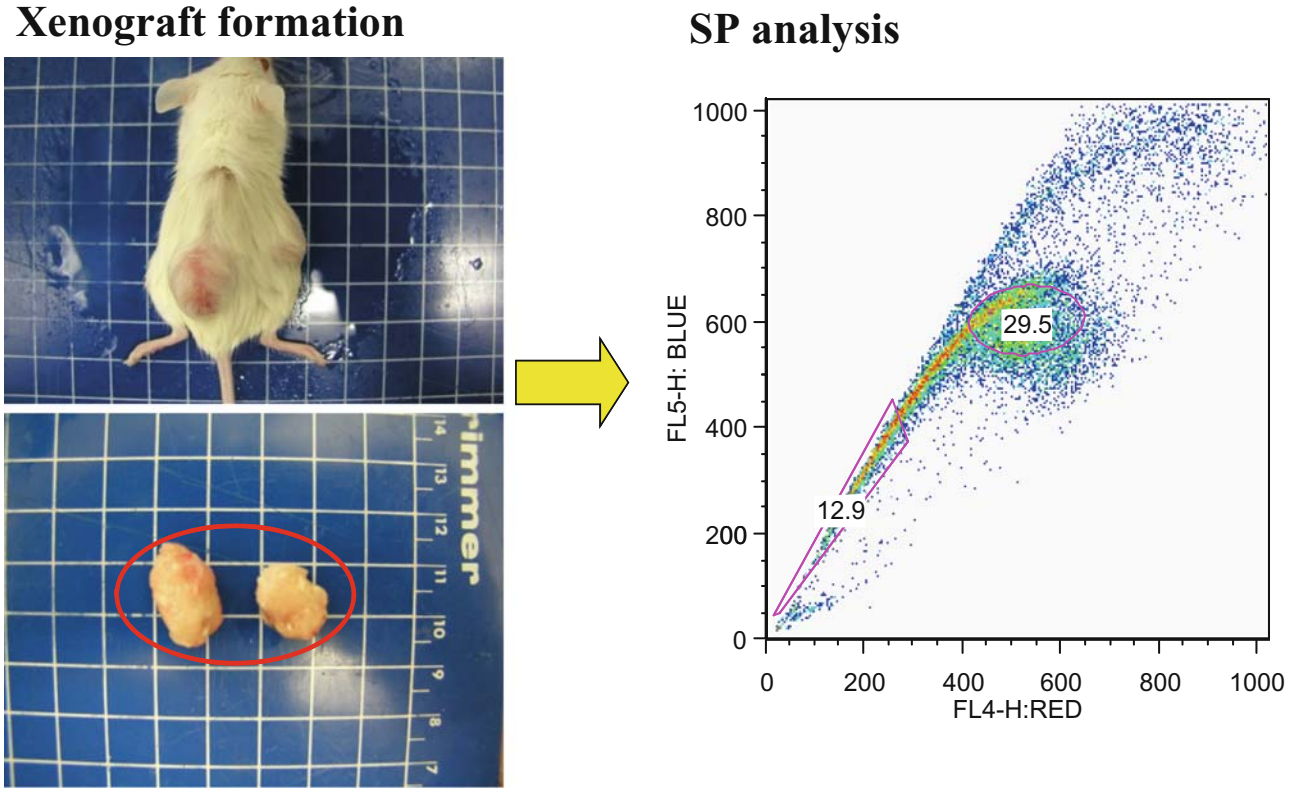

Fig. 4. An in vivo method involving the implantation of candidate CSCs under the skin of NOD/SCID/ILRγ null (NOG) mice suggests tumorigenicity and the possible existence of CSCs (from [39], with permission). *SCID*, Severe combined immunodeficiency; *ILR*, interleukin receptor; *SP*, side population

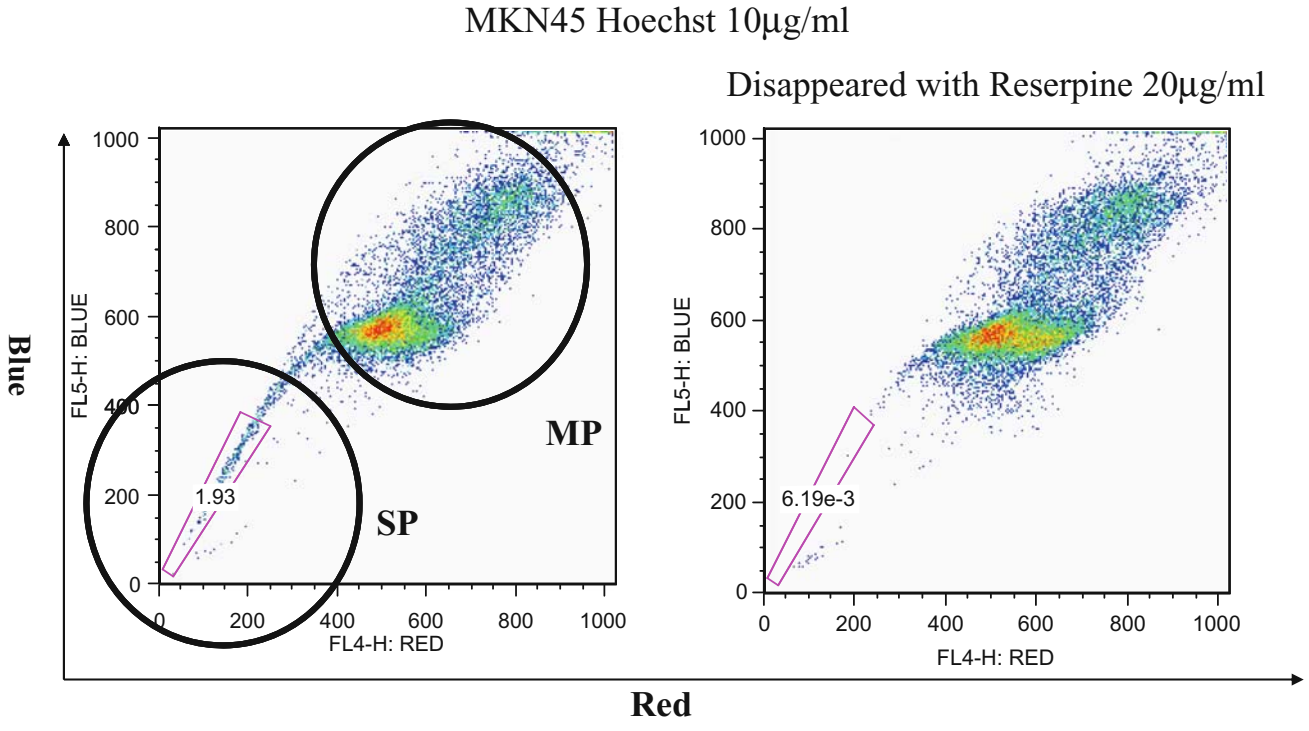

Fig. 5. Side population (SP) analysis using gastric cancer cell lines. The existence of SP cells in human gastric cancer has been confirmed, and many studies suggest that the SP cell fraction derived from a tumor in solid cancers will be enriched for TICs

subepithelial myofibroblasts in the normal mouse colon were derived from the transplanted cells. Thus, the underlying mechanism supporting stem cell renewal and differentiation inside the niche remains a critical question in understanding the NSC's capacity for generating multiple cell lineages in a tissue [55–59]. Complete understanding of such phenomena might greatly assist the development of new treatment modalities for solid malignant tumors.

Contribution of bone marrow stem cells (BMSCs) to normal gastric cell lineages and gastric carcinogenesis

Every tissue consists of normal differentiated cells derived from undifferentiated stem cells. Morphological identification of stem cells in each tissue is difficult due to the paucity of lineage-specific phenotypic markers. Stem cells can upregulate cell turnover in response to increasing regenerative demand evoked by tissue damage or disease. During stem cell production of the entire specialized cell repertoire of the stomach, they must maintain a balance between self-renewal and the rapid differentiation of committed cells, and their proliferation, senescence, and apoptosis.

Certain adult stem cells give rise to multiple cell lineages within foreign tissues, a phenomenon termed "plasticity" or "transdifferentiation". For example, transplanted adult BMSCs contribute to several nonhematopoietic lineages, including epithelial and mesenchymal lineages in the mouse and human GI tract [60–64]. Bone marrow–derived cells (BMDCs) are thought to be the most primitive uncommitted adult stem cells, and they possess differentiative capacity similar to that of BMSCs, possessing a wide range of plasticity and ability to migrate through peripheral organs following inflammation and tissue injury. The differentiative pattern and growth regulation of BMDCs or BMSCs may well depend largely on local environmental signals and cues [62, 65, 66].

These principles have been demonstrated in the fumarylacetoacetate hydrolase (FAH–/–)-deficient mouse, which develops a fatal metabolic liver disease resembling human type I hereditary tyrosinemia. The mouse can be rescued by transplantation of hematopoietic BMSCs, which migrate to the liver and differentiate into FAH-synthesizing hepatocytes [67]. In addition, lethally irradiated female mice injected with a single male hematopoietic BMSC demonstrated donor-derived epithelial cells in the esophagus, stomach, small intestine, and colon 11 months after transplantation [64]. In patients who received a peripheral blood stem cell transplant, epithelial cells of donor origin were observed throughout the GI mucosa [64]. In GI biopsies from female patients who suffered graft versus host disease following a bone marrow transplant from a male donor, transplanted BMSCs contributed to a population of myofibroblast cells, the intestinal subepithelial myofibroblasts (ISEMF). These cells are located in the lamina propria subjacent to the epithelial mucosa and regulate intestinal epithelial cell function via epithelial/mesenchymal interactions. Transplanted BMSCs contribute to ISEMFs in both the mouse and human small intestine and colon, and 6 weeks post-transplant, almost 60% of all ISEMFs in the mouse colon were of donor origin [62]. Transplanted BMSCs also transdifferentiate to form fibroblasts and smooth muscle cells in the lamina propria and mucosal layers, indicating that transplanted adult BMSCs contribute to multiple specialized adult GI mesenchymal lineages in diseased tissue. Additionally, perivascular smooth muscle cells and endothelial cell lineages derived from transplanted cells were observed in newly formed blood vessels in inflamed colons, suggesting a potential role of transplanted BMSCs in vasculogenesis. It is possible that a bone marrow cell might differentiate into a myofibroblast stem cell, and this highlights the potential for bone marrow cells as regulators of epithelial cell function via mesenchymal-epithelial paracrine interactions. These results substantiate the importance of the adult BMSC in the treatment of human inflammatory disease, and suggest that bone marrow cells might contribute to multiple cell lineages in the regeneration of the GI tract. Detailed information regarding tissue regeneration and clonal expansion of BMSCs is currently lacking for the GI tract. Further investigations into the mechanisms of adult stem cell transdifferentiation and the origins of both epithelial and mesenchymal lineages in the GI tract may enhance our understanding of the biological mechanisms of normal GI system function, including the genetic and epigenetic regulation of events via cellular signaling pathways in nonmalignant and malignant diseases.

Houghton et al. [4] demonstrated in 2004 that BMDCs might also represent a potential source of malignant cells in gastric cancer, while epithelial cancers are believed to originate from the transformation of tissue stem cells. Normal BMDCs are frequently recruited to sites of tissue injury and inflammation. Houghton et al. [4] showed that chronic infection of C57BL/6 mice with *Helicobacter* induced repopulation of the stomach with BMDCs, and subsequently these cells progressed through metaplasia and dysplasia to intraepithelial cancer. These findings suggest that epithelial cancers can originate from marrow-derived sources. These data have broad implications for the multistep model of cancer progression [4]. Subsequently, many studies (described below) demonstrated the possible relationship of BMDCs to carcinogenesis [68, 22]. Previous studies showed that bone marrow–derived endothelial

progenitor cells can contribute directly to angiogenesis in tumor formation [67–71]. In addition, BMDCs may indirectly influence carcinogenesis as cancer-associated fibroblasts [72, 73]. Bone marrow–derived human mesenchymal stem cells may increase the metastatic potency of human breast cancer by stimulation of the de novo secretion of the chemokine CCL5 [74]. Houghton et al. speculated that the plasticity of BMDCs might contribute to epithelial cancers, particularly those associated with chronic inflammation [4]. The model of gastric cancer in *Helicobacter felis*-infected C57BL/6 mice represents an ideal system for evaluating the effects of chronic inflammation on BMDC recruitment and engraftment in the stomach. In this model, inflammation is maximal 2 to 3 months after infection, and the inflammation subsequently continues at a moderate level for the remainder of the life of the animal. With persistent *Helicobacter* infection, the gastric mucosa progresses through a series of dramatic changes, including metaplasia and dysplasia, and ends in invasive gastric cancer after 12 to 18 months of infection [75–77]. In this model, chronic inflammation and tissue injury may be associated with BMDC engraftment within the gastric epithelium, and this environment is also strongly linked with the progression of inflammation-associated cancer. BMDCs may also contribute to established cancers through cell mimicry or cell fusion, or they may initiate cancer directly. Recent studies have suggested that cell fusion is a possible mechanism for bone marrow–derived hepatocytes or intestinal cells [78–82]. Interestingly, acute gastric infection with *Helicobacter* species, acute ulceration, or drug-induced parietal cell loss does not lead to the recruitment of BMDCs, while severe chronic inflammation may lead to BMDC-related carcinogenesis. The latter process probably upregulates proinflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor-α (TNFα), and chemokines such as CXCL12 (also known as SDF-1α), contributing to the recruitment of progenitors. Recent studies have also shown that activated myofibroblasts (alpha smooth muscle actin [α-SMA]–expressing stromal cells) could be a source of CXCL12, mediating progenitor recruitment [83, 84]. In addition, in 2008, Oguma et al. [85] demonstrated that TNFα, derived from recruited macrophages, potentiated Wnt/beta-catenin signaling and gastric carcinogenesis by activating Akt signaling and GSK3beta phosphorylation independent of the nuclear factor (NF)-kappaB pathway in activated epithelial cells.

In summary, these observations suggest that *Helicobacter*-mediated chronic inflammation regulates the "penetrance" of initiating oncogenic mutations in the GI tract, leading to GI carcinogenesis.

Signaling pathways regulating the differentiation of NSCs

Recent developments are improving our understanding of the molecular pathways that regulate the proliferation and differentiation of stem cells to mature GI cells. Several major signal transduction pathways such as Wnt/β-catenin, the T-cell factor/lymphocyte enhancer factor (Tcf/LEF) DNA binding protein family, the *Cdx-1* and *Cdx-2* homeobox genes, transforming growth factor (TGF)-β, and Smad, Notch, and sonic hedgehog have already been reported to modulate cell proliferation and differentiation. However, our understanding is incomplete. Below, several pathways are discussed in regard to epithelial cell proliferation and differentiation, in both normal and neoplastic GI mucosa, as determined by the stem cell niche/microenvironment.

The Wnt/β-catenin pathway is a major contributor to malignant transformation, as 85% of human sporadic colorectal tumors are reported to have an *APC* mutation [86]. This *APC* mutation renders the GSK3β/Axin/APC complex incapable of destabilizing β-catenin, thereby leading to an accumulation of nuclear β-catenin/Tcf/LEF complexes, and a subsequent increase in target gene transcription and cell proliferation which can lead to tumor formation [87, 88]. The Wnt protein activates the cytoplasmic phosphoprotein through its receptor "frizzled", causing inhibition of GSK3β and a resultant accumulation of cytosolic β-catenin [89]. The β-catenin then translocates to the nucleus and interacts with the Tcf/LEF family of DNA binding proteins, converting them from transcriptional repressors to activators. This step activates downstream target genes with carcinogenic potential in the GI tract, such as *c-myc*, *tcf-1*, *cyclin D1*, *c-jun*, urokinase-type plasminogen activator receptor, fibronectin, *CD44*, or the matrix metalloproteinase *matrilysin* [87–90]. When the Wnt signal is removed, APC extracts β-catenin from the nucleus, and Tcf/LEF activation is suppressed [91]. The Tcf/LEF DNA binding protein family consists of four proteins: Tcf-1, LEF1, Tcf-3, and Tcf-4. They are essential in establishing the stem cell population within the niche of small-intestinal crypts, and are likely activated by Wnt signaling from the underlying mesenchymal cells which form the stem cell niche [91]. The mammalian homeobox proteins Cdx-1 and Cdx-2 also appear to play an important role in intestinal epithelial stem cell transcriptional regulation [92–96]. The TGF-β family (TGF-β and Smad) and notch signaling may also regulate GI epithelial cell proliferation or differentiation [97–102].

The sonic hedgehog (Shh) pathway provides important signals in gastric cell differentiation. The *SHH* gene encodes a morphogenetic signaling protein with an

important regulatory role in GI development [103]. Gastric epithelial cells in the stomachs of both adult mice and humans express high levels of Shh. This observation has led to proposals that Shh promotes gland cell differentiation with negative regulation of progenitor cell and gland cell proliferation [104, 105]. Mice with a targeted homozygous deletion of Shh fail to develop gastric epithelium, resulting in mucosal defects [106]. Fox1 is thought to be a downstream target of the Shh signaling pathway, as verified by its inhibition by bone morphogenetic protein 4 (BMP4), a member of the TGF-β superfamily [107]. When Shh signaling in mice was blocked with cyclopamine, increased gastric gland cell proliferation and levels of expression of the Shh receptor "patched" (Ptc) were observed. Furthermore, transcriptional targets of Shh (transcription factor *HNF3β* [*FOX2a*], *BMP4*, and islet factor-1 [*ISL1*]) were decreased [103]. In the mouse, Shh, Ptc, HNF3β, and BMP2 are expressed within gastric epithelial cells [104, 105], whereas Fox1 and BMP4 are expressed in myofibroblasts [105, 107], providing further evidence of epithelial-mesenchymal interactions in the regulation of GI cell proliferation and differentiation. The observed negative regulation of epithelial cell proliferation by Shh was unexpected, as Shh generally increases cell proliferation [107]. However, BMPs are known inhibitors of cell proliferation, and the observed increase in proliferation when Shh signaling is blocked with cyclopamine could result from the downstream inhibition of BMP4.

The GATA family of transcription factors is important in the development of many endodermal structures in a variety of organisms. GATA-4 is vital for gastric epithelial morphogenesis, as mice with a targeted homozygous deletion of the *GATA4* gene show defects in gastric gland morphogenesis and fail to form terminally differentiated adult epithelial gastric lineages. The increased expression of Shh in the gastric epithelial cells of these mice indicates that GATA-4 interacts with Shh to regulate epithelial cell proliferation [108].

Clinical significance of stem cell research in the field of solid cancers

Here, we focus on four clinical aspects: (1) recurrent disease and CSCs, (2) chemoresistance of CSCs, (3) prognostic outcome after surgical therapy or chemo-(radio) therapy, and (4) novel therapeutic approaches.

Considering tumor recurrence, even after curative surgery with complete resection of the tumor, as proven by histological investigation, recurrent disease with local or metastatic lesions may be found after surgery. On the contrary, even after noncurative surgery with residual cancer cells, as proven by histological investigation, the treatment may result in a curable outcome without regrowth of the tumor at the lesion. Thus, relapse is likely due to CSCs, which can survive in local or distant organs, forming one or more tumors even after curative surgery. Considering metastasis, the survival of metastasizing epithelial cancer cells is difficult because blood and lymphatic fluid do not supply an appropriate microenvironment. With regard to BMDCs and BMSCs, epithelial cell metastasis may involve a mesenchymal-epithelial transformation of CSCs with self-renewal and differentiation. This would enable CSCs to migrate through the hematogenous or lymphatic system, survive for several years, and regrow at a local site or distant organ.

The treatment of solid tumors is made difficult by CSCs' chemoresistance. Two mechanisms of resistance are recognized: inherent and acquired resistance to anticancer drugs. CSCs are known to overexpress ABC transporter genes such as *MDR1* [109–112] and *BCRP1* [113–115], suggesting that CSCs themselves are more chemoresistant to anticancer drugs than mature cancer cells (Fig. 6). Thus, the chemosensitivity of a tumor cell might be determined by the number of CSCs within the tumor, even before or after exposure to therapeutic drugs.

Following treatment, it is not possible to predict the outcome, as some patients will be cured while others will relapse. Those who achieve complete cure likely had all CSCs eliminated by therapy and/or surgery (Fig. 7). And, even a small population of CSCs might mean cancer recurrence to death, while the mature cancer cells cannot survive in inappropriate microenvironments such as the peritoneum, hematogenous or lymphatic system, or other organs.

According to novel therapeutic approaches, Bao et al. [116] reported that glioblastoma stem cells were generally resistant to radiation therapy, but that such radioresistance could be reversed with a specific inhibitor of Chk1 and Chk2 checkpoint kinases. Piccirillo et al. [117] utilized BMP4, which can trigger a significant reduction in the stem-like, tumor-initiating precursors of human glioblastomas, effectively suppressing tumor growth and reducing mortality in mice after the intracerebral grafting of human glioblastoma cells. These new therapies targeting brain CSCs may be applicable to other CSCs, including gastric cancer.

Conclusion

CSCs are defined as a unique subpopulation in tumors which possesses the ability to initiate tumor growth and sustain self-renewal as well as having metastatic potential. Evidence strongly indicates the existence of

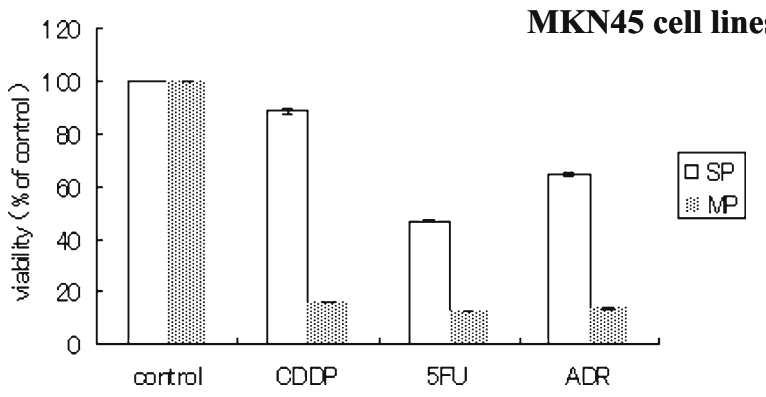

Fig. 6. Resistance to anticancer drugs in SP cells. Our study showed chemoresistance of TICs derived from a human cancer cell line. CSCs are chemoresistant, while mature cancer cells show sensitivity to some anticancer drugs. *CDDP*, Cisplatin; *5FU*, 5-fluorouracil; *ADR*, adriamycin. From [39], with permission

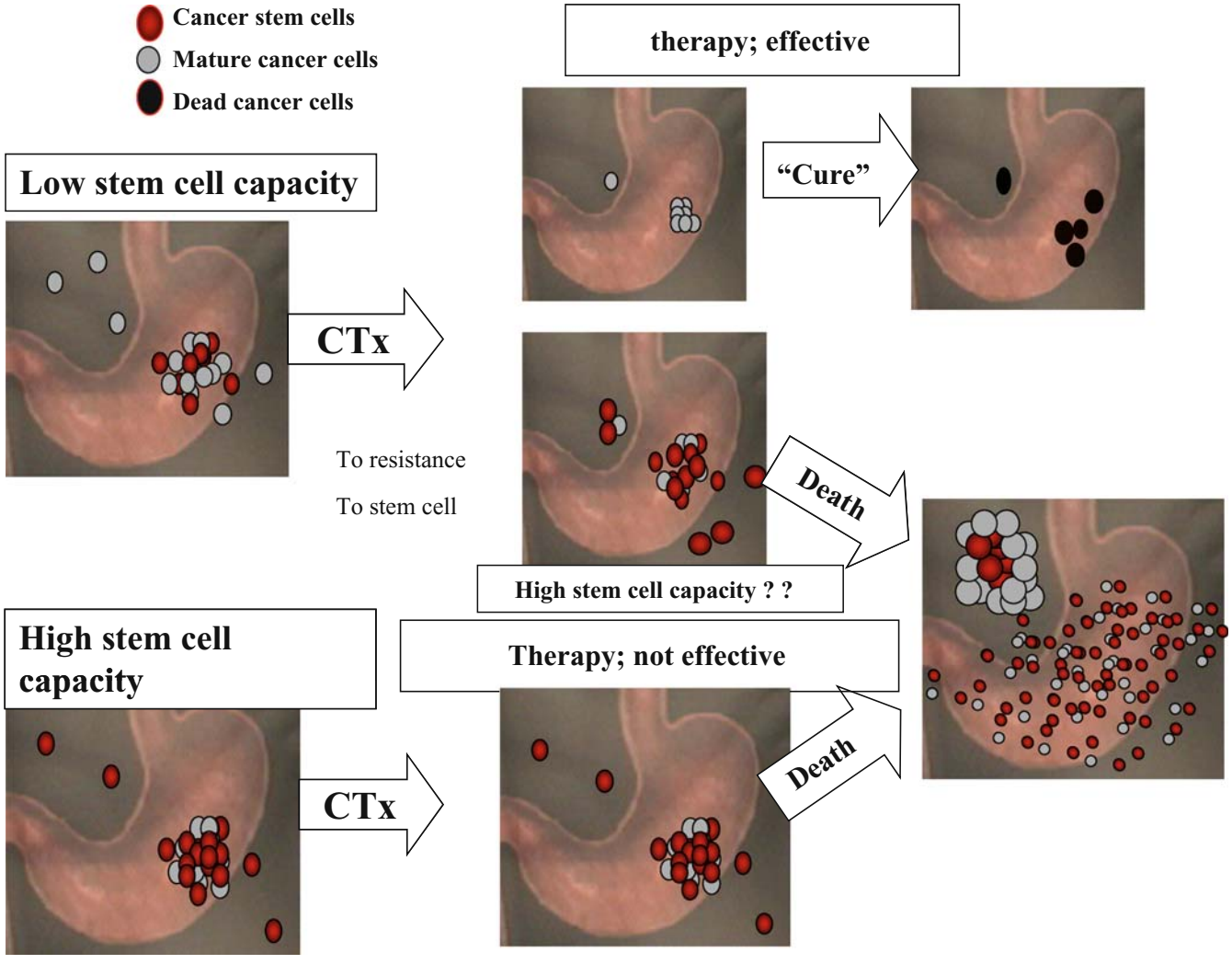

Fig. 7. At the initiation of cancer treatment, the outcome cannot be predicted. CSC theory suggests that curative treatment requires complete elimination of CSCs by means of surgery or chemo-(radio) therapy (*CTx*). Even a small residual population of CSCs greatly increases the chance of relapse. The greater the number of CSCs in a tumor, the greater the difficulty in achieving a cure

CSCs in solid tumors in a wide variety of organs, including gastric cancer. The origin of human gastric CSCs has yet to be elucidated, but data obtained from a mouse model of *Helicobacter*-induced gastric cancer have implicated BMDCs as a potential candidate. These novel findings will lead to new insights in inflammation-associated cancers of every organ, including the hierarchy ranging from CSCs down to mature gastric cancer cells. Although most recent cancer research and therapeutic modalities consider tumors as a mass of comparatively homogeneous cells, it is important to recognize tumor heterogeneity and seek a therapeutic approach for targeting CSCs. Further studies focused on the identification and characterization of CSCs in gastric cancer may lead to novel diagnostic and therapeutic tools, dramatically improving the prognosis of gastric cancer patients.

References

1. Adami HO, Hunter D, Trichopoulos D. A textbook of cancer epidemiology. 1st ed. New York: Oxford University Press; 2002.
2. Bruckner HW, Morris JC, Mansfield P. In: Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. Cancer medicine. 6th ed. Ontario: BC Decker; 2003.
3. Saikawa Y, Kitajima M. In: Bland KI, Sarr MG, Buchler MW, Csendes A, Garden OJ, Wong J, editors. General surgery, principles and international practice. 2nd ed. Berlin New York Heidelberg Tokyo: Springer; 2009. p. 567–76.
4. Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, et al. Gastric cancer originating from bone marrow-derived cells. Science 2004;306:1568–71.
5. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. Am J Anat 1974;141:537–62.
6. Bjerknes M, Cheng H. Clonal analysis of mouse intestinal epithelial progenitors. Gastroenterology 1999;116:7–14.
7. Karam SM, Leblond CP. Dynamics of epithelial cells in the corpus of the mouse stomach. I. Identification of proliferative cell types and pinpointing of the stem cell. Anat Rec 1993; 236:269–79.
8. Brittan M, Wright NA. Stem cell in gastrointestinal structure and neoplastic development Gut 2004;53:899–910.
9. Potten CS, Booth C, Pritchard DM. The intestinal epithelial stem cell: the mucosal governor. Int J Exp Pathol 1997;78: 219–43.
10. Holzer H. Cell lineages, stem cells and the "quantal cell cycle concept". In: Potten CS, Lord BI, Schofield R, editors. Stem cells and tissue homeostasis. Cambridge: Cambridge University Press; 1978. p. 1–27.
11. Wright NA. Epithelial stem cell repertoire in the gut: clues to the origin of cell lineages, proliferative units and cancer. Int J Exp Pathol 2000;81:117–43.
12. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Entero-endocrine cells. Am J Anat 1974;141:503–19.
13. Wright NA, Alison MR. The biology of epithelial cell populations, vol. 2. Oxford: Oxford University Press; 1984.
14. Withers HR, Elkind MM. Microcolony survival assay for cells of mouse intestinal mucosa exposed to irradiation. Int J Radiat Biol 1970;17:261–7.
15. Matsuyama M, Suzuki H. Differentiation of immature mucous cells into parietal, argyrophil and chief cells in stomach grafts. Science 1970;169:385–7.
16. Brittan M, Wright NA. Gastrointestinal stem cells. J Pathol 2002;197:492–509.
17. Karam SM, Li Q, Gordon JI. Gastric epithelial morphogenesis in normal and transgenic mice. Am J Physiol 1997;272: G1209–20.
18. Karam SM, Straiton T, Hassan WM, Leblond CP. Defining epithelial cell progenitors in the human oxyntic mucosa. Stem Cells 2003;21:322–36.
19. Kayahara T, Sawada M, Takaishi S, Fukui H, Seno H, Fukuzawa H, et al. Candidate markers for stem and early progenitor cells, Musashi-1 and Hes1, are expressed in crypt base columnar cells of mouse small intestine. FEBS Lett 2003 30;535:131–5.
20. Akasaka Y, Saikawa Y, Fujita K, Kubota T, Ishikawa Y, Fujimoto A, et al. Expression of a candidate marker for progenitor cells, Musashi-1, in the proliferative regions of human antrum and its decreased expression in intestinal metaplasia. Histopathology 2005;47:348–56.
21. Takaishi S, Okumura T, Tu S, Wang SS, Shibata W, Vigneshwaran R, et al. Identification of gastric cancer stem cells using the cell surface marker CD44. Stem Cells 2009;27:1006–20.
22. Takaishi S, Okumura T, Wang TC. Gastric cancer stem cells. Int J ClinOncol 2008;26:2876–82.
23. Houghton J, Morozov A, Smirnova I, Wang TC. Stem cells and cancer. Semin Cancer Biol 2007;17:191–203.
24. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645–8.
25. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730–7.
26. Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. Annu Rev Cell Dev Biol 2007;23:675–99.
27. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003;100:3983–8.
28. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003;63:5821–8.
29. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 2005;65:10946–51.
30. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res 2005;65:9328–37.
31. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007;445:106–10.
32. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. Nature 2007;445:111–5.
33. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A 2007;104:10158–63.
34. Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroenterology 2007;132:2542–56.
35. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. Cancer Res 2007;67:1030–7.
36. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 2007;1:313–23.

37. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S A 2007;104:973–8.
38. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells: perspectives on current status and future directions — AACR Workshop on Cancer Stem Cells. Cancer Res 2006;66:9339–44.
39. Fukuda K, Saikawa Y, Ohashi M, Kumagai K, Kitajima M, Okano H. Tumor initiating potential of side population cells in human gastric cancer. Int J Oncol 2009;34:1201–7.
40. Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. Nat Rev Cancer 2006;6:425–36.
41. Vargo-Gogola T, Rosen JM. Modelling breast cancer: one size does not fit all. Nat Rev Cancer 2007;7:659–72.
42. Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, et al. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. Nat Med 1997;3:1337–45.
43. Larderet G, Fortunel NO, Vaigot P, Cegalerba M, Maltère P, Zobiri O, et al. Human side population keratinocytes exhibit long-term proliferative potential and a specific gene expression profile and can form a pluristratified epidermis. Stem Cells 2006;24:965–74.
44. Decraene C, Benchaouir R, Dillies MA, Israeli D, Bortoli S, Rochon C, et al. Global transcriptional characterization of SP and MP cells from the myogenic C2C12 cell line: effect of FGF6. Physiol Genomics 2005;23:132–49.
45. Setoguchi T, Taga T, Kondo T. Cancer stem cells persist in many cancer cell lines. Cell Cycle 2004;3:414–5.
46. Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, et al. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian inhibiting substance responsiveness. Proc Natl Acad Sci U S A 2006;103:11154–9.
47. Mitsutake N, Iwao A, Nagai K, Namba H, Ohtsuru A, Saenko V, et al. Characterization of side population in thyroid cancer cell lines: cancer stem-like cells are enriched partly but not exclusively. Endocrinology 2007;148:1797–803.
48. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105–11.
49. Li L, Xie T. Stem cell niche: structure and function. Annu Rev Cell Dev Biol 2005;21:605–31.
50. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. Am J Physiol 1999;277:C183–201.
51. Lin H. The stem-cell niche theory: lessons from flies. Nat Rev Genet 2002;3:931–40.
52. Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. Nature 2001;414:98–104.
53. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. Nature 2002;416:545–8.
54. Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002;416:542–5.
55. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003;422:897–901.
56. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature 2003;422:901–4.
57. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci U S A 2003;100:12313–8.
58. Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM. Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. Proc Natl Acad Sci U S A 2003;100:2088–93.
59. Weimann JM, Johansson CB, Trejo A, Blau HM. Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. Nat Cell Biol 2003;5:959–66.
60. Brittan M. Bone marrow-derived stem cells contribute to multiple cell lineages in experimental colitis. J Pathol 2003;201(Suppl):1A.
61. Brittan M, Hunt T, Jeffery R, Poulsom R, Forbes SJ, Hodivala-Dilke K, et al. Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. Gut 2002;50:752–7.
62. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105:369–77.
63. Korbling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl J Med 2002;346:738–46.
64. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–9.
65. Okamoto R, Yajima T, Yamazaki M, Kanai T, Mukai M, Okamoto S et al. Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. Nat Med 2002;8:1011–7.
66. Avital I, Moreira AL, Klimstra DS, Leversha M, Papadopoulos EB, Brennan M, et al. Donor derived human bone marrow cells contribute to solid organ cancers developing after bone marrow transplantation. Stem Cells 2007;25:2903–9.
67. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;6:1229–34.
68. Dittmar T, Seidel J, Zaenker KS, Niggemann B. Carcinogenesis driven by bone marrow-derived stem cells. Contrib Microbiol 2006;13:156–69.
69. Kopp HG, Ramos CA, Rafii S. Contribution of endothelial progenitors and proangiogenic hematopoietic cells to vascularization of tumor and ischemic tissue. Curr Opin Hematol 2006;13:175–81.
70. Li B, Sharpe EE, Maupin AB, Teleron AA, Pyle AL, Carmeliet P, et al. VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. FASEB J 2006;20:1495–7.
71. Spring H, Schuler T, Arnold B, Hämmerling GJ, Ganss R. Chemokines direct endothelial progenitors into tumor neovessels. Proc Natl Acad Sci U S A 2005;102:18111–6.
72. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG, et al. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 2002;110:341–50.
73. Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, et al. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. Cancer Res 2004;64:8492–5.
74. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 2007;449:557–63.
75. Wang TC, Goldenring JR, Dangler C, Ito S, Mueller A, Jeon WK, et al. Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with Helicobacter felis infection. Gastroenterology 1998;114:675–89.
76. Boivin GP, Washington K, Yang K, Ward JM, Pretlow TP, Russell R, et al. Pathology of mouse models of intestinal cancer: Consensus report and recommendations. Gastroenterology 2003;124:762–77.
77. Cai X, Carlson J, Stoicov C, Li H, Wang TC, Houghton J. *Helicobacter felis* eradication restores normal architecture and inhib-

its gastric cancer progression in C57BL/6 mice. Gastroenterology 2005;128:1937–52.
78. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003;422:897–901.
79. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature 2003;422:901–4.
80. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003;425:968–73.
81. Camargo FD, Finegold M, Goodell MA. Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. J Clin Invest 2004;113:1266–70.
82. Rizvi AZ, Swain JR, Davies PS, Bailey AS, Decker AD, Willenbring H, et al. Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. Proc Natl Acad Sci U S A 2006;103:6321–5.
83. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005; 121:335–48.
84. Li HC, Stoicov C, Rogers AB, Houghton J. Stem cells and cancer: evidence for bone marrow stem cells in epithelial cancers. World J Gastroenterol 2006;12:363–71.
85. Oguma K, Oshima H, Aoki M, Uchio R, Naka K, Nakamura S, et al. Activated macrophages promote Wnt signalling through tumour necrosis factor-alpha in gastric tumour cells. EMBO J 2008;27:1671–81.
86. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159–70.
87. Mei JM, Hord NG, Winterstein DF, Donald SP, Phang JM. Differential expression of prostaglandin endoperoxide H synthase-2 and formation of activated beta-catenin-LEF-1 transcription complex in mouse colonic epithelial cells contrasting in Apc. Carcinogenesis 1999;20:737–40.
88. Wielenga VJ, Smits R, Korinek V, Smit L, Kielman M, Fodde R, et al. Expression of CD44 in Apc and Tcf mutant mice implies regulation by the WNT pathway. Am J Pathol 1999;154: 515–23.
89. Polakis P. The oncogenic activation of beta-catenin. Curr Opin Genet Dev 1999;9:15–21.
90. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. Cell 2000;103:311–20.
91. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, et al. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 1998;19: 379–83.
92. Subramanian V, Meyer B, Evans GS. The murine Cdx1 gene product localises to the proliferative compartment in the developing and regenerating intestinal epithelium. Differentiation 1998;64:11–8.
93. Mizoshita T, Inada K, Tsukamoto T, Kodera Y, Yamamura Y, Hirai T, et al. Expression of Cdx1 and Cdx2 mRNAs and relevance of this expression to differentiation in human gastrointestinal mucosa — with special emphasis on participation in intestinal metaplasia of the human stomach. Gastric Cancer 2001;4:185–91.
94. Mallo GV, Rechreche H, Frigerio JM, Rocha D, Zweibaum A, Lacasa M, et al. Molecular cloning, sequencing and expression of the mRNA encoding human Cdx1 and Cdx2 homeobox. Down-regulation of Cdx1 and Cdx2 mRNA expression during colorectal carcinogenesis. Int J Cancer 1997;74:35–44.
95. Lickert H, Domon C, Huls G, Wehrle C, Duluc I, Clevers H, et al. Wnt/(beta)-catenin signaling regulates the expression of the homeobox gene Cdx1 in embryonic intestine. Development 2000;127:3805–13.
96. Ee HC, Erler T, Bhathal PS, Young GP, James RJ. Cdx-2 homeodomain protein expression in human and rat colorectal adenoma and carcinoma. Am J Pathol 1995;147:586–92.
97. Miyazono K. Transforming growth factor-beta signaling and cancer. Hum Cell 2000;13:97–101.
98. Fiocchi C. TGF-beta/Smad signaling defects in inflammatory bowel disease: mechanisms and possible novel therapies for chronic inflammation. J Clin Invest 2001;108:523–6.
99. Massague J. TGF-beta signal transduction. Annu Rev Biochem 1998;67:753–91.
100. Takaku K, Oshima M, Miyoshi H, Matsui M, Seldin MF, Taketo MM. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. Cell 1998;92:645–56.
101. Yang Q, Bermingham NA, Finegold MJ, Yang Q, Bermingham NA, Finegold MJ. Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. Science 2001;294: 2155–8.
102. van Den Brink GR, de Santa Barbara P, Roberts DJ. Development. Epithelial cell differentiation — a Mather of choice. Science 2001;294:2115–6.
103. Fukuda K, Yasugi S. Versatile roles for sonic hedgehog in gut development. J Gastroenterol 2002;37:239–46.
104. van den Brink GR, Hardwick JC, Tytgat GN, Brink MA, Ten Kate FJ, Van Deventer SJ, et al. Sonic hedgehog regulates gastric gland morphogenesis in man and mouse. Gastroenterology 2001;121:317–28.
105. van den Brink GR, Hardwick JC, Nielsen C, Xu C, ten Kate FJ, Glickman J, et al. Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. Gut 2002;51:628–33.
106. Mahlapuu M, Enerback S, Carlsson P. Haploinsufficiency of the forkhead gene Foxf1, a target for sonic hedgehog signaling, causes lung and foregut malformations. Development 2001;128: 2397–406.
107. Wicking C, Smyth I, Bale A. The hedgehog signalling pathway in tumorigenesis and development. Oncogene 1999;18:7844–51.
108. Jacobsen CM, Narita N, Bielinska M, Syder AJ, Gordon JI, Wilson DB, et al. Genetic mosaic analysis reveals that GATA-4 is required for proper differentiation of mouse gastric epithelium. Dev Biol 2002;241:34–46.
109. Cowie FJ, Pritchard-Jones K, Renshaw J, Pinkerton CR. Multidrug resistance modulation in rhabdomyosarcoma and neuroblastoma cell lines. Int J Oncol 1998;12:1143–9.
110. Haber M, Bordow SB, Haber PS, Marshall GM, Stewart BW, Norris MD. The prognostic value of MDR1 gene expression in primary untreated neuroblastoma. Eur J Cancer 1997;33: 2031–6.
111. Haraguchi N, Utsunomiya T, Inoue H, Tanaka F, Mimori K, Barnard GF, et al. Characterization of a side population of cancer cells from human gastrointestinal system. Stem Cells 2006;24:506–13.
112. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA 1998; 95:15665–70.
113. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 1998;58:5337–9.
114. Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59: 8–13.
115. Kawabata S, Oka M, Soda H, Shiozawa K, Nakatomi K, Tsurutani J, et al. Expression and functional analyses of breast cancer resistance protein in lung cancer. Clin Cancer Res 2003;9: 3052–7.

116. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444: 756–60.

117. Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 2006;444:761–5.