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Review

Review

Identifying therapeutic targets in gastric cancer: the current status and future direction

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Abstract

Gastric cancer is the third leading cause of cancer-related death worldwide. Our basic understanding of gastric cancer biology falls behind that of many other cancer types. Current standard treatment options for gastric cancer have not changed for the last 20 years. Thus, there is an urgent need to establish novel strategies to treat this deadly cancer. Successful clinical trials with Gleevec in CML and gastrointestinal stromal tumors have set up an example for targeted therapy of cancer. In this review, we will summarize major progress in classification, therapeutic options of gastric cancer. We will also discuss molecular mechanisms for drug resistance in gastric cancer. In addition, we will attempt to propose potential future directions in gastric cancer biology and drug targets.

Key words: gastric cancer, targeted therapy, cancer stem cell, Wnt, hedgehog

Introduction

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-related deaths [1]. According to GLOBOCAN 2012, an estimated 951,600 new gastric cancer cases and 723,100 deaths occurred in 2012 [2]. In general, 70% of these cases occurred in Eastern Asia (especially in Korea, Mongolia, Japan, and China), whereas the incidence rates are low in Northern American and most parts of Africa [2].

Gastric cancer is a solid tumor, with complex genetic and environment interactions involved [3]. Clinically, the preferred means of therapy is surgical resection with total or partial gastrectomy depending on the size and location of the primary tumor. Chemotherapeutic interventions have been carried out in the neoadjuvant, adjuvant, or primary treatment options [4]. Bernards *et al.* [5] reported from 4797 cases of non-cardia gastric cancer patients in south Netherlands from 1990 to 2011 that the incidence of metastatic patients was actually increased from 24% in 1990 to 44% in 2011. Despite an increased proportion of palliative chemotherapy accepted from 5% to 36%, the median survival remained between 15 and 17 months ($P = 0.1$). While some patients initially respond to chemotherapy, almost all patients with advanced gastric cancer eventually relapse. Therefore, chemotherapy drug resistance becomes a major barrier to achieve effective gastric cancer treatment.

In this review, we will summarize recent progress on gastric cancer classification (pathological and molecular classification) and treatment options. We will also discuss possible molecular mechanisms responsible for drug resistance in gastric cancer.

Pathological Classification of Gastric Cancer

Common used pathological classification of gastric cancer includes Borrmann, Lauren, and WHO classification, based on tissue morphology and cell biology features. As early as 1923, German pathologist Dr Borrmann proposed a general form of gastric cancer typing [6], based on morphological characteristics in mucosal surface and invasiveness. According to Borrmann classification, gastric cancer is divided into four types: type I (nodular type), type II (ulcer localized), type III (infiltration ulcer), and type IV (diffuse infiltrative type, also known as 'gastric linitis plastica').

In 1965, Dr Lauren divided gastric cancer into intestinal, diffuse, and mixed subtypes, later known as the Lauren classification [7]. The

intestinal subtype of gastric cancer has glandular morphology, and is more commonly associated with inflammation and salty diets as a high-risk factor. It is believed that the intestinal type of gastric cancer is developed from a cascade of morphological changes including gastritis, metaplasia, and dysplasia, while diffuse subtype has no regional differences in occurrence [8]. The prognosis of the intestinal subtype is better than the diffuse subtype.

In 1990, a consensus for gastric cancer classification was established by WHO [9]. In this classification, gastric cancer is divided into gastric epithelial tumors and carcinoid tumors, and epithelial tumors included gastric adenocarcinoma (papillary adenocarcinoma, tubular adenocarcinoma, poorly differentiated adenocarcinoma, mucinous adenocarcinoma, and signet ring cell carcinoma), adenosquamous carcinoma, undifferentiated carcinoma, and uncommon carcinomas. In 2010, WHO classification further divided gastric cancer into four subtypes: tubular, papillary, mucinous, and poorly cohesive gastric cancer (including signet ring cell carcinoma) [10].

Molecular Classification of Gastric Cancer

Although pathological classification has been commonly used in the clinic for many years, their use in clinical management for gastric cancer is not significant. Thus, there is an urgent need to find a new classification for gastric cancer for better clinical management of this deadly disease.

Based on gene expression pattern, Cancer Genome Atlas Research Network [11] divided 37 gastric cancer cell lines into Genomic intestinal type (G-INT) and Genomic diffuse type (G-DIF) in 2011. The G-INT cell lines were more sensitive to 5-fluorouracil (5-Fu) and oxaliplatin, while the G-DIF cell lines were more sensitive to cisplatin. Further studies have validated this classification with 521 cases of gastric cancer samples. The G-INT type of gastric cancer is found to have a significantly better prognosis.

Further, based on the comparison of gene expression patterns among 248 gastric cancers, Dr Lei's group divided gastric cancer into proliferative, metabolic, and mesenchymal subtypes in 2013 [12]. The proliferative subtype is characterized with high levels of genomic instability, TP53 mutations, and DNA hypomethylation. The metabolic subtype cancers are more sensitive to 5-Fu, with better prognosis after treatment. The mesenchymal subtype contains cells with cancer stem-cell features, and is more sensitive to PI3K–AKT–mTOR inhibitors. These studies clearly help guiding individualized treatment of gastric cancer.

In 2014, as part of The Cancer Genome Atlas (TCGA) project, a comprehensive molecular classification of gastric cancer was proposed by the TCGA research network [11]. Tissues and blood samples of 295 primary gastric cancers who had not received chemotherapy were collected and performed with single nucleotide polymorphism array, somatic copy-number analysis, whole-exome sequencing, mRNA sequencing, miRNA sequencing, array-based DNA methylation profiling, and reverse-phase protein array. By calculating and integrating a large number of data, a new molecular classification divides gastric cancer into four subtypes: Epstein–Barr virus (EBV) positive subtype, characterized by recurrent *PIK3CA* mutations, extreme DNA hypermethylation, and amplification of *JAK2*, *CD274*, and *PDCD1LG2*; microsatellite unstable subtype, which displays elevated mutation rates, including mutations of genes encoding targetable oncogenic signaling proteins; genomically stable subtype, enriched for the diffuse histological variant and mutations of *RHOA* or fusions involving RHO-family GTPase-activating proteins; and chromosomal instability subtype, marked with aneuploidy and focal amplification of receptor tyrosine kinases (RTKs). These molecular subtypes exhibit specific genomic features, which will facilitate the development of clinical trials to explore therapies in defined sets of gastric cancer patients and improve survival ultimately. While the link of this molecular classification to clinical treatment and patient outcomes remains to be seen, the view of gastric cancer as a single disease has already been challenged.

Clinically, gastric cancer can be divided into four stages [13], which are closely associated with patient outcomes and widely used for patient treatment options. Stage I disease has tumors mostly within the stomach mucosa (IA has no lymph node spread whereas IB has one to two lymph node spread in stomach). In Stage II disease, the tumor may grow into outer covering of stomach (serosa layer) or has more than three lymph node spreads. In Stage III disease, tumor has grown in the serosa layer and has lymph nodes near the stomach, but all tumors are still within the stomach. In Stage IV disease, the tumor has spread into distal organs such as lung and liver. Patients with Stage IV disease are not curable through surgery, and are mostly treated with chemotherapy with or without radiation therapy.

Gastric Cancer Chemotherapy

Complete surgical resection remains the only curative therapy for early gastric cancer, while perioperative and adjuvant chemotherapy, considered as multimodality treatment, can improve the survival of gastric cancer [14,15]. Chemotherapy has been used during the past three decades, and 5-Fu, cisplatin, and epirubicin continue to serve as the first-line therapeutics according to NCCN guidelines [16]. Nevertheless, novel chemotherapeutic agents, including taxanes (docetaxel and paclitaxel) and oral fluoropyrimidines (capecitabine and S-1), as well as oxaliplatin and irinotecan were emerging in recent years [17].

5-Fu is an analog of uracil with a fluorine atom substituted at the carbon-5 position of the pyrimidine ring in place of hydrogen. It fulfilled the expectations of biochemical, pharmacologic, and clinical activity of anticancer drugs. The 5-fluorinated pyrimidines, synthesis firstly by Heidelberger *et al.* [18], have become useful in the treatment of human solid tumors, including breast, gastric, colorectal, pancreatic cancers, and squamous cell carcinomas arising in the head and neck [19]. There are at least four primary mechanisms of action for 5-Fu. First, incorporation of fluorouridine triphosphate into RNA interferes with RNA synthesis and function; secondly, fluorodeoxyuridine monophosphate inhibits thymidylate synthase, leading to the depletion of thymidine 5′ monophosphate and thymidine 5′ triphosphate and the accumulation of deoxyuridine monophosphate and deoxyuridine triphosphate; thirdly, incorporation of fluorodeoxyuridine triphosphate and deoxyuridine triphosphate into DNA may affect DNA replication and stability; furthermore, genotoxic stress caused by 5-Fu can trigger programmed cell death pathways [20,21]. Capecitabine is an orally administered pro-drug of 5-Fu, which is absorbed through the gastrointestinal tract as an intact molecular and formatted to 5-Fu by cascade reaction [22]. S-1, another kind of oral 5-Fu, has demonstrated antitumor activity against gastric cancer when used either as a single agent or in combination with other chemotherapies. S-1 monotherapy has been adopted as the standard chemotherapy regimen for inoperable and recurrent gastric cancer in East Asian countries, especially in Japan [23]. The combinations of S-1 with other cytotoxic drugs have been found to be promising, with response rates of 40% and higher and relatively favorable safety profiles [24].

Cisplatin (*cis*-diammine-dichloro-platinum) was first used as an anticancer drug in the 1960s, which opened a new era in cancer

treatment [25]. The biological actions of cisplatin are due to displacement reactions, which cause cisplatin to become stably bound to DNA, RNA, proteins, and other biomolecules. Cisplatin-induced DNA damage can cause a series of cellular defects, including inhibition of DNA synthesis, suppression of RNA transcription, effects on the cell cycle, and induce the apoptosis. The detailed mechanisms are described as follows: (i) cisplatin covalently binds to DNA to form DNA–protein and DNA–DNA inter-strand and intra-strand cross-links and disrupts DNA function; (ii) cisplatin-induced DNA damage activates cell cycle checkpoints which results in cell cycle arrest; (iii) cisplatin-induced DNA damage causes an activation of P53 and MAPK pathway; and (iv) P53 promotes apoptosis by inhibition of anti-apoptic Bcl-2 and consequent caspase activation [26,27]. Beside cisplatin, multiple platinum derivatives are used in clinical chemotherapy. Among them, carboplatin and oxaliplatin have received worldwide approval for clinical use [28].

Epirubicin is another synthase potent anticancer agent, displaying clinical activity against a wide variety of solid tumors. It exerts anti-proliferation and cytotoxic activity in cancer cells [29]. Epirubicin forms a complex with DNA by intercalation of its planar rings between nucleotide base pairs, with consequent inhibition of nucleic acid (DNA and RNA) and protein synthesis. Such intercalation triggers DNA cleavage by topoisomerase II, resulting in cytocidal activity. Epirubicin also inhibits DNA helicase activity, preventing the enzymatic separation of double-stranded DNA, and interfering with replication and transcription. Epirubicin is also involved in oxidation/reduction reactions by generating cytotoxic free radicals [30].

The taxanes (paclitaxel and docetaxel) represent a novel class of antineoplastic agents that interfere with microtubule function leading to altered mitosis and cellular death. Paclitaxel is originally extracted from a yew tree, docetaxel is a semisynthetic analog of paclitaxel that differs at two positions in its chemical structure and this small alteration makes it more water-soluble [31]. Several studies have focused on the use of taxanes in advanced gastric cancer as a single agent or in combination. V325 study reported that the combination of docetaxel, cisplatin, and 5-Fu was shown to significantly improve the time to progression, the survival time and the response rate in untreated advanced gastric cancer patients compared with cisplatin and 5-Fu treatment [32]. Further study demonstrated that paclitaxel plus 5-Fu and docetaxel plus 5-Fu appear to have similar efficacy against advanced or recurrent gastric cancer [33].

Gastric Cancer Target Therapy

Improving molecular characterization in gastric cancer may provide better treatment targets in selecting patients for specific treatment options. Some potential biomarkers are pending clinical validation, such as HER-2 (human epidermal growth factor receptor-2), MET (mesenchymal-epithelial transition factor), and FGFR-2 (fibroblast growth factor receptor-2) [34]. Furthermore, the application of chemotherapy with novel targeted agents plays an important role for the multimodal management of gastric cancer.

At present, HER-2 is the only predictive biomarker for gastric cancer responsiveness to targeted agents [34]. HER-2, a transmembrane tyrosine kinase receptor encoded by the *ErbB2* gene, is a member of the HER family. HER-2 overexpression is observed in 7%–34% of gastric cancer samples [35,36]. Based on the Phase III trial using trastuzumab for gastric cancer (ToGA), patients with HER-2 overexpression in the tumor have better overall survival compared with the chemotherapy alone group (13.8 months vs. 11.1 months) [37].

Currently besides HER-2, targeting approaches to MET and FGFR-2 are most used in clinically advanced cancers [38]. MET is activated by hepatocyte growth factor (HGF). In gastric cancer, both MET and its ligand HGF are overexpressed [39]. MET interaction with multiple signaling pathways involved in tumor growth, invasion, and metastasis [40]. MET inhibitor, ribotumumab, is a monoclonal antibody that binds to and neutralizes HGF, preventing the binding of HGF to MET. A Phase II clinical trial showed that patients with high MET expression have improved median overall survival when treated with rilotumumab plus chemotherapy when compared with chemotherapy plus placebo (11.1 months vs. 5.7 months) [41]. FGFR-2 that belongs to the RTK superfamily regulates cell proliferation, differentiation, and motility. Its frequent gene amplification is linked to tumor formation in gastric cancer [42]. Dovitinib is one of anti-FGFR2 therapy drugs and can inhibit cell growth in *FGFR2*-amplified gastric cancer cell lines and xenografts [43,44].

Drug Resistant Mechanisms

Overall chemotherapy drugs can extend the lifespan of gastric cancer patients. However, the benefit is limited because most cancer cells eventually become irresponsive to chemotherapeutic drugs. Several mechanisms are involved in chemotherapy resistance (**Fig. 1**). Such mechanisms include decreased intracellular drug accumulation and/or increased drug efflux, increased nucleotide excision-repair activity, evasion of apoptosis, and some signaling pathways activation. The crosstalk between tumor and the tumor microenvironment (TME) and the presence of cancer stem-cell population may also be responsible for chemotherapy resistance.

Drug transportation and metabolism

The effect of the drug efflux plays an important role in chemotherapy resistance [45]. There is a list of membrane transport proteins, such as

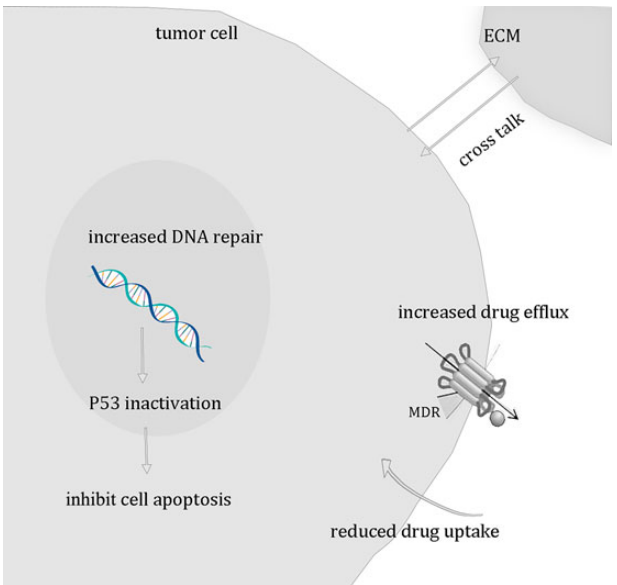

Figure 1. Current known mechanisms for chemotherapy resistance in gastric cancer Currently, the molecular basis underlying chemotherapy resistance in gastric cancer remains largely elusive. There are several known mechanisms reported in gastric cancer, such as defects in molecule transport, apoptosis regulation, and TME. Because *P53* gene mutation is very common in gastric cancer, P53-mediated apoptosis is not functional, which may play a role in drug resistance via defective apoptosis.

multidrug resistance protein, multidrug resistance-associated protein 1, breast cancer resistance protein, and so on [46,47]. These proteins can transport chemotherapy drugs out of cells and prevent them to work inside cells. Of these, ABC transporters may play a more significant role [48]. Menon and Povirk [49] found that ABC transporters in a group of NCI60 cells showed that more than half of the members of ABC transporters were linked with drug resistance.

The abnormal activation and inactivation of drugs are also important. This process may depend on the different types of drugs. The inactivation of platinum is related with sulfur-contained glutathione [25]. The 5-Fu cannot be metabolized into an active ingredient in the absence of appropriate intracellular enzymes. Oral capecitabine only works when it is metabolized to 5-Fu by thymidine phosphorylase, however, methylation of this enzyme-encoding gene can lead to drug resistance [50,51].

Repair of DNA damage

Chemotherapeutic drugs can induce DNA damage either directly or indirectly. DNA damage can induce cell cycle arrest, allowing the damaged cells to repair. Because of the mutations of oncogenes or tumor suppressor genes, some tumor cells can affect cell cycle arrest. In the presence of wild type p53, DNA damages trigger cell cycle arrest [49]. Mismatch repair (MMR) system is the key to maintain the integrity of the genome [52]. *MLH1* and *MSH2* mutations lead to microsatellite instability, while the absence of MMR is related with multiple chemotherapy drugs. High methylation of *MLH1* leads to cisplatin resistance [53]. Topoisomerase II is a critical enzyme that is involved in DNA replication and repair. Reduced topoisomerase II expression or function can contribute to resistance to agents [54].

Inhibition of apoptosis

Drug resistance can also be a result of failed apoptosis following DNA damages or other cellular injuries. Alterations of survivin and XIAP, which regulate apoptosis, promote resistance to chemotherapy drugs [55]. P53 can eliminate the damaged cells by promoting apoptosis through the induction of pro-apoptotic genes, such as *FAS* and *Bax*, and the down-regulation of anti-apoptotic *Bcl2* [56]. Apoptotic inhibitors directly or indirectly impact the activities of caspases, which are the direct effectors of apoptosis. For cisplatin, caspase-3, -8, and -9 are critical, and their activation is attenuated in resistant cells [57]. The inhibition of caspase-3 and caspase-8 activation may be due to down-regulation of the apoptosis pathway owing to the lack of Fas signal following cisplatin treatment [58].

PI3K/MAPK signaling activation

PI3K/Akt signaling pathway, involved in regulating cell growth, differentiation, migration, and development, is frequently activation in gastric cancer [59]. Amplification of PIK3CA is commonly detected and is associated with a poor prognosis in gastric cancer [60]. Persistent PI3K signaling is a significant component of acquired resistance to upstream inhibitors [61]. Studies performed *in vitro* and *in vivo* using small molecule inhibitors of the PI3K/Akt pathway together with standard chemotherapy have been successful in attenuating chemotherapeutic resistance [62]. Yokoyama *et al.* [63] reported that elevated Akt expression and Akt phosphorylation are detected in gastric cancer, and pretreatment of BGC-823 and SGC-7901 cells with wortmannin, a PI3K inhibitor, blocks Akt phosphorylation and attenuates resistance to etoposide and doxorubicin [64].

Activated Ras/MAPK signaling exists in many types of cancer. When activated, the Ras/MAPK pathway contributes to post-translational modification of p53 [65]. The Ras/MAPK pathway also results in activation of other transcription factors, such as c-Myc, c-Fos, and c-Jun [66]. In recent years, many studies suggest that MAPK signaling is implicated in the response of tumor cells to chemotherapeutic drugs, and the mechanism may be through regulation of resistance-associated gene and protein expression. Thus, inhibiting Ras/MAPK signaling may sensitize tumor cells to chemotherapy [63,67].

Tumor microenvironment

TME is consisted of extracellular matrix, cancer-associated fibroblasts, immune and inflammatory cells, and vascular cells. TME not only provides a refuge for tumor cells to escape from chemotherapy drugs, but also provides the conditions evading from apoptosis and emerging secondary resistance [68]. TME facilitates the development of drug resistance by intercellular and cell-ECM adhesion, cell communication, mechanical alterations, and phenotypic transitions [69]. Integrins, cell surface adhesion molecules, play a role in connecting cells and the ECM. Integrin-mediated ECM adhesion could alter cellular response to chemotherapy drugs. The expression levels of β1-integrin are a predictor for trastuzumab to treat HER-2 positive gastric cancer patients [70]. Inhibitors of IL-6 and MMP-1 can affect doxorubicin treatment efficiency.

Cancer stem cells

Accumulating evidence in recent years strongly indicates that cancer stem cells may be an important mechanism of drug resistance. Cancer stem cells are naturally resistant to chemotherapy through their quiescence, their capacity for DNA repair, and ABC-transporter expression based on the tumor-stem-cell concept [71]. Existing anticancer drugs are mostly focused on tumor cells, but not cancer stem cells, which can differentiate into new tumor cells and result in tumor recurrence, leading to resistance to chemotherapy. Cancer stem cells with tumor-initiating capability can be identified by expression of a distinct set of marker proteins, such as the ABC family transporter ABCG2, CD133, CD24, CD44, epithelial cell adhesion molecule, or aldehyde dehygrogenase (ALDH) [72]. Some critical signaling pathways, including hedgehog, Wnt, and Notch, may regulate cancer stem cells in chemotherapy resistance (**Fig. 2**). In gastric cancer, ALDH high cells have stronger resistance to 5-Fu and cisplatin; further, Notch1 and Sonic hedgehog expression are also increased in ALDH high cells [73]. Druker *et al.* [74] isolated tumor sphere cells from gastric cancer cell lines that showed an increased chemotherapy resistance, expressed CSC-related markers (CD44, CD24, and CD133), and had higher tumorigenic capacity *in vivo*. Hedgehog signaling was activated in the tumor sphere cells, and blocking hedgehog pathway with cyclopamine strongly enhanced the sensitivity to chemotherapeutic drugs in tumor sphere cells [75]. CD44(+) cells sorted from gastric cancer cell lines that were resistant to 5-Fu and cisplatin, had significantly more malignant properties, and had up-regulation of hedgehog pathway proteins. Moreover, hedgehog signaling inhibitor, vismodegib, can reverse chemotherapy resistance in CD44(+) cells [76]. Based on these and other studies, CSC-targeted treatment approaches appear to be promising to overcome drug resistance.

Perspective

Discovery of BCR-ABL tyrosine kinase inhibitor STI571 (other names include imatinib mesylate, Gleevec) by Novartis scientists and successful clinical trials in CML patients by Druker *et al.* [74,77] established a major milestone for targeted therapy. It becomes clear now that gastric

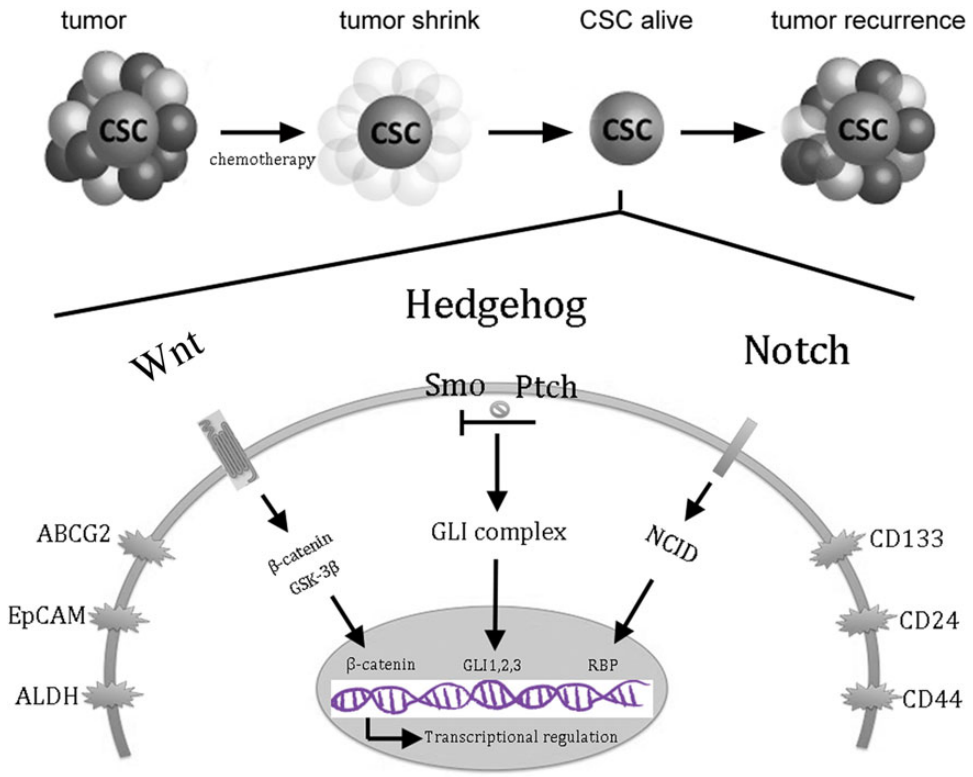

Figure 2. Pathways regulating cancer stem cells Wnt, hedgehog, and Notch are known pathways involved in embryonic development, and their activities are induced in cancer. Increasing evidence indicates that these three pathways may be involved in gastric cancer. In fact, beta-catenin gene mutations have been found in gastric cancer.

cancer is not a single disease, but a collection of many distinct groups of cancer with specific gene signature. Classification of gastric cancer based on gene expression has taken a major step toward better strategic design of targeted cancer treatment options. One important direction in the near future is to translate the gene signature in subsets of gastric cancer into clinically effective therapeutic treatments. This type of study has already achieved impressive outcomes in non-small cell lung cancer using specific inhibitors for EGFR and ALK kinases [78–80]. Both chemotherapy and targeted therapy encounter drug resistance, thus understanding drug resistance mechanisms will enable us to design better ways to treat gastric cancer. In addition to use traditional approaches for drug resistance studies, next generation sequencing will provide quick and sufficient information for genome wide alterations, but this type of study will be more effective through close collaboration between bioinformatics scientists and biologists. Up to now, limited mouse models for gastric cancer have been generated, and many will take over 1 year to see the phenotype after *Helicobacter pylori* or *Helicobacter felis* infection. Thus, physiologically relevant and robust preclinical models for gastric cancer are urgently needed. Just like the progress in lung cancer in the last 10 years, we anticipate major and significant advance in our understanding of gastric cancer biology and novel strategies in treatment of this deadly cancer.

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