Chapter 3
Cancer Stem Cells as Therapeutic Targets for Gastrointestinal Cancers

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Abstract Gastrointestinal cancers are the most severe malignancies in the world and tend to be the most prominent cause of cancer mortality. Gastrointestinal (GI) cancer leads to malignant gastrointestinal tract diseases and accessory gastrointestinal organs, namely the esophagus, uterus, kidney, pancreas, small intestine, colon rectum, and anus. And it is believed that "cancer stem cells (CSCs)" are responsible for tumor growth and drug resistance; therefore, radiation tolerance, aggressive growth, metastasis, and tumor relapse are the main causes of cancer-related deaths. Because gastrointestinal CSCs are also considered as resistant to traditional treatments, effective and innovative treatment of cancer is crucial. So, targeting gastrointestinal CSCs is quite difficult. CSCs in a gastrointestinal tumor are identified for the first time in colorectal cancer. Many gastrointestinal cancers are identified later in the esophagus, stomach, liver, and pancreas. Consequently, current basic and translational studies are primarily designed at gaining a better understanding of the biology and these approaches are used to target CSCs. Therefore, recent developments and advancements in the field of GI CSCs can continue to provide new insights into gastrointestinal cancer and its treatment approaches for GI cancer eradication. Hence this chapter reflects on the modern advancements by using CSCs as the main target to eradicate gastrointestinal cancers. Knowledge about CSCs can help to develop new clinical strategies and markers for gastrointestinal cancers.

Keywords Cancer stem cells · Gastrointestinal cancer · Esophagus · Pancreas

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Abbreviations

ALDH1	Aldehyde dehydrogenase 1
BetA	Betulinic acid
CaMK2	Calmodulin kinase
Cdc42	Cell division control protein 42
CRC	Colorectal cancer
CSCs	Cancer stem cells
CXCR4	C-X-C chemokine receptor type 4
DHH	Desert Hedgehog
DVL	Dishevelled phosphorylation
EGF	Epidermal growth factor
ESA	Epithelial specific antigen
GI	Gastrointestinal cancer
GLI	Glioma-associated oncogene
GPR	G-protein-coupled receptor
GSK-3	Glycogen synthase kinase 3
IFNα	Interferon-α
IHH	Indian Hedgehog
IL-6	Interleukin-6
JNK	Jun kinase
Lgr5	Leucine-rich repeat-containing G-protein-coupled receptor 5
MSCs	Mesenchymal stem cells
OS	Overall survival
PDGF	Platelet-derived growth factor
RAC	Ras-related C3 botulinum toxin substrate
SHH	Sonic Hedgehog
TCF	T-cell-specific transcription factor
TGF-β	Transforming growth factor-β
TNF	Tumor necrosis factor

1 Introduction

Cancer is associated with high mortality and morbidity and among all cancers, gastrointestinal cancers remain a great burden worldwide [1]. Despite critical developments in anticancer treatments over the past few decades, patients' overall survival (OS) rate remains insufficient [2]. The high mortality rates in gastrointestinal cancers are due to late diagnosis, high morbidity, and accessibility of not many focused-on treatments [3]. The incidence rate of colorectal cancer (CRC) is recorded in the top 10 for the tumor; however, five gastrointestinal cancers, including colorectal, pancreatic, hepatic, biliary, and esophageal cancers, are in the top 10 for

tumor death rates in the USA [4]. The use of a few therapeutic methods such as surgery, endoscopic care, chemotherapy, and radiation will improve the recovery of gastrointestinal cancer patients. The adequacy of these drugs depends on the cancer state, metastasis, radiation/chemotherapy tolerance, and recurrence, all of which are believed to be induced by CSCs. Therefore, additional medicinal choices need to be developed for these diseases. But recent postulation suggests that cancer stem cells (CSCs) are a small subpopulation of cells which have self-regeneration and uncontrolled proliferation capacity that causes cancers. The cancer stem cells are found in several forms of tumors and could be effective therapeutic targets [5]. In addition to their self-renewal ability, CSCs have the potential to metastasize and recur to cancer [6, 7]. This (stochastic) theory of clonal evolution proposes that many cancers are usually driven by CSCs via self-renewal property, leading to an increase in the population of CSCs that can be further changed through genetic or epigenetic changes [8, 9]. CSCs have been known for years in a wide range of solid tumors including GI cancers [10]. The $CD44^{+}CD24$ cancer stem cells of breast cancer were first reported in solid tumors [7]. The main gastrointestinal CSC study was reported in $CD133^{+}$, $CD44^{+}$, $ALDH1^{+}$, and CRC fraction [4, 11]. These cells are crucial for tumor improvement and harbor the transformations needed to start a tumor. But reports demonstrate that CSCs may originate from differentiated mature cells, progenitor cells, and/or pools of transdifferentiated stem cells [10, 11]. It was also suggested that the cell fusion, chromosomal rearrangement, and/or horizontal gene transfer processes that often include tissue repair processes can also play an important role in tumor initiation, growth, and origin of CSCs [6, 11]. The deregulation of the key regulatory signaling pathways like Hedgehog, TGF-β, Wnt, and Notch is involved in normal homeostasis of the tissue, which is also involved in the advancement and development of CSCs in tumors [12, 13].

2 Cancer Stem Cells in Gastrointestinal Cancers

The current treatment methods (including chemotherapy, radiotherapy, and other specific therapies) only affect the rapidly dividing segregated cancer cells, while cancer stem cells (CSCs) or otherwise known as tumor-initiating cells, which are considered to be the vital origin cells of cancer, will usually avoid and endure these treatments [14]. CSCs become the best target for cancer due to their capacity for self-renewal and uncontrolled proliferation ability [15]. But there is a need for possible application of CSCs in the therapy of GI cancer. In addition, inadequate diagnosis of colorectal cancer (CRC) and metastasis of the liver are the major reasons for GI cancer mortality [16]. Early diagnosis and distant metastasis of any primary cancer are based on specific and accurate tumor markers. Dr. ZY Jiao has demonstrated the importance of colorectal CSCs, which helps in metastasis of liver in patients with CRC, and he reported the use of colorectal CSCs in the prevention of metastasis and also improvements in therapeutic treatments [17]. From a therapeutic standpoint, the complete eradication of colorectal CSCs would be an ideal approach. Betulinic acid

(BetA) is a broad-acting natural compound introduced by Dr. Lisette Potze et al. in this special issue [18]. The authors have demonstrated that BetA can induce a quick method for eradicating CSCs of the colon by reducing their clonogenic ability [18]. Indeed, this complex permits other experimental studies in future, especially animal studies [18]. Mesenchymal stem cells (MSCs) contain self-renewal potential to retain multi-potency nature. These MSCs show immunomodulatory effects during inflammatory conditions [19]. MSCs secrete numerous factors, which dcreases inflammation, improves tissue repairing capacity, promotes angiogenesis. These roles may further be improved by altering other genes found in MSCs [19]. As a result, increasing numbers of researchers are trying to improve the therapeutic effectiveness by using genetic engineering methods in MSCs. Many MSC-based cell therapies have shown to be reliable and useful in certain diseases, such as cirrhosis, graft-versus-host disease, and osteoarthritis, but the efficacy has been lacking in most of the diseases [20]. Further investigations to study the beneficial ability of MSCs in GI cancers are probably warranted. In this respect, Dr. YL Zhou et al. reported the issues and the trials related to this method for the understanding of the beneficial ability of MSCs in gastric cancer [21].

Generally, suitable reports have shown up-to-date information on common GI cancers in the field of CSCs for readers. The information in this report certainly provides clinicians and translation researchers with insights into improved curative methods for GI cancers.

2.1 Colorectal Cancer

CRC cells that expressed CD133 were first reported to have a CSC phenotype in 2007 [35]. Lgr5, a marker of intestinal stem cells that express upon upregulation of the Wnt signals, leads to the transformation of intestinal stem cells into CSCs [36]. Additionally, tumorigenicity reduction in CRC cells by Lgr5 knockdown was observed in some studies [37]. CD44 or CD166 is a colon CSC marker [38]; other CSC markers are ALDH1, Lgr5, and EpCAM [39]. Those are not only classified as CSCs, but they are also still found in regular stem cells. Cancer stem cells were known to express Dclk1 but they do not express in regular intestinal stem cells [40]. Epigenetic pathways generally regulate CSCs, wherein promoter methylation regulates CD133 marker [41], and in turn Lgr5's DNA methylation is involved in CRC tumorigenesis [42]. Furthermore, studies reported that regulatory stemness gene expression was inhibited by miRNAs [43]. Cellular niches, a microenvironment formed by adjacent cells such as vascular endothelial cells or fibroblasts, play an important role in the development and maintenance of ordinary stem cells. Myofibroblasts stimulate colon-stemming CSCs by secreting growth factors in hepatocytes or type I collagen [44]. Alternatively, endothelial vascular cells which secrete Jagged-1 stimulate the CSC phenotype in CRC [45]. A method was reported from existing CRC cell lines which produce CRC-like stem cells [46]. In brief, a group of identified factors (OCT3/4, SOX2, and KLF4) were retrovirally transfected and induced pluripotent stem cells from CRC cells, and these induced cells had CSC

properties. This methodology will promote the study on colon CSC which supports the growth of new therapies focused on CSC.

2.2 Pancreatic Cancer

The most severe histological type of pancreatic cancer is pancreatic ductal adenocarcinoma. It is a complex genetic disease and its progression includes the sequential growth of numerous genetic mutations, containing inactivated CDKN2A, SMAD4, and TP53 and active KRAS which are already detectible in premalignant lesions. Met^+, $CD133^+$, $CXCR4^+$, and $CD24^+CD44^+EpCAM^+$ are unique markers in pancreatic CSC isolation [47]. An alternate CSC recognition strategy in PDAC is focused on the activity of enhanced ALDH1 and improved efflux capability in Hoechst 33342. In CSCs of pancreas many signaling pathways like the Hedgehog, Notch, Wnt, and phosphatidylinositol-3 kinase/Akt (protein kinase B) are activated. Hedgehog inhibition reduced pancreatic CSC phenotypes and tumorigenesis [48]. Moreover, several miRNAs are important when regulating the phenotypes of CSC. However, in PDAC patients miR-221 and microRNA-21 are overexpressed, and downregulation occurs simultaneously with antisense oligos that leads to reduced development, chemoresistance, and metastasis [49]. An increased expression of miR-21 is associated with inadequate diagnosis in patients of PDAC. In contrast, tumor suppressors, miR-34, miR-200a, and miRNAs, are reduced in PDAC, and their repair activity inhibits cancer [50]. Mutant KRAS control in PDAC cancer stem cells is a really difficult process [51]. Although the ablation of KRAS contributed to the regression of the tumor, PDAC cells developed resistance and showed tumorigenic capacity with elevated expressions of CD133 and CD44 [4]. Endured KRAS ablation in CSC-like cells was strongly mitochondrial and showed inhibition of tumorigenesis and elevated sensitivity to inhibitors of oxidative phosphorylation.

2.3 Liver Cancer

HCC and intrahepatic cholangiocarcinoma are two major liver cancers. Cholangiocytes and hepatocytes are differentiated by progenitor cells called bipotential hepatic cells; these two kinds of cancers originate from the progenitor cells, while the latest studies reported that a trans-differentiation process from intrahepatic cholangiocarcinoma results in the development of cholangiocytes from hepatocytes [52]. In this chapter, we discuss the CSCs within HCC. Most normal hepatocytes multiply and maintain liver function after surgical removal and cause severe liver injury. In contrast, hepatic progenitor cells are induced in chronic liver diseases and are differentiated into cholangiocytes or hepatocytes. Because HCC usually progresses with chronic liver diseases, regularly expressed hepatic

progenitor cell markers are present in this manner, in which hepatic progenitor is related to hepatocarcinogenesis. The pharmacological blockage of interleukin-6 decreases a link between HCC and chronic inflammation in hepatitis [53]. Hepatic CSC marker isolation involves EpCAM, CD44, CD90, CD13, OV6, CD24, and CD133 [54]. Most normal progenitor hepatic cells express markers present on it, in which $OV6^+$ cells and $CD90^+$ are metastatic in nature, whereas $CD133^+$, $EpCAM^+$, $CD13^+$, and $CD24^+$ are chemoresistant [55, 56]. In turn, nonmetastatic $EpCAM^+$-co-injected cells metastasize from metastatic $CD90^+$ cells into the lungs [57]. Hepatocarcinogenesis includes many signaling pathways like Hedgehog, Wnt, P53, Akt, insulin-like growth factor-1 receptor, Notch, and TGF-β. Such pathways are triggered in common and chronic liver diseases. For example, cell cycle signaling regulators are Wnt signals and CD24- and STAT3-mediated Nanog regulator triggered by EpCAM [4, 53]. Protein nestin is a class IV intermediate filament that controls CSC tumorigenesis in liver and cellular plasticity in a p53-dependent manner. Likewise, liver CSCs' self-renewal process is controlled by a transcription factor Twist2 which is CD24 dependent [58, 59]. A study reported that $CD133^+$ HCC cells upregulate families like miR-181, let-7, and miR-130b, while downregulating miR-150, which regulates phenotypes of cancer stemness. The self-renewal and tumorigenesis regulated by increasing miR-130b levels lead to decrease in tumor protein P53 expression stimulating nuclear protein-1 [60]. Inhibition of let-7 or miR-181 reduces invasive capacity and motility [61]. Overexpression of miR-150 substantially decreases $CD133^+$ liver CSCs [62].

2.4 Esophageal Cancer

Esophageal adenocarcinoma and ESCC are two subtypes of cancer. Esophageal cancer treatment is done by the combined use of chemotherapy drugs or with radiation in any case, if ordinary medications are not truly successful. ESCC cell line as a single clone source for isolating esophageal CSCs [63]. There have been similar characteristics of stem cells, the ESCC cells being more radioresistant than their parental cells. High-level expression was observed in b1-integrin, b-catenin, and Oct3/4 in SP cells which are radioresistant cancer cells of the esophagus [64]. The latest findings show the connection between the miR-296 [65] and miR-200c [66] miRNA expression, in the chemoresistance of ESCC. The esophageal tumorigenesis involves many genetic alterations. PIK3CA inhibition decreases CSC proliferation in ESCC. In cells that have a PIK3CA mutation, the inhibition of phosphatidylinositol-3 kinase was more successful than controls. Likewise, WNT10A overexpression enhances the ability to self-renew and causes a higher CSC population, indicating invasion, and WNT10A mediates migration in ESCC [67]. CD44, aldehyde dehydrogenase 1, and Lgr5 are helpful in esophageal CSC categorization. High level of CD44 expression in cancer cells shows characteristics of EMT. The initiation of EMT via TGF-β by the receptor epidermal growth factor plays a vital role in this signaling [68].

2.5 Gastric Cancer

The first discovered CSCs in GC occurred when cell lines of GC were analyzed. [69] EpCAM and CD44 are two markers used for isolating cancer stem cells from GC cell lines or resected tumors. In addition, CD54 and CD44 present in peripheral blood of GC patients help in the isolation of gastric CSCs. $Lgr5^+$ stem cells present in the stomach were the source for isolating gastric CSC [70]. Increased levels of $Lgr5^+$ in patients of GC show median survivability [71]. Induction of hyperplasia and manipulation of all progenitor cells and $Lgr5^+$ stem cells are due to *Helicobacter pylori* colonization [72]. Gastric CSCs are believed to derive from regular stem cells in the tissue. *Helicobacter pylori* leads to chronic infection; however, induction of inflammation led to the regenerating gastric tissue made with bone marrow, although acute inflammation does not contribute to the induction of bone marrow-derived cells [73]. In the pyloric gland, stem cells that express villin and $villin^+$ gastric stem cells may be transformed into GC cells [74]. KLF4 may play a crucial role in the initiation and progression of GC in gastric $villin^+$ stem cells [74]. Furthermore, CSC candidate markers might be ALDH1, CD90, CD71, and CD133. By inducing EMT, microRNAs may control the properties of gastric CSCs [75].

3 Signaling Pathways of Cancer Stem Cells in GI Cancer

Some major signaling mechanisms involved in CSCs are TGF-β-signals, Hedgehog, Notch, and Wnt/β-catenin; these pathways have been associated with CSC maintenance in GI cancers [11, 76] (Table 3.1).

3.1 Wnt Signaling

Self-renewal of gastrointestinal epithelial cells controls the growth and reproduction by the Wnt signaling pathway which is crucial in embryogenesis [77]. Epigenetic and genetic changes observed in GI cancers are due to abnormality in the pathway of Wnt [78, 79]. This pathway has also been used in recent years to control stem cell biology in adult gastrointestinal organs [80]. The noncanonical (Wnt/calcium), canonical (Wnt/β-catenin), and noncanonical planar cell polarity (PCP) are the three branches classified in the Wnt pathway [77]. The canonical pathway requires Wnt ligand binding to the receptor Frizzled (FZD) as well as the low-density lipoprotein receptor associated with protein 5/6 co-receptor (LRP5/6) to activate intracellular signaling by β-catenin nuclear translocation. When a Wnt ligand binds to the FZD receptor the signaling process begins and induces dishevelled phosphorylation (DVL) that further recruits Axin to deconstruct the degradation complex and it tends to monitor and control β-catenin and trigger a β-catenin T-cell-specific

Table 3.1 Unique markers of gastrointestinal cancer stem cells

Tumor type (references)	Regulatory pathways	Markers of cancer stem cells according to tumor type
Colorectal cancer [22–25]	Wnt signals, epigenetic pathways	$Lgr5^+$/$GPR49^+$ $CD133^+$/ $CD44^{+/}ALDH1^+$ $CD44^+/CD24^+$ $EpCAM^+/CD44^+$ $CD166^+$
Metastatic colon [26]	EGF signaling, Ras-ERK, PI3K/Akt kinase pathway PI3K/Akt and, STAT3-dependent signaling	$CD133^+/CD26^+$
Gastric cancer [27]	Wnt/β-catenin and (NF)-kB	$CD44^+$
Liver cancer [28–31]	Hedgehog, Wnt, P53, Akt, insulin-like growth factor-1 receptor, Notch, and TGF-β	$CD13^+$ $D90^+/CD45^-$ $EpCAM^+$ $CD133^+/CD49^+$
Pancreatic cancer [32, 33]	Hedgehog, Notch, Wnt, and phosphatidylinositol-3 kinase/act (protein kinase B)	$CXCR4^+$ $CD133^+$/CD44+/$CD24^+$/ ESA^+
Esophageal cancer [34]	TGF-β	$CD44^+/ALDH1^+$

ESA epithelial specific antigen, *ALDH1* aldehyde dehydrogenase-1, *Lgr5* leucine-rich repeat-containing G-protein-coupled receptor 5, *EpCAM* epithelial cell adhesion molecule, *GPR* G-protein-coupled receptor, *CXCR4* C-X-C chemokine receptor type 4

transcription factor (TCF)-lymphoid enhancer-binding factor (LEF) transactivation complex [81, 82]. Without Wnt ligand binding, cytoplasmic β-catenin is phosphorylated by a degradation complex and degrades within the proteasomes. This degradation complex is constituted by the tumor suppressor adenomatous polyposis coli (APC), the scaffolding protein AXIN, CK1 (casein kinase 1), and GSK-3 (glycogen synthase kinase 3). In general, noncanonical Wnt pathways are associated with differentiation, cell polarity, and migration. By recruiting and activating DVL, Wnt ligands bind to the FZD receptor in the noncanonical PCP pathway and activate various GTPases such as Ras homologous gene family member A (RhoA), Ras-related C3 botulinum toxin substrate (RAC), and cell division control protein 42 (Cdc42). Wnt ligands bind to both the FZD receptor and alternative receptors of the tyrosine kinase family also known as RYK (receptor-like tyrosine kinase) or ROR (tyrosine kinase-like orphan receptor) in the noncanonical calcium-dependent Wnt signal. This signaling pathway promotes cell migration and canonical Wnt signaling inhibition through intracellular calcium flux and calmodulin kinase (CaMK2), Jun kinase (JNK), and PKCα activation. Notch activation can also enhance active β-catenin levels by regulating the endo-lysosomal degradation of β-catenin after translation [83]. The equilibrium between β-catenin delocalization distinction and self-renewal in several adult CSCs is mainly regulated by the "canonical" Wnt/β-catenin pathway [11]. This process allows the control of stem

cells (SCs) and their instability may lead to the expansion of CSCs. A recent study shows that CD133 and EpCAM have been described as specific transcription targets for hepatocellular carcinoma (HCC) in the signaling of Wnt/β-catenin [55]. In particular, the depletion of EpCAM in HCC stem cells interfered with proliferation, colony formation, and migration [55]. Further, β-catenin siRNA knockdown inhibits CSCs [84]. Wnt/β-catenin signaling activation occurs in the intestine after Apc mutation leading to the disease of the familial adenomatous polyposis (FAP) [85]. One of the early incidents during carcinogenic cases in the most intermittent colorectal cancers was due to Apc gene complete impairment. In addition, intense Apc mutant polyposis mice (Apc1322 T) was associated with increased Lgr5 expression, and other stem cell markers like Bmi1, CD44, and Musashi1 [86]. Deleting the main gene Wnt CD44 in Apcmin/+ mice also reduces intestinal tumorigenesis [87]. All these findings related to Wnt signaling provide information on gastrointestinal tumorigenesis for cancer stem cell model and it also helps in maintaining the CSC role to enhance progression of cancer.

3.2 Signaling of Transforming Growth Factor-β (TGF-β)

In all the signaling pathways the transforming growth factor-β (TGF-β) shows a key role in regulating the gastrointestinal epithelial cells' development, differentiation, survival, and fate [88]. TGF-β functions as a tumor suppressor in a normal and healthy environment, by cell proliferation inhibition, autophagy suppression, and apoptosis-triggering processes. Change in their response to TGF-β develops tumors and use it as a powerful promoter of cell motility, invasion, metastasis, and CSC preservation [89]. TGF-β is an important inducer in the transformation of mesenchymal-epithelial (EMT) by regulating transcriptional activation of the protein family Snail and TWIST, the EMT system's key regulators [90, 91]. One of the most frequently altered signaling pathways is TGF-β signaling in GI cancers [92]. And it plays an important role in the maintenance of CSCs in human kidney, pancreatic, gastric, and colorectal cancers [4]. Kim et al. recently stated its importance of control in the development of colon cancer by stimulating nuclear translocation of β-catenin showing a correlation between TGF-β1 and ALDH1 [11].

3.3 Notch Signaling Pathway

The Notch pathways play an important role including cell homeostasis and differentiation during embryogenesis, and are of major importance in many areas of cancer biology, from CSC to angiogenesis, and tumor immunity [93, 94]. The pathway of Notch signaling is usually complicated and multidimensional, imitating its functions in various functional processes [95, 96]. Notch mediates a number of biological processes through four Notch receptors (Notch-1–4) and five Notch

ligands such as Delta-like ligands 1, 3, and 4, and Jagged-1 and Jagged-2 [97]. Cell-to-cell contact for canonical Notch signaling is usually necessary for the activation of Notch, where Notch can be separated by multiple enzymes through a series of proteolytic enzyme cleavages, resulting in the release and activation of target gene Notch [97]. Notch's key genes include NF-κβ, c-Myc, cyclin D1, Akt, and mTOR and the endothelial vascular (VEGF) growth factor [98]. The different GI cancers express Notch receptors and ligands differently. Also, noncanonical Notch signaling has begun to be delineated independently of ligand-receptor interaction and some of its roles are essential for GI cancer [99]. Cross talk with Wnt and/or Hedgehog (HH) signaling may also be used to determine the overall effect of signaling in Notch adding an additional layer of complexity [13]. For example, Notch signaling activation as a suppressor may have occurred in HCC tumor but it can play an oncogenic role in both colon and pancreatic cancer [100]. In fact, the key role of Notch signaling in the extending of CSCs has been demonstrated. In pancreatic CSCs, Notch-1 and -2 are overexpressed and associated with reduced CD44 [101].

3.4 JAK–STAT3 Pathway

The signal transducer and transcription activator 3 (STAT3) significantly contribute to the regulation of cell-related processes mediated to producing and progressing cancer, including proliferation, angiogenesis, cell survival, and immune function [102]. In many GI cancer types, including colorectal cancer, dysregulated STAT3 has been identified [103]. Consider that the activation cycle for the STAT3 starts with Janus kinases (JAKs) which are phosphorylated to specific signals such as interleukin-6 (IL-6), interferon-α (IFNα), tumor necrosis factor (TNF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) [104]. Nevertheless, the JAK–STAT3 mechanisms are of interest not only for cancer-related immune cells but also for CSCs [104]. In this way, CSCs of hepatocellular cancers $EpCAM^+$/$CD133^+$ and $ALDH^+$/CD133 and CSC of colon cancers demonstrate the increased activity of IL-6/STAT3 which is a causative interplay in the niche spreading in CSCs [11, 105]. Recent evidence suggests that STAT3-signaling feedback activation plays an important role in mediating drug resistance to a wide spectrum of anticancer treatments and IL6/STAT3 pathway inhibitors can be used to eradicate CSCs [11, 105].

3.5 Hedgehog (HH) Signaling Pathway

The regulation of cell destiny specifications and the patterns are due to the Hedgehog (HH) signaling which is involved in embryonic development, normal tissue repair, and EMT [106]. In mammals with HH ligands, there are three proteins: sonic Hedgehog (SHH), Indian Hedgehog (IHH), and desert Hedgehog (DHH). Such

proteins bind to the transmembrane receptor Patched-1, which induces the internalization and prevents its suppression of the transmembrane protein Smoothened (SMO) and thereby activates the signaling pathway [107]. Subsequent signaling by SMO leads to activation and nuclear localization of transcription glioma-associated oncogene (GLI) factor that helps to produce HH target genes such as c-myc, cyclin D1, VEGF, BCL2, and Split (HES) family protein enhancer [108]. The development, survival, and angiogenesis involve such target genes [109]. CSCs are influenced by the HH signaling, according to the emerging results from gastrointestinal tumors [48]. Activated HH signals have been identified in the CSCs, which was demonstrated later by relatively high colorectal cancer expression of GLI1, GLI2, PTCH1, and Hedgehog interacting protein (HIP) [110]. Moreover, in colorectal cancer the progression of CCSs with the target gene SNAIL1 is linked to EMT and involved in metastasis [110]. In addition, cyclopamine and siRNA of SMO, GLI1, and GLI2 inhibited the HH pathway activation, reduced the tumor cell growth, and induced apoptosis [111]. EMT-clonogenic growth possibilities have also been studied in pancreatic CSCs and cyclopamine inhibits each functional property and leads to the formation of the metastatic disease [112]. In fact, CD133 + liver CSCs are strongly expressed as genes participating in the hedgehog pathway [113].

3.6 mTOR Pathway

Recent reports have shown that the mTOR pathways are important for GI cancer pathogenesis [114]. PIK3 mutations occur in a number of cancers including gastric and colorectal cancers [115]. Many human cancers, like GC, are related to poor prognosis of Akt activation [114]. Akt1 and Akt2 were particularly observed for gastric, pancreatic, and colorectal cancers [116]. mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) are increased during cancer progressions in hepatic, pancreas, gastric, and colorectal cancers. They also control EMTs, motility, and metastasis [117, 118]. Radioresistance is also associated with EMT and CSC phenotypes by activating the PI3 K/Akt/mTOR signals [119]. Recent studies of colon cancer cells have also shown that PI3 K/Akt/mTOR inhibits proliferation of the CSC in colon and lowers stemness, as observed by CD133 and Lgr5 expression [120]. mTOR inhibition also reduces ALDH1, a marker for colorectal CSCs [121]. Similarly, the inhibition of mTORC2 has led to decreased EpCAM expression in the liver CSCs that have little or no tumorigenicity [122]. Matsumoto et al. and Yang et al. also show that mTOR inhibition increases $CD133^{+}$ subpopulation and activates the CD133 shift to the $CD133^{+}$ in vitro population, by using gastrointestinal tumor cells [123].

Classic signaling pathways described above play an important role in GI and CSC self-renewal [124]. Considering the growing reports that GI cancer is a disease driven by multipotent, self-renewing CSCs, it is essential that we understand how these signaling pathways synchronize events and the development and evolution of

CSC. This will lead to a more successful early diagnosis of cancer and the development of treatment approaches to reduce recurrence and/or cancer therapy.

4 Therapeutic Resistance Mechanisms

Chemotherapy and radiotherapy are key components of GI cancer treatment, namely esophageal, gastric, hepatic, pancreatic, and rectal cancer. Unfortunately, following such therapies, disease recurrence and worsening frequently occur, with increasing evidence including CSCs for these adverse effects. Mechanisms by which CSCs survive when establishing standard cancer therapies include increased DNA repair, dormancy maintenance, drug efflux, and redox capacity.

4.1 Senescence Maintenance

Radiation and traditional chemotherapy kill cancer cells by causing DNA damage that most effectively induces cell death in rapidly dividing cells with inactive replication of DNA. While this effectively helps to separate cancer cells rapidly, CSCs are often quiescent, reducing the cytotoxic effects of radiation and chemotherapy on these cells. In addition, stemlike $CD44^+$ cells sustain a quiescent condition in prostate cancer [14]. This CSCs engage in DNA repair mechanisms in this quiescent state before progressing into the mitotic phase of the cell cycle [125]. Likewise, in experiments with cell lines of gastric, colon, and esophageal cancer, chemotherapy treatment enriches a stemlike population of cells with a quiescent state [126]. In the EC9706 line of esophageal cancer cells, this subgroup of stem cells display increased resistance to DNA damage compared to non-stem cancer cells [127]. These examples together highlight the demand for innovative methods of targeting and killing CSCs that do not rely on cell proliferation.

4.2 DNA Damage Checkpoint Repair

As above, the proliferation rate of CSCs provides an innate defense against genotoxic cancer therapies. Similarly, CSCs also increased DNA repair efficiency, further reducing the efficacy of these conventional cancer therapies. Glioma treatment with ionizing radiation for example enriches CD133 + CSCs. CD133 + glioma cells express greater checkpoint activation of ataxia-telangiectasia-mutated (ATM) Rad17, Chk1, and Chk2 checkpoint proteins in response to DNA damage, resulting in cell cycle arrest. This radioresistance is opposed by inhibition of checkpoint kinases [128]. Similarly, other DNA damage response targets including ATM, ATR, Chk1, and PARP1 have increased levels of glioblastoma CSCs [129].

4.3 Increased Redox Capacity

Radiation therapy induces DNA damage by reactive oxygen species (ROS) production, where ROS reaches the cell's antioxidant capacity [130]. CSCs produce extremely ROS scavengers, such as glutathione (GSH), which results in resistance to DNA damage induced by ROS. To support this, GSH pharmacological reduction induces radiosensitivity in CSCs [131]. The CD44 variant isoforms (CD44v), strongly expressed in carcinomas of the epithelial form and GI CSCs, are implicated in intracellular GSH control [132]. CD44v-expressed cells play a vital role in the tumor-beginning process and have increased resistance to H_2O_2, cisplatin, and docetaxel in order to play a crucial role in the initiation of tumors. This indicates that the control and enhancement of the cellular antioxidant ability of CSC CD44v play a role not only in tumorigenesis but also in resistance to chemotherapeutic treatment strategies and in maintaining ROS levels below those of non-stem cells [133].

4.4 Efflux of Drug

In addition, preventing or repairing chemoradiation-induced DNA damage, CSCs have the capability to export toxic chemicals and drugs. This is induced by proteins that transport from the cell membrane, the most common being the ATP-binding cassette (AC) transporter family. Such hydrophobic chemotherapy drugs are eliminated by transport proteins from the cytosol to the extracellular space [134]. Studies of CSCs from various types of solid cancers revealed superior efflux ability compared to non-cancer stem cells. For example, two drug transporters, MDR1 and BRCP1, are overexpressed by $CD133^+$ glioma CSCs compared with $CD133^-$ cells [126, 135]. In fact, enhanced production of the MDR1 transporter protein ABCG2 in colorectal CSCs in these cells conferred chemoresistance [136].

5 Targeting of CSCs

CSCs are armed with numerous mechanisms for avoiding conventional cancer therapy, limiting the effectiveness of these therapeutic strategies and enabling CSCs to promote the recurrence of metastatic diseases. So ideal antitumor therapies should target both the proliferating population of cancer cells and CSCs. In this case, therapies designed to eliminate CSCs have attempted to remove these cells through either activate differentiation or targeted eradication [137, 138].

5.1 Differentiation Induction

Differentiation therapies are based on the principle that differentiation of CSCs contributes to a loss of self-renewal capabilities and properties of drug resistance, hence making them susceptible to regular therapies. The best known example of treatment designed to induce differentiation of CSCs is the treatment of APL. ATRA, a retinoid, has helped turn APL into one of the most treatable leukemia forms. ATRA targets PML/RAR-α, a differentiation suppressor, which induces cell differentiation [139]. Retinoids were also used in head-and-neck and lung cancer in addition to their use in APL, where differentiation induction was used as a chemical prevention tool for precancerous lesions [140]. In GI cancer ATRA induces differentiation in the cell lines of colon cancer [141]. Furthermore, ATRA treatment of patient xenografts resulting from stomach cancers causes CSC differentiation and apoptosis, decreasing tumorigenicity [142]. Clinically, ATRA substantially improves precancerous gastric dysplasia when combined with omeprazole and sucralfate [143]. Furthermore, the use of ATRA in traditional gastric cancer treatment increases survival rate compared to standard therapy alone [144]. While ATRA has failed to demonstrate therapeutic effectiveness in the diagnosis of other GI tumors, ATRA provides a framework and proof of concept for the future use of differentiation therapy in specific cases. Certain compounds cause differentiation in comparison to ATRA, like PPAR-γ agonists. PPAR-γ is an important regulator of differentiation in many cell types, particularly those involved in lipid homeostasis, controlling preadipocyte and fibroblast terminal differentiation [145]. Most of the work with PPAR-γ has been done in cancer cell lines to date. PPAR-γ agonists, such as pioglitazone, exit the cell cycle by inducing terminal differentiation [146]. Of interest to GI, Sarraf et al. worked on tumors in colonic adenocarcinoma and he showed the enhanced expression of PPAR-γ in adenocarcinoma. Additionally, treatment with PPAR-γ agonist troglitazone leads to the differentiation of colon cancer cells, measured by increased carcinoembryonic antigen expression (CEA) [147]. In addition, knockdown of PPAR-γ in mice promoted tumor growth by decreasing cancer cell differentiation [148]. Such findings showed that there are mechanisms that can be controlled for cell differentiation in GI tract cancers. Nevertheless, significant work is required in this field before therapies reach the clinic since most experiments have been performed in vitro in cancer cell lines.

5.2 Targeted Elimination

Besides therapies that induce differentiation, treatment of CSCs can also be performed by targeting stem cells for elimination. As previously described, CSCs have distinct surface marker expression profiles, creating opportunities for pharmacotherapeutic strategies targeting these different CSC properties. Targeted

removal may also be done through targeting pathways that give therapeutic resistance or survival benefits to CSCs.

6 Targeting of Cell Surface Receptors

The classification of CSCs by cell surface markers is observed in many GI cancers, including the pancreatic liver and colon [54, 149, 150]. Identification and selective targeting of CSC-specific cell surface markers enable the administration of highly potent cytotoxic agents with minimal systemic toxicity, as compared to traditional chemotherapeutics that target all rapidly dividing cells. There are two possible ways to remove such markers: immunotherapy and drug carriers.

6.1 Immunotherapy

Targeting CSCs that use the immune system provides benefits compared to standard therapies. First, along with other antibody treatments, immune cells exhibit antigen-specific cytotoxic activity, providing a more focused approach to CSC targeting. Second, in conventional treatments, such as chemotherapy and radiation, toxicity depends in part on the phase of the cell cycle [151]. Since CSCs are relatively quiescent compared to non-CSCs, immunotherapeutic strategies can provide a way of eliminating CSCs irrespective of proliferation status [152]. Finally, immunotherapy can produce long-lasting memory responses that might be effective in challenging a cancer relapse. Numerous immunotherapy strategies are listed below, targeting CSCs in gastrointestinal malignancies.

6.1.1 Chimeric Antigen Receptor T-Cell Therapy

With the recent US FDA approval of tisagenlecleucel and axicabtagene for refractory B-cell malignancies, chimeric antigen receptor (CAR)T cells have turned into an exciting immunotherapeutic approach to cancer treatment [153]. In addition, CAR T cells are T cells engineered to express an artificial receptor that consists of a targeting domain generated from an antibody linked to intracellular signals [153]. Therefore, CARs derived from antibodies which target surface antigens on CSCs represent a potential therapeutic approach. Details of a phase I study of CAR T cells guided against CD133 in patients with hepatocellular, pancreatic, and colorectal carcinomas have recently been reported [154]. Of the 23 cases, 3 had limited recovery and 14 had stable illnesses. Analysis of biopsied tissues also shows that $CD133^+$ cells were decreased. While EpCAM is a less selective CSC marker, CAR T cells target this marker. Ang et al.'s report found that anti-EpCAM CAR T cells increased survival with human rectal tumors in xenograft mouse models

[155]. Clinical trials are currently ongoing to test anti-EpCAM CAR T cells in various gastrointestinal malignancies (NCT03013712).

6.1.2 Vaccines

Ning et al. found that vaccination with dendritic cells pulsed with CSC lysates identified by high dehydrogenase expression (ALDH) was capable of generating in vivo CSC-specific T cells and antibody responses [156]. In addition, dendritic cells pulsed with tumor lysates from ALDH cells showed increased inhibition of tumor growth compared to whole-tumor lysates and reduced metastases in squamous cell and melanoma tumor models. It indicates that autologous tumor cell vaccines targeting CSCs may have a greater effect on antitumors. Phase I/II clinical trials delivering CSC vaccines in the liver (NCT02089919), colorectal (NCT02176746), and pancreatic (NCT02074046) cancers were performed based on the Ning et al. methodology. Reports of these studies are still to be published.

6.1.3 Other Immune Cells

Although most efforts targeting CSCs have based on exploiting the adaptive immune system, it is increasingly recognized that innate immune cells often identify CSCs. Tallerico et al.'s in vitro experiments showed that natural killer (NK) cells in colorectal tumors preferentially lysed CSCs over non-CSCs [157]. Cytotoxic effect against CSCs was associated with enhanced expression of NK-activating ligands NKp30 and NKp44 and reduced expression on CSC surfaces of inhibitory ligands such as MHC class I compared to non-CSC cells. In melanoma and glioblastoma, similar reports of CSC-specific killing by NK cells were published [157, 158]. In addition, recent studies by Ames et al. have shown NK cells' ability to target pancreatic CSCs in vivo. The adoptive migration of NK cells was observed using mice with human pancreatic tumor xenografts to decrease percentages of ALDH high cells which act synergistically with radiation to inhibit tumor growth [159, 160]. However, γδ T cells in various malignancies often show anti-CSC activation [161]. In colorectal cancer, Todaro et al. documented that zoledronate caused the aggregation of metabolites of mevalonate in CSCs, making them targets for the destruction of cells by γδ T cells [161]. Alternative methods using NK and γδ T cells for targeting CSCs are independent of antigen processing and expression of MHC.

6.2 Drug Carriers

Apart from immunological strategies, the delivery of cytotoxic agents on the surface of cells is a possible method for the eradication of CSCs. One such successful

therapy is the development of aptamers, which are small single-stranded DNA or RNA, about 20 times smaller than antibodies. These aptamers bind to their targets with great affinity and are internalized by cells [162]. In 2010 Shigdar et al. created the first RNA aptamer targeting a CSC surface marker. This aptamer was designed to interact with the molecule of epithelial cell adhesion (EpCAM), a transmembrane glycoprotein commonly overexpressed in both solid tumors and CSCs. It was associated with several cell lines of cancer including KATO III (gastric carcinoma) and T47D (cell line of colon adenocarcinoma) [163]. More recently, two RNA aptamers were found to target CD133AC133 epitope, a CSC marker in the colon and pancreas [164, 165]. Among other cells, HT-29 (human colorectal cancer cell line) and Hep3B (hepatocellular carcinoma cell line) internalized these CD133 aptamers. They also demonstrated a superior ability to penetrate HT-29 tumor spheres compared to an antibody CD133 [166]. A recent study showed that an aptamer directed at the CD44 surface receptor, a specific CSC marker, has been internalized via breast cancer cell lines [167]. While this report did not show the internalization of the aptamer in GI cells, it is important to note that CD44 was previously used as a marker for GI CSCs [165]. As aptamers are usually less immunogenic and have low toxicity, they hold excellent potential as a drug delivery mechanism with less systemic toxicity than conventional therapies. The exciting potential of these conjugates has not been proved to be effective in vivo at this point and further work is required before systematic delivery [168]. Antibody-drug conjugates are a promising therapeutic choice, like nanocarriers such as aptamers, which would enable cytotoxic agents to be administered to specific cells in the absence of systemic toxicity. Antibody-drug conjugates require internalization accompanied by lysosomal processing and cleavage to activate the drug. It causes only those cells which show the antigen to be given therapy [169]. Such conjugates can be used together with normal chemotherapy and radiation to produce improved results. In addition, this concept was used in the treatment of acute myeloid leukemia, where a gemtuzumab ozogamicin conjugate targeting $CD33^{+}$ leukemia cells was paired with conventional chemotherapy to increase survival rate [170]. Antibody-drug conjugates targeting CSC surface markers are under study. Two antibody conjugates have recently been established that target LGR5, a marker of CSCs in colon cancer. In a mouse model, a study shows within in vivo antitumor efficacy and safety. Although much more work should be done until therapies such as these are safe for humans, this study provided evidence of the idea that antibody-drug conjugates can be targeted at CSC surface markers [171].

6.3 Targeting Resistance Mechanisms

Another potential mechanism for eradicating CSCs is to target the machinery that mediates standard therapy resistance. Two fields where this has been examined in CSCs include inhibition of ABC transporters and targeting of antioxidant systems.

6.3.1 Transporters

As previously described, ABC transporters allow CSCs to avoid conventional chemotherapy by effluxing chemotherapeutic agents. Therapy designed to disrupt these transporters makes CSCs sensitive to standard chemotherapy. The best studied technique for inhibiting the role of ABC transporters is through direct modulators, three generations of which exist. Despite showing promise versus leukemia cells in vitro, in phase I clinical trial the first known modulator, verapamil, failed to improve vinblastine toxicity [172]. Second-generation inhibitors seem to be optimistic, but resulted in lower clearance of chemotherapy and increased toxicity in clinical trials [173]. Third-generation inhibitors have shown more promise as a possible multidrug resistance therapy [173]. Certain approaches targeted at transcriptional regulation of ABC carriers or signaling pathways involving ABC carriers are in their infancy and will need further improvement [173].

6.3.2 Antioxidant Systems

Another therapeutic approach to disarm mechanisms of resistance to CSCs is through targeting antioxidant systems, increasing oxidative stress in radiation and chemotherapy setting. The most important potential target is GSH, a metabolite that defends the cells from oxidative damage [174]. In squamous head-and-neck carcinoma, inhibition of xCT, a cysteine transport mediator required for the synthesis of GSH, leads to apoptosis in CD44v-expressing stemlike cells [175]. CD44v interacts and stabilizes xCT, promoting cysteine uptake enabling synthesis of GSH. Subsequently, CD44v ablation destabilizes xCT and decreases GSH. CD44v ablation in a mouse model of gastric cancer resulted in a loss of cell surface expression and a decline in intracellular GSH, thereby suppressing tumor growth [176]. These studies show that extracting aspects of the cell defense system from ROS will influence cell viability.

6.4 Antitelomerase Therapy

The shortening of telomeres is a major regulator of cell death. In most tissues, telomerase that helps maintain the length of the telomere is suppressed before birth and maintains normal telomere-dependent cell mortality. Lifetime telomerase activity is relegated to the selection of stem cell populations, thus allowing immortality. Unlike ordinary stem cells, CSCs remain immortal and capable of self-renewal, largely due to telomerase expression which enables them to avoid replicative senescence. Besides CSCs, most tumor cells express any level of telomerase activity [177]. This makes telomerase an outstanding target therapy because it can influence all differentiated cancer cells and CSCs. There are currently two methods of guiding

telomerase therapy. The first BIBR1532 antitelomerase compound was effective but it failed to advance to the clinical trial level. More recently, the GRN163L compound has progressed to the clinical trial level and has proven effective in multiple tissues of mouse xenografts [178]. In the field of GI cancer, GRN163L showed the efficacy of human hepatoma impairing tumor growth in vivo in mouse xenografts. When GRN163L was granted prechemotherapy, chemosensitivity to doxorubicin was increased in vitro [179]. There is also a decrease in telomerase expression in cells isolated from surgical specimens of the Barrett's esophagus treated with GRN163L. In addition, telomere shortening is observed resulting in eventual in vitro apoptosis. In vivo treatment with GRN163L decreased the volume of tumors in a mouse xenograft model [180]. In fact, GRN163L enhanced the sensitivity to radiation in the esophageal squamous cell carcinoma cell lines, leading to increased apoptosis [181]. Although these studies suggest promise for a potential therapeutic for GI cancer, to date 19 clinical trials using GRN163L have been conducted, but none have been targeted for GI cancers [182]. The second antitelomerase treatment method is through immunotherapy. Vaccines targeted at TERT, a catalytic component of telomerase, will require $CD8^+$ T cells to destroy tumor cells while largely avoiding toxicity to normal tissues with little to no expression of telomerase [183]. There was potential in the area of pancreatic cancer therapy that GV1001, a telomerase vaccine, would prove effective in patients with advanced-stage pancreatic cancer. However, given in combination with chemotherapy, in a phase III clinical trial, GV1001 showed no improvement in overall survival [184]. Further study in the field of telomerase immunotherapy and telomerase-inhibiting therapy is required to understand its potential for targeting CSCs.

7 Targeting Tumor Microenvironment

Specific activation of CSCs is a first-line clinical technique for combating these cells. Furthermore, other therapeutic approaches are also suggested as knowledge of the tumor microenvironment is rapidly increasing, which could establish a gap for developing and protecting CSCs from cancer therapy. Tumor microenvironment cells comprise fibroblast, myofibroblast, adipocyte, mesenchymal stem cells, and immune cells, such as macrophages and neutrophils, as well as endothelial cells forming the blood vessel walls and moving through the tumor [185]. CXCR4, the stromal cell receptor associated with factor-1 (CXCL12/SDF-1α), makes tumor progression, angiogenesis, and drug tolerance easier. However, expression of CXCR4 is a prognostic factor in several GI carcinomas, including gastrointestinal carcinoma [185]. The association of adhesive tumor/stroma will disrupt CXCR4 antagonists, such as analogs plerixafor (AMD3100) and T14003, which can make stem cells responsive to cytotoxic drugs [186]. In both clinical studies and gastrointestinal cancer mouse models, a new approach to targeting the CXCR4-CXCL12 axis is being investigated [185]. Developing more efficient anticancer methods often means inhibiting the angiogenic process needed to vascularize and grow tumors.

Likewise, the development and observation of antiangiogenic agents that could interact with a VEGF-VEGFR pathway were carried out to effectively combat tumor growth in in vivo animal models, including anti-VEGF or VEGFR antibodies, VEGFR antagonists, and soluble truncated VEGFR form [187].

8 The New Approach to Preclinical Therapy Assessment

In our opinion, preclinical evaluation of efficient treatment of CSC involves confirmation, and this test can be conducted in a variety of ways, each representing different intensity levels, and which more accurately demonstrates clinical conditions. Grafting and cell culture models are the conventional way to test the success of the treatment against CSCs. These initiatives may be inaccurate because culturally adapted cells cannot imitate actual primary CSC properties.

9 Cell Line, Patient-Derived Xenograft (PDX), and Tumoroids

The traditional new drug screening by using cell line-derived xenograft or syngeneic mouse models could not predict the successful development of oncological drugs because 97% of novel treatments were successful in in vivo xenograft studies but were unsuccessful in clinical trials [188]. In contrast, a small fraction of fresh tumor tissue from the patient is transplanted into an immunodeficient mouse tumor model [188]. This procedure allows for faster cell movement and effective tumor growth and position control. PDX models can be preferable to traditional line xenografts because they are similar to parental tumors. Detailed examination of PDX mice reveals that histology and gene expression profiles are maintained with SNPs and copy number variants, and PDX models efficiently screen the drug efficacy [188].

Another new cell culture technology, known as “organoids” and “tumoroids,” was recently developed and allowed to derive from adult stem cells and tumors (especially CSCs), respectively [189]. The structures are the same as organ/tumor in vivo and can develop fast and in relatively great amounts in structural and developmental processes. While many works have been done on the production of tissue repair organoids/tumoroids, more detailed implementation includes high-throughput therapeutic testing, from cell signals and analyses to palliative chemotherapy sensitization and to optimization of treatment protocols in personalized medicinal products. Therefore, without the complications associated with organism growth, gene knockout and knock-in can be done. This form of preclinical models in vitro helps researchers to predict clinical reaction trends and to conduct customized clinical trials.

10 Conclusion

Many gastrointestinal tumors are likely to generate a small population of self-regenerating cells called CSCs. Moreover, it does not show enough evidence in order to determine the relationships between CSCs sorted according to different methods. As previously mentioned, supposed CSC is isolated by their markers. Anticancer therapy is usually assessed for its ability to shrink tumors. If these treatments do not eliminate CSCs, there could be a relapse and tumors may establish more resistance through CSCs. Targeted therapies against these molecules could provide new ways of eradicating malignant cancer phenotypes without disturbing ordinary stem cells. CSC-targeted therapy has arisen as a method of treatment that could revolutionize cancer therapy and have a significant impact on reducing recurrence and metastatic diseases. Furthermore, many CSC-targeted therapies are more specific and would allow less systemic toxicity than traditional chemotherapy and radiation therapy. There are several major obstacles to implementing CSC-targeted therapies. Many of the treatments mentioned above are not specific to CSCs but are typically inherent in stem cells. In fact, there is a huge amount of cross-talking between signaling pathways, and the impact of interrupting such paths in normal cell populations remains unclear. Therapies targeted at CSC-specific cell surface markers offer an interesting opportunity to avoid this issue, as they can provide selective therapy with reduced systemic toxicity. This can be accomplished in many forms, including drug carrier mechanisms and immunotherapies, such as vaccinations and CAR-T-cell therapies. Stem cells have only been identified in a small number of GI cancers. Therefore, there is the possibility of stem cell subsets that do not express known markers. In addition, there are potential surface marker profiles between stem cells that may vary across various patients. The substantial effort will be required to reliably identify stem cell markers among various GI cancer profiles before these therapies can progress to a clinically impactful stage. More work is needed to recognize CSCs and consider their survival mechanisms, resistant therapeutic properties, and cell signaling pathways. However, this is an exciting therapeutic approach that will involve a lot of research and investment in the coming years to fulfill its promise of revolutionizing cancer therapy.

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