Therapeutic targeting of gastrointestinal cancer stem cells

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Gastrointestinal cancers remain a tremendous burden on society. Despite advances in therapy options, including chemotherapy and radiation, cancer mortality from recurrences and metastases occur frequently. Cancer stem cells (CSCs) drive disease recurrence and metastasis, as these cells are uniquely equipped to self-renew and evade therapy. Therefore, cancer eradication requires treatment strategies that target CSCs in addition to differentiated cancer cells. This review highlights current literature on therapies targeting CSCs in gastrointestinal cancer.

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Gastrointestinal (GI) cancer encompasses tumors arising from the proximal esophagus to the distal rectum, including cancers of the hepatic and biliary tracts, which are continuous with the lumen of the bowel. GI cancers are a societal burden with 319,160 new cases and 160,820 GI cancer-related deaths projected in the USA in 2018 [1]. Despite advances in surgical technique, endoscopic interventions, chemotherapy and radiation therapy, these cancers continue to take a tremendous toll on society with numerous patients suffering from disease recurrence and progression. In that context, cancer stem cells (CSCs), which reside within heterogeneous tumors, have emerged as major drivers of cancer recurrence and progression [2,3]. Indeed, stem cells are paramount to disease recurrence as they possess qualities which allow them to resist standard chemotherapies and radiation therapy.

Identification of CSCs

Tumors consist of a complex ecosystem comprised of numerous cell types, which arise from mutations among subclones or epigenetic modifications in epithelial cells [2–8]. Within this heterogeneous cell population resides a small subpopulation of CSCs. These cells are largely responsible for sustaining a tumor by providing a renewable clonal population that possesses the unique abilities of self-renewal, differentiation capacity and marked tumorigenicity [2,3,9,10].

CSCs were initially described in hematological malignancies, with the first characterization of these cells made by Lapidot *et al.* in 1994. This landmark study revealed that a small subpopulation of acute myeloid leukemia cells expressing the cell surface marker CD34, but not CD38, (CD34⁺, CD38⁻ cells) potently induced leukemia when transplanted into severe combined immunodeficiency (SCID) mice, in contrast to other tumor cells [11]. Further investigation of CD34⁺, CD38⁻ cells not only confirmed their potent tumorigenicity, but also revealed a capacity for self-renewal and multilineage differentiation [12], characteristics that have since become the standard for CSC identification [13,14]. Subsequent studies demonstrated these same characteristics in subpopulations of cells in solid tumors, suggesting the existence of CSCs in tumors of brain [15], breast [16], skin [17], colon [18,19], pancreas [20] and prostate [21]. Moreover, there is mounting evidence that CSCs possess unique attributes that confer resistance to chemo- and radiotherapy, suggesting a central role for these cells in cancer recurrence, metastasis and progression. Thus, targeting CSCs represents a critically important component of cancer therapy, with the potential

to revolutionize cancer treatment and decrease cancer recurrences. The lack of current therapies effectively targeting these cells highlights the profound clinical need for advances in this important chemotherapeutic field [22].

Mechanisms of therapeutic resistance

Chemo- and radiotherapy are mainstays in the treatment of GI cancers, including esophageal, gastric, hepatic, pancreatic and rectal cancer. Unfortunately, disease recurrence and progression frequently occur despite these treatments, with growing evidence implicating CSCs for these adverse outcomes. Mechanisms by which CSCs survive in the setting of standard cancer therapies include increased DNA repair, maintenance of dormancy, drug efflux and redox capacity.

Maintenance of senescence

Radiation and conventional chemotherapies target cancer cells by inducing DNA damage, which most potently initiate cell death in rapidly dividing cells engaged in active DNA replication. Although this effectively targets rapidly dividing cancer cells, CSCs are often quiescent, minimizing the cytotoxic effects of radiation and chemotherapy on these cells. Indeed, in prostate cancer, stem-like CD44⁺ cells maintain a quiescent state [23]. In this quiescent state, these CSCs engage DNA repair mechanisms prior to progressing into the mitotic phase of the cell cycle [22–24]. Similarly in experiments with cell lines from gastric, colon and esophageal cancer, treatment with chemotherapy enriches for a stem-like cell population which displays a quiescent state [25–27]. In the esophageal cancer cell line EC9706, this subgroup of stem cells displays increased resistance to DNA damage compared with nonstem cancer cells [27]. Together, these examples highlight the demand for novel methods of targeting and killing CSCs that do not rely on cellular proliferation.

DNA damage checkpoint repair

As described above, the rate of proliferation of CSCs provides an innate defense against genotoxic cancer therapies. In addition, CSCs possess an increased capacity for DNA repair, further minimizing the efficacy of these traditional cancer therapies. For example, treatment of glioma with ionizing radiation enriches CD133⁺ CSCs. Compared with CD133[−] glioma cells, 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. Inhibition of checkpoint kinases opposes this radioresistance [28]. Similarly, glioblastoma CSCs have increased levels of many DNA damage response targets including ATM, ATR, Chk1 and PARP1. Inhibition of ATM, ATR or PARP1 increases radiation susceptibility [29].

Increased redox capacity

Radiation therapy induces DNA damage through the generation of reactive oxygen species (ROS), when ROS exceed the antioxidant capacity of the cell [30,31]. CSCs highly express ROS scavengers, such as glutathione (GSH), resulting in resistance to ROS-mediated DNA damage. Supporting this, pharmacologic reduction of GSH induces radiosensitivity in CSCs [30,32]. CD44 variant isoforms (CD44v), which are highly expressed in epithelial type carcinomas and GI CSCs, are involved in the regulation of intracellular GSH [33]. In addition to playing a critical role in tumor initiation, CD44v-expressing cells have increased resistance to H₂O₂, cisplatin and docetaxel. Taken together, this suggests that CSC CD44v regulation and enhancement of cellular antioxidative capacity plays a role in not only tumorigenesis, but also resistance to chemotherapeutic treatment strategies and the maintenance of ROS levels below those of nonstem cells [31,34].

Drug efflux

In addition to avoiding or repairing DNA damage induced by chemoradiation, CSCs possess the ability to export toxic chemicals and drugs. This is driven by cell membrane transport proteins, the most well-known being the ATP-binding cassette (AC) transporter family. These transport proteins eliminate hydrophobic chemotherapy drugs by excretion from the cytosol to the extracellular space [35]. Studies of CSCs from multiple types of solid cancers have demonstrated superior efflux capacity compared with nonstem cancer cells. For example, CD133⁺ glioma CSCs overexpressed two drug transporters, MDR1 and BRCP1, compared with CD133[−] cells [36,37]. Additionally, increased expression of MDR1 transporter protein ABCG2 in colorectal CSCs conferred chemoresistance in these cells [38].

Targeting CSCs

CSCs are armed with numerous mechanisms to evade conventional cancer therapy, limiting the efficacy of these therapeutic strategies and allowing CSCs to drive disease recurrence and metastatic disease. Thus, ideal antitumor therapies should target both the proliferating cancer cell population as well as CSCs. In that context, therapies intended to eradicate CSCs have attempted to eliminate these cells by either induction of differentiation or targeted eradication [39,40].

Differentiation induction

Therapies aimed at inducing differentiation are built upon the principle that differentiation of CSCs leads to a loss of self-renewal capabilities and drug resistance properties, thus making them susceptible to standard therapies. The best-known example of therapy aimed at inducing differentiation in CSCs is in the treatment of APL. ATRA, a retinoid, has helped to make APL one of the most treatable types of leukemia. ATRA targets PML/RAR- α , a suppressor of differentiation, inducing cellular differentiation [41]. In addition to their use in APL, retinoids also have been used in head-and-neck and lung cancer, where induction of differentiation has been utilized as a chemopreventative mechanism for precancerous lesions [39].

In GI cancer, ATRA induces differentiation in colon cancer cell lines [42]. Similarly, ATRA treatment of patient-derived xenografts from gastric cancers induces CSC differentiation and apoptosis, reducing tumorigenicity [43]. Clinically, when combined with omeprazole and sucralfate, ATRA significantly improves precancerous gastric dysplasia [44]. Additionally, incorporating ATRA into standard therapy for gastric cancer improves survival compared standard therapy alone [45]. Although ATRA failed to demonstrate clinical efficacy in the treatment of other GI cancers, its aforementioned utility in specific cases provides a framework and proof of principle for the potential use of differentiation therapy.

In addition to ATRA, other agents induce differentiation, including PPAR- γ agonists. PPAR- γ is an important regulator of differentiation in many cell types, most notably those involved in lipid homeostasis, controlling the terminal differentiation of preadipocytes and fibroblasts [46]. A majority of the work with PPAR- γ to date has been performed in cancer cell lines. Agonists of PPAR- γ , such as pioglitazone, exit cells from the cell cycle by inducing terminal differentiation [47]. Of particular interest for GI tumors is the work done by Sarraf *et al.* which revealed increased PPAR- γ expression in colonic adenocarcinoma. Additionally, treatment with the PPAR- γ agonist troglitazone led to colon cancer cell differentiation, measured by increased expression of carcinoembryonic antigen (CEA) [48]. Moreover, knockdown of PPAR- γ in mice promoted the growth of tumors by decreasing differentiation among cancer cells [49]. These studies reveal that there are pathways which can be targeted in cancers of the GI tract to toggle differentiation among cells. However, significant work in this area is required prior to therapies advancing into the clinic, since most studies have been carried out in cancer cell lines *in vitro*.

Targeted elimination

In addition to therapies which induce differentiation, treatment of CSCs also can be accomplished by targeting stem cells for elimination. As described previously, CSCs have unique surface marker expression profiles, creating opportunities for pharmacotherapeutic strategies that target these CSC-specific properties. Targeted elimination also may be accomplished by attacking mechanisms which afford CSCs with therapeutic resistance or survival advantages.

Targeting cell surface receptors

To date, cell surface markers have been utilized to identify CSCs within numerous GI cancers, including pancreatic [20,50], liver [51] and colon [18,19]. Identification and selective targeting of CSC-specific cell-surface markers allow for the administration of highly potent cytotoxic agents with limited systemic toxicity, in contrast to conventional chemotherapeutics that target all rapidly dividing cells. Two potential modes of eradication exist which use these markers as targets: immunotherapies and drug carriers.

Immunotherapy

Targeting CSCs utilizing the immune system offers clear advantages compared with standard treatments. First, much like other antibody therapies, immune cells exert cytotoxic effects in an antigen-specific manner, offering a more focused approach toward targeting CSCs. Second, in conventional treatments, such as chemotherapy and radiation, toxicity is partly dependent on cell cycle phase [52]. Since CSCs are relatively quiescent compared

with non-CSCs, immunotherapeutic approaches may offer a way to eliminate CSCs independent of proliferation status [53]. Finally, immunotherapy may produce long-lived memory responses that could be effective in challenging a cancer relapse. Numerous immunotherapeutic approaches targeting CSCs in gastrointestinal malignancies are described below.

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 become an exciting immunotherapeutic approach for treating cancers [54]. In brief, CAR T cells are T cells engineered to express an artificial receptor composed of a targeting domain derived from an antibody connected to intracellular signaling domains [54]. CARs derived from antibodies targeting surface antigens on CSCs therefore represent a possible therapeutic approach. Recently, results from a Phase I trial testing CAR T cells directed against CD133 in patients with hepatocellular, pancreatic and colorectal carcinomas were published [55]. Of 23 patients, 3 underwent partial remission and 14 exhibited stable disease. Analysis from biopsied tissues also revealed reduction of CD133⁺ cells. Although Epcam is a less-selective CSC marker, CAR T cells targeting this marker also have been developed. A report by Ang *et al.* found that anti-Epcam CAR T cells increased survival in xenograft mouse models with human rectal tumors [56]. Clinical trials testing anti-Epcam CAR T cells in various gastrointestinal malignancies are currently in progress (NCT03013712).

Vaccines

Vaccines stimulating anti-CSC responses have also been reported. Ning *et al.* found that vaccination using dendritic cells pulsed with lysates from CSCs identified by high expression of aldehyde dehydrogenase (ALDH^{High}) was capable of generating CSC-specific T cell and antibody responses *in vivo* [57]. Moreover, dendritic cells pulsed with tumor lysates from ALDH^{High} cells, compared with whole tumor lysates, displayed increased inhibition of tumor growth and reduced metastases in squamous cell and melanoma tumor models. This suggests that autologous tumor cell vaccines targeting CSCs may lead to greater antitumor effects. Based on the approach by Ning *et al.*, Phase I/II clinical trials administering CSC vaccines have been conducted in liver (NCT02089919), colorectal (NCT02176746) and pancreatic (NCT02074046) cancers. Results from these trials have yet to be published.

Other immune cells

Although most efforts targeting CSCs have focused on exploiting the adaptive immune system, it is becoming increasingly appreciated that CSCs also are recognized by innate immune cells. *In vitro* experiments by Talerico *et al.* found that natural killer (NK) cells preferentially lysed CSCs over non-CSCs in colorectal tumors [58]. Cytotoxic activity toward CSCs was associated with increased expression of NK activating ligands NKp30 and NKp44 and decreased expression of inhibitory ligands such as MHC class I on CSC surfaces compared with non-CSC cells. Similar reports of CSC-specific killing by NK cells have been reported in melanoma and glioblastoma [59,60]. Moreover, recent studies by Ames *et al.* demonstrated the ability of NK cells to target pancreatic CSCs *in vivo*. Using mice with human pancreatic tumor xenografts, adoptive transfer of NK cells was found to reduce percentages of ALDH^{High} cells and work synergistically with radiation to suppress tumor growth [61,62]. Interestingly, $\gamma\delta$ T cells also exhibit anti-CSC activity in multiple malignancies [63,64]. In colorectal cancer, Todaro *et al.* reported that zoledronate induced accumulation of mevalonate metabolites in CSCs making them targets for elimination by $\gamma\delta$ T cells [65]. Use of NK and $\gamma\delta$ T cells may present alternative methods for targeting CSCs independent of antigen processing and MHC expression.

Drug carriers

In addition to immunologic approaches, cells surface marker-targeted delivery of cytotoxic agents is a potential strategy for the eradication of CSCs. One such promising therapy is the creation of aptamers, which are small single stranded DNA or RNA, nearly 20-times smaller than antibodies. These aptamers bind with high affinity to their targets and are internalized by cells [66–68]. The first RNA aptamer targeting a CSC surface marker was created in 2010 by Shigdar *et al.* This aptamer was designed to interact with epithelial cell adhesion molecule (EpCAM), a transmembrane glycoprotein commonly overexpressed in solid tumors as well as CSCs. It interacted with multiple cancer cell lines, including KATO III (gastric carcinoma) and T47D (colon adenocarcinoma cell line) [68]. More recently, two RNA-aptamers targeting the AC133 epitope of CD133, a marker of CSCs in colon and pancreas, were identified [18–20,65,67]. These CD133 aptamers were internalized by both HT-29 (human colorectal cancer

cell line) and Hep3B (hepatocellular carcinoma cell line) cells, among others. Additionally, they displayed superior ability to penetrate HT-29 tumor-derived spheres compared with a CD133 antibody [67]. Recently, an aptamer directed at the CD44 surface receptor, a common CSC marker, was internalized by breast cancer cell lines [68]. Although this study did not demonstrate internalization of the aptamer in GI cells, it is important to note that CD44 has been utilized as a marker of GI CSCs previously [69]. As aptamers are typically less immunogenic and have low toxicity, they have excellent potential as a drug-delivery system with less systemic toxicity than standard therapies. In spite of the exciting potential of these conjugates, at this point, they have not proven to be efficacious *in vivo* and further work is needed prior to systemic delivery [70].

Similar to nanocarriers such as aptamers, antibody–drug conjugates are a promising therapeutic option, which would allow cytotoxic agents to be delivered to targeted cells in the absence of systemic toxicities. Antibody–drug conjugates require internalization followed by lysosomal processing and cleavage in order to activate the drug. This allows for delivery of therapy to only those cells which display the antigen [40]. These conjugates can be used in conjunction with standard chemotherapy and radiation for improved results. Indeed, this concept has been utilized in the treatment of acute myeloid leukemia, where a gemtuzumab–ozogamicin drug conjugate directed at CD33⁺ leukemia cells has been combined with standard chemotherapy to improve survival [71]. Antibody–drug conjugates directed at CSC surface markers are currently under investigation. Recently, two antibody–drug conjugates were developed targeting LGR5, a marker of CSCs in colon cancer. In a mouse model, one displayed antitumor efficacy and safety *in vivo*. Although there is much further work prior to therapies such as this being safe for use in human subject, this study did provide proof of concept that antibody–drug conjugates can be targeted at CSC surface markers [72].

Targeting resistance mechanisms

Another potential mechanism to eradicate CSCs is by targeting the machinery mediating resistance to standard therapies. Two areas where this has been explored in CSCs include inhibition of ABC transporters as well as targeting antioxidant systems.

Transporters

As described earlier, ABC transporters afford CSCs the ability to evade traditional chemotherapy by effluxion of chemotherapeutic agents. Therapy aimed at disrupting these transporters sensitizes CSCs to standard chemotherapy. The best investigated strategy to inhibit the function of ABC transporters is by direct modulators, of which there are three generations. In spite of showing promise versus leukemia cells *in vitro*, the first known modulator, verapamil, failed to improve the toxicity of vinblastine in a Phase I clinical trial [73,74]. Second generation inhibitors appeared to be promising, yet caused decreased clearance of chemotherapy and increased toxicity in clinical trials [75,76]. Third generation inhibitors have shown much more promise as a potential therapy for multidrug resistance [75,76]. Other strategies targeting transcriptional regulation of ABC transporters or signaling pathways involving ABC transporters are largely in their infancy and will require further development [75,76].

Antioxidant systems

Another therapeutic approach to disarm CSCs resistance mechanisms is through targeting antioxidant systems, increasing oxidative stress in the setting of radiation and chemotherapy. The most common potential target is GSH, a metabolite which protects cells against oxidative injury [31,77]. In squamous cell carcinoma of the head and neck, inhibition of xCT, a mediator of cysteine transport required for GSH synthesis, leads to apoptosis in CD44v-expressing stem-like cells [77]. CD44v interacts with and stabilizes xCT, promoting cysteine uptake allowing for GSH synthesis. Consequently, CD44v ablation can destabilize xCT and decrease GSH. In a mouse model of gastric cancer, CD44v ablation resulted in a loss of cell surface expression and decrease in intracellular GSH, suppressing tumor growth [31]. These studies reveal that eliminating aspects of the cellular defense system against ROS can impact cell viability.

Antitelomerase therapy

Telomere shortening is a major regulator of cell mortality. In most tissues, telomerases, which help maintain telomere length, are suppressed prior to birth maintaining normal telomere-dependent cell mortality. Telomerase activity throughout life is relegated to select populations of stem cells, thus allowing for immortality. CSCs, much like normal stem cells, remain immortal and capable of self-renewal, largely due to expression of telomerase, allowing

Figure 1. Targeting cancer stem cells. (A) Traditional chemotherapy targets tumor cells undergoing rapid division. CSCs possess qualities such as overexpression of drug transport channels, depicted here, permitting efflux of chemotherapeutic agents. Other modes employed by CSCs to evade therapy include increased redox capacity, increased DNA checkpoint repair and maintenance of a senescent state. By evading standard therapies, CSCs generate new populations of rapidly dividing tumor cells, leading to disease recurrence. (B) Ideal therapies target both the bulk tumor population as well as CSCs, preventing recurrence. CAR-T cells, shown here, represent one example of directed therapy that could target both cell populations. CAR-T cells can be programmed to recognize cell surface receptors expressed on CSCs, leading to their elimination and thus removing them as drivers of disease recurrence. Numerous additional methods of CSC-targeted therapies currently under investigation are discussed in more detail. CAR: Chimeric antigen receptor; CSC: Cancer stem cell.

them to escape replicative senescence. In addition to CSCs, most tumor cells express some level of telomerase [78]. This makes telomerase an excellent target for therapy, as it can affect differentiated cancer cells as well as CSCs.

Currently, there are two methods for directing therapy at telomerase. One is by direct inhibition of the enzyme itself. The first antitelomerase compound BIBR1532, showed promise, but failed to advance to the clinical trials stage. More recently, compound GRN163L has advanced to the clinical trial stage and proven to be effective in mouse xenografts of multiple tissue types [78]. In the field of GI cancer, GRN163L has shown efficacy in a mouse xenograft of human hepatoma impairing tumor growth *in vivo*. *In vitro*, chemosensitivity to doxorubicin was increased when GRN163L was given prechemotherapy [79]. Also, in cells purified from surgical specimens of Barrett's esophagus treated with GRN163L, a decrease of telomerase activity occurs. Additionally, telomere shortening is observed, leading to eventual apoptosis *in vitro*. *In vivo*, treatment with GRN163L decreased tumor volume in a mouse xenograft model [80]. Additionally, GRN163L increased radiation sensitivity in esophageal squamous cell carcinoma cell lines, leading to increased apoptosis [81,82]. Although these studies suggest promise for a potential GI cancer therapeutic, to date, 19 clinical trials have been opened utilizing GRN163L, yet none have been targeted at GI cancers [83].

The second method of antitelomerase therapy is by immunotherapy. Vaccines directed at TERT, a catalytic component of telomerase, would allow for the killing of tumor cells by CD8⁺ T cells, while largely avoiding toxicity to normal tissue with little to no telomerase expression [78]. In the field of pancreatic cancer therapy, there was hope that GV1001, a telomerase vaccine, would prove efficacious in patients with advanced stage pancreatic cancer. However, given in combination with chemotherapy, GV1001 showed no improvement in overall survival in a Phase III clinical trial [84]. Further research is needed in the area of telomerase immunotherapy as well as telomerase inhibiting therapy, to realize its potential to target CSCs.

CSC signaling pathways

One last therapeutic approach aimed at GI CSCs is to target the signaling pathways implicated in self-renewal and increased CSC survival. The pathways that are largely responsible for CSC self-renewal include Notch/ δ , Hedgehog and Wnt/ β -catenin [40,85–87]. Targeting these pathways is difficult as many of them are diverse, composed of varying receptors and ligands which are heterogeneous among different tumor types. Targeting these pathways requires a specific understanding of the role of the pathway in each tumor type as well as the development of a readout for successful pathway inhibition [87].

Notch

The notch signaling pathway is one of the most diverse of those implicated in CSC signaling. The two classes of prominent notch-signaling inhibitors being investigated are γ -secretase inhibitors and anti-DLL4 antibodies. γ -secretase inhibitors are those that prevent the final proteolytic cleavage of the notch receptor [87]. A Phase II clinical trial was carried out with R04929097, a selective inhibitor of γ -secretase, as a single agent for patients with metastatic colorectal cancer which showed minimal activity [88]. Also, it failed to show efficacy as a monotherapy in a Phase II trial for metastatic pancreatic cancer [89]. Although R04929097, the most tested of the γ -secretase inhibitors, has not shown therapeutic effect versus GI cancers to this point, it has shown efficacy in other tumor types [90]. These studies investigated notch inhibitors as monotherapies, thus they do not discount the possibility that γ -secretase inhibitors may be a potential option to target CSCs in combination with other standard therapies.

The second major way of targeting the notch pathway is with anti-DLL4 antibodies, which disrupt the formation of capillary networks necessary for tumor proliferation. Currently, there are numerous ongoing clinical trials involving anti-DLL4 antibodies, including Phase I trials for combination therapy in locally advanced pancreatic cancer and metastatic colorectal cancer. However, results from these studies are not available at this time. Beyond targeting DLL4 and γ -secretase, there are other monoclonal antibodies being developed that target a variety of notch receptors [87]. Although it appears that targeting notch may have some potential in therapeutic treatment of stem cells, further work is necessary in this setting.

Hedgehog

The hedgehog pathway plays a significant role in stem cell maintenance as well as in the differentiation and fate of many cell types [40]. The best studied anti-HH agent is vismodegib, a cyclopamine-competitive agonist. It has been approved by the FDA for the treatment of advanced basal-cell carcinoma [87,91]. In spite of its promise in basal-cell carcinoma, it has not improved progression-free or overall survival in clinical trials as combination therapy

in patients with gastric, gastroesophageal junction tumors or metastatic pancreatic adenocarcinoma [92,93]. In spite of the lack of clinically available agents to target GI CSCs, there is promise regarding anti-HH agents and their ability to effect CSCs. *In vitro*, vismodegib decreased proliferation and self-renewal of CD133⁺ glioblastoma CSCs, justifying its investigation as a stem cell-targeting agent [94].

Wnt/ β -catenin

The Wnt/ β -catenin pathway is comprised of a family of proteins that regulates stem cell renewal and maintenance. Moreover, it plays a vital role in tumorigenesis when dysregulated [40,86,87]. This signaling pathway is of particular importance to colorectal cancer where mutations in APC or β -catenin, upregulate Wnt signaling, leading to tumor initiation and progression [86,95]. Currently, there are numerous agents in development targeting the Wnt pathway. Ongoing Phase I and II clinical trials using porcupine inhibitors, a membrane-bound O-acyltransferase which regulates secretion of Wnt proteins, are under investigation in metastatic colorectal cancer. Three Wnt pathway antibodies, including OMP18R5 (anti-frizzled-1/2/5/7/8), OMP-54F28 (Frizzled-8-Fc decoy fusion protein) and OMP131R10 (anti-R-spondin 3 antibody), are in Phase I trials as combined therapies in pancreatic cancer, hepatocellular carcinoma and metastatic colorectal cancer among other non-GI cancers [86,87]. In that context, OMP-54F28 decreases CSC frequency in patient-derived xenografts of ovarian cancer, in striking contrast to standard therapy with paclitaxel which enriches for CSCs [96]. Other mechanisms of Wnt directed therapies, including those aimed at downstream effectors, also are currently being developed. However, these largely remain in the preclinical stage [86]. Given the evidence that targeting Wnt signaling has shown effects on CSCs in xenograft studies, as well as having numerous agents currently in clinical trials, it appears to be a promising potential therapeutic direction for targeting CSCs.

Future perspective

As cancer continues to have a tremendous impact on modern day society, it is clear that more advanced therapies are needed to prevent metastatic and recurrent disease. CSC-targeted therapy has emerged as a treatment method which could revolutionize cancer therapy and has an enormous impact on reducing recurrence and metastatic disease. Additionally, many therapies aimed at targeting CSCs are more specific and would allow for less systemic toxicity than traditional chemotherapy and radiation therapy.

In spite of the promise of these therapeutic options, it is clear that there are not available therapies to eradicate CSCs in GI cancer at the present time. There are several major barriers to developing therapies targeted at CSCs. One large barrier is that the safety of such drugs remains a concern. Many of the therapies described above target aspects that are not unique to only CSCs, but rather inherent to stem cells in general. Therapies directed at differentiation, telomerases and cell-signaling pathways would likely have effects not only on CSCs, but also on normal stem cell populations. Additionally, there is a large amount of cross-talk between signaling pathways, and the effect of interrupting these pathways in normal cell populations remains unclear.

Therapies directed at cell surface markers specific to CSCs provide an interesting opportunity to avoid this conundrum as they have potential to provide targeted therapy with minimal systemic toxicity (Figure 1). As described, there are numerous potential ways to accomplish this, including drug carrier systems and immunotherapies, such as vaccines and CAR-T cell therapies. One of the largest problems to overcome when using this approach is the identification of CSCs via reliable cell surface markers that are expressed exclusively among CSCs. Stem cells have been identified only in a small number of GI cancers. Further, there is the potential for subsets of stem cells that do not express known markers. Moreover, there is a potential surface marker profiles among stem cells may vary across different patients. A substantial effort will be required to reliably identify stem cell markers among diverse profiles of GI cancers before these therapies can be advanced to a clinically impactful stage.

Further work is needed in identifying CSCs, understanding their survival mechanisms, therapeutic resistant properties and cell-signaling pathways. However, it is evident that this is an exciting therapeutic approach and one that will demand a large amount of research and investment in the coming years to realize its promise to revolutionize cancer therapy.

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Executive summary

- Despite advancement in chemo- and radiation-therapies, gastrointestinal (GI) cancer remains a tremendous health and economic burden on society.
- Cancer stem cells (CSCs) are a population of cells residing in a heterogenous tumor biome which are uniquely capable of self-renewal and differentiation.
- CSCs possess attributes enabling them to evade therapy and drive metastatic and recurrent disease.
- Ideal cancer therapeutics would target both the differentiated cancer cell population as well as CSCs.

Targeting CSCs

- CSCs may be eliminated by either induction of differentiation or targeted eradication.

Differentiation therapy

- Differentiation induction leads to a loss of self-renewal capabilities and drug resistance properties, making CSCs susceptible to treatment.
- Differentiation therapy has been successful in treatment of acute promyelocytic leukemia providing a framework for use in GI cancer therapy.

Targeted eradication

- CSCs express a unique profile of cell surface receptors, allowing for their identification in many cancer types.
- Immunotherapy such as chimeric antigen receptor T cell therapies and vaccines directed at these receptors have been proposed as potential solutions with clinical trials in both areas presently underway.
- Drug carriers such as aptamers and antibody–drug conjugates are in their infancy but provide a mechanism of delivering directed therapies.
- CSCs may be targeted by attacking their machinery for therapeutic resistance including drug transporters and antioxidant systems, but these methods are largely untested clinically.
- At last, CSC signaling pathways such as notch, hedgehog and Wnt/ β -catenin may be targeted to eradicate CSCs.

Future perspective

- As cancer continues to have large impacts on society, future therapies must be directed not only at bulk tumor cell populations, but also at CSCs.
- Many potential mechanisms of CSC eradication exist; however, few have shown clinical efficacy.
- The safety of drugs attacking CSCs remain a concern, as many would target general stem cell populations as well as CSCs.
- Targeting cell surface receptors appear to be a promising therapy which would largely avoid systemic toxicity, yet this will require further research into identifying stem cell surface profiles.

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