REVIEW ARTICLE
Journal of Digestive Cancer Reports 4(1):1-9, 2016
ISSN 2288-1581
http://www.gicancer.or.kr

Chemoquiescence with Molecular Targeted Ablation of Cancer Stem Cells in Gastrointestinal Cancers

Jong-Min Park[1], Young-Min Han[1], Migyeong Jeong[1], Eun Jin Go[1], Napapan Kangwan[3], Woo Sung Kim[1], Ki Baik Hahm[1,2]

[1]*CHA Cancer Prevention Research Center, CHA University, Seongnam,*
[2]*Digestive Disease Center, CHA University Bundang Medical Center, Seongnam, Korea,*
[3]*Department of Biochemistry, University of Phayao, Phayao, Thailand*

The abundance of multi-drug resistance ATPase binding cassette and deranged self-renewal pathways shown in cancer stem cells (CSCs) played a crucial role in tumorigenesis, tumor resistance, tumor recurrence, and tumor metastasis. Therefore, elucidation of CSCs biology can improve diagnosis, enable targeted treatment, and guide the follow up of GI cancer patients. In order to achieve chemoquiescence, seizing cancer through complete ablation of CSCs, CSCs are rational targets for the design of interventions that will enhance responsiveness to traditional therapeutic strategies and contribute in the prevention of local recurrence as well as metastasis. However, current cancer treatment strategies fail to either detect or differentiate the CSCs from their non-tumorigenic progenies mostly due to the absence of specific biomarkers and potent agents to kill CSCs. Recent advances in knowledge of CSCs enable to produce several candidates to ablate CSCs in gastrointestinal (GI) cancers, especially cancers originated from inflammation-driven mutagenesis such as Barrett's esophagus (BE), *Helicobacter pylori*-associated gastric cancer, and colitis-associated cancer (CAC). Our research teams elucidated through revisiting old drugs that proton pump inhibitor (PPI) and potassium competitive acid blocker (p-CAB) beyond authentic acid suppression, chloroquine for autophage inhibition, sonic hedgehog (SHH) inhibitors, and Wnt/β-catenin/NOTCH inhibitor can ablate CSCs specifically and efficiently. Furthermore, nanoformulations of these molecules could provide an additional advantage for more selective targeting of the pathways existing in CSCs just like current molecular targeted therapeutics and sustained action, while normal stem cells intact. In this review article, the novel approach specifically to ablate CSCs existing in GI cancers will be introduced with the introduction of explored mode of action.

Key Words: Cancer stem cells, GI cancer, Chemoquiescence, Sonic hedgehog, Molecular targeted therapy

INTRODUCTION

Stem cells (SCs) are characterized by the capacity for self-renewal and the ability to differentiate into diverse specialized cell types, of which concept has been extended from the embryonic stem cells (ESCs) and adult stem cells to the definition of CSCs.[1] Though CSCs are defined as malignant cells possessing normal stem cell capacity within tumor cells, many studies of CSCs have demonstrated their rapid growth and high metastatic potential, while normal stem cells are thought to be slow-growing and self-renewing, and lack in functional capacities such as cell migration and attachment.[2] Recent evidence suggests the existence of CSCs in a wide variety of solid tumors. Though CSCs are a small subpopulation of cells within cancers, they are featured with enhanced capabilities of self-renewal, differentiation, and tumorigenesis. When CSCs transplanted into an animal host, using a regulatory network composed of Wnt/β-catenin, Notch, TGF-β, and SHH signaling pathways, they played as tumor initiating cells responsible for tumor recurrence, unresponsiveness to chemoradiotherapy, and metastasis after some latency period (Fig. 1). Therefore, in order to achieve chemoquiescence, terminology telling a state free from tumor resistance to chemotherapy, tumor recurrence, and tumor metastasis, it will be essential to do a definitive

Received: April 5, 2016, Accepted: June 16, 2016
Corresponding author: **Ki Baik Hahm**, MD, PhD
CHA Cancer Prevention Research Centerand Digestive Disease Center, CHA University Bundang Medical Center, 59 Yatap-ro, Bundang-gu, Seongnam 13496, Korea
Tel: +82-31-780-5005, Fax: +82-31-881-7185
E-mail: hahmkb@cha.ac.kr

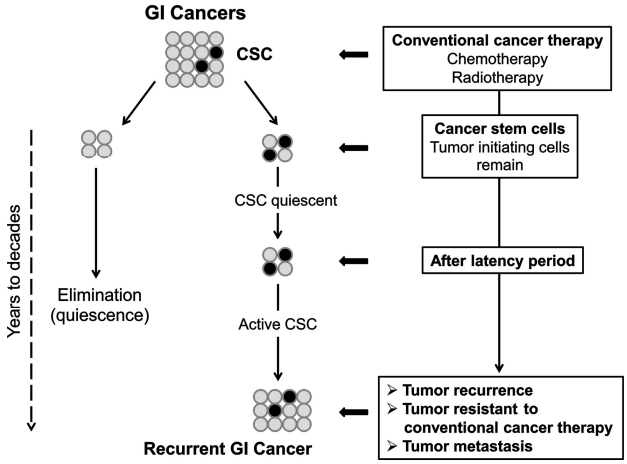

Fig. 1. CSCs implicated in recurrent, resistant, and metastatic GI cancers. CSCs are tumorigenic cancer cells that possess the characteristics associated with normal stem cells, but are responsible for cancer relapse and metastasis featured with characteristics quite different from non-tumorigenic cancer cells. In order to achieve chemoquiescence, CSCs should be ablated, ultimate therapeutic target in oncology. Current anti-cancer therapies inhibit cancer cell growth and make cancer cells to die, unfortunately target normal cells as well as do nothing for resistant cells. Although initial treatments appear to be successful, a relapse generally occurs at a later date. This relapse and resistance to therapy occurs because most traditional and mainstream therapies can't eliminate CSCs. Therefore, it is essential to target these CSCs in order to prevent tumor relapse and to provide an efficient and less toxic treatment for cancer therapy.

assessment of putative CSCs and to do specific ablation of CSCs existing in GI cancers. The ultimate proof of the relevance of CSCs in tumor development and in the clinical management of GI cancers can be achieved with that specific targeting of CSCs can improve patient's outcomes, a goal strongly awaited by scientists, oncologists and cancer patients.

Targeting cancer stem cells (CSCs) in GI cancer; why they are important?

As defined above (Fig. 1), CSCs are tumorigenic cancer cells that possess the characteristics associated with normal SCs, therefore, can generate multiple types of cells found in specific cancer and have the potential for self-renewal like normal SCs. Since CSCs are hypothesized to be tumorigenic and responsible for cancer relapse and metastasis and showed characteristics quite different from non-tumorigenic cancer cells, CSCs attracted the enormous attentions as ultimate therapeutic target in oncology. Upon the questions how CSCs act on tumor is contentious, two representative models of CSCs, one is hierarchical model hypothesizing only subset of cancer cells can induce new cancer and the other is clonal evolution model, are arguing that all cancer cells have potential to proliferate indefinitely and account for tumor heterogeneity and tumor forming subpopulation.[3,4] The premalignant SCs are postulated to acquire accumulation of mutations and become CSCs.[4] Recently, a revised model that unifies the above mentioned hierarchical model and clonal evolution model has raised featuring that instead of distinct model, all factors interact with one another and influence tumor heterogeneity and generation of CSCs.[4] Stimulated with these hypothetical facts, the first modern evidence for a role of SCs in cancer came in 1994 with a study of human acute myeloid Leukemia (AML),[5] in which an AML-initiating cell population was identified from AML patients by transplantation into severe combined immune-deficient mice. Human CSCs were first identified in solid tumors including breast[6] and brain cancer,[7] followed with subsequent reports regarding identification of CSCs in colon, pancreas, lung, prostate cancer, malignant melanoma, and glioblastoma. Besides the ability to newly form tumors, the possibility of migrating CSCs and acquisition of EMT characteristics was proposed as a way of malignant tumor progression.[8] If the CSCs are strongly involved in tumor formation and metastasis, regulation of CSCs is one of the keys in eradicating tumors.[4] Instead of chemotherapeutics which only focus on killing of highly proliferative cancer cells yet do not consider functional heterogeneity of CSCs, profiling and targeting CSCs would be more effective way to treat cancer. Since fundamentals of CSCs are the regeneration of a tumor that resembles the original tumor from which the CSCs was derived, the proportion of CSCs within a tumor may correlate with the severity of the cancer,[9] and the ability to resist cancer therapies, finally leading to recurrence and metastasis. Therefore, more clinical evidence and research is mandatory to identify the CSC-specific surface markers, by which an effort to understand the regulation of CSC tumorigenic capacity and discovery of ablating agents should be continued.

CSCs in GI cancer; Are they instigators and propagators?

Though the existence of CSC is yet not clarified in clinical oncology, several experimental evidences strongly support the concept of CSCs. As much as CSCs, SC niches are specialized microenvironments located within each tissue because SCs reside in the niche. Like well-acknowledged tumor microenvironment, the local tissue environment of SC niche contributes to the onset and progression of tumorigenesis. The

mechanism by which the niche regulates self-renewal, differentiation, tumorigenesis and metastasis of CSCs is same as with SCs not intervened carcinogenesis.[10] Initially, CSCs were believed to represent a very small fraction of the total cell population in a solid tumor, but as progressed, many as 25% of cancer cells may have the properties of CSCs.[11] Furthermore, pre-metastatic niche formation is initiated in small niche, but may increase niche size to enhance metastasis. Recently, Wang X et al.[12] unveiled the role of the PCNA-associated factor-Wnt signaling axis as key molecule that modulates cancer cell stemness and Mery B et al.[13] stressed that in order to successfully eradicate CSCs in head and neck cancer, CD44, polycomb ring finger protooncogene BMI1, CD133, ALDH1 should be targeted. EMT is associated with a cancer stem cell phenotype, particularly in breast cancer,[14] in detail, a downstream effector of EMT in breast cancer is the receptor tyrosine kinase Axl, which is clearly important for invasion and metastasis and upstream regulators of EMT such as TGF-β and relevant microRNAs in combination with treatments that kill the bulk of the tumor cells would therefore seem a promising approach to effective chemotherapy. The microRNA family, miR-103/miR-107, could be another target by which to block EMT. As principle markers suggestive of CSCs as tumor propagating cells, ALDH encoding detoxifying enzymes, of which high expression can be detrimental to tumor eradication; Bmi1 suppressing the INK4A and ARF genes as polycomb complex protein; CD24, a sialoglycoprotein that acts as a ligand for p-selectin enabling cells to bind to platelets and facilitates tumor invasion through interacting with endothelia; CD44, a transmembrane glycoprotein that binds hyaluronan; CD90 a glycosylphosphatidylinositol-anchored protein known as Thy-1; CD105, a type I integral membrane protein; CD117 known as c-kit; a tyrosine kinase receptor for SCF; CD133, which is the first identified member of the prominin family of pentaspan membrane proteins; CD166, activated leukocyte cell-adhesion molecule (ALCAM); EpCAM, a glycosylated type I integral membrane protein expressed by many tumor cells involved in components of Wnt signalling stimulating cell proliferation after cleavage of EpCAM intracellular domain; SP (side population) cells, efflux fluorescent dyes such as Hoechst 33342 and DyeCycle Violet, which is a phenotype that usually depends on expression of the ATPase binding cassette (ABC) superfamily of membrane transporters.[15] As possible therapeutic targets and strategies based on targeting intracellular pathways active in TPCs, the active Wnt, EpCAM, hedgehog (HH), and Notch pathways, ABC transporters, CD133, and ALDH activity, and CXCR4-CXCL12 and CD44-HA interactions have all been implicated in self-renewal and proliferation involved in invasion and metastasis as well as direct destruction of endothelial cells that maintain vascular niches of tumor promoting cells. If tumor initiating or tumor promoting cells (all are part of CSCs) are the roots of cancer, then these are the cells that must be specifically eliminated for a successful therapy. Therefore, highly specific therapeutics must be developed to target CSCs and TPCs.

Targeting CSCs in GI cancer; features according to type of GI cancers

Barrett's esophagus (BE) and BE-associated adenocarcinoma (BAA)

BE is defined as any metaplastic columnar epithelium in the distal esophagus which replaces normal squamous epithelium and which predisposes to cancer development. While BE has been defined pathologically since the 1950's,[16] and identified as a risk factor for BAA since the 1970's,[17] our understanding of the molecular events giving rise to this condition remains limited. What is known about the intestinal features of BE and how well it recapitulates the intestinal epithelium includes stem identity and their function to identify new insights to explain clinical relevance.[18] Though there has not yet been any definitive identification of SCs functionally or based on broadly cell markers, several groups have identified populations of cells expressing intestinal SC markers such as Lgr5, doublecortin and CaM kinase-like-1 (DCAMKL-1), and sex determining region Y-Box 9 (SOX-9). Though the SCs for BE had not yet been identified, the expression of these markers provides the clue to identify. Though still controversial, label retaining cell (LRC) populations have been implicated as SCs by some. In a very novel *in vivo* study in humans, a rare slow-cycling LRC population, representing <0.1% of whole epithelial cells, has been detected in the crypt base in BE, whereas >99% of these LRC do not express lineage-specific markers such as defensin-5, MUC2 and chromogranin A. In near future, much more work needs to be carried out to identify stem cell populations giving rise to BE.

Helicobacter pylori (H. pylori) infection is responsible for activation of CSCs as class I

carcinogen Gastric cancer is one of the most common cancers of digestive system globally and *H. pylori* infection is believed to be a major risk factor as class 1 carcinogen defined by International Agency for Research Cancer. The multiple mechanisms of *H. pylori*-induced gastric carcinogenesis and their progressions are intervened, including inflammation-induced

gastric atrophy, tumor microenvironment, DNA nitration and oxidation induced by mutagenic factors, *H. pylori*-induced epigenetic modifications, *H. pylori*-induced disruption of the balance between proliferation and apoptosis, and *H. pylori*-induced cancer cell invasion, all essential steps implicated in gastric carcinogenesis. Furthermore, *H. pylori* may also affect the biological function of CSCs.[19] The ability of *H. pylori* and its oncoprotein CagA to reprogram epithelial cells and activate properties of stemness show the sophisticated relationship between *H. pylori* and progenitor cell transformation in the gastric mucosa.[20] Within the last years, it became clear that gastric self-renewal and carcinogenesis are intimately linked during *H. pylori*-associated chronic inflammatory conditions because gastric cancer is now regarded as a disease resulting from dysregulated differentiation of stem and progenitor cells influenced by an inflammatory environment, plausible condition that various gastric epithelial stem cells relevant to *H. pylori* infection contribute to self-renewal and these dysregulated routes are known to gastric adenocarcinomas including CSCs.[21] Experimental studies have highlighted the role of bone marrow-derived cells (BMDCs) and particularly mesenchymal stem cells (MSCs), in the neoplastic process in about a quarter of the cases and possibly an epithelial-mesenchymal transition (EMT) in the other cases. Different studies have confirmed that chronic *H. pylori* infection induces a chronic inflammation and subsequent damage of the gastric epithelial mucosa, leading to BMDC recruitment. EMT induces the emergence of $CD44^+$ cells possessing MSC and SC properties, resulted in metaplastic and dysplastic lesions to give rise to the emergence of CSCs and adenocarcinoma.[22] Choi YJ et al.[23] explored that *H. pylori* infection may trigger the TGF-β1-induced EMT pathway and lead to the emergence of CSC. Interestingly, its eradication may prevent the carcinogenesis of gastric cancer by inhibiting these two events. In conclusion, the recent identification of normal SCs and gastric CSCs has greatly improved our understanding of the molecular and cellular etiology of *H. pylori*-induced gastric cancer and will aid in the development of effective therapies to treat patients.[24]

Colitis-associated cancer (CAC) arising from longstanding inflammatory bowel disease

Colorectal cancer (CRC) is one of the most commonly diagnosed and lethal cancers worldwide. It is a multistep process that requires the accumulation of genetic and epigenetic aberrations. There are several issues concerning colorectal carcinogenesis that remain unanswered such as the cell of origin and the type of cells that propagate the tumor after its initiation. There are two models of carcinogenesis: the stochastic and the CSC model in CRC and CAC development. According to the stochastic model, any kind of cell is capable of initiating and promoting cancer development, whereas the CSC model suggests that tumors are hierarchically organized and only CSCs possess cancer-promoting potential. Moreover, various molecular pathways, such as Wnt/Notch as well as the complex crosstalk network between tumor microenvironment and CSCs, are involved in CRC and CAC. In colon cancer, also the identification of CSCs remains controversial due to the lack of widely accepted specific molecular markers yet. Since CSCs are responsible for tumor relapse, because conventional drugs fail to eliminate the CSC reservoir, the design of CSC-targeted interventions is a rational target, which will enhance responsiveness to traditional therapeutic strategies and reduce local recurrence and metastasis as in other GI cancers. This review discusses the implications of the newly introduced CSC model in CRC, the markers used up to now for CSC identification, and its potential implications in the design of novel therapeutic approaches.[25] The traditional, so-called "stochastic model" of tumor development, which assumes that each cancer cell is tumorigenic, has been deeply challenged during the past decade by the identification of CSCs, leading to the development of the hierarchical model of tumorigenesis which assumes that only CSCs have the ability to initiate tumor growth, both at primary and metastatic sites. As in esophageal and gastric cancer, the elimination of all CSCs is fundamental to eradicate tumors and that failure to do so might be responsible for the occurrence of relapses or metastases frequently observed in the clinical management of CRC cancer patients. CSC population in human CRC may derive from transformation of quiescent, normal long-term SCs or could result from the dedifferentiation of more mature cell.

Targeting CSCs in GI cancer; chemoquiescence in order to seize GI cancer

Many therapeutic approaches are on the horizon by which to target CSCs in cancer, which is a challenging prospect given that these cells seem to be particularly resistant to current therapies, of which achievement is termed as "chemoquiescence". As the term "chemoprevention" had been put forward as key in the cancer prevention before the emergence of CSCs theory, chemoquiescence is the best way to seize cancer completely, completed by ablating CSCs during chemoprevention. The terminology chemoprevention was first defined by Sporn MB and Hong WK in Science publication that the prevention of

chemical carcinogenesis by vitamin A and its synthetic analogs retinoids in the early 1970s and identified that natural or synthetic retinoids to prevent and reverse carcinogenesis in many epithelial tissues. This is the modern day concept of chemoprevention and the first proposed[26] and some government program have been created to grow vegetable consumption and reduce cancer occurrence.[27] Our authors group also focused on the discovery of several chemotherapeutic agents. For example, the plant-derived products were confirmed about reducing carcinogenesis, but some products have high toxicity. Phytochemical product can be chemoprevention agents in the process of carcinogenesis, i.e., initiation, promotion and progression, is reported from many laboratories. Tea polyphenol epigallocatechin-3-gallate (EGCG), curcumin from the plant Curcuma longa, resveratrol from red wine, luteolin in several green vegetables had been put forward as potential chemopreventive agent based on confirmed mechanism of inhibiting carcinogenesis, attenuating inflammation, inducing apoptosis and cell cycle arrest.[28-31] Among chemopreventive agents explored by our group that Korean red ginseng, diallyl-sulfide or S-allyl cysteine, cancer preventive kimchi, NSAIDs/aspirin,[32-34] some were additionally proved to be specifically targeted CSCs, reaching to the conclusion that phytochemicals could be taken on a long-term will be valuable tools in tumor formation or tumor recurrence by CSCs, described in detail below. As examples, Balic A et al.[35] demonstrated that chloroquine (CQ), anti-malarial agent, *in vitro* treatment significantly reduced CSCs, decreased tumorigenicity and invasiveness *in vivo*. Our group also identified CQ significantly inhibited tumorsphere formation in a dose dependent manner. Furthermore, combination treatment with CQ and gemcitabine was able to remove tumors, ameliorated survival, and inhibited CXCR4 and hedgehog (HH) signaling. Napapan K et al.[36] in a recent publication, demonstrated *in vitro* treatment with SHH inhibitors (cerulenin, cyclopamine, itraconazole) significantly inhibited TNF-β-stimulated IL-6 and IL-6 receptor/gp130 signaling through STAT3. Furthermore, *in vivo* administration of these SHH inhibitors significantly prevented azoxymethane-initiated, dextran sodium sulfated-promoted CAC in mice. SHH inhibitors significantly reduced colosphere formation and tumorigenesis of tumorsphere.

Potential candidates for ablating CSCs in GI cancer

Current anti-cancer therapies inhibit cancer cell growth and render cancer cells to die. Although initial treatments appear to be successful, a relapse generally occurs at a later date. This relapse and resistance to therapy occurs because most traditional and mainstream therapies can't eliminate CSCs. Therefore, it is essential to target these CSCs in order to prevent tumor relapse and to provide an efficient and less toxic treatment for cancer therapy.[37] The resistance of CSCs to conventional cancer treatments such as chemotherapeutic agents and radiation therapy is considered a formidable problem because remaining CSCs presumably can trigger relapse after the cease of treatment. Therefore, development of new therapeutic strategies based on the CSC model has therefore become a key goal in the challenge to achieve complete eradication of cancer. To date, four strategies have been considered in this review (Fig. 2).[38]

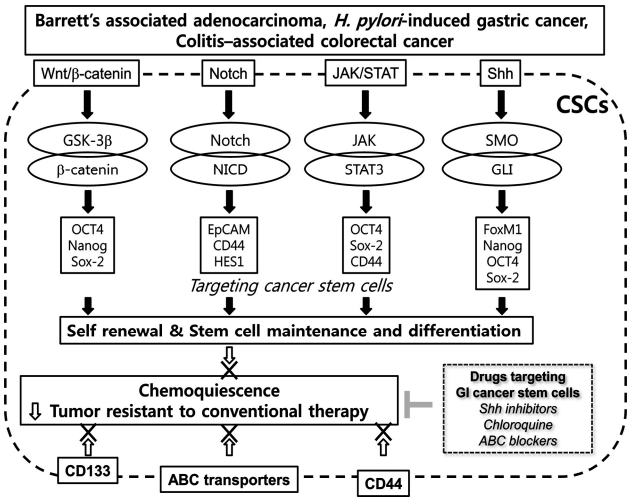

Fig. 2. Hope of eliminating CSCs for chemoquiescence of GI cancers The resistance of CSCs to conventional cancer treatments such as chemotherapeutic agents and radiation therapy is considered a formidable problem because remaining CSCs presumably can trigger relapse after the cease of treatment. Therefore, development of new therapeutic strategies based on the CSC model has therefore become a key goal in the challenge to achieve complete eradication of cancer. In this review article, four strategies have been introduced to eliminate CSCs, targeting ABC transporter, autophagy, proliferative signals, and hedgehog singalings against three inflammation-based GI cancers, BAA, CAG-associated cancer, and CAC.

SHH inhibitors

The HH signaling pathway is a key mediator of segmental patterning to proper development of embryonic cells through controlling the proliferation, migration and differentiation of target cells.[39,40] In mammals, three types hedgehog homologues are present, DHH (desert HH), IHH (indian HH) and SHH (sonic HH), of which SHH is the best studied and these signaling proteins has different effects depending its concentrations in different parts of the embryo.[41-44] The SHH pathway is the important regulator during vertebrate embryonic

development such as organization of brain and the growth of digits on limbs.[39] Recent studies suggested that it controls adult SCs involved in cell division, maintenance and regeneration of adult tissue,[45] but the presence of CSC was found in many human tumors including glioblastoma, breast cancer, pancreatic adenocarcinoma, multiple myeloma and chronic myeloid leukemia.[46-52] Therefore many pharmaceutical companies challenged to develop drugs to target HH signaling against cancer. In the inactive state, the absence of HH leads to prevent highly expression and activation of Smoothened (*Smo*) by the cell-surface transmembrane protein *Ptch*1 while *Gli* 1/*Gli* 2 (among the *Gli* family, zinc-finger transcription factors, acts as activators to transcription) are phosphorylated and removed through proteosomal degradation, but in the active state, the extracellular HH binds to *Ptch*1, initiating *Smo* activation which allowing *Gli1* family activation it means that released *Gli* 1/2 from the Smo protein complex translocate to the nucleus, leading to transcriptional activation of HH associated gene.[53,54] Potential therapeutic agents hasbeen focused that target events such as HH and *Smo* activation and downstream *Gli* proteins in development. Many studies suggested that self-renewal of CSCs to the maintenance of the malignant clone are required HH signaling regulates this process including tumor microenvironment[55-57] and several investigators reported that HH signaling modulates human tumor-derived CSCs.[50] When the tumor progression, CSCs appear in disease progression and HH signaling seem to play an important role, such as for controlling the function of CSCs during metastasis of solid tumor.[56] Perhaps, HH signaling is believed to be a critical role as the Notch/Wnt pathway in cancer.[58,59] Especially, HH and *Gli* pathway (HH- Gli) is required in every step of primary human colon carcinogenesis, essential step for the survival and proliferation. Accor- ding to Varnat et al., the HH-GLI is activated in colorectal cancer epithelial cell, which has been modulated the tumor growth and rate of $CD133^+$ CSCs. In addition, there were increased levels of expression of HH-Gli1 signaling components in advanced metastatic CRC compared with non-metastatic CRC, which were dependent on increases in the HH- Gli1 activity.[60] As cyclopamine is the first phytochemical found in plant *Veratum califomicum*, commonly called the corn lily, which is a drug that acts to suppress the HH pathway and cyclopamine plays to inhibit SHH pathway,[61] many researchers are studied the therapeutic effects using cyclopamine in basal cell carcinoma, medulloblastoma, rhabdomyosarcoma, glioblastoma and multiple myeloma, etc.[61] According to a recent study related to colon CSCs with cyclopamine, HCT116 cells-derived CSCs could see that a lot of those expressing a markers of CSC, SHH downstream gens and EMT as compared to cells with conventional culture. We also confirmed cyclopamine treatment significantly decreased the CSCs stemness marker, SHH downstream genes, and EMT markers.[62] When using the HCT116 cells-derived CSCs in xenograft model, cyclopamine is reduced intact tumor sheet and tumor size and increased apoptosis of tumor.[36]

Chloroquine (CQ)

Several studies suggested that CQ, (N′-(7-chloroquinolin-4-yl)-N, N-diethyl-pentane-1, 4-diamine), anti-malaria agent and anti-autoimmune disease agent, has been evaluated as an autophagy inhibitor during numerous late-stage cancers treatment.[63] Although it is unknown clearly whether it acts solely by inhibiting cancer cell autophagy, CQ was found to have direct effects on different types of malignancies. CQ's anticancer activity is believed to rely on induction of apoptosis, inhibition of autophagy, interaction with nucleotides, elimination of CSC, normalization of the vasculature, enhancing cross presentation, and immune suppression.[64] Therefore, several research groups including ours have discovered CQ as potential candidates to control tumor dormancy or CSCs.[65] In oncology, CQ has been identified as a CSC targeting agent for other aggressive cancers including liver cancer, breast cancer, and CML based on autophagy blocking actions.[66-69] Since autophagy is activated in various GI cancer cells following different anticancer therapies and can activate the survival of cancer cells against hypoxic and nutrient deprived tumor microenvironment by providing catabolites required for repair and by reducing toxic substances and cytoplasmic acidification, autophagy inhibition with CQ can cause a stop of cell recycle needed for CSCs survival, make CSCs more sensitive to the tumor microenvironment, and be useful in improving anti-cancer treatments.[66] Recently, Wei et al. reported that the inhibition of photodynamic therapy (PDT)-induced autophagy by CQ substantially facilitated apoptosis of CSCs existing at CRC and decreased the ability of colonosphere formation *in vitro* as well as tumorigenicity *in vivo*.[70] These results suggested that targeting autophagy by CQ could be used to elevate the PDT sensitivity of CSCs as novel therapeutic approaches for GI cancer treatment. Besides, CQ was identified as a potential CSC inhibitor through *in silico* gene expression signature analysis of the CSCs population. Choi et al., in their recent publication, showed CQ eliminated the CSCs in triple negative breast cancer through inhibition of the Janus-activated kinase 2 (Jak2)-Signal transducer and activator of transcription 3 (STAT3) signaling pathway by reducing the expression of Jak2 and DNA methyltransferase 1

(DNMT1).[71] In pancreatic cancer, Balic et al. showed that CQ significantly decreased CSCs, leading to diminished *in vivo* tumorigenicity and invasiveness of pancreatic cancers.[35] CQ strongly promotes γ-irradiation (γ-IR) induced cell death in highly radio-resistant CSCs of glioma via induction of strong apoptosis and inhibition of autophagy.[72] As results, triple com binations of CQ, γ-ray irradiation, and a PI3K/Akt inhibitor permit reduction of the CQ dose required to trigger cell death. These findings emphasized that CQ can decrease CSCs via autophagy-dependent and autophagy-independent mechanisms on the tumor cells as well as tumor stroma. Moreover, the addition of CQ to the standard of care may greatly and safely potentiate current anti-cancer treatments.

ATPase binding cassette (ABC) blockers; PPI and p-CAB

CSCs are featured with high levels of ABC transporters. Expression of such transporters is commonly associated with multi-drug resistance (MDR) as they provide for a unique defense of cells against chemotherapeutic drugs by significantly decreasing the cellular accumulation of cytotoxic agents. Notably, since CSCs are quiescent, CSCs spend most of their time in G0 cell cycle phase, and have high DNA repair capacity, this is an additional reason for their chemoresistance. MDR cells may repopulate the tumor after the therapy as a result of selection when some of the cancer cells accumulate necessary genetic or epigenetic changes that confer drug resistance. Such resistant cells acquire selective advantage over the rest of the cells, which allows them to evade the therapy. According to the CSCs theory as explained above, tumors already contain small population of intrinsically resistant tumor initiating cells (TICs). Exposure to the chemotherapeutic drugs eliminates the drug sensitive stromal tumor cells, leaving the CSCs. Mutations in the surviving CSCs and their progenies can lead to the development of MDR phenotype. Drug resistance of CSCs has been also associated with overexpre- ssion of other drug efflux transporters. For example, P-glycoprotein (Pgp), also designated ABCB1, is one of the most important efflux transporters related to CSCs resistance. Studies have shown that higher expression of CD133 in CSCs or transfected cells was accompanied with an elevated ABCB1 efflux activity. Angeastro et al. found that Pgp/ABCB1 was up-regulated and displayed functional drug efflux activity in many $CD133^+$ glioma cells in comparison with the parental cells. Though the mechanism behind the increase in ABC transporter expression and activity in CSCs is not clear, recent study pointed out that cisplatin treatment selects for MDR $CD133^+$ cells by activating Notch signaling pathway.

CONCLUSION

Ever since the first experimental identification of CSCs, the CSC model has been a major topic of debate as a result of uncertainties concerning the properties of these cells such as defining cell surface markers and plasticity. However, it might be certain that the introduction of the CSC concept has resulted in important advances in cancer research. For instance, the CSC-based hierarchical model has provided a better understanding of the tumor heterogeneity and the goal of development of anti-cancer drugs will change from reduction of tumor size to targeting subpopulations of tumor cells with a high tumorigenic potential. Future studies of CSCs will need to expand beyond the xenograft approach, in which cells derived from patients tumor are transplanted into immune-compromised mice, PDX (patient derived xenograft) model and include the development and characterization of mouse tumor models that recapitulate aspects of tumor heterogeneity and the microenvironment in order to provide further insight into the complexity of CSCs observed in actual human tumors. In this review, we explored the potential of SHH inhibitors, PPI inhibitor, p-CAB, and CQ as future potential specifically targeted CSCs existing in GI cancer and hope for chemoquiescence can be come true with well-designed clinical trials in a near future.

ACKNOWLEDGMENTS

Supported by This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014 R1A1A2058732)

REFERENCES

1. Yu Z, Pestell TG, Lisanti MP, Pestell RG. Cancer stem cells. Int J Biochem Cell Biol 2012;44:2144-2151.
2. Saikawa Y, Fukuda K, Takahashi T, Nakamura R, Takeuchi H, Kitagawa Y. Gastric carcinogenesis and the cancer stem cell hypothesis. Gastric Cancer 2010;13:11-24.
3. Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell 2014;14:275-291.
4. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105-111.
5. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645-648.
6. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ,

Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003;100:3983-3988.
7. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003;63: 5821-5828.
8. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. Nat Rev Cancer 2005;5:744-749.
9. Diehn M, Clarke MF. Cancer stem cells and radiotherapy: new insights into tumor radioresistance. J Natl Cancer Inst 2006;98:1755-1757.
10. Todaro M, Francipane MG, Medema JP, Stassi G. Colon cancer stem cells: promise of targeted therapy. Gastroenterology 2010;138:2151-2162.
11. Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. Science 2007;317:337.
12. Wang X, Jung YS, Jun S, et al. PAF-Wnt signaling-induced cell plasticity is required for maintenance of breast cancer cell stemness. Nat Commun 2016;7:10633.
13. Mery B, Guy JB, Espenel S, et al. Targeting head and neck tumoral stem cells: From biological aspects to therapeutic perspectives. World J Stem Cells 2016;8:13-21.
14. Alison MR, Lim SM, Nicholson LJ. Cancer stem cells: problems for therapy? J Pathol 2011;223:147-161.
15. Alison MR, Islam S, Wright NA. Stem cells in cancer: instigators and propagators? J Cell Sci 2010;123:2357-2368.
16. Johnstone A. Oesophagitis and Peptic Ulcer of the Oesophagus: The Mackenzie Davidson Memorial Lecture. The British journal of radiology 1955;28:229-240.
17. Naef AP, Savary M, Ozzello L. Columnar-lined lower esophagus: an acquired lesion with malignant predisposition. Report on 140 cases of Barrett's esophagus with 12 adenocarcinomas. J Thorac Cardiovasc Surg 1975;70:826-835.
18. Nakagawa H, Whelan K, Lynch JP. Mechanisms of Barrett's oesophagus: intestinal differentiation, stem cells, and tissue models. Best Pract Res Clin Gastroenterol 2015;29:3-16.
19. Meng W, Bai B, Sheng L, et al. Role of Helicobacter pylori in gastric cancer: advances and controversies. Discov Med 2015; 20:285-293.
20. Amieva M, Peek RM, Jr. Pathobiology of *Helicobacter pylori*-Induced Gastric Cancer. Gastroenterology 2016;150:64-78.
21. Hoffmann W. Current Status on Stem Cells and Cancers of the Gastric Epithelium. Int J Mol Sci 2015;16:19153-19169.
22. Bessede E, Dubus P, Megraud F, Varon C. *Helicobacter pylori* infection and stem cells at the origin of gastric cancer. Oncogene 2015;34:2547-2555.
23. Choi YJ, Kim N, Chang H, et al. *Helicobacter pylori*-induced epithelial-mesenchymal transition, a potential role of gastric cancer initiation and an emergence of stem cells. Carcinogenesis 2015;36:553-563.
24. Zhao Y, Feng F, Zhou YN. Stem cells in gastric cancer. World J Gastroenterol 2015;21:112-123.
25. Vaiopoulos AG, Kostakis ID, Koutsilieris M, Papavassiliou AG. Colorectal cancer stem cells. Stem Cells 2012;30:363-371.
26. Sporn MB, Dunlop NM, Newton DL, Smith JM. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 1976;35:1332-1338.
27. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003;3:768-780.
28. Khan N, Bharali DJ, Adhami VM, et al. Oral administration of naturally occurring chitosan-based nanoformulated green tea polyphenol EGCG effectively inhibits prostate cancer cell growth in a xenograft model. Carcinogenesis 2014;35:415-423.
29. Papi A, Farabegoli F, Iori R, et al. Vitexin-2-O-xyloside, rapha satin and (-)-epigallocatechin-3-gallate synergistically affect cell growth and apoptosis of colon cancer cells. Food Chem 2013; 138:1521-1530.
30. Yang SF, Yang WE, Chang HR, Chu SC, Hsieh YS. Luteolin induces apoptosis in oral squamous cancer cells. J Dent Res 2008;87:401-406.
31. Lim do Y, Jeong Y, Tyner AL, Park JH. Induction of cell cycle arrest and apoptosis in HT-29 human colon cancer cells by the dietary compound luteolin. Am J Physiol Gastrointest Liver Physiol 2007;292:G66-75.
32. Choi KS, Song H, Kim EH, et al. Inhibition of Hydrogen Sulfide-induced Angiogenesis and Inflammation in Vascular Endothelial Cells: Potential Mechanisms of Gastric Cancer Prevention by Korean Red Ginseng. J Ginseng Res 2012;36: 135-145.
33. Park JM, Han YM, Kangwan N, et al. S-allyl cysteine alleviates nonsteroidal anti-inflammatory drug-induced gastric mucosal damages by increasing cyclooxygenase-2 inhibition, heme oxygenase-1 induction, and histone deacetylation inhibition. J Gastroenterol Hepatol 2014;29 Suppl 4:80-92.
34. Jeong M, Park JM, Han YM, et al. Dietary prevention of *Helicobacter pylori*-associated gastric cancer with kimchi. Oncotarget 2015;6:29513-29526.
35. Balic A, Sorensen MD, Trabulo SM, et al. Chloroquine targets pancreatic cancer stem cells via inhibition of CXCR4 and hedgehog signaling. Mol Cancer Ther 2014;13:1758-1771.
36. Kangwan N, Kim YJ, Han YM, et al. Sonic hedgehog inhibitors prevent colitis-associated cancer via orchestrated mechanisms of IL-6/gp130 inhibition, 15-PGDH induction, Bcl-2 abrogation, and tumorsphere inhibition. Oncotarget 2015;
37. Bandhavkar S. Cancer stem cells: a metastasizing menace! Cancer Med 2016;
38. Sugihara E, Saya H. Complexity of cancer stem cells. Int J Cancer 2013;132:1249-1259.
39. Ingham PW, McMahon AP. Hedgehog signaling in animal development: paradigms and principles. Genes Dev 2001;15: 3059-3087.
40. Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. Genes Dev 2008;22:2454-2472.
41. Krauss S, Concordet JP, Ingham PW. A functionally conserved homolog of the Drosophila segment polarity gene hh is expressed in tissues with polarizing activity in zebrafish embryos. Cell 1993;75:1431-1444.
42. Echelard Y, Epstein DJ, St-Jacques B, et al. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 1993;75: 1417-

1430.
43. Riddle RD, Johnson RL, Laufer E, Tabin C. Sonic hedgehog mediates the polarizing activity of the ZPA. Cell 1993;75: 1401-1416.
44. Scholpp S, Wolf O, Brand M, Lumsden A. Hedgehog signalling from the zona limitans intrathalamica orchestrates patterning of the zebrafish diencephalon. Development 2006; 133:855-864.
45. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. Nature 2004;432:324-331.
46. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol 2007;17:165-172.
47. Bar EE, Chaudhry A, Lin A, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. Stem Cells 2007;25:2524-2533.
48. Dierks C, Beigi R, Guo GR, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell 2008;14:238-249.
49. Liu S, Dontu G, Mantle ID, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res 2006;66:6063-6071.
50. Feldmann G, Dhara S, Fendrich V, et al. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. Cancer Res 2007;67:2187-2196.
51. Peacock CD, Wang Q, Gesell GS, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci USA 2007;104:4048-4053.
52. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature 2009;458:776-779.
53. Ruiz i Altaba A, Mas C, Stecca B. The Gli code: an information nexus regulating cell fate, stemness and cancer. Trends Cell Biol 2007;17:438-447.
54. Apionishev S, Katanayeva NM, Marks SA, Kalderon D, Tomlinson A. Drosophila Smoothened phosphorylation sites essential for Hedgehog signal transduction. Nat Cell Biol 2005;7: 86-92.
55. Yauch RL, Gould SE, Scales SJ, et al. A paracrine requirement for hedgehog signalling in cancer. Nature 2008;455:406-410.
56. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704-715.
57. Rasheed ZA, Yang J, Wang Q, et al. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. J Natl Cancer Inst 2010;102:340-351.
58. Pannuti A, Foreman K, Rizzo P, et al. Targeting Notch to target cancer stem cells. Clin Cancer Res 2010;16:3141-3152.
59. Takahashi-Yanaga F, Kahn M. Targeting Wnt signaling: can we safely eradicate cancer stem cells? Clin Cancer Res 2010; 16:3153-3162.
60. Manicke NE, Dill AL, Ifa DR, Cooks RG. High-resolution tissue imaging on an orbitrap mass spectrometer by desorption electrospray ionization mass spectrometry. J Mass Spectrom 2010;45:223-226.
61. Taipale J, Chen JK, Cooper MK, et al. Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. Nature 2000;406:1005-1009.
62. Batsaikhan BE, Yoshikawa K, Kurita N, et al. Cyclopamine decreased the expression of Sonic Hedgehog and its downstream genes in colon cancer stem cells. Anticancer Res 2014; 34:6339-6344.
63. Maes H, Rubio N, Garg AD, Agostinis P. Autophagy: shaping the tumor microenvironment and therapeutic response. Trends Mol Med 2013;19:428-446.
64. Pascolo S. Time to use a dose of Chloroquine as an adjuvant to anti-cancer chemotherapies. Eur J Pharmacol 2016;771: 139-144.
65. Vazquez-Martin A, Lopez-Bonetc E, Cufi S, et al. Repositioning chloroquine and metformin to eliminate cancer stem cell traits in pre-malignant lesions. Drug Resist Updat 2011;14:212-223.
66. Song YJ, Zhang SS, Guo XL, et al. Autophagy contributes to the survival of CD133+ liver cancer stem cells in the hypoxic and nutrient-deprived tumor microenvironment. Cancer Lett 2013;339:70-81.
67. Cufi S, Vazquez-Martin A, Oliveras-Ferraros C, Martin-Castillo B, Vellon L, Menendez JA. Autophagy positively regulates the CD44(+) CD24(-/low) breast cancer stem-like phenotype. Cell Cycle 2011;10:3871-3885.
68. Gong C, Bauvy C, Tonelli G, et al. Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. Oncogene 2013;32:2261-2272, 2272e 2261-2211.
69. Bellodi C, Lidonnici MR, Hamilton A, et al. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. J Clin Invest 2009;119:1109-1123.
70. Wei MF, Chen MW, Chen KC, et al. Autophagy promotes resistance to photodynamic therapy-induced apoptosis selectively in colorectal cancer stem-like cells. Autophagy 2014;10: 1179-1192.
71. Choi DS, Blanco E, Kim YS, et al. Chloroquine eliminates cancer stem cells through deregulation of Jak2 and DNMT1. Stem Cells 2014;32:2309-2323.
72. Firat E, Weyerbrock A, Gaedicke S, Grosu AL, Niedermann G. Chloroquine or chloroquine-PI3K/Akt pathway inhibitor combinations strongly promote gamma-irradiation-induced cell death in primary stem-like glioma cells. PLoS One 2012;7:e47357.