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Investigating the molecular mechanism of cancer stem cells (CSCs) in treatment of gastrointestinal cancers

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Abstract

Cancer stem cells (CSCs) are involved in tumor formation, drug and radiation resistance, invasive growth, metastasis, and tumor progression and are major causes of cancer-related mortality. Gastrointestinal cancers are one of the most common malignancies and causes of cancer death worldwide. Because gastrointestinal cancer stem cells are thought to be resistant to common treatments, new and effective treatments are needed. Cancer stem cells have been reported in colorectal, esophageal, gastric, liver and pancreatic cancers. Given that, understanding the formation of cancer stem cells and identifying control pathways and investigating the molecular mechanism of signaling involved in these cells and their role in cancer treatment leads to the development of diagnostic and therapeutic methods in basic and clinical cancer research. In this study, the functional role and molecular mechanisms of cancer stem cells in the treatment of gastrointestinal cancers are investigated.

Keywords: Cancer stem cells, Molecular mechanism, Gastrointestinal cancers

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Introduction

Gastrointestinal cancer is the development of tumors from the proximal esophagus to the distal rectum, including cancers of the liver and bile ducts that extend to the intestinal lumen. Despite advances in surgery, endoscopy, chemotherapy, and radiation therapy, patients with these cancers continue to suffer from recurrence and progression. Cancer stem cells (CSCs) found in heterogeneous tumors are known to be major contributors to cancer recurrence and progression. CSCs are very important in disease recurrence because they have properties that make them resistant to chemotherapy and radiation therapy (1, 2).

Cancer stem cells are responsible for tumor growth, drug and radiation resistance, invasive growth, metastasis, and tumor recurrence, which are major causes of cancer-related deaths. Because gastrointestinal CSCs are thought to be resistant to conventional therapies, effective and new cancer treatment is necessary (3). CSCs are derived from normal adult normal stem cells, progenitor cells, and differentiated adult cells. Thus, signal transduction pathways in CSCs that play an important role in self-renewal are similar to those involved in normal fetal growth (4).

These pathways include Wnt, Hedgehog, and Notch signaling in addition to the polycomb group protein pathways. In addition, growth factors such as fibroblast growth factor, insulin-like growth factor-1, and TGF - β may also play a role in controlling CSCs. Proinflammatory cytokines facilitate the production of CSCs, indicating a possible link between cytokines and inflammation. Hypoxia also plays an important role in regulating self-renewal in normal cells and CSCs (5-7).

Chemotherapy and radiotherapy are the main treatments for cancers of the gastrointestinal tract including the esophagus, stomach, liver, pancreas and rectum. Unfortunately, there is a recurrence and progression of the disease with these treatments. The mechanism that CSC performs in the treatment of cancer includes increased DNA repair, incubation, and drug release and redox capacity. CSCs are armed with multiple mechanisms to escape conventional cancer treatment, so this limits treatment options and allows CSCs to cause disease recurrence and metastasis. Therefore, ideal antitumor therapies should target both proliferating cancer cells and CSCs. In this regard, induction and differentiation therapies are targeted to eliminate CSCs (8, 9). Combining several treatments such as surgery, endoscopy, chemotherapy and radiation therapy may improve survival in patients with gastrointestinal cancer. However, the effectiveness of these treatments depends on the cancer status, metastasis, radiation/chemotherapy resistance, and recurrence, which are thought to be due to CSC. Therefore, new treatment options for these diseases must be developed (10).

In this study, we investigated the role and application of cancer stem cells and the molecular mechanisms of signaling involved in gastrointestinal cancers.

Cancer stem cell characteristics, tumor heterogeneity, and treatment resistance

Intratumor and intratumor heterogeneity are two types of tumor heterogeneity. Tumor heterogeneity can be caused by the origin cells. PDAC and pancreatic neuroendocrine neoplasm, for instance, are two main pancreatic tumor histological categories. Pancreatic neuroendocrine malignancy is further split into two types: well enough and inadequately pancreatic neuroendocrine carcinoma (PanNEC). Different driver genes can display heterogeneity among PanNEC, PDAC, and PanNET. KRAS, SMAD4, CDKN2A, and TP53 are among the major driver gene alterations discovered in PDAC. PanNEC has mutations in the KRAS, TP53, and RB1 genes, whereas PanNET has mutations in the MEN1, DAXX/ATRX, and mTOR pathway genes, which are completely different from those found in PDAC and PanNET. In addition, the origins of PDAC, PanNEN, and PanNEC are murky. PDAC can be caused by intralobular duct precursor cells or acinar cells with exocrine secretion. PanNETs can come from the -cell lineage, islet cell precursors, or the -cell lineage. Originating PanNEC cells could be undifferentiated progenitor cells with stem cell-like features (11).

CSCs are divided into subpopulations with different roles, developmental processes, and gene expression patterns (12, 13). CSC populations can be isolated and identified using cell surface markers. Hematopoietic and embryonic stem cells provide the majority of the

indicators. Nanog, Sox2, Oct4, and c-Myc are among the markers that have been considered favored stemness markers. Various indicators have been identified to characterize CSC populations in many cancer types (Table 1); for example, the combination of CD24 and CD44 markers define a common CSC population for colorectal cancer, liver cancer, pancreatic cancer, and other cancer types. Surprisingly, this population also describes the breast cancer mesenchymal-like CSC population. Furthermore, the expression of most CSC indicators differs among tumor types and even within the same subtype of patients. CD24, for example, was shown to be considerably lower in oral squamous cell carcinoma and considerably larger in pancreatic intraepithelial neoplasia (14).

Due to the obvious absence of uniformity, indicator, EpCAM-in pure populations, and using various markers to enrich CSCs optimally could help. Indeed, EpCAM, CD166, and CD44 were more reliable than CD133 alone as indicators of colorectal cancer.

Table 1. Representative markers of gastrointestinal CSCs.

Gastrointestinal cancer	**Factors**
Gastric cancer	CD44+, Lgr5+, CD44+/CD24+, CD133+, CD44+/Snail1+/VIMENTIN+/E-cadherin+, Snail+, CD44v8-10+, Frizzled7+
Colorectal cancer	E-cadherin-, CD44v2+, CD44v6+, ALDH, CD133+, CD44+/CD24+, CD166+, CD133+/CD44+/ALDH1+
Esophageal cancer	CD44+, B7H4+, CD133+/CXCR4+, WASH+, Numb+, ALDH1A1+, ALDH1+
Liver cancer	CD13+, CD133+, Lin28B+, SALL4+/ EpCAM+, CD90+/CD45−, CD44+/CD90+, SOX9, β-catenin+/GEP
Pancreatic cancer	CD44+/CD24+/EpCAM+, CD133+/ CXCR4+, Pakt+/ SOX9+, CD133+/ CXCR4+, FAM83A+, ALDH1A1+, CD133+/CD44+/CD24+/ESA+

Although they tend to retain activation of one or more important and highly conserved signaling pathways essential in the differentiation and pluripotency of stem cell phenotypes, CSCs exhibit many characteristics of ESCs. CSCs, like ESCs, which mature into blastocysts and supply nourishment for fetal growth, can generate and sustain tumor growth. They can make tumor cells from a variety of stem cells as well as normal somatic cells. They also have potential transcription factors and surface indicators in common. They're also rich in developmental signaling pathways that control embryonic cell characteristics, proper organogenesis, and cell lineage differentiation, all of which could play a role in the onset and advancement of poorly differentiated cancers. Tumor cells have been found to contain five primary signaling pathways that confer embryonic stemness (15).

The Hedgehog, Hippo, Notch, TGF-, and Wnt/-catenin pathways were among them. All of these routes are crucial for CSCs to be able to self-renew and transform into similar daughter cells, preserving their immortality and allowing them to differentiate into different types of cells. Furthermore, these pathways are involved in the development, migration, and resistance of gastrointestinal cancers. Because CSCs are so diverse, the expression of stemness pathways fluctuates over time and in different types of gastrointestinal tumors. Activation of CSC pathways has also been found in tumor cells that exhibit specific CSC markers. In pancreatic cancer, for example, overexpression of Notch1 and Notch2 has been related to higher expression of CD44 and EpCAM Wnt signaling has been demonstrated to be active in CD44+ gastric CSCs to preserve self-renewal and tumor growth. In CD133+ hepatocellular carcinoma (HCC) CSCs, Notch and Jagged be strongly expressed (16).

Biomarkers and signaling pathways specific to CSCs are important in differentiating molecular categories with stem-like characteristics. Variable subtypes have different levels of expression and activation of CSC biomarkers and signaling pathways, which has led to research into potential new paths of therapeutic strategies (Figure 1).

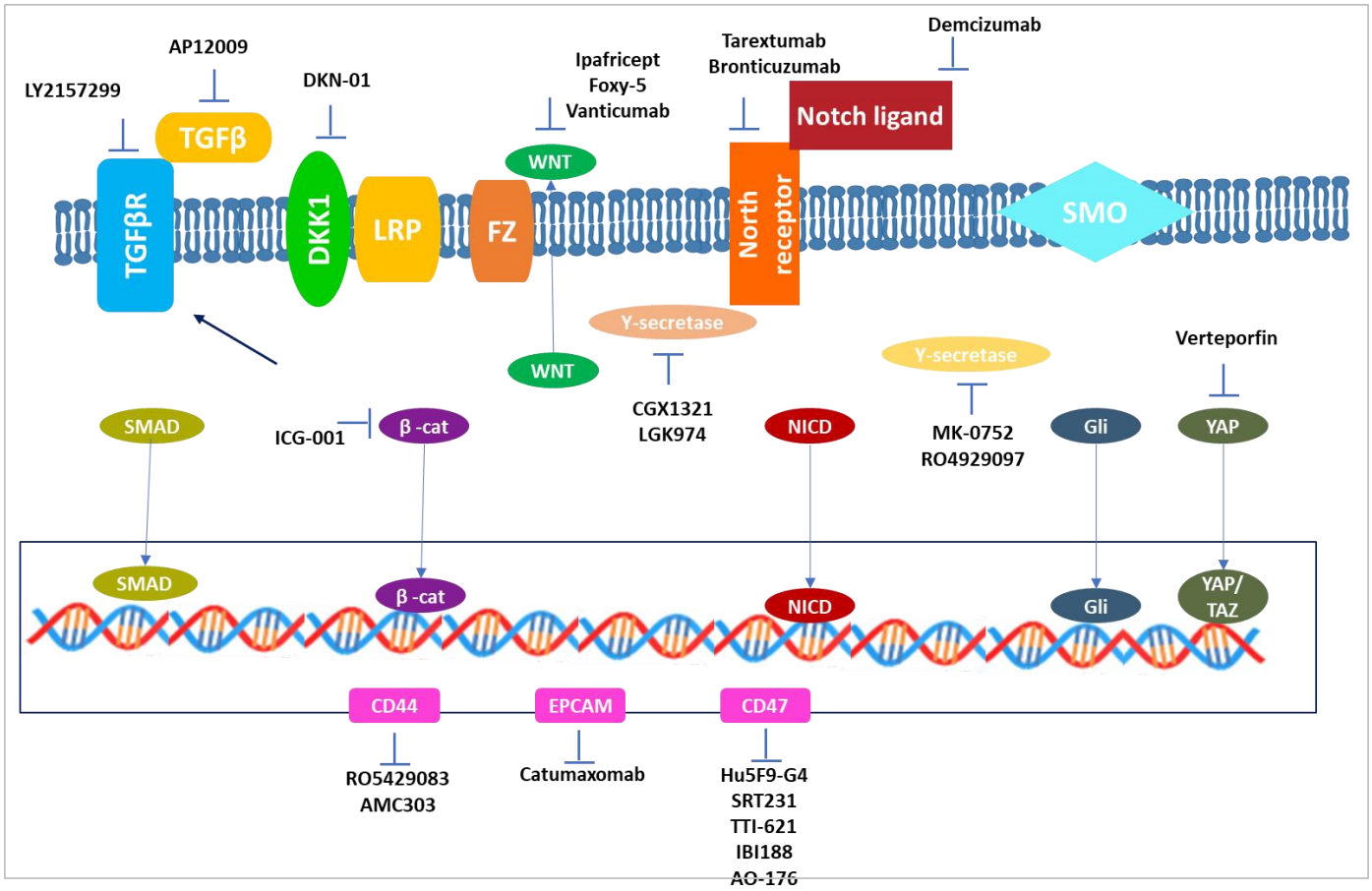

Figure 1. Treatments that target CSCs. Anti-CSC medicines that target developmental pathways and CSC-associated surface indicators have been identified.

Tumor plasticity

One of the key processes contributing to intratumor heterogeneity has been suggested as cancer cell plasticity. In response to microenvironmental stimuli, cancer cells can transition between a nontransformed differentiated state and a tumorigenically transformed undifferentiated or CSC state. Multilineage interconversion, dedifferentiation, and transdifferentiation are all examples of stem cell plasticity (17). Normal stem cells, progenitors, and/or differentiated somatic cells can all give rise to CSCs. CSCs can develop into cancer cells, dedifferentiate back to their original lineage cells, and/or transdifferentiate into other lineages (18, 19). Malignant transformation is fueled by abnormally activated plasticity, which allows tumors to adapt to the restrictions of tumor development and therapeutic resistance. CHD1L was discovered to be a possible clinical developmental lineage oncogene in HCC in a prior investigation. CHD1L expression is active during embryonic development but gradually diminishes after terminal differentiation. CHD1L expression, on the other hand, is abnormally elevated in HCC. Elevated liver ancestral precursor markers and decreased hepatic lineage differentiation markers accompany this dynamic expression pattern. Further CHD1L inhibition may impede poorly differentiated HCC and make patients more susceptible to chemotherapeutic treatments (20).

The link between CSCs and EMT has been discovered by providing proof. But it is still debatable if EMT is required for CSCs, it is highly significant in CSCs. Firstly, intermediate mesenchymal states are reversible at earlier stages of development, depending on microenvironmental signals, and EMT in tumor cells may be temporary, resulting in a more plastic CSC phenotype, poorer patient survival, and more drug resistance. Six separate EpCAM-cell populations

characterized by the CSC markers CD61, CD106, and CD51, for instance, displayed this intermediate EMT state and produced metastases more efficiently (21). Furthermore, an increase in EMT master transcription factors not only increases the metastatic potential and increases tumor starting capacity (22). The EMT phenotype is strongly associated with most gastrointestinal cancer subtypes with stem cell characteristics.

In gastrointestinal cancers, molecular subgroups with CSC features have been described.

Within tumors, gastrointestinal malignancies are exceedingly variable, and molecular subtypes have been identified to help classify them. Many cancers' transcriptomic, genomic, and/or epigenomic profiling provides the foundation for molecular categorization. Different biological bases, such as immunology, metabolism, and stemness, are reflected in these diverse molecular subtypes. CSCs, in particular, are a key cause of intratumor heterogeneity. Integrative molecular subclassification analyses from a CSC perspective may be promoted to obtain a consensus molecular classification in patient prognosis and therapy decisions.

Colorectal cancer CSC features categorization

Colorectal cancer stemness-based subtyping has received a lot of attention, just like other gastrointestinal malignancies. C1 (21%) is characterized by the suppression of pathways associated with EMT, C2 (19%) is characterized by suppression of the Wnt pathway, C3 (13%) is characterized by suppression of EMT, C4 (10) is described by increased expression of EMT and genes linked to stem cell-like signatures, C5 (27%) is categorized by up-regulation of Wnt pathway genes, and C6 (10) is characterized by upregulation of the EMT pathway (23). The transit-amplifying subtype is a heterogeneous subtype with a high concentration of stem cell-relevant genes and the Wnt pathway, which may be separated into two categories based on the differential cetuximab response (CS-TA and CR-TA). Another stem-like subset is described by Wnt signaling target gene overexpression and the presence of mesenchymal and myoepithelial stem-cell characteristics, but also reduced expression of differentiation markers, whereas the goblet-like and enterocyte subsets are enriched in well-differentiated genes with few stem cell characteristics and low Wnt marker expression (24). Using meta-gene profiles to detect five primary subsets: surface crypt-like, lower crypt-like, CIMP-H-like, mesenchymal, and mixed, in contrast to standard molecular categorization based on gene expression profiling. Whenever the mesenchymal subtype and mixed subtypes are enriched for high expression of the EMT/stroma gene module, the surface crypt-like and lower crypt-like subtypes are well distinguished with low expression of the EMT/stroma gene subsystem (25).

Gastric cancer CSC features categorization

The following four characteristics of the mesenchymal subtype are CSC-like. For starters, this subtype is significantly linked to the activation of the CSC pathway. Second, as compared to other varieties, it has high CD44 and low CD24 levels, which is similar to the QM-PDA subtype of PDAC. Third, it maintains an undivided state, which is a crucial characteristic of CSCs. Finally, genes expressed at low levels in HCC with hepatic stem cell features have a considerable overlap with hypermethylated gene sets (26). Furthermore, the proliferative subtype has increased activity for some carcinogenic pathways: RAS, E2F, and MYC. MSI, MSS/EMT, MSS/p53+, and MSS/p53 are four patient subtypes of gastric cancer, where MSS refers to microsatellite stable tumors. The MSS/EMT module has a strong relationship with the EMT signature (27).

Esophageal cancer CSC features categorization

Esophageal cancer is divided into two categories based on histology: esophageal adenocarcinomas (EACs) and esophageal squamous cell carcinomas (ESCCs) (ESCCs). Molecular classification studies of esophageal cancer are still scarce, in contrast to studies on other gastrointestinal tract malignancies. ESCC1, ESCC2, and ESCC3 are the three ESCCs. SOX2 and TP63 amplification is common in ESCC1 malignancies. SOX2 is a pluripotent stem cell transcription factor that promotes squamous epithelia formation and maintenance. ZNF750 and NOTCH1 mutations, inactivation of the histone demethylases KDM6A and KDM2D, deactivation of the PIK3CA

inhibition PIK3R1 and PTEN, and CDK6 amplification are all more common in ESCC2 tumors. The last category, ESCC3, has mutations that predict RTK/RAS/PI3K pathway activation (28). A further investigation discovered two unique ESCC subgroups. Subtype I contain a highly activated immune response pathway, whereas subtype II contains pathways involved in ectoderm development. Subtype II has an abundance of epithelial development genes such as E2F4, JUN, KRT5, and KRT14. In Subtype II ESCC, PDPN and SIX1 have high expression levels, and SIX1 can maintain or increase PDPN-positive CSCs. They uncovered potential ESCC subset-specific diagnostic markers, including EYA2 and FOXA1 for subtype I and KRT14 and LAMC2 for subtype II, that could aid in ESCC therapeutic practice (29).

Treatments based on subtypes and clinical relevance

Colorectal cancer subtypes

In De Sousa et al. study (30) evaluated the clinical characteristics of CCS1 and CCS3 cancers and discovered that CCS1 tumors had an excellent prognosis. At an early stage of adenomas, CCS3 tumors had malignant potential and were resistant to anti-EGFR treatment The prognosis was better in the surface crypt-like and lower crypt-like categories. CIMP-H-like and mesenchymal subtypes were linked to poor overall survival (OS), with the former additionally being linked to short relapse survival (SAR). There was a trend toward the worse OS in the mixed subgroups (25). Type A has the greatest prognosis, Type B has an intermediate prognosis but can benefit from adjuvant 5-FU treatment, and Type C has the worst survival and resistance to 5-FU-based chemotherapy, according to molecular categorization. Four consensus molecular subtypes were discovered to be connected to clinical characteristics while analyzing the existence of core subtype gene expression patterns among current CRC subtyping techniques (31).

Gastric cancer subtypes

Gastric adenocarcinomas are divided into three types: proliferative, metabolic, and mesenchymal. There were no significant variations in survival between the three groupings, according to the analysis of survival data. 5-FU therapy did not affect patients with tumors of the proliferative and mesenchymal subtypes. PI3K-AKT-mTOR inhibitors were selectively responsive to mesenchymal-subtype gastric cancer cells, probably because this subtype of cells mimics CSCs. PI3K-AKT-mTOR inhibitors are also effective in prostate cancer and glioblastoma, according to this report (32, 33). Another distinguishing aspect of the mesenchymal subtype is the presence of high levels of CD44. CD44 is a well-known CSC surface biomarker that is abnormally expressed in a variety of malignancies as CD44s or CD44v (variant isoform). CD44 overexpression is strongly linked to a malignant phenotype and poor clinical outcomes. Sorafenib and 5-FU sensitivity was reduced in CD44-positive cancer cells. Targeting CD44 for cancer treatment could be a promising method. Antibodies to CD44 and inhibition of the HA-CD44 balance are two treatments that can successfully decrease CSC characteristics in a variety of malignancies (34).

Esophageal cancer subtypes

Esophageal cancer molecular categorization studies are still restricted. Four subtypes are therapeutically significant. Subtype 1 was made sensitive to the cell cycle checkpoint inhibitor CHFR. Furthermore, CDK4/6 inhibitors were beneficial across all subtypes, although CDK2 inhibitors were more successful in patients with subtype 4 (35).

Conclusion

The most common genetic alterations and tumor subtypes are gradually becoming more well-known, and their clinical significance is becoming clearer. The biological characteristics and clinical characteristics of gastrointestinal malignancies show substantial differences, which are most likely due to heterogeneity. In clinical terms, heterogeneity is mostly responsible for tumor development, metastasis, therapeutic resistance, and relapse. The occurrence of molecular subtypes causes molecular heterogeneity. CSC features are unquestionably linked to molecular categories due to CSCs' substantial effect on heterogeneity. CSCs mimic embryonic stem cells in appearance, implying the importance of developmental cues in cancer onset and resistance to treatment. As a result, combining the molecular subtypes linked to stemness features could reveal additional information about treatment

resistance. Other, more putative therapeutic, like scRNA-seq and appropriate preclinical models, should be developed and employed in the precise evaluation of intra- and intertumoral heterogeneity. More precise targeting of tumor-initiating and driving events based on subtype-specific biomarkers could be a unique therapeutic technique in the treatment of gastrointestinal cancer. Eventually, comprehensive tumor and liquid biopsy procedures should be developed to identify characteristic molecules that enable the whole molecular profile to be defined and patient categorization to be determined. In conclusion, we present an overview of molecular categorization from a CSC perspective that may aid in the therapeutic management of patients with gastrointestinal malignancies, resulting in better results.

Author contributions

SEN, **FN**, and **SV** wrote and compiled this article. **AAS** wrote and edited the manuscript comprehensively. All authors confirmed the final version of the paper.

Conflict of interest

The authors declare that they have no conflicts of interest.

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