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Targeting Colorectal Cancer Stem Cells as an Effective Treatment for Colorectal Cancer

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Abstract

As one of the common cancers that threaten human life, the recurrence and metastasis of colorectal cancer seriously affect the prognosis of patients. Although new drugs and comprehensive treatments have been adopted, the current treatment effect on this tumor, especially in advanced colorectal cancer, is still not satisfactory. More and more evidence shows that tumors are likely to be a stem cell disease. In recent years, the rise of cancer stem cell theory has provided a new way for cancer treatment. Studies have found that a small number of special cells in colorectal cancer tissues that induce tumorigenesis, proliferation, and promote tumor migration and metastasis, namely, colorectal cancer stem cells. Colorectal cancer stem cells are defined with a group of cell-surface markers, such as CD44, CD133, CD24, epithelial cell adhesion factor molecule, LGR5, and acetaldehyde dehydrogenase. They are highly tumorigenic, aggressive, and chemoresistant and thus are critical in the metastasis and recurrence of colorectal cancer. Therefore, targeting colorectal cancer stem cells may become an important research direction for the future cure of colorectal cancer.

Keywords

CRC, CSC, CCSCs, targeted therapy, drug resistance

Abbreviations

5-Fu, 5-fluorouracil; AKT, silk/threonine protein kinase; ALDH, acetaldehyde dehydrogenase; BMP, bone morphogenetic protein; CCSCs, colorectal cancer stem cells; CRC, colorectal cancer; CSC, cancer stem cell; EGF, epidermal growth factor; EGFR1, EGF receptor 1; EpCAM, epithelial cell adhesion factor molecule; HMGA1, high mobility group protein A1; IL-6, interleukin 6; PAI-1, plasminogen activator inhibitor 1; STAT3, signal transducer and activator of transcription 3; TERT, telomerase reverse transcriptase; TGF-β, transforming growth factor β

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Colorectal cancer (CRC) is one of the most common malignancies in the digestive system. The incidence of CRC in the United States between 2009 and 2013 was as high as 40.7 per 100 000, and the mortality rate from 2010 to 2014 was 14.8 per 100 000.[1-3] It is the third largest malignant tumor in the United States and is also widely prevalent throughout the world. At present, the treatment plan for CRC is still mainly combined with surgery, radiotherapy, and chemotherapy.[4] In recent years, with the development of endoscopy and various assistive technologies, although the mortality rate of patients has decreased, there is still no effective method to prevent the recurrence and metastasis of CRC.[5] Hence, targeted therapy for the key pathogenic factors of CRC has become a major breakthrough, and the birth of cancer stem cell (CSC) theory has opened a new window for cancer treatment.[6]

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Table 1. Surface Markers of Cancer Stem Cells.

Name of Marker	Cancer	Function	References
EpCAM	Colon cancer, liver cancer	Chemoresistance, tumorigenesis, invasiveness, self-renewal	18,21-23
CD133	Colon cancer, lung cancer, gastric cancer, ovarian cancer, pancreatic cancer, liver cancer	Chemoresistance, self-renewal, tumorigenesis, invasiveness, proliferation, differentiation, angiogenesis, resistant to apoptosis	18,21,22
CD166	Colon cancer	proliferation, tumorigenesis, self-renewal, chemoresistance	23
CD44	Breast cancer, prostate cancer, colon cancer, lung cancer, gastric cancer, ovarian cancer, pancreatic cancer, liver cancer	Self-renewal, chemoresistance, metastasis, invasiveness, tumorigenesis	18,21-24
CD90	Gastric cancer, head and neck cancer, liver cancer	Metastasis, chemoresistance, tumorigenesis, self-renewal	25
CD26	Colon cancer	Self-renewal, tumorigenesis, invasiveness	26
ALDH	Breast cancer, prostate cancer, colon cancer, lung cancer, ovarian cancer	Self-renewal, tumorigenesis, invasiveness, metastasis	18,27
Aurora-A	Colon cancer	Proliferation, chemoresistance, EMT, invasiveness, metastasis	28
CXCR4	Lung cancer, colon cancer	Metastasis, chemoresistance, tumorigenesis, self-renewal	29,30
Lamin A	Colon cancer	chemoresistance, tumorigenesis, self-renewal	31
CD24	Colon cancer, gastric cancer, prostate cancer	Metastasis, chemoresistance, tumorigenesis, self-renewal	18

Abbreviations: ALDH, acetaldehyde dehydrogenase; EMT, epithelial–mesenchymal transition; EpCAM, epithelial cell adhesion factor molecule.

Cells that have self-renewal capacity in tumors and that produce heterogeneous tumor cells are called cancer stem cells (CSCs).[7] Cancer stem cells play an important role in the occurrence, development, recurrence, and metastasis of tumors.[8] Traditional treatment methods can only reduce the volume of tumors and kill differentiated cells in tumors, whereas CSCs can resist radiotherapy and chemotherapy, eventually leading to tumor recurrence and metastasis.[9] The research currently focuses on the observation of colorectal cancer stem cells (CCSCs) biological characteristics.[10-12] With the development of stem cell theory, research on targeting CSC therapy has received increasing attention.

Discovery of CCSCs

The German pathologist Rudolf Virchow first proposed the theory that "every cell comes from another cell" in 1858.[13] Makino *et al*[14] first proposed the CSC hypothesis in 1956 and believed that tumors were produced by CSCs. In the 1950s and 1960s, researchers found that a small number of cells in leukemia and myeloma cells can form clones *in vitro* and *in vivo*.[15-17] This small number of cells is considered to be CSCs. In 2007, O'Brien *et al*[18] purified and expressed CD133-positive human CRC primary cells and transplanted them into the renal capsule of immunodeficient mice. These cells were found to maintain self-renewal and differentiation while forming tumors and therefore are called colorectal cancer stem cells (CCSCs).

Since these cells account for a small proportion of tumor tissues, identifying and understanding the characteristics of CCSCs in the past is difficult. However, with the development of molecular biology technology and related research, a variety of biomarkers have been available for the isolation of CCSCs. More cell-surface markers related to CCSCs have been discovered.[4,19,20] It has been validated in different studies, which provides a basis for further study of the characteristics of CCSCs.

Cell-Surface Markers of CCSCs

Several cell-surface markers (solid tumors) have been used for the identification and isolation of CSCs and CCSCs (Table 1). In 2007, O'Brien *et al*[21] demonstrated that all CCSCs were CD133 expressing, while CD133-negative cells were unable to form tumors and functionally confirmed the rationality of CD133 as a surface marker for CCSCs. Haraguchi *et al*[22] found that $CD133^+CD44^+$ cells were tumorigenic, while $CD133^+CD44^-$ and $CD133^-CD44^-$ cells were not tumorigenic, and CD44 was further introduced into CD133 cells as a screening marker for CCSCs. Huang *et al*[27] found that acetaldehyde dehydrogenase (ALDH) was better as a CCSCs marker than CD133 and CD44. Dalerba *et al*[24] successfully isolated human CCSCs using CD44 and epithelial cell adhesion factor molecule (EpCAM) as a surface marker. Tumor cells of $CD44^+/EpCAM^{high}$ were found to have the characteristics of CSCs and were verified in nonobese diabetic/severe combined immunodeficient mice, and the value of CD166 as a synergistic marker was also found.[23] Pang *et al*[26] isolated $CD26^+$ cells from CRC specimens and injected them into the cecal wall of mice and found that $CD26^+$ cells were stronger in invasiveness and drug resistance than $CD26^-$ cells.

Studies have shown that the serine-threonine kinase Aurora-A is highly expressed in CCSCs, and downregulation of this gene leads to decreased tumorigenicity and metastasis of CCSCs, indicating that Aurora-A is used as a potential biomarker for CCSCs.[28] Dessein *et al*[29] found that drug-resistant CRC cells have stronger invasiveness and distant metastasis and drug resistance. These cells express CXCR4 highly, and the

macrophage migration inhibitor MIF, which is a key ligand of CXCR4, promotes cell malignancy. Behavior found that the CXCR4-MIF axis plays an important role in the regulation of CCSCs and may be a candidate for screening CCSCs.[32] In addition, studies by Willis *et al*[31] showed that Lamin A has potential value for identifying CCSCs.

Currently reported surface markers of CCSCs have a certain scientific basis, but a highly specific surface marker or method has not been found to isolate and identify CCSCs. The markers of CCSCs newly screened in the future are likely to be important genes and products in the signaling pathway that plays an important role in regulating the cancer stemness.

Biological Characteristics of CCSCs

A study of $ALDH^+$ CCSCs populations revealed that insulin-like growth factor 1 overexpresses the constitutively activated mutant of the silk/threonine protein kinase (AKT) in a β-catenin-dependent manner, resulting in increased stem cell numbers and tumor growth.[33] In addition, $ALDH^+CD133^+$ CCSCs express high levels of phosphorylated signal transducer and activator of transcription 3 (STAT3).[34] Curcumin and its analog GO-Y030 can inhibit this change, reduce the expression of target genes downstream of STAT3, and induce apoptosis of CCSCs to inhibit tumor formation.[35] Other studies have shown that interleukin 6 (IL-6) can enhance the tumorigenic ability of cancer cell by inducing the expression of Notch homolog 1 and CD44 in CCSCs of HCT-116 and HT-29 CRC cell lines.[36] Interferon γ and tumor necrosis factor α can also stimulate CCSCs in C26 mice and accelerate tumor growth.[25] In addition, inhibition of hypoxia-inducible factor signaling pathway in CCSCs can effectively reduce the tumorigenic ability of CCSCs in an inflammatory environment *in vivo*.[37] Leng *et al*[30] found that the overexpressed kruppel-like factor 4 in DLD-1 cell line can induce somatic cells to produce pluripotent stem cells and promote tumor formation *in vitro* and *in vivo*.

The Wnt signaling pathway is highly activated in CCSCs and inhibition of the reactive protein in this pathway will block signal transduction, thereby affecting the growth of CCSCs and achieving the purpose of treating CRC.[38-40] High levels of Yap polypeptides are thought to be important factors in promoting the proliferation of CCSCs.[41] High expression of Yap can cause the expansion of intestinal stem cell population and promote cancer cell proliferation, while the elimination of Yap can attenuate β-catenin and Notch signaling and inhibit cell proliferation and survival.[42] In the HCT116 cell line, epidermal growth factor (EGF) was also found to be essential for maintaining stem cell proliferation.[43-46] Inhibition of autophosphorylation of EGF receptor (EGFR1) and downstream signaling pathway protein AKT and extracellular signal-regulated kinase ERK1/2 decreases proliferation and induces apoptosis in CCSCs.[47] Conditional inactivation of the telomerase reverse transcriptase (*TERT*) gene in colonic epithelial cells of newborn mice can induce overexpression of colonic crypts in mice, causing an increase in mucosal thickness and an increase in the number of goblet cells.[48] This change in the differentiation status of selective crypts suggests that deficiency of TERT induces the proliferation and behavior of CCSCs to lead to tumorigenesis.

The study found that 5hmC is abundant in normal colon tissues of rats and humans, but significantly decreased in colon cancer tissues and CCSCs, indicating that 5hmC plays an important role in tissue differentiation.[49-51] Another study found that activation of bone morphogenetic protein (BMP) signaling pathway in $CD133^+$ stem cells can promote terminal differentiation and apoptosis of CCSCs.[52] The CCSCs of immunodeficient mice express high expression of the tyrosine protein kinase receptor EphB2, which exhibits strong tumor production and long-term self-renewal potential, but gradually silences in cell differentiation.[53]

The high mobility group protein A1 (HMGA1) is a key transcription factor in the molecular mechanism of colon cancer metastasis.[54-57] Previous study found that HMGAI-induced CCSCs express Twist 1 protein in transgenic mice, inhibit the expression of E-cadherin, and promote tumor metastasis and development; while knockout of HMGA1 blocks anchorage-independent growth and transplantation of tumor cells.[58] The phenomenon of liver metastasis of colon cancer is also related to the involvement of transforming growth factor β (TGF-β).[59-61] Transforming growth factor β promotes the growth of primary tumor and the occurrence of liver metastasis, which may be related to the activation of Smad molecules in CCSCs induced by TGF-β and the enhancement of cell invasion and transendothelial migration.[62] Other studies have found that CCSCs of colon cancer cell lines HT29 and HCT-116 secrete high levels of plasminogen activator inhibitor 1 (PAI-1), which can significantly stimulate the migration of these 2 cell lines, while antibodies against PAI-1 can block this effect.[63]

Colorectal cancer stem cells are closely related to the resistance of colon cancer to chemotherapy drugs.[64-66] Acetaldehyde dehydrogenase 1-positive HT29 and HT29-taxol cell lines lowly expressed miR-125a/b and highly expressed ALDHIA3 and Mcl1. The downregulation of miR-125a/b caused overexpression of ALDH1A3 and Mcl1 gene expression, increased cancer cell viability and stemness, and decreased cell apoptosis to play a key role in the resistance of chemotherapy drugs.[67] Studies have found that BMP4 can attenuate the chemical resistance of CCSCs to treatment and enhance the antitumor effect of 5-fluorouracil (5-Fu) and oxaliplatin.[68] The apoptotic protein inhibitor BIRC6 is an important regulator of the resistance of tumor stem cells to oxaliplatin and cisplatin in patients with CRC with liver metastasis, and the treatment with BIRC6 may help eliminate CCSCs and enhance the drug sensitivity of CRC cells to oxaliplatin and cisplatin.[69] In another study, the expression of miR-451 in CRC cells with stem cell characteristics was downregulated in patients with CRC who did not respond to irinotecan-based first-line drug therapy, while the recovery of miR-451 reduced the expression of adenosine triphosphate-binding cassette B1.[70] The expression of miR-451 leads to the sensitivity of cancer cells to irinotecan, suggesting that downregulation of miR-451 expression in CCSCs is an important factor in drug resistance for patients with CRC.

Table 2. Ongoing Clinical Trials of CSC-Targeted Agents.

Trial	Target	Status
NCT02753127	BBI-608 (STAT3 inhibitor) + FOLFIRI I metastatic colorectal cancer	Enrolling phase 3
NCT01553851	GSK1120212 (MEK1/2 inhibitor) in oral cavity squamous cell cancer	Phase 2 complete
NCT01190345	Bevacizumab (anti-VEGF) + conventional therapy in breast cancer	Phase 2
NCT01579812	Metformin (type 2 antidiabetic) + conventional therapy in ovarian, fallopian tube, and primary peritoneal cancer	Phase 2
NCT01624090	Mithramycin (RNA synthesis inhibitor) in lung, esophageal, mesothelioma, breast cancer	Phase 2
NCT01861054	Reparixin (inhibitor of CXCR1 and CXCR2) in breast cancer	Phase 2
NCT01195415	Vismodegib (Hedgehog inhibitor) + Gemcitabine in advanced pancreatic cancer	Phase 2
NCT00645333	MK-0752 (γ-secretase inhibitor) + Docetaxel in metastatic breast cancer	Phase 2
NCT01088815	GDC-0449 (Hedgehog inhibitor) + conventional therapy in metastatic pancreatic cancer	Phase 2
NCT02370238	Paclitaxel + Reparixin in metastatic triple negative breast cancer	Phase 2
NCT02279719	BBI608 (STAT3 inhibitor) + Sorafenib or BBI503 (Nanog inhibitor) + Sorafenib in advanced hepatocellular carcinoma	Phase 2
NCT01951690	VS-6063 (FAK inhibitor) in KRAS mutant non-small cell lung cancer	Phase 2 complete
NCT02541370	Cocktail CD133 CAR-T and CART-EGFR	Phase 2
NCT02049489	ICT-121DC vaccine	Phase 2
NCT02915445	EpCAM-targeted CAR-T cells	Phase 1
NCT03013712	EpCAM-targeted CAR-T cells	Phase 2
NCT02216409	Hu5F9-G4 (monoclonal antibody)	Phase 1
NCT02953782	Combination Hu5F9-G4 and Cetuximab	Phase 2
NCT01567202	Dendritic cell-based vaccine	Phase 2

Abbreviations: CSC, cancer stem cell; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion factor molecule; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor.

Targeting the Treatment of CRCSCs

The results of research on CSCs indicate that clinically used chemotherapy drugs and radiation therapy cannot effectively eliminate CSCs. Colorectal cancer stem cells are not sensitive to the treatment of 5-Fu and oxaliplatin, which may be the key factors leading to tumor metastasis and recurrence after chemotherapy.[71] Therefore, research on targeting CCSCs has gradually become a research hotspot in the treatment of CRC in recent years (Table 2).

Targeting the Cellular Signal Pathway of CCSCs

The signal pathways of CSCs mainly refer to their self-renewal pathways, including Wnt, Hedgehog, and Notch pathways.[72-74] Almost all intestinal tumors have mutations in the Wnt pathway, involving changes in the tumor suppressor genes *APC* and *Axin2*, or the oncogene β-catenin.[75] The activation of Wnt pathway in CRC has made it a target for molecular intervention. The use of small molecule inhibitors to inhibit β-catenin activity in the nucleus or to block β-catenin aggregation has been confirmed by *in vitro* experiments.[68] In addition, treatment with β-catenin antisense primers reduced β-catenin expression and cancer cell stemness, and significant inhibition of tumor growth was observed in animal models of CRC.[76]

Hedgehog can activate the Hedgehog signaling pathway by binding to its receptor, and a highly expressed transcription factor Gli1 is found in CD133-positive CRC cells, which acts to activate Hedgehog, but not in CD133-negative cells.[77] By knocking out the *SMO* gene and blocking the Hedgehog pathway in CRC cell lines, it is possible to inhibit the stemness of CCSCs *in vitro* and promote apoptosis of CRC cells.[78] In addition, blocking the Hedgehog pathway of CCSCs can also inhibit tumor growth *in vivo*.[79]

The Notch pathway has been shown to be one of the important pathways for CCSCs to maintain stem cell characteristics.[80] In an animal model of CRC, the polyclonal antibody 21M18 was used to block the role of DLL4 in the Notch pathway, and the proportion of tumor stem cells was reduced, and tumor growth was significantly inhibited.[81] Further studies have shown that in the CRC metastasis model, although the treatment of imatinib can inhibit tumor growth, the proportion of $ESA^{+}CD44^{+}CD166^{+}$ CCSCs is significantly increased.[56] Combined application of imatinib and hDLL4 antibodies can significantly inhibit tumor growth and reduce the proportion of CCSCs, which is superior to antibody therapy alone.[82]

Promote the Differentiation of Stem Cells in CRC

Chemotherapy drugs for CRC are mainly directed to cells that are rapidly proliferating and isolated, while tumor stem cells are in a resting state in the cell cycle, which may be the main reason for their escape chemotherapy drugs.[83-85] Therefore, it has become another major research direction to promote the further differentiation of CSCs to make them sensitive to traditional chemotherapy.[86,87] Bone morphogenetic protein 4 can cause undifferentiated tumor cells to lose tumorigenicity, and tumors that lose tumorigenicity will undergo apoptosis, change cell proliferation cycle, or cell morphology changes.[88]

Study on the Microenvironment of CSCs

The stem cell microenvironment affects the ability of stem cells to proliferate, migrate, and invade. The results of previous study showed that both malignant tumors were congenital and acquired matrix changes.[89] Treatment for the microenvironment of tumor cells may achieve significant results. CD133-positive CCSCs are capable of autocrine IL-4 and alter the microenvironment of CCSCs, making them insensitive to the chemotherapy drug 5-Fu or oxaliplatin.[90] Conversely, when IL-4 antibodies are administered, tumor growth is significantly inhibited and sensitivity to chemotherapeutic drugs is increased. This phenomenon was also confirmed in animal models, and chemotherapy with IL-4 antibody treatment can significantly inhibit tumor growth.[91]

Colorectal Cancer Stem Cells and Tumor Immunotherapy

Tumor immunotherapy controls tumor recurrence and metastasis by improving the body's antitumor immunity.[92] After years of research, solid tumor immunotherapy has made some progress, but its clinical efficacy has not yet reached the goal of radical tumor treatment. The reason may be that the antigen applied by tumor immunotherapy is directed against tumor-differentiated cells, but the role of CSCs in tumor recurrence and metastasis is not significant.[93] Therefore, further efforts to improve the efficacy of tumor immunotherapy should focus on treatment of CSCs. Studies have found that CSCs express high tumor-associated antigens and are used as antigen-induced DC vaccines to treat tumors, which can stimulate the action of antigen-specific T cells and prolong survival.[94] Moreover, studies have shown that CSC antigens are superior to tumor cells in inducing antitumor immunity.[95-97]

If the tumor originates from the canceration of normal stem cells, treatment of CSCs is bound to result in damage to normal tissue stem cells.[98-101] This contradictory state seems to indicate that treatment of CSCs can cause damage to the entire body.[8,100,102] However, due to the different sensitivity of normal tissue stem cells and CSCs to treatment, the treatment of CSCs can kill tumor stem cells to the greatest extent.[70,103-105] Just as the current chemotherapy drugs, although it causes serious side damage to the body, its role in controlling tumors is still positive.

Outlook

Traditional treatments for CRC include surgery, preoperative or postoperative adjuvant radiotherapy, or chemotherapy. After years of research on tumors, we have realized that tumors are a systemic chronic disease. Surgical treatment can remove tumors, and the adjuvant radiotherapy and chemotherapy can reduce the volume of the solid tumor to a certain extent and increase the survival rate of the patient. However, the theory of CSCs makes us more aware that chemoradiotherapy can only target differentiated tumor cells, but has little effect on CSCs that cause tumor recurrence and metastasis. In particular, recent studies have shown that changes in the tumor microenvironment can cause differentiated tumor cells to return to a state similar to stem cells. Tumor cells may have a phenomenon of returning to the ancestors, and the treatment of CSCs alone cannot cure the tumor. Therefore, future treatment methods should adopt a comprehensive treatment mode that minimizes tumor burden. By combining drugs targeting CCSCs with traditional chemotherapeutic drugs, both tumor metastasis and drug resistance are prevented, which will improve the efficacy of treating colon cancer at this stage. Colorectal cancer stem cells targeted inhibitors as an emerging treatment for colon cancer; although there are still many unclear mechanisms yet to be discovered, it is expected that these drugs will prevent colon cancer recurrence and treat colon cancer metastasis in the future.

Authors' Note

Yu-Shui Ma and Wen Li contributed equally to this work. QLL and DF designed and supervised research. All authors interpreted the data and contributed to the final version of the manuscript. Our study did not require an ethical board approval because it did not contain human or animal trials.

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