Review

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Posted Date: 17 October 2023

doi: 10.20944/preprints202310.0988.v1

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Review

Colorectal Cancer Stem Cells and Targeted Agents

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Abstract: Once discovered, cancer stem cells have become a hot topic in the research of cancer therapy. These cells possess stem cell-like self-renewal and differentiation capacities and are important factors that dominate cancer metastasis, therapy- resistance and recurrence. What's worse, their own characteristics make them difficult to be eliminated. Colorectal cancer is the third most common cancer and the second leading cause of cancer death worldwide. Targeting colorectal cancer stem cells (CRCSCs) can inhibit colorectal cancer metastasis, enhance therapeutic efficacy, and reduce recurrence. Here, we introduced the origin, marker proteins, identification, cultivation and research techniques of CRCSCs, summarized the signaling pathways that regulate the stemness of CRCSCs, such as Wnt, JAK/STAT3, Notch, and Hh signaling pathway. In addition to these, we also reviewed anti-CRCSC drugs targeting signaling pathways，markers，and other regulators in recent years. These will help researchers gain insight into the current agents targeting to CRCSCs, explore new cancer drugs, and propose potential therapy.

Keywords: colorectal cancer stem cells; LGR5; Wnt signaling pathway; single-cell omics technology

1. Introduction

In 1994, John and Bonnet isolated and identified cancer cells with stemness from leukemia cells, and proposed the concept of "leukemia stem cells (LSCs)"[1]. This is the first confirmation of the existence of stem cells in cancer, a major breakthrough in the field of cancer stem cells (CSCs) research. In 2003, Dontu and colleagues isolated CSCs from breast cancer cells [2], which was the first time to prove the existence of CSCs in solid tumors. In the following years, CSCs were found in brain tumor, prostate cancer, lung cancer, colorectal cancer and other tumors [3-6]. Nowadays, the theory of CSCs has gained consensus and has attracted high attentions in cancer treatment research. CSCs are a small population of cancer cells with stemness like stem cells. They can fulfill self-renewal through symmetrical division and asymmetric division to produce daughter cells with stemness or normal cancer cells [7]. Besides, CSCs can form cancer cells with different degrees of differentiation and reconstitute the complete cancer cell repertoire of the original cancer [8, 9]. And normal cancer cells without CSC properties can dedifferentiate back into CSCs through a bidirectional interconversion process [10]. This is a major reason for cancer cell heterogeneity [11]. For a good clinical outcome, cancer cells with or without properties of CSCs must be eradicated. During the development of cancer, CSCs are important factors that lead to metastasis, therapy-resistance, and recurrence [12-14]. CSCs are often accompanied by an epithelial to mesenchymal transition phenotype, and they interact with stromal cells, endothelial cells, and others to promote angiogenesis, stem-like cancer cell differentiation, and accelerate metastasis [15]. The cell cycle of CSCs arrests in G0 phase, so they are resistant to cycle specific chemotherapy drugs [16]. Due to their DNA synthesis asynchrony and enhanced DNA repair, CSCs are resistant to DNA damaging drugs [16]. And CSCs are highly expressed in drug transporters and anti-apoptotic proteins such as Bcl-2, which endows them with the ability to pump chemotherapy drugs out of the cell and resist programmed cell death [16]. Recent research suggested that resting cancer stem cells can evade immune surveillance and lay the seeds for cancer recurrence [17, 18]. This makes CSCs more difficult to eliminate than cancer cells

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Colorectal cancer (CRC) is the third most common malignant tumor. In recent years, with the popularization of early screening for colorectal cancer and the advancement of treatment methods, the mortality rate of colorectal cancer has decreased [19]. However, metastasis and recurrence are still the leading causes of death in most end-stage CRC patients. Reducing metastasis and recurrence remains an urgent problem in CRC therapy. Colorectal cancer stem cells (CRCSCs) may be the initial cells of colon cancer [20], promoting colon cancer metastasis [21, 22], and also being one of the main culprits of therapy-resistance and recurrence [23](Figure 1).Eliminating CRCSCs can promote colon cancer therapeutic effects[24-26].Here, we reviewed the origin and identification of colorectal stem cells, and summarized the potential therapeutic targets of CRCSCs and the current research status of agents targeting CRCSCs. These will help researchers gain insight into the current agents targeting CRCSCs, explore new drugs, and propose potential therapy.

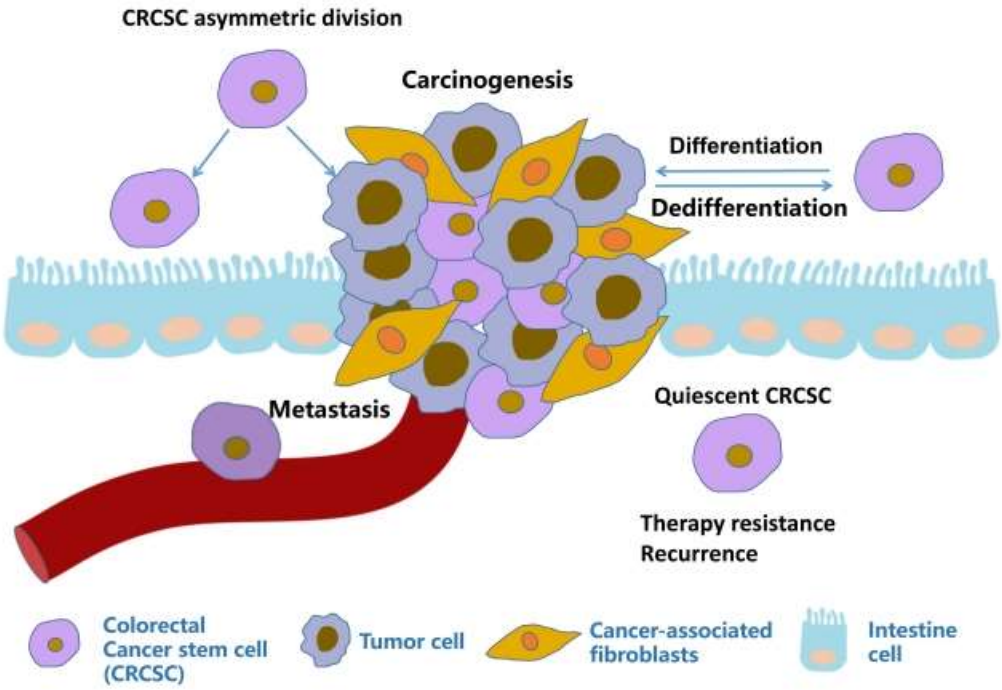

Figure 1. CRCSCs promote metastasis, therapy-resistance and recurrence.

CRCSCs not only divide into CRCSCs, but can also produce ordinary cancer cells through proliferation or differentiation. Due to their quiescent state, high differentiation activity, and other properties, CRCSCs can promote metastasis, therapeutic resistance, and recurrence.

2. Colorectal cancer stem cells

2.1. Origin of CRCSCs

Researchers generally believe that CSCs have two main origins, derived from normal cells that acquire mesenchymal or traits [27] or transformed from normal adult stem cells [28]. The same holds true for the origin of CRCSCs. Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) expressed selectively in the intestinal crypt-base columnar cells [29]. Meanwhile，this is the first proved marker of CRCSCs. In mouse models, genetic inactivation of the key colorectal cancer (CRC) driver gene Adenomatous Polyposis Coli (Apc) in LGR5+ cells precipitated rapid tumor induction [30]. By downregulating β-Catenin and YAP signaling pathways, Protein kinase C ζ(PKC ζ) can inhibit intestinal stem cell function. And PKC ζ deficiency can lead to an increase in stem cell activity in organoid cultures. Tumorigenic activity increased in LGR5+PKC ζ deficiency mice [31]. These evidence suggests that CRCSCs seem to originate from intestinal stem cells. However, selective and effective killing of Lgr5+ cells had no impact on primary tumor growth [24], and the migratory cells

 doi:10.20944/preprints202310.0988.v1

that seed and colonize distant organs were frequently LGR5- at dissemination [32]. Recent research using single cell sequencing technology have shown that subpopulations both of LGR5+ and LGR5- cancer cells have elevated rDNA transcription and protein synthesis characteristic of functional stem cell activity [33] and that lineage conversion between cell types can be driven by the combination of key CRC driver genes and microenvironmental extracellular signaling [34].Vazquez and colleagues also confirmed that the intestine contains two types of stem cells, LGR5+ crypt-base columnar stem cells (CBCs) and LGR5 regenerative stem cells (RSC) using single cell sequencing technology. The two stem cell populations can coexist during tumorigenesis, exhibit dynamic plasticity, and complement each other to achieve homeostasis. The relative abundance of CBC-RSC is related to epithelial mutation and microenvironment signal destruction [35]. With the advancement of research technology, it is certain to uncover the origin of CRCSCs.

2.2. Identification of CRCSCs

The sorting of cancer stem cells mainly relies on flow cytometry and magnetic activation sorting. The most commonly used basis is cancer stem cell marker proteins. Previous studies have found that CSCs have specific markers, including CD133, ALDH1, CD44, EpCAM, etc [36]. CSC markers vary with the type of tumors. There are also some biomarkers for CRCSCs. The marker proteins located on the cell membrane include LGR5 [37], CD133[38, 39], CD44[40]、CD26[41]、CD24[42]、CD29 [43]、CD166 [44] and EpCAM [45]. Acetaldehyde dehydrogenase1 (ALDH1) is an intracellular enzyme that oxidizes aldehydes and mediates the control of differentiation pathways. It is currently widely used as a marker for identifying and isolating various types of normal stem cells and CSCs [44, 46]. Oct4[47]、Sox2[48] and Nanog[49] are transcription factors as marker located in the nucleus. (Figure 1)

CSCs sorting methods include flow cytometry and magnetic activated cell sorting. By combining fluorescent labeled antibodies with cancer stem cell markers, flow cytometry can select CSCs expressing related markers from cancer cells. Side population (SP) cells, characterized by the ability to efflux the fluorescent dye hoechst33342 out of the cell, appear non-fluorescent when tested by flow cytometry. SP cell sorting method is mainly used to sort CSCs with strong drug resistance [50]. Magnetic activated cell sorting utilizes antibodies attached to magnetic beads to bind to CSC markers, adsorbing the corresponding cancer stem cells onto a separation column, while unbound cells pass through the separation column. Cancer stem cells with positive surface labeling can be obtained by elution from the separation column[51, 52] Single-cell omics technology is a powerful tool for exploring CSCs[53, 54]. Single-cell omics technology can characterize and type CSCs in tumors, and establishing a stemness model has prospective clinical implications for prognostic evaluation [35, 55]

2.3. Cultivation of CRCSCs

It is worth emphasizing that although the research results of cancer stem cells have broad prospects for practical clinical applications, they are still in the initial stage. To successfully unleash the enormous potential of cancer stem cell research achievements, there are still many urgent issues to be addressed. To understand the physiological activity of CSCs, the first step is to obtain them. For solid tumor, the commonly used method to enrich cancer stem cells is non-adhesive culture with serum-free culture [56, 57]. CSCs with self-renewal capacity are able to survive under non-adherent conditions and maintain clonogenic activity, whereas non-CSCs undergo anoikis by loss of anchorage.

Three dimensional（3D） culture has emerged as a cell culture method in vitro in recent years. By using hydrogel to mimic extracellular matrix and applying different culture conditions, 3D culture can create a similar microenvironment in vivo[58]. Different gel materials have different porosity, permeability, surface chemical and mechanical properties, which will have different effects on cell growth and differentiation [59]. 3D culture can be used to enrich stem cells or study cell differentiation [60]. Organoid is an advanced version of three-dimensional culture, which is a 3D micro cell cluster formed by directional differentiation of stem cells [61]. Organoids have the ability

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to self-renewal, self-organized, and can highly mimic the structure and function of organs in vivo. They have been widely used in the study of organ diseases, drug toxicity and cancer therapy [62, 63].

3. Agents targeting CRCSCs

Clearing all cancer cells can cure cancer, but some CSCs are drug-resistant and quiescent，require targeted therapy. Several agents targeting CSCs have been approved for clinical trial [64]. CRCSCs also have various types of targeted drugs

3.1. Targeting CRCSC markers

Marker proteins are targets for rapid screening of CRCs. In order to enhance the specificity of therapeutic strategies, researchers often choose ligands or antibodies against CSC surface makers (**Table 1**). MCLA-158 is an EGFR and Lgr5 targeting bispecific antibody with strong growth inhibitory effects on CRC organoids. Simultaneously, it exhibits strong anti-tumor activity in xenograft models derived from patients with high expression of LGR5 and EGFR [65]. In mouse orthotopic xenograft models derived from CRC patients, MCLA-158 treatment not only reduced the size of the primary tumor, but also effectively suppressed metastasis, including KRAS mutant tumors resistant to cetuximab. Currently, researchers are conducting clinical trials of MCLA-158 in various solid tumors (NCT03526835) [65]. Catumaxomab is the first T cell binding bispecific antibody approved by the European Medicines Agency (EMA) in 2009 for the treatment of malignant ascites [66]. Catumaxomab is a three functional bispecific antibody that binds to EpCAM on cancer cells and CD3 on T cells. It also binds to FcγR to recruit immune helper cells [66]. Catumaxomab can effectively eliminate CD133+/EpCAM+CSC in malignant ascites of patients with advanced ovarian cancer, gastric cancer and pancreatic cancer, which indicates that it has potential therapeutic application it has potential therapeutic application in eradicating CSCs of epithelial cancer[67, 68]. Similar to catumaxomab, solidomab is also a bispecific antibody targeting EpCAM and CD3. Solidomab treatment effectively eradicated EpCAM+ CSCs from colon or pancreatic cancer patients inoculated into NOD / SCID mice [69, 70].

Table 1. Agents targeting to CRCSC markers and Wnt pathway.

Agents	Targets of CRCSCs	Reference
MCLA-158	EFGR and LGR5	[65]
Catumaxomab	EpCAM	[67, 68]
Solidomab	EpCAM	[69, 70]
CD133-directed CAR T cells	CD133	[71]
Cetuximab	EFGR	[72]
CD133-targeted oncolytic virus	CD133	[73]
NCB0846	Wnt pathway	[74]
Epigallocatechin gallate	Wnt pathway	[75, 76]
XAV939	Wnt pathway	[77]
Phenethyl isothiocyanate and sulforaphane	Wnt pathway	[78, 79]
Salinomycin	Wnt pathway	[80]
JIB04	Wnt pathway	[81]
CBB1003	Wnt pathway	[82]
YW2065	Wnt pathway	[83]
LF3	Wnt pathway	[84]

 doi:10.20944/preprints202310.0988.v1

Dickkopf-2	Wnt pathway	[85]
ICG-001	Wnt pathway	[86]
4-Acetyl-antroquinonol B	Wnt pathway and JAK-STAT pathway	[87, 88]
Diallyl trisulfide	Wnt pathway	[89]
36-077	Wnt pathway	[90]
Evodiamine	Wnt and Notch pathway	[91]
Farnesyl dimethyl chromanol	Wnt pathway	[92]
FH535	Wnt pathway	[93]

In addition to antibodies, there are oncolytic virotherapy and CSCs vaccines for targeted marker therapies. Oncolytic viruses are a class of viruses with tumor killing functions. Oncolytic virotherapy is an emerging new tumor treatment that utilizes oncolytic viruses to selectively destroy tumor cells while leaving normal cells intact. Using the properties of oncolytic viruses combined with receptors on tumor cells, researchers have screened or engineered oncolytic viruses that target cancer stem cells [94]. Due to the characteristics of virus vectors, oncolytic virotherapy can trigger immunogenic cells death, release tumor related antigens and elicit anti-tumor immune response, which can exert stronger anti-cancer effect [94]. The oncolytic viruses with CD133-targeting moti effectively infected and killed CD133+ CRCSCs, and inhibited the growth of CRC xenotransplantation model [73]. Oncolytic virotherapy is a potential therapy, but it still needs further research. CSCs vaccines are also a type of immunotherapy under research. For example, B16F10 CD133+/CD44+ CSCs vaccine can effectively inhibit melanoma growth in mice and reduce CSC population within tumors [95]. Although no cancer stem cell vaccine has entered clinical trials at this time, the efficacy of a vaccine targeting metastatic CRC has been reassuring and has raised hope [96].

3.2. Targeting signaling pathway

Multiple signaling pathways are involved in the self-renewal, proliferation, apoptosis, and angiogenesis processes of CRCSCs. Currently, it is believed that specifically targeting cell signaling pathways to inhibit the effects of CRCSCs is a major development direction for CRC therapy.

3.2.1. Wnt signaling pathway

The Wnt pathway plays a critical role in controlling epithelial stem cell self-renewal, and its dysregulation will cause colorectal carcinogenesis [97, 98]. The canonical Wnt pathway downstream signaling is regulated by the level of β-catenin (**Figure 2**). TRAF2- and NCK-interacting kinase (TNIK) is an essential activator of Wnt target genes [97]. The inhibitory activity of TNIK inhibitors such as NCB0846 on CRCSCs has been confirmed [74]. Epigallocatechin gallate (EGCG), is one kind of the catechins from green tea. It has been proven to effectively inhibit stem cells from various cancers [99, 100]. EGCG can inhibit the stemness of CRC cells by downregulating the expression of markers such as CD133, CD44, NANOG, OCT4, ALDH1 and Wnt/β-catenin signaling pathway [75, 76]. The small molecule inhibitor XAV939 significantly downregulated CSC markers in colon cancer cells and increased apoptosis induced by chemotherapy drugs [77]. Phenethyl isothiocyanate (PEITC) and sulforaphane are a natural product present in many cruciferous vegetables with anti-cancer activities in clinical trials [78, 101]. Importantly, PEITC inhibited the properties of CRCSCs through downregulation of the Wnt/β-catenin pathway, and decreased the percentage of CD133+ cells [78, 79]. Salinomycin, an anti-bacterial polyether isolated from Streptomyces albus, selectively eliminated CD133+ cells in CRC [102]. Salinomycin induced caspase activation, increased DNA damage and caused disruption of the Wnt/β-catenin/TCF complex and apoptosis of human CRCSCs, and decreased tumor growth and expression of CSC-related Wnt genes, including LGR5 [80, 103]. In addition to these, there are many drugs that reduce CSC stemness by targeting the Wnt signaling

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pathway, such as pan-inhibitor of histone demethylases JIB04 [81], lysine-specific demethylase 1 inhibitor CBB1003 [82] and so on. (**Table1**)

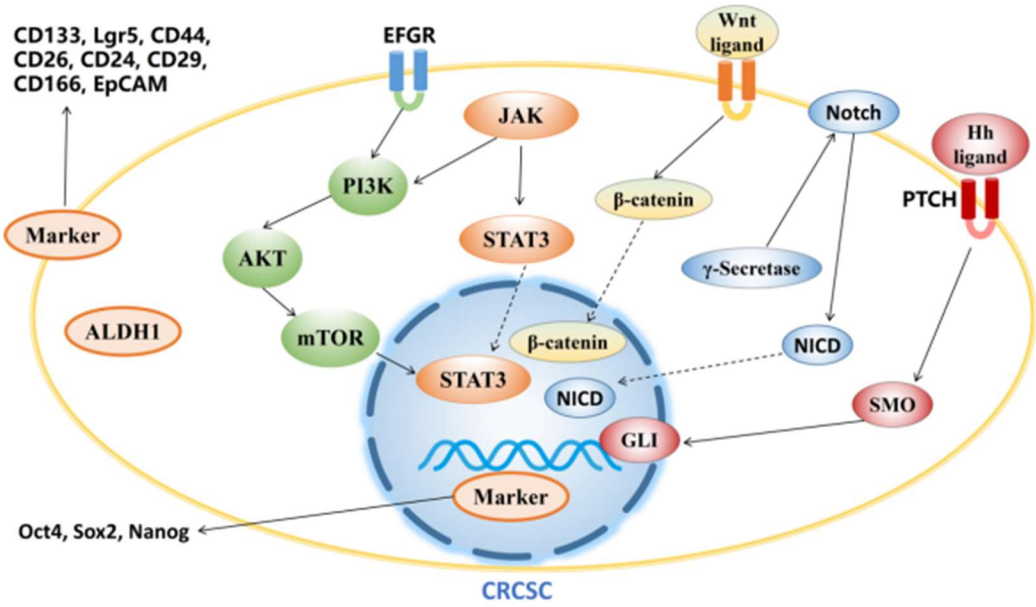

Figure 2. Marker proteins and regulators in pathways in CRCSCs.

Marker proteins and regulators in the pathway are the most prominent targets in CRCSC therapy.

3.2.2. Hedgehog signaling pathway

The Hedgehog (Hh) signaling pathway plays an essential role in the growth and differentiation of gastrointestinal tissue [104]. In canonical Hh signaling, Hh ligands (sonic Hh, Indian Hh, or desert Hh) bind to the patched (PTCH) transmembrane receptor, which leads to the release of smoothened (SMO) and consequent activation of a downstream cascade. In this process, GLI protein will be activated and become transcriptional activators of the downstream targets of the Hh signaling pathway.The Hh-GLI pathway is involved in maintaining the self-renewal ability of CRCSCs [105, 106]. (**Figure 2**)

Vismodegib (also named Ericdge, GDC-0449) is a Hedgehog signaling pathway inhibitor used in clinical practice approved by the US Food and Drug Administration for the treatment of basal cell carcinoma. Vismodegib targets a subpopulation of CSCs in basal cell carcinoma[107].Studies have shown that vismodegib can inhibit the stemness of CRCSCS and the expression of markers CD44 and ALDH1 [108]. Cyclopamine is a natural alkaloid that can inhibit the Hh-GLI signaling pathway by inhibiting SMO. After cyclopamine treatment, the mRNA levels of CSC markers and genes related to Hh signaling, including PTCH1, SMO, and GLI1, decreased in stem cells derived from HCT116 [109].Given the regulation of CRCSCs by Hh signaling pathway, more new inhibitors are being developed.(Table 2)

Table 2. Agents targeting to signaling pathway.

Agents	Targets of CRCSCs	Reference
Vismodegib	SMO of Hedgehog pathway	[108]
Cyclopamine	SMO of Hedgehog pathway	[109]
RO4929097	γ-secretase of Notch pathway	[110]
Anti-DLL4	DLL4 of Notch pathway	[111]
Honokiol	γ-secretase of Notch pathway	[112]

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Quercetin	γ-secretase of Notch pathway	[113]
α-Mangostine	Notch pathway	[114]
BEZ235	PI3K/Akt/mTOR pathway	[115]
LY294002	PI3K/Akt/mTOR pathway	[116]
Piplartine	PI3K/Akt/mTOR pathway	[117]
Rapamycin	mTOR of PI3K/Akt/mTOR pathway	[118]
Metformin	mTOR of PI3K/Akt/mTOR pathway	[119]
Atractylenolide I	PI3K/Akt/mTOR pathway	[120]
Taselisib	PI3K/Akt/mTOR pathway	[121]
Buparlisib	Akt of PI3K/Akt/mTOR pathway	[122]
Miransertib	Akt of PI3K/Akt/mTOR pathway	[122]
MK-2206	Akt of PI3K/Akt/mTOR pathway	[123]
Torkinib	mTOR of PI3K/Akt/mTOR pathway	[118]
Curcumin and GO-Y030	STAT3 of JAK/STAT3 signaling pathway	[124]
Napabucasin	STAT3 of JAK/STAT3 signaling pathway	[125]

3.2.3. Notch signaling pathway

Notch signaling is involved in the regulation of cell differentiation, proliferation, and tumorigenesis [130]. The pathway consists of four receptors (Notch1-4) and five ligands (Jagged-1, Jagged-2, Delta-1, Delta-3, Delta-4) and DNA binding proteins. The interaction between receptors and ligands will initiate protein cleavage cascade reactions, leading to the activation of Notch target genes [131]. Gamma secretase inhibitors (GSIs) can inhibit Notch signaling by preventing the proteolytic cleavage of Notch receptors [132] (**Figure 2**). However, RO4929097, one of the GSIs, failed to achieve excellent results in clinical trials [110]. More GSIs are under investigation. DLL4 is an activator protein of the non-canonical Notch signaling pathway.DLL4 antibody was confirmed to be effective against both KRAS wild-type and mutant CRC cells, effectively eradicating CRCSCS and enhancing the antitumor effect of irinotecan [111, 133].In addition, Honokiol, Quercetin, and others have also been shown to have the ability to inhibit CRCSC stemness [112, 113]. (**Table 2**)

3.2.4. PI3K/Akt/mTOR signaling pathway

The PI3K/Akt/mTOR signaling pathway plays a crucial role in cell metabolism and proliferation, and is closely related to the CRCSC phenotype [134]. Studies have demonstrated that components of the PI3K / Akt signaling pathway are overexpressed in CRC in vitro and in vivo [123, 135]. The combination of PI3K and MEK inhibitors can induce CRCSC death and regression of tumor xenografts [136]. BEZ235, a dual pathway inhibitor of mTOR and PI3K, could inhibit the proliferation of CRCSCs and the expression of its markers CD133 and LGR5, thus suppressing the stemness of CRCSCs [115]. LY294002 is a PI3K inhibitor based on the flavonoid quercetin. LY294002 blocked Akt phosphorylation through the PI3K/Akt signaling pathway and inhibited liver CSC proliferation and tumorigenicity in vitro and in vivo [116]. Treatment with LY294002 resulted in decreased

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proliferation, spheroid formation, and self-renewal properties, together with reduced Akt phosphorylation and cyclin D1 expression in CRCSCs in vitro [116]. Piplartine is an alkaloid amide isolated from peppers. It was reported to inhibit stemness properties in leukemia and oral cancer [117, 136]. In combination with auranofin, piplartine reduced the expression levels of surface marker CD44v9, eliminated CRCSCs, and inhibted CRC growth [117]. Rapamycin is a mTOR inhibitor and used clinically as an immunosuppressive drug, It can decrease the spheroid-forming ability and ALDH1 activity in CRC cell lines [118]. Cotreatment with 5-FU and oxaliplatin, rapamycin reduced CRCSCs subpopulation. Metformin, was also reported to reduce the CSC population in different types of cancer [137]. Metformin can not only reduce the proliferation of CSC population in mouse xenografts [119], but also effectively reduce CSCs in patients with colorectal and other gastrointestinal cancers in a pilotclinical trial [138]. There are also many drugs that targeting PI3K/Akt/mTOR signaling pathway to inhibit CRCSCs, such as Atractylenolide I, Taselisib and so on[120, 121].

3.2.5. JAK/STAT3 signaling pathway

JAK / STAT signaling is closely related to cancer growth and metastasis .In cancer cells, JAK / STAT signaling can be activated by multiple mechanisms, most notably STAT3 activation [139]. High STAT3 activity was found in CRC-SCs, but not in normal colon epithelial cells [140]. The latest study revealed that the JAK2 / STAT3 signaling pathway promoted the persistence and radio-resistance of CRCSCs [141]. Curcumin is a polyphenol from Curcuma longa, and GO-Y030 is a novel curcumin analog. Curcumin and its analog GO-Y030 as drug candidates to eliminate CRC-SCs by suppressing STAT3 activity [124]. Napabucasin, also named BBI608，is an orally administered STAT3 inhibitor with anti-CSC activity against various types of cancer [142, 143]. It showed a significant survival benefit compared to placebo in CRC clinical trials [125].These preclinical and clinical data indicate that napabucasin is a promising anti-CSC drug in CRC therapy. It is the most advanced drug in development that targets cell signaling pathways to eradicate CRC-SCs and the only one with published results from phase III clinical trials. Napabucasin may be the first anti-CRC drug approved for clinical use targeting CSCs. (**Figure 2**)

There are other signaling pathways such as TGF-β, Hippo et al regulating CSCs stemness. Various signaling pathways do not operate independently and often act in crosstalk to influence cancer progression [22, 104, 128, 144, 145]. (**Table 2**)

3.3. Other agents targeting CRCSCs

FBXL5 E3 ligase plays an important role in maintaining the stemness of CDCSCs. Anandamide uptake inhibitor AM404 can suppress FBXL5 expression and inhibit CRCSC dedifferentiation, migration, and drug resistance [146]. Prexasertib, also named LY2606368, is an investigational checkpoint kinase inhibitor. By inhibiting checkpoint kinase (CHK) 1, LY2606368 affected DNA replication in most CRCSCs[261]. ASR352 and NSC30049 are all CHK1 inhibitor [147, 148]. RAB5/7, which is associated with the endo lysosomal pathway, plays an important role in the survival and maintenance of CSCs through the mitophagic pathway. Mefloquine, an anti-malaria drug, has been identified as a new inhibitor of RAB. In the PDX model of colorectal cancer, mefloquine can target RAB5/7 to inhibit the mitophagic pathway and induce mitochondrial-induced apoptosis, thereby exerting anti-tumor effects without significant side effects [149]. At present, there are many other types of CCSCs antagonists, such as pitavastatin [150], histone deacetylase inhibitor trichostatin A [151], and inhibitors of the post-translational sumoylation modification pathway [152], among others. They may play an important role in targeting CRCSCs in future. (**Table 3**)

Table 3. Agents targeting CRCSCs.

Agents	Targets of CRCSCs	Reference
AM404	FBXL5	[146]

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LY2606368	Checkpoint kinase 1	[153]
ASR352	Checkpoint kinase 1	[147]
NCS30049	Checkpoint kinase 1	[148]
Mefloquine	RAB5/7	[149]
Pitavastatin	-	[150]
Trichostatin A	histone deacetylase	[151]
Dabrafenib	BRAF	[154]
Mithramycin A	SP1	[155]
Parthenolide	USP47	[156]
Gambogic acid	ZFP36	[157]

4. Future prospect

Despite significant progress in research on therapeutic drugs for CRCSCs, cancer treatment still faces many challenges. Tumor microenvironment (TME) plays a major role in determining cell fate and behavioral choices [158, 159]. Under the complex interaction of TME, reversible transformation can be achieved between tumorigenic and non-tumorigenic cells. This is the reason why it is difficult to completely remove CSCs [160]. Cancer-associated fibroblasts (CAFs) play a significant positive role in the development and transfer of CRCSCs [161]. Tumor is an entity composed of multiple heterogeneous cells. Different subtypes of CSCs may have different resistance mechanisms, and therefore, each cancer subtype may require unique therapies [162]. The plethora of contributing factors in cancer and the complex regulatory network, making it difficult to eradicate cancer with a single therapeutic intervention.

Fortunately, researchers never give up. In order to achieve effective treatment, more extensive and in-depth research has been conducted in molecular and cellular aspects, including the synergistic targeting of CRCSCs and TME in cancer treatment. Fibroblast activation protein (FAP) is a type II membrane-bound glycoprotein which is overexpressed in CAFs and activated fibroblasts at wound healing/inflammatory sites. FAP inhibitor has been developed to target CAFs to improve TME [163]. In response to the problem of tumor stem cell heterogeneity, anti CSC drugs with diverse targets have been or are currently being developed. And many of them have been incorporated into clinical or preclinical trials. In the face of different responses of different patients to therapeutic approaches, prognosis prediction and personalized treatment are the best solutions. Single cell omics and organoid technology can assist in achieving this goal. Using large-scale omics technologies can subtype cancer and build predictive models for treatment response [35, 55]. In vitro culture of patient derived tumor organoids can enable prediction of drug sensitivity and resistance, and achieve precision treatment [164]. In summary, in the face of different treatment responses in patients, the heterogeneity of cancer stem cells, and the complex regulatory mechanisms of cancer, researchers have been struggling to decipher them.

5. Conclusions

CRCSCs are a small group of stem cells in colon cancer that have unlimited proliferation, self-renewal, and differentiation, playing an important role in drug resistance, metastasis, and recurrence. CSCs are like seeds of cancer, which cannot be ignored in cancer treatment. The advancement of modern medical technology has given us a certain level of understanding of colon cancer stem cells, but we have not fully understood them. Regarding the current situation of CCSCs targeted inhibitors, it is important to strengthen the synergistic effect between drugs. By combining drugs targeting CCSCs with other treatment methods, we can prevent cancer metastasis and recurrence while reducing the occurrence of drug resistance, which will improve the effectiveness of current CRC treatment. Cancer and the tissue involved are a whole, and treatment should adopt a systematic

approach, striving to completely eliminate the seeds to prevent metastasis and recurrence. CRCSCs targeted inhibitors, as an emerging treatment method for CRC, although there are still many unclear mechanisms to be discovered, it can be expected that in the future, these drugs will play an undeniable role in preventing colon cancer metastasis and recurrence. Certainly, a complete cancer treatment not only requires targeted treatment for CRCSC, but also targeted combination therapy for non-CRCSC and TME, as well as the entire tumor. In order to benefit all patients, personalized therapy is the ultimate goal. Single-cell omics technology and organoid technology have contributed to a deeper understanding of the different aspects of cancer stem cells and to the development of more effective treatments for cancer. Achieving this goal still requires considerable attempts and collaboration from researchers.

Supplementary Materials: Not Applicable.

Author Contributions: X.B. proposed the theme of the manuscript. H.Z. and R.H searched, consulted literatures, and wrote the manuscript, while Z.W. and J.X. revised the manuscript. H.Z. and R.H made the same contribution to the manuscript. All authors read and approved the final manuscript.

Funding: This research was supported by Shenzhen Science and Technology Program (JCYJ20210324133602007), Construction Funds of Key Medical Disciplines in Longhua District, Shenzhen (MKD202007090211) and. Inflammation and Immune Mediated Diseases Laboratory of Anhui Province Open Project (IMMDL20220009).

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Not Applicable.

Acknowledgments: We would like to thank the Support from the Shenzhen "Healthcare San Ming Project".

Conflicts of Interest: The authors declare no conflict of interest.

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