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REVIEW ARTICLE

Cancer stem cells in colorectal cancer and the association with chemotherapy resistance

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Abstract

The incidence and mortality of colorectal cancer (CRC) have always been among the highest in the world, although the diagnosis and treatment are becoming more and more advanced. At present, the main reason is that patients have acquired drug resistance after long-term conventional drug treatment. An increasing number of evidences confirm the existence of cancer stem cells (CSCs), which are a group of special cells in cancer, only a small part of cancer cells. These special cell populations are not eliminated by chemotherapeutic drugs and result in tumor recurrence and metastasis after drug treatment. CSCs have the ability of self-renewal and multidirectional differentiation, which is associated with the occurrence and development of cancer. CSCs can be screened and identified by related surface markers. In this paper, the characteristic surface markers of CSCs in CRC and the related mechanism of drug resistance will be discussed in detail. A better understanding of the mechanism of CSCs resistance to chemotherapy may lead to better targeted therapy.

Keywords Colorectal cancer · Cancer stem cells · Surface markers · Chemotherapeutic resistance

Introduction

According to cancer statistics, colorectal cancer (CRC) is the third most common malignant tumor in the world [1]. Since the mid-1990s, the incidence of CRC has been increasing in people under 50 years of age, especially from 2012 to 2016, the incidence rate increased by 2.2% annually [2]. Simultaneously, the age of death was becoming younger and younger, and the mortality of young and middle-aged people increased year by year [2]. CRC-related death is one of the leading causes of cancer-related deaths[3]. At the moment, surgical treatment is the main treatment for the early non metastatic CRC patients, while the advanced CRC patients need to add new adjuvant therapy, such as preoperative or postoperative radiotherapy and chemotherapy [4]. Nevertheless, continuous chemotherapy will produce multidrug resistance (MDR) of cancer cells. Due to the existence of MDR, the effect of chemotherapy is not significant and the prognosis cannot be effectively improved. The formation of MDR is a complex process. Although it has been known that the mechanism of MDR may be achieved through the intrinsic (genetic or epigenetic) or acquired (host factors) of tumor cells, the specific mechanisms involved are still unknown [5]. In recent years, more and more evidences point out that the occurrence of MDR is closely related to the existence of cancer stem cells (CSCs) [6].

Bonnet et al. found that injecting tumor cells from patients with acute myeloid leukemia into immune-deficient mice can lead to homologous tumors in mice. Therefore, the hypothesis of the existence of CSCs has been supported [7]. According to the cancer stem cell hypothesis, CSCs are subsets of cells with similar characteristics to normal stem cells [8]. These cells can produce the same cells through asymmetric division, and can also differentiate into cancer cells, which is considered to be the main reason for tumor initiation. Therefore, CSCs are defined as a group of self-renewal, unlimited proliferation, multidirectional differentiation potential, and driving tumor development. Now, two mechanisms have been proposed to induce the generation of CSCs: the first is the carcinogenic mutation of normal stem

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cells, which leads to the uncontrolled proliferation of cells and the second is the dedifferentiation of ordinary cancer cells and their transformation into stem cell-like cells [9]. Due to the characteristics of CSCs, it is found that they have the ability of antichemotherapy drugs, antiradiation, and the ability to cause distant metastasis and recurrence after treatment. CSCs have been confirmed in various solid tumors, including breast cancer [10], prostate cancer [11], CRC [12], etc. Although CSCs account for only a small part of tumor tissue, they are essential for the occurrence and development of tumors.

In normal intestinal epithelium, there are four different cell lines: goblet cell, intestinal cell, endocrine cell, and Pan's cell. In addition, there is a kind of crypt base columnar cells (CBCs) in the intestinal epithelium. These cells are undifferentiated cells, which are real intestinal stem cells. CBCs have the ability of asymmetric division and proliferate and differentiate into intestinal cells, goblet cells, and endocrine cells when they move upward through the crypt [9]. The mutation of CBCs plays an important role in carcinogenesis, and in the process of mutation accumulation, CBCs obtain more immature phenotype and higher value-added rate [13]. The surface markers of CSCs can be used to isolate and identify themselves and share some of the surface markers with normal stem cells. CSCs can be screened and identified from original patient samples or established CRC cell lines according to the confirmed cell surface markers [14, 15]. In the process of tumor treatment, tumor stem cell surface markers can be used as novel therapeutic targets to target tumors at the stem cell level, so as to make the tumor sensitive to chemotherapy drugs and reduce the tumor metastasis and recurrence.

The adenosine triphosphate binding cassette (ABC) transporter family with transport function, signal pathways in various signal transduction processes, noncoding RNAs (ncRNAs), and epithelial–mesenchymal transition (EMT) play an important role in CRC stem cells (CRC-SCs)-related drug resistance. This review will elaborate the possible drug resistance mechanism of colorectal cancer through these aspects.

The surface markers of CRC-SCs

The surface markers of CSCs can be used for the differentiation and isolation of themselves, and they partially share surface markers with normal stem cells [16]. Based on the fact that CSCs have the same cell surface markers as normal stem cells, these markers are often used to isolate CSCs from original patient samples or to establish CRC cell lines [17]. In the process of tumor treatment, tumor stem cell surface markers can be used as late-model therapeutic targets to target tumors at the stem cells level, so as to reduce tumor metastasis and recurrence. In the following paragraphs, we will explore in detail some of the more typical surface markers in CRC-SCs.

LGR5

The leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is a target gene of Wnt signaling pathway and belongs to the G protein-coupled receptor (GPCR) family [18]. McClanahan et al. first found highly expressed LGR5 in CRC using RT-PCR [19]. Then, Barker et al. introduced APC (adenomatous polyposis coli) mutation into LGR5-positive cells in mouse model, resulting in the formation of intestinal and colonic adenomas, proved that $LGR5^+$ CSCs mutated may be tumor origin [20]. LGR5 is a widely expressed stem cell marker in the gastrointestinal tract of mice [21]. Since the structure of normal human intestinal crypt is similar to that of mice, it is confirmed that some cells express LGR5 at the base of human crypt, and these cells have the function of stem cell characteristics [22]. Therefore, LGR5 is one of the most accepted stem cell markers. Furthermore, it is found that $LGR5^+$ cells are widely expressed in intestinal adenomas and CRC, but not limited to the base of intestinal crypt. However, it should be noted that a large number of $LGR5^+$ cells only represent a large number of cells with stem cell potential, but not all of them are functional stem cells [22]. The chemoresistance of CRC-SCs is also related to LGR5, which can be achieved by increasing the expression of LGR5 [23]. Wu et al. purified Chemokine receptor 4 (CXCR4) and LGR5 double-positive cells in CRC cell lines and found that LGR5/CXCR4 double-positive cells have a large tumorigenesis ability and are more resistant to chemotherapy [24]. Additionally, in vivo experiments using Irinotecan, some rapidly proliferating $LGR5^+$ CRC-SCs are transformed into slowly proliferating $LGR5^-$ CRC-SCs and then into a quiescent state to reduce cell death. After removing the drug, CRC-SCs status returns to LGR5-positive and reconstructs the tumor [25]. These results indicate that LGR5-positive and LGR5-negative conversion ability of CRC-SCs may lead to chemotherapeutic resistance.

CD44

CD44 is a cell surface protein with three domains: extracellular, transmembrane, and intracellular. It interacts with a variety of extracellular matrix components in the tumor microenvironment, among which hyaluronic acid is the main ligand of CD44 [26]. It plays an important role in cell movement, interaction between cells, adhesion of cytoskeleton to extracellular matrix, and cell migration [27, 28]. Injection of small amounts of $CD44^+$ CRC cells leads to tumor formation in nude mice, and in in vitro experiments, $CD44^+$ CRC cells are cultured to form tumor microsphere characteristic

of stem cells [9]. Convincing data suggest that $CD44^+$ tumor cells have stem cell properties, and CD44 can be used as a surface marker for the separation and identification of CSCs. CD44 plays an important role in promoting the occurrence and development of CRC and regulating the characteristics of tumor stem cells, including drug resistance of CRC.

The CD44 family is encoded by a single gene, which contains 19 exons, of which 10 are designated as mutant exons (V1-V10) [29]. The study found that different indirect variants of CD44 are overexpressed in CRC [30], but it is worth noting that the variant 6 (CD44v6) recently identified as CRC-specific CSCs surface marker has long been considered as a major variant in CRC [31]. CRC-SCs with high expression of CD44v6 exhibit higher spherical formation capacity and stronger drug resistance. Toden et al. selected two spheroid-derived CSCs (SDCSCs) cells in CRC: $CD44v6^+$/$CD44v6^-$, treated with 5-FU or oxaliplatin, found that $CD44v6^+$ SDCSCs form more spheres, demonstrating that $CD44v6^+$ CSCs are much more sensitive to chemotherapeutic agents than $CD44v6^-$ CSCs and show greater tumorigenicity [30]. However, the mechanism of CD44v6 leading to chemoresistance in CSCs remains to be further explored. Then, overexpressing CD44 stem cells are also confirmed to be resistant to chemotherapeutic drugs in other tumors. Yoon et al. confirmed that $CD44^+$ gastric cancer cells are highly resistant to 5-FU or cisplatin chemotherapy in vitro culture [32]. Treatment options for CD44v6 inhibitors targeting subsets of CSCs in clinical models of pancreatic cancer have also proved effective [33].

CD133

CD133, a glycoprotein with five transmembrane domains located in cell processes, was first detected in mouse neuroepithelial stem cells and subsequently in human hematopoietic stem cells [34]. It plays an important role in the binding of specific membrane structure and participates in the interaction between cells [35, 36]. Therefore, CD133 is widely regarded as a stem cell marker. It is not limited to normal stem cells, but also has a marker expression of CD133 in CSCs [37, 38]. Experimental data showed that transplantation of $CD133^+$ cells into immunodeficient mice induces the same tumor formation in CRC, whereas transplantation of $CD133^-$ cells does not [39]. CD133 is closely related to the phenotype of CSCs. It is widely used in the screening and identification of CSCs and can maintain the characteristics of stem cells, so as to control the occurrence and development of tumors. Furthermore, the expression of CD133 can be achieved by regulating a variety of intracellular and extracellular cytokines. Tumor microenvironment, as an extracellular regulatory factor, plays an important role in the regulation of CD133 expression, among which hypoxia is the most important control condition [40]. The expression of CD133 is upregulated in anoxic environment, which promotes the dryness of CSCs [41]. Hypoxia-inducible factor 1α (HIF-1α) is one of the major regulators of tumor biological behavior in the context of hypoxia and is positively correlated with CD133 expression in the post-chemoradiotherapy hypoxia environment, as a predictor of poor prognosis and low survival rate in CRC patients [42]. The chemotherapeutic resistance of CRC is associated with high expression of CD133. A study suggests that the drug resistance of $CD133^+$ CSCs may become of the upregulation of anti-apoptotic protein regulated by high expression of interleukin-4, such as cFlip, Bcl-xL, and Ped, so as to avoid the apoptotic response caused by chemotherapeutic agents [43].

Nevertheless, using CD133 alone to identify CRC-SCs is a topic of debate because not all $CD133^+$ cells have stem cell properties, and CD133 alone is not a good prognostic factor for CRC [44]. In fact, the expression of CD133/CD44 is more significantly correlated with disease-free survival (DFS) and is a better predictor of cancer risk [45].

ALDH

Aldehyde dehydrogenase (ALDH) is an enzyme widely found in all organisms, with different isomer distribution in different organs and tissues [46]. The main function of ALDH is to catalyze the oxidation of endogenous and exogenous aldehyde substrates of organisms to form corresponding carboxylic acids, which are manifested in various biological functions, among which the most important one is cellular detoxification [47]. The data have convincingly shown that members of the ALDH1 family are strongly active in normal tissue stem cells and are considered markers of the disease [47]. $ALDH^{high}$ cells have been found to exhibit stem cell-like characteristics in tumors, such as self-renewal, multidifferentiation, and drug resistance [48–50]. In CSCs, high expression of ALDH activity is previously thought to be primarily attributable to the ALDH1A1 subtype, although other subtypes are involved but of little significance [51]. Interestingly, however, recent studies have demonstrated that ALDH1B1 expression is also increased in normal colorectal stem cells and that the expression of the ALDH1B1 isoenzyme protein is higher in CRC-SCs than ALDH1A1 [52]. ALDH1, as a surface marker of CSCs, can be successfully screened and identified in tumors and is more specific in CRC than CD44 and CD133 [53]. ALDH can detoxify the active aldehydes formed by reactive oxygen species (ROS), while chemotherapy has the effect of increasing the level of ROS, so it is inferred that the high expression of ALDH in the CSCs may have protective effects on cells [54]. Cyclophosphamide (CPA) is a CRC conventional chemotherapeutic agent whose metabolites cause apoptosis in rapidly dividing cells [55]. Nevertheless, CRC-SCs

enrich in residual tumors after CPA treatment demonstrating its insensitivity to CPA treatment [56]. A subset of ALDH enzyme can oxidize and inactivate CPA metabolites, especially ALDH1, which is highly expressed in CRC-SCs [56, 57].

The ABC transporters in CRC-SCs

The largest transmembrane protein superfamily encoded in the human genome is ABC transporters. The ABC transporters are widespread in prokaryotes and eukaryotes and share a conserved sequence, which is where the ATP binds [58]. The bound ATP can be hydrolyzed by ATPase and provides energy for ABC transporters [59]. The ABC transporter family can be divided into seven subfamilies from ABC-A to G according to the similarity or difference of their domains [60]. ABC transporters are widely distributed in normal cells and undertake the transport of a variety of functional peptides, relying on energy from ATP hydrolysis to expel metabolites, foreign bodies, and even toxic substances from cells. It has recently been found that ABC transporters play an important role in pharmacokinetics such as drug absorption, metabolism, and excretion [61, 62]. Now, more than 50 ABC transporters have been found, some of which are overexpressed in CSCs, actively excrete antitumor drugs, reduce the intracellular concentration of drugs, decrease the effect of chemotherapy, and result in MDR [63]. Some studies have shown that ABC transporters can also be used as surface markers of CSCs for the enrichment. In CRC-SCs, the common ABC transporters are as follows: ABCB1, ABCC1, and ABCG2, which are all the submember of the ABC transporter family. Moreover, the transcription level of ABC transporter family members increased in CRC-SCs cells, indicating that ABC transporters are potential targets in the regulatory mechanism of MDR phenotype. Specific roles of several ABC transporter families in the phenotypic regulation of MDR are described in detail below (Fig. 1).

ABCB1

Among the ABC transporter families, the ABCB subfamily is the most variable. And in that subfamily, ABCB1 (multidrug resistance 1/P-glycoprotein), a 170 kDa membrane-bound glycoprotein, is the first member of the ABC transporter family discovered in humans. It may be the most extensive mechanism observed for MDR [64]. ABCB1 is widely found in human normal tissues such as kidney and intestine [65], and further, it is present in the brush-edge membrane of intestinal cells [66]. It has multiple substrate

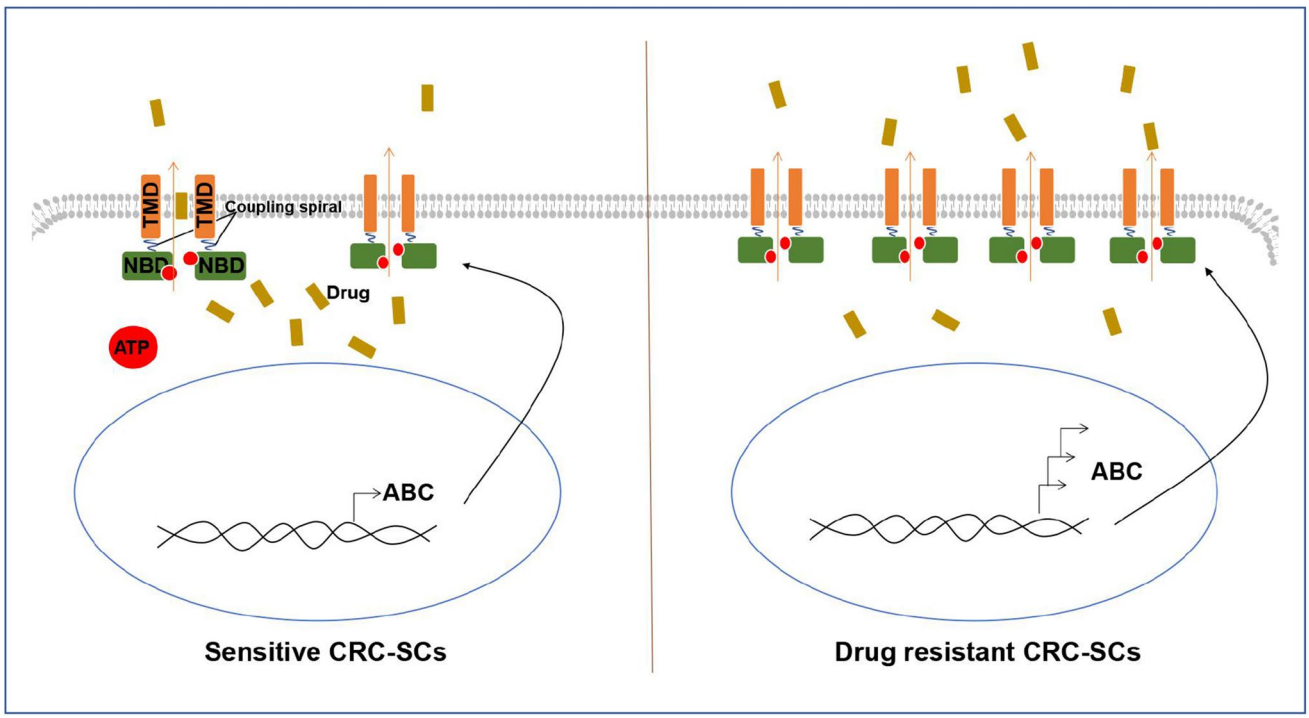

Fig. 1 The ABC transporters in CRC-SCs. Two domains are in the ABC transporter family: TMD and NBD. ATP binding sites are in the NBD domain, which provides energy for ABC to transport drugs when binding ATP. In drug-resistant CRC-SCs cells, the expression of ABC transporter increased, the transport capacity also increased, and the intracellular drug concentration decreased, leading to the occurrence of multidrug resistance

binding sites thus gets extensive substrate recognition ability and high transport capacity to protect cells from cytotoxicity by squeezing foreign matter in cells. Both ATP binding sites of ABCB1 are activated and the two domains alternate into the catalytic cycle [67], so its drug transport capacity is extremely high. Additionally, LGR5-induced CSCs resistance is associated with elevated mRNA and protein levels in ABCB1. The study reported that overexpression of LGR5 in the HT29 cell line results in upregulation of ABCB1 expression levels while no change in ABCG2 expression, indicating a positive correlation between LGR5 and ABCB1. By increasing the expression of LGR5 to upregulate the expression of ABCB1, the accumulation of drugs in cells will be reduced [23]. When ABCB1 is overexpressed in CRC-SCs, it will accelerate the efflux of antitumor drugs, reduce the efficacy of drugs, and give cancer cell resistance phenotype.

ABCC1

Multidrug resistance-associated protein (MRP) is one of the subfamilies of ABC transporter family, among which ABCC1 (MRP1) is the first member of MRP transporter family. ABCC1 is first isolated on the chromosome 16p13.1 of lung cancer stem cells and it is resistant to adriamycin, which belongs to cytotoxic drugs [68]. The primary physiological function of ABCC1 is to block the penetration of cytotoxic drugs in areas similar to the blood–brain barrier and blood–testosterone barrier [69]. ABCC1 is also found in specific human cells, including colon proliferating Paneth cells, bronchial epithelial cells, yolk sac epithelial cells, etc. [70, 71]. It usually locates in the plasma membrane and relies on the energy generated by ATP hydrolysis to transport matter to the outer basement membrane [72]. ABCC1 is a lipophilic transporter characterized by transporting anionoids and transports glutathione conjugates. Studies have shown that it is not sensitive to anthracyclines, but has a transient high level of resistance to methotrexate [73]. In the mouse experiment, it was found that the ABCC1 knockout mice show higher chemotherapy sensitivity in the intestinal tissue, oropharyngeal mucosa, and other parts of tumor tissue. It was confirmed that the expression of ABCC1 in tumor cells is beneficial to the increase of drug efflux rate and the decrease of drug efficacy [69]. Previous studies have shown that ABCC1 is highly expressed in tumors, including CRC [74]. The mRNA and protein levels of ABCC1 are higher in SP cell populations screened and identified in CRC cell lines. Moreover, compared with the non-SP cell population, the SP cell population shows stronger tumorigenic ability and drug resistance [63]. There are some experiments proved that verapamil reduced antitumor drug efflux and increased chemosensitivity of cancer cells by competitively binding lipophilic drug binding sites on ABCC1 [75]. Nevertheless, the specific relationship between ABCC1 and drug resistance of CRC-SCs remains to be further explored.

ABCG2

In 1988, a protein called breast cancer resistance protein (BCRP) was first found in breast cancer cells MCF-7/AdrVp, which is closely related to breast cancer multidrug resistance [76]. The BCRP is the ABCG2 member of the ABC transporter family. ABCG2 is encoded by ABCG2 gene on chromosome 4Q22 and consists of only two domains, namely transmembrane domain (TMD) and nucleotide-binding domain (NBD). However, there are two TMD and two NBD domains in the ABC transporter families, so ABCG2 is a semi-transporter [77]. It protects cells against cytotoxic damage from natural heme metabolites and drugs. According to the literature, ABCG2 is widely expressed in intestinal tract, liver, various progenitor cells, and stem cells under normal physiological conditions. Further studies in tumor tissues have also found that ABCG2 is highly expressed in CSCs, and its abnormal expression is also related to the high tumorigenicity and drug resistance of the cell population [78]. The ABCG2 has a broad resistance spectrum, such as organic anion conjugates, nucleoside analogues, anthracycline drugs, etc., at least in vitro studies [79, 80]. In CRC, there is a group of side population (SP) cells with stem cell characteristics. ABCG2 is overexpressed in SP cells and correlates with its phenotype. Interestingly, sometimes it also has the ability to isolate and identify SP cell populations, similar to cell surface markers. The resistance of SP cells is also related to ABCG2 [81, 82]. Moreover, the expression of ABCG2 is higher in SP cells than in non-SP cells. Similarly, ABCG2 is found to be overexpressed in $CD133^+$ CRC stem cell-like cells. Furthermore, downregulation of ABCG2 expression significantly increase the apoptosis rate of $CD133^+$ CRC-SCs after chemotherapy [8]. Besides, in this experiment, they also found that knockout of ABCG2 by siRNA could significantly improve the chemotherapy efficacy of LS174T and $CD133^+$ CRC cells [8]. Wang's experiments showed that under the treatment of afatinib, the expression of ABCG2 in S1-MI-80 cells decreases in a potency-dependent manner, and then S1-MI-80 is sensitive to tumor drugs, suggesting that the therapeutic effect of chemotherapy drugs is significantly enhanced [83].

Signal pathway in CRC-SCs

In CRC, the signaling pathway controls the growth and increment of cancer cells, promotes the development of tissue morphology, facilitates the occurrence, development, invasion, and metastasis of tumors, and is closely related to the chemoresistance of CSCs. Wnt/β-catenin signaling

pathway plays an significant role in the progression of CRC [84], and the activation rate of Wnt signaling pathway in CSCs is higher than that of non-CSCs [85, 86]. It is speculated that Wnt pathway regulates the characteristics of CSCs. LGR5, the surface marker of CRC-SCs, is also a target of Wnt/β-catenin signaling pathway, which can be activated by this signaling pathway to regulate the growth and proliferation of CSCs [18]. As mentioned above, ABC transporter family makes CSCs resistant to chemotherapy drugs. Previously, the promoter of ABCB1 gene is found containing multiple targets of transcription factor 4 (TCF4) and β-catenin complex [87]. In accordance with the published study, the expression of ABCB1 can be regulated by Wnt pathways, so as to regulate the resistance of CSCs [88]. The use of siRNA to silence β-catenin reduces the transcription of ABC transporters gene, especially ABCB1 and ABCG2, making SW480 cell line sensitive to chemotherapy drugs [85]. ICG-001 is an organic compound and β-catenin/TCF-mediated transcription inhibitor. IC-2 is one of the derivatives of ICG-001, which specifically binds to CREB (cAMP-response element binding) protein and is an inhibitor of Wnt signaling pathway [89]. The sensitivity of DLD-1 cells to 5-FU increases with IC-2; however, the detailed molecular mechanism that IC-2 inhibits Wnt signaling pathway and makes CRC-SCs sensitive to chemotherapy drugs remains to be further studied [90]. In addition, fibroblasts in the microenvironment of CRC tumor tissues activate the Wnt signaling pathway by releasing exosomes, thus activating the stem cell characteristics of CSCs, such as promoting chemotherapy resistance of tumors [91]. Therefore, targeting Wnt signaling pathway inhibitors is a new therapeutic method for CRC, which reduces the generation of chemotherapy resistance of CRC-SCs and improves the efficacy of chemotherapy [92] (Fig. 2a).

Notch signaling pathway is overexpressed in CRC-SCs, which plays an important part in the occurrence and development of CRC [93]. The activation of Notch pathway induces the expression of survival promoting genes related to chemotherapeutic resistance [94], and chemotherapy activates Notch pathway to achieve drug resistance by inducing Notch-1 intracellular domain (NICD) protein [95]. Notch-1 levels are higher in colonic globules and drug-resistant cells than in CRC parent cell lines (HCT116) [96]. Further in vitro experiments, colon globules, drug-resistant cells, and parent cells were injected into nude mice, followed by intraperitoneal injection of γ-secretase inhibitor DAPT. The results showed that tumor size of colon globules and drug-resistant cells are smaller than that of parent cells, indicating that colon globules and drug-resistant cells display greater DAPT-induced growth inhibition [96]. This proves that

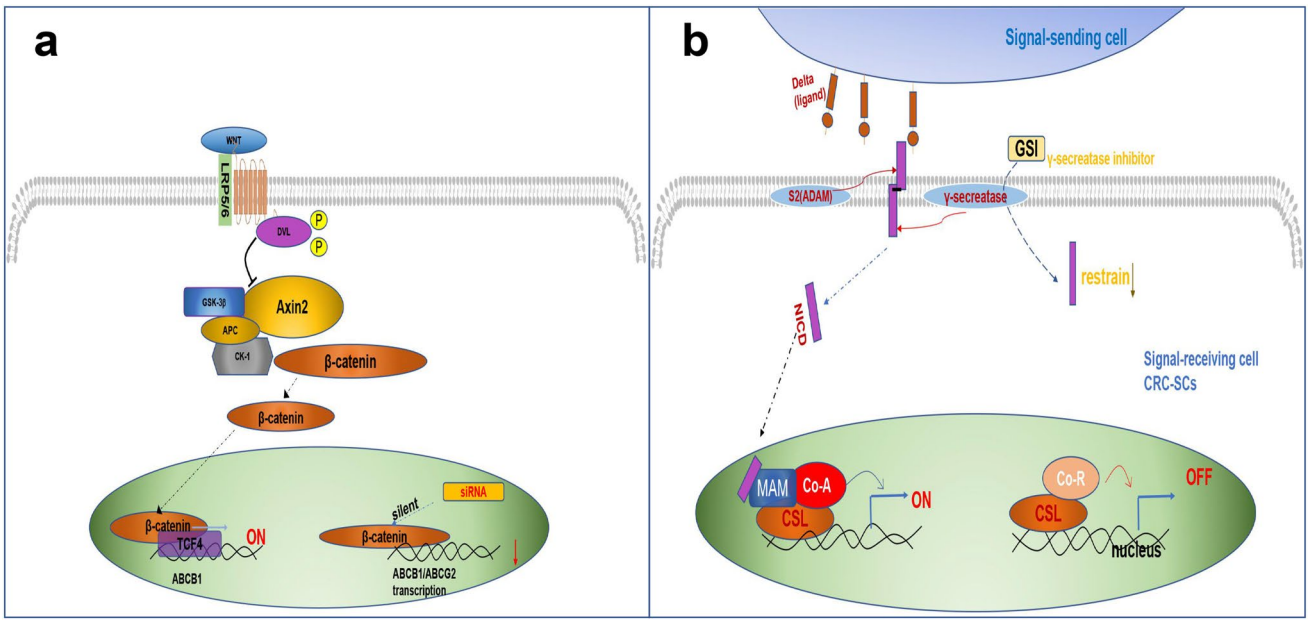

Fig. 2 The signal pathway in CRC-SCs. **a** Wnt signaling pathway. When Wnt ligand binds to CRC-SCs surface receptors, it activates the intracellular signal pathway, makes β-catenin enter the nucleus, activates ABCB1 gene sequence, and starts the mechanism of drug resistance. Silencing β-catenin with siRNA can downregulate the target gene expression of ABCB1 or ABCG2 and reduce their transcription and then make the cells sensitive to chemotherapeutic drugs. **b** Notch signal pathway. CRC-SCs obtain a specific ligand delta from signal transduction cells and bind to two receptors connected together to activate the internal signal pathway. Chemotherapy induces NICD protein to activate Notch pathway to achieve chemoresistance. γ-secretase inhibitor (GSI) can inhibit the activity of γ-secretase, the intermediate product of Notch pathway, thus inhibiting the activity of Notch pathway and making CRC cell lines sensitive to chemotherapy drugs

colonic bulb and drug-resistant cell lines are more dependent on notch signaling pathway (Fig. 2b).

MAPK/ERK pathway also plays an important role in chemotherapeutic resistance of CRC-SCs. For example, KCTD12 (potassium channel specialization domain containing 12) regulates stem cell characteristics of CSCs through MAPK/ERK pathway [97]. It belongs to KCTD family and is also used as an auxiliary subunit of gamma-aminobutyric acid-B receptor (GABAB). Knockout of KCTD12 gene in HT29 cell line results in higher viability of the cells at different concentrations of imatinib and 5-FU [97]. As documented, signaling pathways are particularly important for CRC-SCs to maintain stem cell characteristics, including chemotherapeutic resistance. But right now, the specific regulatory mechanism of signaling pathway in CSCs remains to be explored.

NcRNAs in CRC-SCs

NcRNA is a kind of gene that has noncoding function, but it accounts for over 98% of the whole genome, and more and more evidences show that it plays an important role in the development of tumor tissues [98]. They participate in the occurrence and development of cancer by regulating the expression of transcription factors of important stem cells in cancer and by interacting with CSCs-related signaling pathways, especially microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). miRNAs are a kind of small endogenous ncRNAs, which negatively regulates target genes by cutting mRNA or inhibiting translation [99]. For instance, miR-21 is overexpressed in CRC-SCs, which accelerates the overexpression of programmed cell death 4 (PDCD4), promotes cell apoptosis, and increases drug sensitivity [100]. miR-34a is overexpressed in CRC and directly targets c-kit (a stem cell factor receptor or CD117) to make CSCs sensitive to 5-FU and other chemotherapy drugs [101]. The overexpression of miR-34a inhibits the invasion and migration of tumors, restrains the pellet-forming ability of CRC cells, and decreases the expression of CD44, LGR5, and other stem cell markers [101]. Additionally, the overexpression of miR-34a leads to the reduction of ERK signaling and transformation, which depends on whether the expression of c-kit is inhibited or not [101]. CSCs cell cycle is slow and quiescent during the G0 phase, so as to insensitive chemotherapeutic agents acting on the cell cycle [102]. Cyclin D2 regulates cell cycle and inhibits the maintenance of CSCs characteristics, causing the sensitivity of CRC-SCs to drugs, while suppression of miR-141 can target cyclin D2 and increase its expression [103]. miR-215 is highly expressed in CRC-SCs and targets at denticleless protein homolog (DTL), which slows down the value-added rate of CRC-SCs and enables it to resist the damage of drugs acting on cell cycle and produces drug resistance [104]. The expression of miR-328 in CRC SP cell lines is higher than that in non-SP cell line, and it directly targets ABCG2, which is negatively correlated with the expression of miR-328 [105]. Markedly, ABCG2 is closely related to CRC-SCs resistance. Furthermore, ABCG2 and LGR5 expression levels are negatively correlated with miR-142-3p, and they also directly target miR-142-3p to regulate CRC-SCs resistance [106]. The expression of miR-145 in CRC-SCs is significantly reduced, which inhibits the proliferation of CRC cells and makes them sensitive to chemotherapy drugs by targeting the oncogene Friend leukemia virus integration 1 (FLI1) gene [107]. Additionally, miR-21 also plays a negative role in regulating miR-145 through Ras signaling pathway [108].

LncRNAs are a class of ncRNAs over 200 nucleotides in length. Since no functional role of lncRNA was found in the early stage, it is often referred to as "junk gene." However, subsequent studies have found that lncRNA seems to have the function of regulating cells at the genetic and epigenetic levels, controlling gene expression, and adjusting the proliferation, differentiation, invasion, and migration of tumor cells [109]. Even preclinical studies have found more than 900 lncRNA in mouse embryonic stem cells (mESCs) and human embryonic stem cells (hESCs). Subsequently, the presence of lncRNA has also been found in CSCs, and it plays a major role in maintaining the dryness of CSCs [98]. Silencing lncRNA HOTAIR in $CD133^+$ LoVo cells decreases the invasion and migration ability of the cells, as well as the tumorigenesis ability in vitro, suggesting that HOTAIR expression in $CD133^+$ LoVo is closely related to the self-renewal, metastasis, and infiltration ability of the cells, which are the characteristics of CRC-SCs [109]. LncRNA BCAR4 expression is significantly upregulated in $ALDH^+$-CRC-SCs, and CSCs with high BCAR4 expression show higher expression of surface markers, such as CD44, CD133, and LGR5, as well as higher migration ability and ball-forming ability. BCAR4 functions through the miR-665/STAT3 axis, and STAT3 plays an important role in maintaining the characteristics of CSCs [110]. The p53 mutation, as a tumor suppressor gene, regulates the expression of lncRNA, thus endowing tumor cells with dryness. One of the mutation types, p53-R273H, has the most significant effect. For example, lncRNA 273–31 and lncRNA 273–34 are upregulated in P53-R273H mutant CRC cells. When the two lncRNAs are further knockdown, the number of $ALDH^+$-CRC-SCs decreases, and the resistance to oxaliplatin also reduces [111]. Zhou et al. found a brand new lncRNA and named it cCSC1. Then they found high expression of cCSC1 in CRC and CRC-SCs. When cCSC1 is knockout, the relevant characteristics of CRC-SCs are significantly reduced, whereas the results of high expression of cCSC1 are reversed. By using 5-FU, the apoptotic ratio of cCSC1 is significantly improved after cCSC1 silencing

compared with the control group. This result suggests that the drug resistance of CRC-SCs is related to the expression of cCSC1 [112]. LncRNA CCAT2 also has the ability to regulate the maturation process of miR-145 and the expression of miR-21, so as to regulate the invasion and metastasis of CRC-SCs [113] (Table 1).

EMT in CRC-SCs

In the process of EMT, cells lose epithelial properties, acquire stromal properties, and lose contact and polarity between cells, so as to increase motility and invasiveness [114]. This process has important function in the invasion and metastasis of CRC [115]. E-cadherin is the most important intercellular adhesion medium in epithelial tissue. Low expression of E-cadherin in epithelial cells is a key marker of EMT [116]. EMT phenotype exists in drug-resistant CRC cell lines, suggesting that drug resistance of CRC may be closely related to EMT [115, 117]. Curcumin is a kind of plant drug that has been proved to have antitumor properties [118]. In 5-FU-resistant CRC cell lines, there are some miRNAs that can inhibit EMT, and curcumin would inhibit the EMT process by upregulating these miRNAs, thus increasing the sensitivity of cell lines, such as miR-34a and miR-200c [119]. EMT is also a key step in the generation of CRC-SCs, which indicates that it is strongly linked to CSCs [120]. The combined expression of EMT and CRC-SCs-related markers is associated with the poor prognosis of patients, such as CD44 and CD133 [121]. The regulating factor of EMT also regulates the stem cell characteristics of CRC-SCs [122]. Some studies have displayed that in CRC-SCs microenvironment, cancer-associated fibroblasts (CAFs) secrete exosomes, act on CRC cells, induce dedifferentiation into stem cells, and promote the process of EMT, thus increasing the chemoresistance of CRC [117]. In fact, the specific relationship between EMT and the mechanism of CSCs resistance remains to be further explored and found through experiments.

The quiescence of CRC-SCs

Normal human stem cells can be either proliferative or inactive. During the quiescent phase (G0), metabolic activity of cells are decreases, while stimulated by the extracellular environment, stem cells exit the quiescent phase, re-enter the cell cycle, and regain the ability to proliferate [123]. Therefore, quiescent state is also known as reversible cell cycle stagnation. As CSCs are similar to normal stem cells in nature, they also have a resting period and the ability to switch between resting and proliferating phases [124]. In the resting stage, CSCs' metabolic ability is reduced and can be dormant for a long time. CSCs at the quiescent stage are of great significance, because they may be the main cause of tumor resistance, distant metastasis, and recurrence after treatment [125, 126]. In CRC, CSCs at rest can be screened by a fluorescent tracer PKH26 marker, and these cells grow slowly and show chemotherapy resistance [127]. The exogenous expression of zinc finger E-box-binding homeobox 2 (ZEB2) in CRC-SCs increased the G0/G1 phase of the cells [128]. Dieter et al. found a variety of CSCs subtypes in CRC, including the dormant population, which show resistance to chemotherapy drugs [129]. Up to now, 5-FU and oxaliplatin are still the main drugs for the treatment of end-stage CRC, but they mainly act on restraining the activity of thymidine kinase synthase during DNA replication, thus achieving the killing effect on tumor cells. Hence, the killing effect of CSCs in G0 stage is not significant, and CSCs can induce the recurrence of CRC after receiving some kind of stimulation [130].

Table 1 The ncRNAs associated with CRC-SCs resistance

MiRNA\LncRNA	Expression	Targets	Signal pathway	References
miR-21	↑	PDCD4、TGFβR2	Wnt/β-Catenin	Yu et al. [100]
miR-34a	↑	c-kit	Erk	Siemens et al. [101]
miR-141	↑	cyclin D2	–	Ye et al. [103]
miR-215	↑	DTL	–	Song et al. [104]
miR-328	↓	ABCG2	–	Xu et al. [105]
miR-142-3p	↓	ABCG2、Lgr5、CD133	–	Shen et al. [106]
miR-145	↓	FLI1	Ras	Zhang et al. [107]
lncRNA BCAR4	↑	miR-665、STAT3	–	Ouyang et al. [110]
lncRNA cCSC1	↑	–	Hedgehog	Zhao et al. [111]
lncRNA CCAT2	↑	miR-145、miR-21	–	Yu et al. [113]

In CRC, the expression of ncRNAs related to the resistance mechanism of CSCs, as well as the targeted proteins and the signaling pathways of action

Discussion and conclusion

CRC is one of the most common malignant tumors in the digestive tract, and its related death is still the main cause of cancer-related death [3]. The 5-year follow-up survival rate after surgery plus radiotherapy and chemotherapy is only about 50% [2], and the low survival rate is largely due to its high metastasis and recurrence rate [131]. Additionally, its high metastasis and recurrence rate are considered to be closely related to chemotherapeutic resistance after treatment. Due to the limitations of chemotherapy, such as the toxicity of chemotherapy drugs to normal tissues and the increasing drug resistance during treatment, only 30% of patients with CRC received chemotherapy could achieve the expected efficacy through statistical analysis [132, 133]. The theoretical hypothesis of the existence of tumor stem cells has been gradually confirmed. CSCs are a special group of cells in tumor tissue, which have the characteristics of self-renewal and multidirectional differentiation [134]. It is believed that the presence of CSCs is tightly tied to the emergence of tumor drug resistance [135]. Therefore, in order to provide more effective treatment for CRC patients, it is necessary to further understand the mechanism of drug resistance of CRC-SCs and the potential target of CSCs and improve the chemotherapy effect.

CSCs can be screened and identified by a series of characteristic surface markers, while the more characteristic markers in CRC are LGR5, CD44, CD133, and ALDH. These markers can not only be used to identify the characteristics of CSCs, but also can activate various signaling pathways or serve as targets to change the characteristics of CSCs, including proliferation, migration, invasion, and drug resistance, etc. [136, 137]. ABC transporter superfamilies are overexpressed in CSCs and play an important role in eliminating other chemotherapeutic drugs, which indicates that they are related to drug resistance [63, 138]. Notably, within them, ABCB1, ABCG2, and ABCC1 are common in CRC-SCs. ABCB1 has two ATP binding sites, so it has a stronger ability to transport drugs [67]. ABCC1 is found to be mainly resistant to anthracycline drugs [68], and ABCG2 has a broad resistance spectrum [79, 80]. There is a relationship between the expression of CRC-SCs surface markers and the ABC transporter family. For instance, the upregulation of ABCG2 expression is found in $CD133^+$ CSCs [8]. Besides that, the combined expression of EMT, CD44, and CD133 is also go hand in hand with the poor prognosis of CRC patients [121]. The signaling pathway in CRC-SCs has contacted closely with ABC transporter family. There are multiple targets of β-catenin complex on ABCB1 gene promoter. In turn, β-catenin complex in Wnt pathway also targets ABCB1 activity [139]. On the one hand, miRNA can activate Wnt/β-catenin signal pathway. For example, overexpression of miR-21 downregulates the expression of transforming growth factor TGFβR2 (transforming growth factor β receptor 2) which is involved in cell differentiation, thus activating signal transduction, promoting CSCs differentiation, and achieving sensitization [100, 140]. On the other hand, miRNA has the ability to control the cell cycle and slow down the proliferation of CSCs and target ABCG2 and LGR5 for reference CSCs resistance regulation. More specifically, CSCs are often in a quiescent state to escape apoptosis, which leads to the decline of therapeutic effect of chemotherapy drugs [130]. To sum up, the generation and regulation mechanism of CRC-SCs resistance are mainly interactive. Therefore, it is urgent to find a new treatment scheme to improve the therapeutic effect of CRC patients.

In conclusion, we believe that the mechanism of drug resistance of CRC-SCs is not independent, but a network of interaction. At present, some studies have revealed the mechanisms of drug resistance of CRC-SCs, but there are still many unknown areas to be explored, such as the change of apoptosis mechanism of CRC-SCs, the enhancement of autophagy and the formation of tumor microenvironment balance, etc. In order to obtain an accurate targeted treatment plan for CRC-SCs and improve the sensitivity of chemotherapy, further animal model research, application of more advanced experimental technology and conditions, and scientific exploration and analysis of the mechanism related to the generation and regulation of CRC-SCs resistance are needed (Fig. 3).

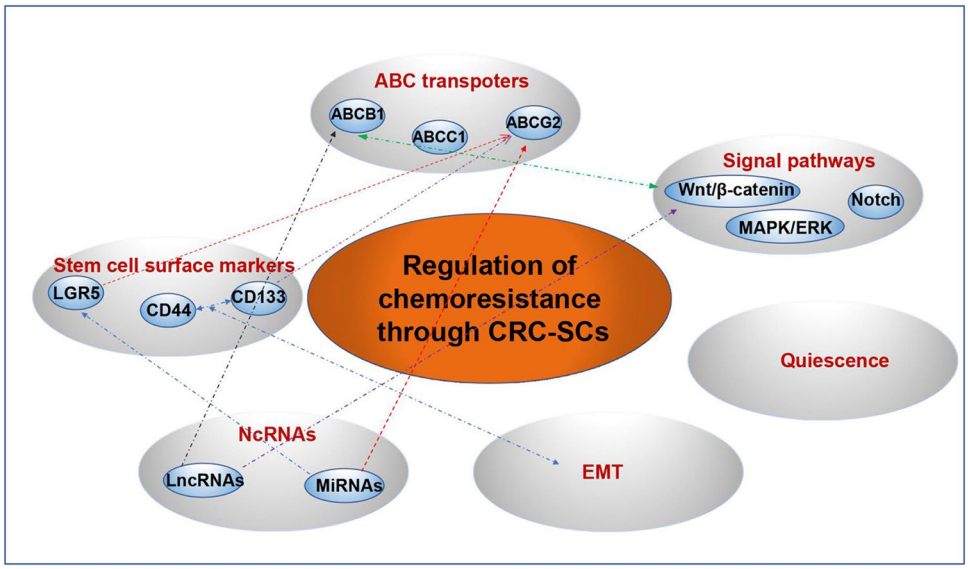

Fig. 3 Network diagram of regulatory mechanism of drug resistance related to CRC-SCs. The regulatory mechanisms exist interact. In the mechanism of drug resistance of CRC-SCs, ABC transporters and ncRNA can be the target of signal pathways. ABC transporters, ncRNA, and stem cell surface markers interact; surface markers and EMT are also related; resting CSCs also play an important role in drug resistance

Author contributions XL and QLH conducted data analysis and drafted manuscripts. ZQL, QZ, RPX, and HBY were involved in research design and data collection. Finally, YLD and WZ revised the manuscript.

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Data availability All data and materials could be found in our published paper.

Declarations

Conflict of interest All authors announce that they have no conflicts of interest.

Consent for publication All listed authors were actively involved in the study, reviewed, and approved the submitted manuscript.

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