Cancer Stem Cells in Patient Survival and Therapies in Cancer

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Abstract

Cancer stem cells (CSCs) are a subpopulation of cancer cells and responsible for stemness properties of cancer cell. It is regarded as one of the major causes of cancer formation, recurrence, and metastasis. Recent studies demonstrated that CSCs are closely related to the prognosis and treatment of many tumors including lung cancer, colorectal cancer, breast cancer, gastric cancer, and melanoma by targeting cell surface markers, signaling pathways, and microRNAs (miRNAs) to affect stemness features of CSCs. In addition, the application of nanotechnology in CSCs also makes it a novel and potential target in therapy of tumor. However, given the limitations of CSCs as mentioned in this paper, its clinical applications as a target of cancer face many challenges. Further research is needed to explore its clinical application as a target for tumor therapy.

Keywords

Cancer stem cells · Cell surface markers · Signaling pathway · MicroRNA · Drug resistance · Survival

Abbreviations

5FUR   5-Fluorouracil-resistant
ABC   ATP-binding cassette

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ABCB	ATP-binding cassette subfamily B
ABCB5	ATP-binding cassette subfamily B member 5
ALDH	Aldehyde dehydrogenase
ALDH1A1	ALDH1 family member A1
ATRA	All-trans retinoic acid
BCRP	Breast cancer resistance protein
BCSCs	Breast CSCs
CAPE	Caffeic acid phenethyl ester
CDDP	Cisplatin
CDF	Difluorinated-curcumin
CRC	Colorectal cancer
CSCs	Cancer stem cells
CSLCs	Cancer stemlike cells
CTCs	Circulating tumor cells
CTL	Cytotoxic T lymphocytes
CTNNBIP1	Catenin beta interacting protein 1
DCs	Dendritic cells
DFS	Disease-free survival
EGCG	Epigallocatechin-3-gallate
EGFR	Epidermal growth factor receptor
EMT	Epithelial-to-mesenchymal transition
EpCAM	Epithelial cellular adhesion molecule
GCSCs	Gastric CSCs
GSI	Gamma secretase inhibitors
HA-SLNs	Solid lipid nanoparticles with hyaluronan
HH	Hedgehog
HHAT	HH acyl transferase
HIF	Hypoxia-inducible factor
HMGA2	High mobility group AT-hook 2
LAC	Lung adenocarcinoma
LGR5	Leucine-rich repeat-containing G-protein-coupled receptor 5
LSCC	Lung squamous cell carcinoma
LSD1	Lysine-specific demethylase 1
MAPK	Mitogen-activated protein kinase
MDR1	Multiple drug resistance
miRNA, miR	MicroRNA
MRP	Multidrug resistance-associated proteins
mTOR	Mammalian target of rapamycin
N2IC	Notch2 intracellular domain
NSCLC	Non-small cell lung cancer
OPN	Osteopontin
OS	Overall survival
PAF	Proliferating cell nuclear antigen-associated factor
PCNA	Proliferating cell nuclear antigen
P-gp	P-Glycoprotein
PRKCI	Protein kinase C iota

PTX	Paclitaxel
Rh123low	Low rhodamine 123
ROS	Reactive oxygen species
SCLC	Small cell lung cancer
SDCSCs	Spheroid-derived CSCs
SIRT1/2	Sirtuin 1 and 2
Smo	Smoothened
SP	Side population
STAT3	Signal transducer and activator of transcription 3
TAM	Tumor-associated macrophage
TNBC	Triple-negative breast cancer
VCR	Vincristine
VEGF	Vascular endothelial growth factor
VM	Vasculogenic mimicry

18.1   Introduction

Cancer stem cells (CSCs) are a minor population of tumor cells with the properties of self-renewal and differentiation, as well as tumorigenic potential. [1] The accumulated evidence suggests that CSCs play an imperative role in metastases, posttreatment recurrence, and resistance to chemoradiotherapy in cancer [2], which closely associated with the worse survival of cancer patients. Meanwhile, these properties also make CSCs become a potential therapeutic target for cancer treatment.

Previous studies have shown that conventional chemotherapy and radiotherapy could not completely eliminate CSCs in cancer patients, resulting in treatment failure. The reason is that CSCs possess the properties of slow cell cycle, detoxification or regulation of cytotoxic outflow, resistance to oxidative stress, and rapid response to DNA damage [3, 4].

Recently, several methods available to target CSCs including specific surface markers of CSCs, specific signaling pathways, tumor microenvironment, or specific microRNA (miRNA, miR) have been reported by accumulating researches. For specific surface markers of CSCs, CD44, CD133, CD24, and ALDH1 are routinely used to identify and validate CSCs [5]. Another approach could be targeting CSCs by their specific signaling pathways. Key cell signaling pathways include Notch, Wnt/β-catenin, hedgehog (HH), human epidermal growth factor receptor (EGFR), and so on [6]. These signaling pathways are of crucial importance in CSCs. Other treatment strategies include targeting the tumor microenvironment or specific microRNA (miRNA, miR). MiRNAs participate in many vital biological processes including cell proliferation and migration, tumor cell aggression, and metastasis. At present, emerging evidences suggest that miRNAs play a critical role in CSCs [7, 8]. Of course, the CSC-targeting strategy certainly goes beyond these methods above.

Therefore, in this article, we have summarized recent advancement on the therapeutic and prognostic role of CSCs in different types of cancer including lung cancer, colorectal cancer (CRC), breast cancer, gastric cancer, and melanoma.

18.2   CSCs in Lung Cancer

Primary lung cancer remains one of the most common malignant tumors, and deaths from lung cancer exceed those from any other malignancy worldwide [9]. A growing number of studies have shown that CSCs exhibited important roles in driving initiation, metastasis, recurrence, and resistance to conventional therapy of lung cancer. Hence targeting CSCs of lung cancer may provide a promising approach to improve the survival and therapies of lung cancer patients in the future.

18.2.1 Targeting Cell Surface Markers of CSCs

Many cell surface and transmembrane proteins are expressed in CSCs of lung cancer including CD24, CD133, ALDH, and so on. These surface markers could not only identify CSC population in lung cancer but also act as an approach to target CSCs.

Elevation of CD133+ and CD24+ cells has been found to be closely related to poor prognosis of cancer treatment. Stem cell surface marker CD24 has been considered as a novel prognostic marker and stem cell marker in non-small cell lung cancer (NSCLC). Overexpression of CD24 was reported to associate with tumor patients with a higher risk of disease progression and tumor-related death. In addition, this study also showed that the overexpression of CD24 could be used as an independent predictor for poor progression-free survival and tumor-specific survival in patients with NSCLC [10]. Therefore, CD24 antigen could provide numerous crucial information to research the biology of NSCLC and may be used as a beneficial tool to promote the development of novel diagnostic and therapeutic ways to eradicate CD24 function in tumor cells. As a specific marker of human hematopoietic stem cells, CD133 has been considered as a marker of CSCs in numerous cancers. In NSCLC, CD133+ cells possess a higher tumorigenicity ability compared with CD133− cells and express genes which confer to cancer cells the property of stemness, adhesion, motility, and drug efflux. And importantly, CD133+ cells of lung tumor are spared by cisplatin treatment [11].

The aldehyde dehydrogenase (ALDH) is another important surface marker of lung cancer with stem cell properties. Compared with the ALDH-CSC population, ALDH+ CSCs show longer telomeres. MST312, a novel telomerase inhibitor, plays an antiproliferative role in lung CSCs and possesses the characteristic of inducing tumor population apoptosis. The previous study demonstrated that MST312 possesses potential antitumor properties in NSCLC in vivo (mouse model): intraperitoneal injection of MST312 (40 mg/kg/day) can reduce the tumor size by more than 70%. In addition, at the end of in vivo treatment, immunohistochemical analysis of ALDH and fluorescence-activated cell sorting analysis of ALDH after removal of

the tumor showed that the population of CSCs was also significantly reduced. Thus, as a antitelomeric therapy mainly by targeting lung CSCs, MST312 may prove to be effective in treating lung cancer [12]. ALDH1-positive CSCs showed stronger proliferative ability, cloning efficiency, and tumorigenicity. And ALDH1, a marker of CSCs, may be employed as a target for the therapy of lung cancer in the future [13]. Huang et al. [14] showed that compared with ALDH1 family member A1 (ALDH1A1)-negative lung cancer cells, lung cancer cells with ALDH1A1-positive possess the property of resistance to gefitinib. Clinical sample studies showed that a significant increase in the proportion of ALDH1A1-positive cells was observed in lung cancer cells that resist to EGFR-tyrosine kinase inhibitor and chemotherapy agents. In addition, a higher proportion of ALDH1A1-positive cells was shown in PC9/gef cells (lung cancer cells showing resistance to gefitinib), compared with lung cancer cells which were sensitive to gefitinib. Another study showed that the expression level of ALDH1A1 was significantly correlated with the poor prognosis of patients with stage I and N0 disease. ALDH tends to select NSCLC stemlike cells with stronger tumorigenicity and self-renewal ability, and those NSCLC carrying tumor cells expressing ALDH1A1 tend to have a worse outcome [15].

18.2.2 Targeting Signaling Pathways of CSCs

Any deregulation of CSC-related signaling pathway will activate CSCs, which eventually results in formation, recurrence, and metastasis of numerous cancer including lung cancer [16]. Dysregulation of various signaling pathways in CSCs is expected to be a novel potential therapeutic target for human cancer.

18.2.2.1 Hedgehog Signaling Pathway

In lung cancer, hedgehog (HH) signaling pathway was found to increase drug resistance in patients and eventually leads to the failure of chemotherapy. Mutations of HH signaling pathway play a critical role in promoting tumorigenesis and activation of CSCs, thereby resulting in lung cancer. A study involving genetically engineered mice showed that the activation of Smoothened (SMO), the component of HH signal molecules, could not only contribute to the formation of cloning in human small cell lung cancer (SCLC) in vitro but also promote the occurrence and development of mouse SCLC in vivo. Furthermore, the key cell-intrinsic role of HH signaling in the progression and maintenance of SCLC has been explored, as Park KS et al. demonstrated that the use of SMO antagonists could suppress the development of SCLC in mice and humans, especially after chemotherapy. And the inhibitor of HH pathway may be a novel potential therapeutic target for human SCLC patients to slow down the further deterioration of the disease and delay the relapse of cancer [17]. GDC-0449, a HH inhibitor, effectively reduces cell growth of SCLC and enhances the inhibitory effect of cisplatin on the growth of lung cancer cells [18]. At present, there is a lack of effective targeted therapy in lung squamous cell carcinoma (LSCC). A study shows that GANT61, a HH-GLI inhibitor, could effectively induce apoptosis of LSCC cells, suggesting that inhibition of HH-GLI

may be employed as a new and effective strategy for the treatment of some patients with LSCC [19]. Protein kinase C iota (PRKCI)-mediated SOX2 is required for HH acyl transferase (HHAT) to initiate and activate the HH pathway. Justilien et al. [20] reported that PRKCI-SOX2-HH signaling pathway is crucial to maintenance of CSC in LSCC. These findings offer a strong rationale for HH inhibitors for treatment of LSCC.

18.2.2.2 Notch Signaling Pathway

Notch signaling pathway is key to maintain a cancer stem or progenitor cell compartment, which is necessary for tumorigenesis in lung cancer.

Notch signaling is one of the most activated pathways in cancer cells and crucial for the correlation between self-renewal of CSCs and angiogenesis. In addition, the growth of lung cells is regulated by it via controlling the fate of normal stem cells. By testing the effect of Notch1 blocking on the growth and viability of lung CSCs, Cai et al. [21] observed that CD44+/CD24– cells isolated from A549 cells possessed stem cell-like properties with high expression of Notch1 and blocking Notch1 by inhibitor DAPT (GSI-IX) or siRNA, both inhibiting the growth capacity of lung CSCs. In a study by Liu et al. [22] in 2014, the difference of Notch signal expression between CD133+ and CD133- cells was compared in the same human lung adeno-carcinoma cell line A549 with CD133 as the marker of stem cells. And in these two cells above, the effects of DAPT combined with cisplatin (CDDP) were detected and compared. The results showed that notch signaling pathway members (Notch1, Notch2, and Hes1) were low expressed in CD133+ cells, and significant drug resistance to CDDP was found in CD133+ cells. Moreover, after combined application of GSI, the inhibitory effect of CDPP was significantly enhanced in both cells above, particularly in CD133+ cells. These findings suggest that the inhibitor of Notch pathway is expected to be a potential therapeutic target for lung cancer. Furthermore, the previous studies [23, 24] showed that the combination of the inhibitor of Src-YAP1, EGFR, and signal transducer and activator of transcription 3 (STAT3) could provide an inhibitory effect beyond its application alone or double in vivo, indicating the importance of combined treatment. Recently, they further investigated whether the expression of CSCs and EMT markers and the activity of ALDH were affected by the inhibition of EGFR. The results showed that combined inhibition of EGFR, STAT3, and Src significantly reduced CSC subsets in the cell model of EGFR mutation. Thus, a single inhibitor of EGFR may increase the number of CSCs; on the contrary, its combination with targeted Src and STAT3 might be beneficial to the treatment of lung cancer [25]. Taken together, these findings suggested that for the treatment of lung cancer, a single inhibitor of signal pathway is insufficient and it would further drive activation of parallel signal pathways, thereby leading to the failure of treatment. In contrast, combined therapy might be beneficial to the treatment of patients, especially for those NSCLC patients with positive EGFR mutations.

18.2.2.3 Wnt/β-Catenin Signaling Pathway

In a 2012 study, trifluoperazine showed an ability to inhibit the generation of CSC tumor sphere and decrease the expression level of CD44/CD133 (CSC markers). It inhibited Wnt/β-catenin signaling in lung cancer sphere with resistance to gefitinib. Furthermore, in animal models of lung cancer metastatic and orthotopic CSC, trifluoperazine has been found to inhibit the development of tumor and increase the sensitivity of gefitinib. Combined application of trifluoperazine, gefitinib, or cisplatin may effectively increase the sensitivity of lung cancer to it [26].

18.2.3 Targeting the miRNAs

Accumulating evidence suggests that as a key regulatory molecule of lung CSC-related metastasis, drug resistance, and tumor self-renewal, miRNA can effectively regulate numerous signal pathways, which participate in the regulation of proliferation, differentiation, apoptosis, cell cycles, and immune response of lung CSCs.

MiRNA plays a critical role in regulating lung CSCs, and these CSCs are closely related to the obstacles in cancer treatment including recurrence, metastasis, and drug resistance of cancer. For instance, as a tumor suppressor gene, miRNA-34a inhibits abnormal cell growth of malignancies including lung cancer [27]. The low expression level of miR-34a is key to promoting the invasiveness of lung CSCs. Moreover, with the recovery of miR-34a activity and the generation of exogenous delivery, this invasive property will also be reduced [28]. Thus, the recovery of miR-34a activity might be employed as an effective strategy for tumor treatment via downregulating the expression of Notch target genes or family members. Qi et al. [29] demonstrated that miR-214 suppressed the expression of catenin beta interacting protein 1 (CTNNBIP1), which also elucidates the mechanism of activation of Wnt/β-catenin signal in lung adenocarcinoma (LAC) tumor stem cells. Moreover, the expression level of CTNNBIP1 is also proportional to the differentiation of cancer cells and could be used to predict the survival of LAC patients. Thus, identifying miR-214-CTNNBIP1 pathways with the ability to regulate the self-renewal and stemness of CSLCs is expected to become a new strategy for the treatment of LAC patients. Recently, Dai et al. [30] identified the important role of miR-150-5p in the recurrence and metastasis of NSCLC. The result showed that the significantly low expression of miR-150-5p was observed in CSCs compared with non-CSCs. Furthermore, there was a significant positive correlation between the low expression of miR-150-5p and the disease deterioration and poor prognosis of NSCLC patients. The suppression of miR-150-5p would lead to the increase of CSC population, stemness, and metastasis of NSCLC cells. On the contrary, the high expression of miR-150-5p would markedly suppress the relapse, metastasis, and tumorigenesis of CSCs via targeting high mobility group AT-hook 2 (HMGA2) and β-catenin signaling in NSCLC. These results showed that as an inhibitor of CSC, the upregulation of miR-150-5p could be expected to a novel potential approach for the inhibition of CSC-induced metastasis and relapse in NSCLC. Taken together, these

findings suggested that miRNAs could significantly affect the biological behavior of lung CSCs by regulating the signaling pathways of LCSCs.

However, targeting CSCs in lung cancer would be a challenge due to heterogeneity of the cells and various genomic pathways involved. Therefore, many studies are focusing on using combination of cellular markers as it increases the specificity of targeted population.

18.3   CSCs in CRC

CRC is one of the most common and fatal malignant tumors in the world. It puts great pressure on medical and health care in all countries, and its incidence is gradually increasing [31]. The metastasis and recurrence in patients with CRC are known to be the main cause of failure of CRC treatment and ultimately leads to the worse prognosis. If we can early detect metastasis of CRC and take appropriate intervention before the disease progression, we can greatly improve the prognosis of CRC patients. Accumulating evidence suggests that CSCs participate in tumor formation, recurrence, metastasis, and resistance to chemoradiotherapy, which might play a crucial role in CRC. In fact, failure to completely eradicate CSCs is a major reason for the failure therapy of cancer [32]. It has been reported that the eradication of CSCs would be useful in increasing CRC patients' survival rates [33]. Thus, colorectal CSC markers can act as effective prediction factor and therapeutic targets.

18.3.1 Targeting Cell Surface Markers of CSCs

CSCs express some specific cell surface macromolecules that can be used for its identification and separation, and these macromolecules or markers can also provide a feasible target for scavenging CSCs. Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), an identification mark of CSCs, is crucial to the development of tissue and the maintenance of adult stem cells in gastrointestinal system. LGR5 overexpression has been reported to be closely related to lymphatic invasion, lymph node metastasis, vascular invasion, tumor depth, and tumor stage in patients with CRC. High level of LGR5 expression was linked to poor disease-free survival (DFS) for CRC patients [34, 35]. Further study [36] demonstrated that the inhibition of LGR5 cell such as selective ablation would suppress the development of primary tumor, but not lead to the regression of tumor. Furthermore, CSCs play a key role in the generation and maintenance of liver metastasis derived from CRCs, which indicate that CSCs may be expected to become a novel potential target for the treatment of metastatic cancers. In addition, Shimokawa and colleagues [37] showed that LGR5-positive (LGR5+) CRC cells could be acted as CSCs in growing cancer tissues. And significant regression of the tumor was observed after eradicating LGR5 + CSCs through ablation in LGR5-iCaspase9 knock-in organoids. Interestingly, the reemerging LGR5 + CSCs are shown to contribute to tumor regrowth after

LGR5 + CSCs' ablation. A previous study [38] showed that LGR5 is effective in the treatment of gastrointestinal tumors, especially colon cancer using different antibody-drug conjugates. Several studies have indicated that LGR5 has been shown to relate to increased drug resistance in gastrointestinal tumors. And in primary colon tumors, overexpression of LGR5 was shown to associate with chemoresistance and lower DFS and overall survival (OS) [39–43]. Hence, LGR5 is expected to be a new prognostic indicator and a potential target for the therapy of CRC. Nevertheless, the mechanism of LGR5 which participates in the tumorigenicity of CRC is not completely understood; therefore, larger, higher-quality studies are needed to illustrate the role of LGR5 in CRC.

CD133, CD24, and CD44 are cell surface markers which have been shown to be linked to stem cells, as well as aggressive cancer types and poor prognosis in CRC. Jing et al. [44] reported that CD44 might be used as an effective indicator to predict liver metastasis and prognosis of colon cancer patients. Du et al. [45] found strong inhibitory effect of knockout CD44 on clone formation and tumorigenicity of xenografts, indicating that CD44 is a robust marker and key to the initiation of tumor. Rao et al. [46] have illustrated that tumor correlated with macrophage (TAM) interacts with CD44-positive cancer cells and secrete osteopontin (OPN) which in turn promoted the clonogenicity and tumorigenicity of CRC. Moreover, tissue microarray data showed that the expression of OPN and CD44v6 (an OPN functional receptor) was inversely related to the survival of patients with CRC. These findings revealed that interaction between OPN and CD44 is crucial to the development of CRC and might be employed as a promising therapeutic target in CRC. However, large-scale, higher-quality studies (such as prospective trials) are needed to verify the results of these studies. Sahlberg et al. [47] reported that overexpression of CD133/CD44 was proportional to the increase of resistance to radiation in colon cancer cells. Liu et al. [48] reported that combined treatment of paclitaxel and siRNA-targeted CD133 cells could effectively reduce the expression of multiple drug resistance-1 (MDR1) in human colon CSCs (CD133+ enriched cell population), which could markedly reduce the resistance to paclitaxel. Jao et al. [49] reported that the overexpression of CD133 was markedly proportional to local relapse and prognosis of patients and could be used as an effective prognostic marker for tumor regression grading in rectal cancer patients after neoadjuvant concurrent chemoradiotherapy. Kanwar et al. [50] observed that difluorinated-curcumin (CDF) combined with 5-fluorouracil and oxaliplatin could effectively inhibit proliferation and induce apoptosis of CSCs via decreasing CD44 and CD166 drug-resistant colon cancer cells, suggesting that the combination of CDF with 5-fluorouracil and oxaliplatin is expected to be a reliable therapeutic approach to inhibit the drug resistance of colon cancer cells via eradicating CSCs. Excepting for the above cell surface markers, ST6Gal-I may also be a promising marker of CSCs. Swindall et al. [51] suggest that ST6Gal-I promotes tumorigenesis and plays a crucial role in maintaining behavior of stemlike cell; thus it might be employed as therapeutic target. Lugli et al. [52] reported that loss of membranous CD44s, CD166, and epithelial cellular adhesion molecule (EpCAM) was linked to tumor progression, invasiveness, and infiltrating tumor growth pattern. Therefore, CD44s, CD166, and

EpCAM possess the potential to predict the survival of CRC patients. Furthermore, Xiang et al. [53] developed a novel therapeutic approach based on the combination of EpCAM aptamer (a new drug delivery system) with doxorubicin which is able to target surface molecules of CSCs. This approach can effectively inhibit the growth of tumor and a prolonged longer tumorigenic latency, thereby improving the prognosis of CRC patients. Wang et al. [54] established a novel therapeutic strategy based on chitosan vesicle entrapment of oxaliplatin, which could eradicate tumor cells and CSCs (more effective than free oxaliplatin) and might be a novel strategy for treatment of CSCs. Recently, ALDH1 is one of putative CSC marker in CRC. Kahlert et al. [55] found that ALDH1 nuclear expression related to shorter survival of patients with CRC. Furthermore, Deng et al. [56] reported that high postoperative ALDH1 expression predicts the recurrence, distant metastasis, and poor prognosis for CRC patients who received neoadjuvant therapy. Also, Goosssens-Beumer et al. [57] suggested that co-expression level of ALDH1, survivin, and EpCAM was a reliable prognostic indicator to predict risk of recurrence and survival of colon cancer patients. However, further validation about these conclusions in clinical trials is warranted.

18.3.2 Targeting Signaling Pathways of CSCs

It has been shown that various signaling pathways including Wnt and Notch can control growth, differentiation, migration, and response to drug treatment of CRC by regulating CSCs. Therefore, targeting CSCs through signal pathway is a potential therapeutic strategy for CRC, but only taking such treatment does not make an effective approach at least today. The combination of conventional therapies such as radiotherapy and chemotherapy and the inhibitor of CSC-specific pathway possesses the potential to improve cancer cure compared with monotherapies [58].

Overexpression of BMI1, a signaling pathway of CSCs, induces tumor progression and metastasis and contributes to the self-renewal of CSCs. Depletion of BMI1 cancer cells can lead to suppression of CSC self-renewal. [59] The STAT3 pathway is crucial to regulate CSC self-renewal, and suppression of this pathway will lead to a decrease in the number of CSCs. Lin et al. [60, 61] reported that CD133/ALDH(+) CSC cell population expressed higher level of pSTAT3, and the effects of STAT3 inhibition in colon CSLCs were examined, which indicate that inhibition of STAT3 in CSLCs might provide a potential therapeutic strategy for CRC. And then they found that GO-Y030 acted as inhibitor of STAT3 phosphorylation and therefore inhibited colon CSCs. Napabucasin, an inhibitor of tumor stemness by targeting STAT3, possesses the ability to inhibit the recurrence and metastasis of numerous cancers [62]. A phase III clinical trial recently aimed to test napabucasin in advanced CRC showed that STAT3 may be a promising target for the therapy of CRC with elevated phosphorylated STAT3 (pSTAT3) expression [63]. Overactivation of Wnt signaling is the main reason for the pathogenesis of CRC. In colon cancer, the suppression of HOXA5 by the Wnt pathway maintains stemness of CSCs, and its

reexpression induces loss of the CSC phenotype, which will prevent tumor progression and metastasis [64].

A previous study revealed that HH-GLI1 was crucial to promote the development, metastasis, and self-renewal of stem cells in advanced colon cancers. Therefore, targeting HH-GLI1 may be used as a therapeutic strategy to reduce tumor size and metastases of colon cancer and eliminate colon CSCs [65]. These results suggest that HH-GLI1 signaling pathway played a key role in the formation of colon CSCs and may be a potential therapeutic strategy for colon cancer, especially for those with refractory and metastatic characteristics.

18.3.3 Targeting the miRNAs

A growing body of evidence suggests that miRNAs are closely related to the invasiveness and metastasis of CRC. And the aggressiveness and stemness of CRC remain a major cause for relapse and metastasis of CRC. Hongdan et al. [66] reported that miR-3210-5p could increase the characteristics of aggressiveness and stem cell-like in colon cancer via decreasing the expression of Axin2 (a regulator of Wnt signaling).Therefore, targeted inhibition of miR-3210-5p may be a promising therapeutic strategy to improve the prognosis of patients with colon cancer. Zhai et al. [67] showed that high expression of miR-140-5p was significantly correlated with the low expression of Smad2 in CRC cell lines, which would lead to the decrease of cell proliferation and invasiveness and the increase of cell cycle arrest. Furthermore, overexpression of miR-140-5p in CSCs abolished tumor formation and metastasis in vivo. The functional and clinical significance of miR-140-5p shows that it can regulate the metastasis and progression of CRC, as well as it has the ability to be a new therapeutic target for CRC in the future. Huang et al. [68] revealed that tRF/miR-1280, a 17-bp fragment derived from tRNA and pre-miRNA, affected Notch signaling pathways supporting the role of CSLCs in CRC progression. They have reported that tRF/miR-1280 could inhibit the metastasis and development of CRC via suppressing Notch signaling pathways. Furthermore, they demonstrated that miRNA with functional activity could be obtained from tRNA, which undoubtedly provides another promising biomarker for the treatment of CRC. A study showed that miR-34a directly inhibited Numb in early-stage colon CSCs and deletion of miR-34a will enhance CSC properties in colon cancer [7].

Colon CSCs have been identified as one of the main reasons for the resistance of colon cancer to chemotherapy. MiRNA is crucial to the progression of colon CSCs and might contribute to reducing drug resistance and increasing sensitivity to chemotherapy [8]. It has been shown that the expression of miR-451 leads to the decrease of colonic bulb self-renewal and tumorigenicity and the increase of its sensitivity to irinotecan by reducing the expression of ABCBA1 (an ATP-binding cassette drug transporter). The above results revealed that miR-451 may be used as a novel marker to predict the relapse and chemoresistance of CRC, especially the response of colon cancer to irinotecan [69]. A study by Xu et al. [70] in stem cell-like side population (SP) cells in CRC showed that the high expression of miR-328 could

improve the chemoresistance and suppressed the invasiveness of SP cells. These findings indicate that miR-328 plays a crucial role in maintaining cancer stemlike SP phenotype and might be a promising target for the treatment of CRC.

18.3.4 Other Strategies Targeting CSCs

Conventional therapies, such as radiotherapy and chemotherapy, play a predominant role in the management of patients with advanced-stage CRC. However, the inherent or acquired chemo- and radiation resistance of CRCs results in failure of treatment, which is partly on account of the enrichment of CSCs with resistance to conventional therapy.

A recent study shows that the combination of MST-312 (a telomerase inhibitor) with flavonoid morin can decrease the stemness of CSCs. In addition, co-treatment of the two drugs above can also enhance the therapeutic effect of 5-FU [71]. Therefore, the combination of flavonoid morin and MST-312 can be used as a novel therapeutic target to improve the prognosis of tumors. Recently, epigallocatechin-3-gallate (EGCG), an active catechin present in green tea, has been found to possess the ability to inhibit the development of CSC in a variety of tumor. Toden et al. [72] reported that EGCG could increase the efficacy of 5-FU and inhibit tumor proliferation in 5-fluorouracil-resistant (5FUR) CRC cell lines. EGCG treatment in these 5FUR cells leads to the inhibition of spheroid-derived CSCs' (SDCSCs) generation and increased sensitivity of 5-FU to SDCSCs. The above findings provide a support for EGCG to enhance the sensitivity of 5-FU by targeting CSCs of CRC and highlight the potential of EGCG as an adjuvant therapy for conventional chemotherapy in patients with CRC.

18.4   CSCs in Breast Cancer

A growing number of studies demonstrated that the formation of breast cancer was thought to be driven by CSCs. Today, acquired and inherent resistance to radiotherapy and chemotherapy represents a main obstacle in therapy of breast cancer. Breast CSCs (BCSCs) not only drive tumor formation, recurrence, and metastasis of cancer but also mediate therapeutic resistance [73]. Thus, developing strategies with effective targeting BCSCs might be beneficial to control tumor relapse, increase DFS, and increase sensitivity to conventional therapy such as chemoradiotherapy.

18.4.1 Targeting Cell Surface Markers of CSCs

Doxorubicin can achieve the therapeutic effect of scavenging BCSCs by decreasing the expression of CD44 in vitro. Moreover, decreased expression of CD44 will increase the sensitivity of CD44+CD24− breast cancer cells to doxorubicin [74]. Interestingly, another study [75] showed that knockout of the CD44 gene

makes BCSCs become non-BCSCs with less tumorigenicity, which changes the cell cycle and some CSC-related gene expression, leading to the loss of stemness and enhancing response to conventional therapy.

The malignant potential of triple-negative breast cancer (TNBC) is also relied upon a subpopulation of BCSCs. CD133 and EpCAM, two BCSC markers, are significantly related to invasiveness of breast tumors, indicating that combined therapy targeting two surface molecules at the same time might be an effective strategy for the therapy of TNBC [76]. GD2 (a glycosphingolipid) is a new CSC-specific cell surface marker. As a key enzyme of GD2 synthesis, GD3 synthase might be a promising therapeutic target for CSCs and may improve the prognosis of breast cancer patients. Furthermore, complete knockout of GD3 could eliminate the formation of tumor in vivo [77]. Hence, developing a therapy approach targeting CSCs may be beneficial to the treatment of patients with breast cancer.

18.4.2 Targeting Signaling Pathways of CSCs

A recent study has revealed that the noncanonical hedgehog inhibitor GANT61 could reduce not only the development of cells but also the number of CSC in triple-negative breast cancer cells and GANT61 could enhance the inhibitory effect of paclitaxel on the growth of these cells [78]. Likewise, GANT61 also possesses the ability to increase the proportion of CSC in ER-positive breast cancer cells excepting for its inhibitory effect on breast cancer cells [79]. These results suggest that GANT61 could be used as a target for the therapy of breast cancer patients through its inhibitory effect on cancer cells and CSCs. Genistein inhibited the breast CSCs and MCF-7 cells' growth and proliferation and promoted apoptosis via the downregulation of hedgehog-Gli1 signaling pathway [80]. These researches offer reasonable and reliable evidence to further explore the clinical application of genistein in the therapy of breast cancer via targeting BCSCs.

The Notch pathway is crucial to stem cell renewal and might be a promising target for BCSC-directed treatment. A preclinical and clinical study [81] shows that treatment with gamma secretase inhibitors (GSI) reduced BCSCs in MC1 and BCM-2147 tumor grafts via suppression of the Notch pathway. In this study, GSI shows the capability of enhancing the efficacy of docetaxel in breast cancer. These results indicate that the inhibitor of the Notch pathway could lead to the decrease of BCSCs. A 2017 study revealed that vitamin D compounds could be acted as a promising preventive drug of inhibiting TNBC via regulating BCSC differentiation and reducing its population through inhibition of Notch pathway [82].

Wnt/β-catenin pathway is critical for regulation of BCSC-mediated metastasis. Survivin was found to contribute to self-renewal of CSCs via driving the activation of PI3K/Akt-dependent Wnt/β-catenin pathway, which mediates the breast cancer metastasis [83]. Some signaling pathways are not only key to maintaining the biology of CSCs but also main reason for breast cancer patients to resist treatment [6]. For instance, a study [84] in 2018 shows that the STAT3 pathway can promote BCSC maintenance and breast cancer chemoresistance. Furthermore, the inhibitor of

JAK/STAT3 blocks BCSC self-renewal, and blocking fatty acid β-oxidation, induced by STAT3 in BCSCs, can resensitize them to chemotherapy. These results indicate that STAT3 pathway may be a promising target for BCSC-directed therapy. Sabutoclax has been reported to reduce sphere formation of drug-resistant cells and eliminated the CSC subpopulation by downregulating the IL-6/STAT3 signaling pathway. When sabutoclax was combined with chemotherapeutic agents, it presented a strong synergistic antiproliferative effect [85]. The study shows the feasibility of combination of sabutoclax and chemotherapy; however, larger and higher-quality studies are needed to verify these findings and determine the potential value of the combination of sabutoclax with conventional therapy in chemotherapy-resistant breast cancer.

18.4.3 Other Strategies Targeting CSCs

18.4.3.1 Antiangiogenic Therapies

The formation of blood vessels in the tumor provides a continuous supply of nutrition and oxygen for the frenzied growth of the tumor. BCSCs is found to favor the generation of novel blood vessels by undergoing dedifferentiation into endothelial cells, which is termed as vasculogenic mimicry (VM) [86]. The presence of the close relationship between VM and CD133(+) expression may be helpful for TNBC relapse and progression [87]. Later, USP44(+) CSCs subpopulation possess the ability to predict the generation and invasiveness of VM and could be used as a reliable prognostic biomarker of worse clinical outcomes in breast cancer patients [88].

The therapy of antiangiogenic drug can effectively suppress the growth of tumor neovascularization, thus inhibiting the development of tumor. Reversely, tumors will inevitably be resistant to antiangiogenic drugs due to the generation of HIF (hypoxia-inducible factor) with the ability to promote formation, metastasis, and aggression of tumor blood vessels and CSC self-renewal. Conley et al. [89] found that the combined administration of HIF-1α-targeted agents like CRLX101 and antiangiogenic drug such as bevacizumab could get a better effect by targeting the CSC populations. In addition, OPN also is critical for angiogenesis and tumor progression of breast cancer. A recent study [90] has revealed the prime role of OPN in controlling breast cancer progression and angiogenesis through ILK and NF-κB-mediated HIF1α-dependent vascular endothelial growth factor (VEGF) expression in response to hypoxia. Compared to the control group, the expression of OPN cells induced the progression and angiogenesis of breast cancer by upregulating the expressions of proangiogenic factors, suggesting that OPN and its controlled signal network may be a promising target for breast cancer therapy.

18.4.3.2 Radiotherapy

It was reported that CSCs show resistance to radiotherapy via enhancing the ability of DNA repairing and reducing the concentration of intracellular reactive oxygen species (ROS), due to the overexpression of ROS scavengers in CSCs [91]. These

BCSCs with low ROS level are proportional to the priority spare and smaller DNA loss after irradiation, resulting in resistance to radiotherapy in breast cancer [92].

Mammalian target of rapamycin (mTOR) activation is critical for sustaining the self-renewing ability of CSCs. In triple-negative MDA-MB-453 and MDA-MB-468 breast tumor cells, rapamycin suppression of mTOR phosphorylation helped to sensitize the resistant breast cancer to low-dose radiation therapy [93]. In addition, proliferating cell nuclear antigen (PCNA)-associated factor (PAF) is a pivotal factor with the ability to regulate the stemness of cancer cell. PAF was overexpressed in breast cancer cells, and its depletion impairs the maintenance of stemness of CSCs, suggesting that PAF may become an effective therapeutic target to restore radiotherapy sensitivity of breast cancer [94].

BCSCs are also accountable for drug resistance by expressing drug efflux proteins like multidrug resistance-associated proteins (MRP), P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP) [95]. ATP-binding cassette (ABC) transporters, a class of drug transporters, possess the ability to promote resistance to drug by relying on ATP to drain the drug out [96]. These ABC efflux pumps provide shelter for CSCs to protect them from various drug treatments [97]. As an ABC transporter encoded by MDR1 gene, P-gp participated in the protecting these tumor cells from anticancer chemotherapeutic drugs. The combination of P-gp inhibitors and chemotherapeutic drugs can maintain the concentration of chemotherapeutic drugs in tumors [96], suggesting that BCSCs expressing P-gp may be significantly associated with the chemoresistance and recurrence of tumor, which could make CSCs a novel therapeutic target and beneficial to current antineoplastic therapy. The high expression of BCRP (a protein of ABC transporter superfamily) will promote the resistance of tumors to some drugs including topotecan, methotrexate, mitoxantrone, doxorubicin, and daunorubicin [98].

Recently, lysine-specific demethylase 1 (LSD1) is considered as a major contributor to EMT, tumor stemness and drug resistance of breast cancer. Circulating tumor cells (CTCs) from patients with metastatic breast cancer were found to be rich in LSD1. Furthermore, targeting LSD1 by pharmacological inhibitor inhibited the stem cell-like and mesenchymal characteristics of the above CTCs. This report suggests that LSD1 might be acted as an effective therapeutic target for the therapy of advanced and drug-resistant breast cancer [99]. Lanzardo et al. [32] reported that downregulation of xCT damaged the formation of tumor sphere and changed the balance of CSCs intracellular redox in vitro. In addition, anti-xCT vaccination was shown to increase CSC chemosensitivity to doxorubicin in vivo; therefore therapeutically targeting xCT may contribute to the therapy of patients with breast cancer. Accumulating evidence suggests that metformin can also act as a promising agent for breast cancer and may be used as an effective (neo-)adjuvant therapy to eradicate CSCs and inhibit tumor aggressiveness [100, 101].

18.5   CSCs in Gastric Cancer

Gastric cancer is one of the five most frequently diagnosed cancers and the third leading cause of tumor-related death worldwide [31]. Gastric CSCs (GCSCs) have been proved to play a critical role in gastric cancer chemoresistance, recurrence, and metastasis. As a result, GCSCs might be expected to be a potential therapeutic target for improving the prognosis of patients with gastric cancer.

18.5.1 Targeting Cell Surface Markers of CSCs

CD44, a surface marker of CSCs, can be used as a key factor to promote the development of GCSCs. In gastric cancer, the presence of CD44+ CTCs has been considered to be related to lymph node metastasis, distant metastasis, and recurrence, which indicate that CD44+ gastric cancer CTCs may be used as a prognosis indicator of gastric cancer [102]. A variant of CD44 (CD44v8–10) has been considered as a major expression of CD44 variant in gastric cancer cells, which may promote tumorigenesis by enhancing the defense against oxidative stress [103]. A preclinical study shows that overexpression of CD44 and ALDH has recently been demonstrated in gastric cancer and all-trans retinoic acid could downregulate the expression of CD44 and ALDH, which eventually leads to the inhibition of the growth of gastric cancer [104]. Another study further illustrated the great potential of CD44 in predicting the prognosis of gastric cancer. Wang et al. [105] showed that the expression of CD44 was proportional to tumor transformation, TNM grading, metastasis, and recurrence of gastric cancer. At the same time, the high expression of CD133 also tended to predict the poor prognosis of patients. However, further research is necessary to verify the potential value of above markers in predicting the prognosis of gastric cancer. As a marker of CSCs, LGR5 is also highly expressed in gastrointestinal tumors. Gong et al. [106] developed two LGR5-targeting antibody-drug conjugates with the ability to induce the apoptosis of gastrointestinal cancer cells with high expression of LGR5, but it had no effect on gastrointestinal cancer cells that do not express LGR5. These findings suggest that it might represent a novel potential therapy targeting CSCs to eliminate the LGR5-positive gastrointestinal tumors and prevent cancer recurrence.

18.5.2 Targeting Signaling Pathways of CSCs

GCSCs make use of various signaling pathways including Notch, Wnt, HH, and so on to regulate the growth, migration, and response to drug therapy of gastric cancer. There was a significant correlation between target genes with different miRNA expression levels of gastric CSCs and several critical biological pathways including the regulation of cell cycle, the property of stemness, and differentiation [107]. Hence, targeting signaling pathways might be a therapeutic strategy to eradicate CSC population of gastric cancer.

A research exploring the relationship between Notch2 signaling pathway and the progression of gastric cancer showed that the expression of N2IC, an activated Notch2 receptor, not only promoted the proliferation of human SC-M1 cells and its development of transplanted tumor but also enhanced the colony generation, migration, aggression, and wound healing ability of SC-M1 cells. Interestingly, these effects will also disappear with the knockout of Notch2. Therefore, the knockout of Notch2 may be an effective strategy to suppress the development of AGS and AZ521 gastric cancer cells [108].

HH signaling pathway is critical to maintain CSC phenotypes and malignant transformation phenotypes in CD44(+) gastric cancer cells. Moreover, the inhibition of HH can block chemotherapy resistance in CD44(+) cells. However, this study also revealed that combination of HH inhibition and chemotherapy may only be effective for a small number of gastric tumor patients with overexpression of CD44 [109].

18.5.3 Targeting the miRNAs

Recent data suggest that miRNAs are associated with gastric cancer and contribute to carcinogenesis due to abnormality in their expression, which in turn affects cell proliferation, apoptosis, motility, and invasion [110]. It has been found that the expression profile of miRNAs in tumor initiation cells (CSCs) was significant differed from that in noncancerous cells [111].

MiR-501-5p, which plays a critical role in promoting the stem cell-like property of gastric cancer, shares a negative correlation with OS of patients with gastric cancer. MiR-501-5p has been shown to be significantly associated with gastric cancer patients possessing the more aggressive traits, suggesting that miR-501-5p represents a promising target for the therapy of human gastric cancer [112]. A study showed that there was a significant negatively correlation between the expression of miRNA-20a and miRNA-92a and the survival of patients with gastric cancer. This study also illustrated miRNA-92a may be an independent prognosis factors in gastric cancer [113]. Interestingly, miRNA can regulate stemness properties of CSCs of gastric cancer by regulating signaling pathway, thereby affecting the prognosis and therapies of patients with gastric cancer. A study [114] showed that the miR-23b suppressed gastric tumorigenesis such as development, invasion, migration, and metastasis through Notch2 pathway. MiR-132 has also been shown to possess the potential to promote the cisplatin resistance in gastric cancer patients by regulating SIRT1/CREB/ABCG2 signaling pathway [115]. The miRNAs are significantly associated with numerous cellular processes such as differentiation, proliferation, motility, and apoptosis in malignancies including gastric cancer, which suggest that miRNAs may be an effective approach to target CSCs and will ultimately possess the ability to treat gastric cancer patients and affect the prognosis of gastric cancer patients.

18.6   CSCs in Melanoma

In addition to playing a role in hematopoietic cancer and solid tumors (such as brain, breast, colon, pancreas, lung), CSCs have recently been found to be correlated with tumorigenesis, metastasis, and drug resistance of melanoma [116–120].

18.6.1 Targeting Cell Surface Markers of CSCs

Numerous stem cell markers have been found in drug-resistant melanoma cells and clinical specimens like CD133, CD20, ABCB5, ALDH1, and so on. Rappa et al. [121] have shown that in childhood malignant melanoma, CD133 + CSCs may be associated not only with lymph node and/or visceral metastasis but also with lower proliferative Ki-67 index which is one of the reasons for drug resistance. In addition, the overexpression of stem cell-associated markers, nestin, and CD133 in circulatory melanoma cells is related to the worse clinical outcome in melanoma patients. However, further validation in a large study with sufficient follow-up, similar sample sources, and including patients in stages II and III is warranted. [122] Lai et al. [123] have revealed that CD133+ and ABCB5+ subpopulations were co-localized in melanomas in perivascular niches and as stem cell-like cells, CD133+ cells could promote the development of tumor via promoting VM and the morphogenesis of a specialized perivascular niche in melanoma. Furthermore, CD133 knockdown melanoma cells are related to the poorer tumor growth in vivo. Luo et al. [120] reported that the expression level of ALDH was associated with the drug resistance of human melanoma stem cells, thus regulating the proliferation and survival of cancer cells. And the inhibition of ALDH by silencing ALDH1A can not only result in cell cycle arrest, apoptosis, and inhibited cell variation in vitro but also inhibit tumorigenicity in vivo. These results suggested that ALDH was not only a marker of CSC but also a promising target for the therapy of melanoma. However, further research on the molecular mechanisms of regulating CSCs of these isozymes and genes is warranted. Vincristine (VCR) is widely used in melanoma treatment; however, it has been found ineffective to treat the specific CSCs of melanoma. Song et al. [124] found that VCR-containing immunoliposomes combined with CD20 antibody (VCR-Lip-CD20) were 1.85 times more effective than VCR-Lip and VCR in melanoma. Significantly, the results also showed that VCR-Lip-CD20 could selectively kill CD20+ melanoma cells in populations of WM266-4 cells both in vitro and in vivo. These findings indicate that VCR-Lip-CD20 may be expected to be an efficient target to kill CD20+ melanoma cells.

18.6.2 Targeting Signaling Pathways of CSCs

Accumulating studies have shown that there are many signal pathways and potential therapeutic targets in numerous malignant tumors like melanoma. It was reported

that inhibiting HH pathway by interfering SMO or GLI1 drastically attenuates the self-renewal and tumorigenicity of melanoma stem cells with overexpression of ALDH; thus SMO and GLI1 may possess the potential to become a new and effective approach for the targeted therapy of human melanoma [125]. It has been also reported that the overexpression of Notch4 contributes to the aggression and metastasis of melanoma stem cells, which indicates a poor prognosis [126]. Kumar et al. [127] have shown that the expression of CD133+ CSCs which was regulated by Notch1 pathway could activate its regulated signaling network, promoting the development, metastasis, and angiogenesis of melanoma. Furthermore, eradication of Notch1 by blocking or ablation could also suppress the expression level of CD133, which in turn inhibits the cell migration and angiogenesis of melanoma. However, these findings only verified in mice experiments, and further studies are necessary to verify the value of these findings in human melanoma. BRAF and NRAS mutations are reported to occur in about 70% of melanoma, and a study [128] shows the combination of γ-secretase inhibitors (block the activation of Notch signal) and BRAF inhibitors (block the activation of BRAF-MEK-ERK signal) is more effective in the therapy of melanoma with BRAF/NRAS mutation. A study in 2016 showed that andrographolide could attenuate melanoma growth and lung metastasis by abrogation of Notch1-mediated CD133-dependent p38 mitogen-activated protein kinase (MAPK) activation pathway in CD133+ melanoma cells. Mechanistically, Notch1 upregulates MAPK activation through CD133 resulting in the development, lung metastasis, and angiogenesis of melanoma. In contrast, inhibition of Notch1 and MAPK pathways inhibits cell migration and angiogenesis of melanoma [129]. Excepting for these signaling pathways, low rhodamine 123 (Rh123low) cells are enriched for stem cell-like activities, and Rh123low cells possess the characteristic of relative stillness and drug resistance in melanoma CSCs. A study reported that PI3K/Akt pathway was the key to maintain Rh123low in melanoma stem cell compartment [130].

18.6.3 Targeting the miRNAs

Several reports have suggested that miRNAs played a crucial role in development of tumor, angiogenesis, and metastasis in numerous tumors including melanoma. A preliminary report showed that several miRNAs (miR-21, miR-10b, miR-200c, miR-520c, and miR-373) were significantly upregulated in melanoma sphere, suggesting that these miRNAs might regulate the potential of metastasis and stemness in melanoma [131]. Noman et al. [132] have shown that miR-210, as a hypoxia-regulated miRNA in lung cancer and melanoma, could reduce the lysis of tumor cells by antigen-specific cytotoxic T lymphocytes (CTL). And low expression of miR-210 could also enhance the CTL-mediated lysis of tumor cells in lung cancer and melanoma. Thus, miR-210 could be a promising prognostic marker and therapeutic target. Moreover, a study [133] suggested that miR-33b directly binds to HMGA2 3′ untranslated region to suppress its expression and suppresses EMT and migratory potential of melanoma cells. Forloni et al. [128] found that overexpression

of miR-146a enhances the proliferation property of human melanoma cells in culture and the formation of tumor in mice by targeting Notch signaling and BRAF-MEK-ERK signaling, while knockout of miR-146a showed opposite results. Oncogenic DNp73 is a dominant-negative variant of tumor suppressor gene p73, which confers to tumor cells the characteristic of increased stemlike properties by attenuating expression of miR-885-5p [134]. It was reported that augmentation of miR-9 markedly inhibited the cell proliferation and migratory capacity of melanoma cells. In contrast, knockout of miR-9 or anti-miR-9 miRNA inhibitor could enhance not only the expression of Snail1 but also the cell proliferation and migration capacity of melanoma. Mechanistically, miR-9 binds to the $3'$ noncoding region of NF-κB and attenuates its expression, thus preferentially inhibiting Snail1 and finally inhibiting the proliferation and metastasis of melanoma cells [135]. Taken together, these findings suggested that miRNAs may regulate the proliferation and metastasis of melanoma cells by modulating CSC properties and may be a promising prognostic marker and therapeutic target.

18.6.4 Targeting the Microenvironment of CSCs

Tumor progression also depends on their microenvironment. The tumor microenvironment like fibrous cells, immune cells, inflammatory cells, and blood vasculature (angiogenesis of tumor) is necessary for CSC survival since it could not only produce and maintain CSCs but also protect them from attacks by the immune system and lead to enhanced migration and recolonization as secondary tumors [136, 137].

TAMs play multifaceted roles in the growth of tumor, especially related to the aggression and angiogenesis of tumor. CSCs may regulate the surrounding niche by regulating the expression of OPN in TAM. Kale et al. [138] have shown that macrophage related to melanoma modulated tumor microenvironment by secreting OPN, triggering the angiogenesis and development of melanoma. Therefore, inhibition of OPN and its regulated signaling network may be a potential approach to eliminate melanoma through the manipulation of TAMs.

Besides, hypoxic microenvironment is linked to the worse clinical outcome of tumor and controls the number of CSCs by stabilizing HIF [139]. It has been reported that HIF1α and HIF2α drove the aggression of melanoma and related to the metastases of melanoma patients [140]. MFG-E8, a powerful angiogenic factor, increased tumor angiogenesis and the development of melanoma under hypoxic conditions via enhancing the expression of VEGF and ET-1 in MSC and M2 polarization of macrophages [141]. However, this finding which is based on a tumor model of bone marrow chimeric mice does not represent that MFG-E8 has a similar effect in vivo, and further researches are necessary to verify findings above. Another study [142] showed that hypoxia can downregulate the expression of miR-340-5p, while the low expression of miR-340-5p is negatively related to the expression of ATP-binding cassette subfamily B member 5 (ABCB5, a marker of

melanoma stem cells, is a key transmembrane transporter closely related to tumor chemoresistance).

18.6.5   Other Strategies Targeting CSCs

In addition to the above targeted CSC therapy, there are also some other methods targeting CSCs to treat melanoma. Melanoma stem cells can also display drug resistance via enhancing drug efflux, which is mediated through ATP-binding cassette subfamily B (ABCB). El-Khattouti et al. [143] revealed that caffeic acid phenethyl ester (CAPE), a bioactive molecule, could enhance the expression of E2F1 and apoptosis in CD133(−) melanoma, but not in CD133(+). Interestingly, the knockdown of ABCB5 was shown to enhance the sensibility of CD133(+) cells to CAPE. Therefore, combination of ABCB5 inhibitor and CAPE can eliminate chemoresistance in melanoma-specific CSCs. A study [144] showed that the novel sirtuin 1 and 2 (SIRT1/2) inhibitor tenovin-6 could effectively eliminate CSCs and induce apoptosis in uveal melanoma. The approach of targeting tumor stem cells can also be used in tumor immunological therapy. Lu et al. [145] developed a vaccine by using CSC lysate-pulsed dendritic cells (DCs), which could target the CSC populations of numerous cancers including melanoma.

As a CSC marker, the high expression of CD44 is significantly related to the development and metastasis of various human cancers like melanoma. CD44 can bind specially to hyaluronic acid (HA), a pericellular matrix component. Shen et al. [146] reported that solid lipid nanoparticles coated with hyaluronic (HA-SLNS) possess the ability to target paclitaxel (PTX) transport to B16F10 melanoma cells with high expression of CD44 and significantly improve their intracellular transferring efficiency. Furthermore, it will cause numerous CD44(+) cells to induce apoptosis in vitro, and the growth and lung metastasis of melanoma were also significantly inhibited. In addition, this strategy could be markedly beneficial to the survival of patients with no adverse events. Moreover, there was a significant positive correlation between the expression of differentiation inhibitor/DNA binding (Id) proteins 1 and 3 (both depend on BMP4/7) and the development and poor survival of patients with malignant melanoma. Moreover, the interaction between HA-CD44 and BMPR contributes to the expression of Id1/3 protein relying an BMP4/7 and poor survival in patients with melanoma [147]. These results suggest that targeting CD44 is a potential and effective approach for melanoma therapy by elimination of CSCs.

18.7   Application of Nanomedicine in Targeting CSCs

Although targeting CSCs is one of the most promising therapeutic approaches, the traditional methods of targeting CSCs have several flaws including poor water solubility, poor pharmacokinetics, and poor stability of CSC-specific agents [148–150]. At present, for the existing technology, it is a challenging task to target drugs

into a small amount of CSCs in tumor tissue. In recent years, nano-transport technology offers a novel approach to effectively exert the efficacy of drugs via controlling the release of drugs, prolonging the effective time of drugs, and improving the biological distribution of drugs. And this technique can effectively solve the obstacle of eradicating CSCs and improve the therapeutic effect of targeted CSC drugs. It has been proved that CSC-targeted inhibitors alone are not particularly effective in inhibiting tumor growth. The double-targeted nano-drugs containing CSC-targeted inhibitors and conventional antineoplastic drugs can not only effectively eliminate CSCs and tumor cells but also possess lower toxicity than that of free drugs [151–153]. For example, through nanotechnology, Sun et al. [154] wrapped all-trans retinoic acid (ATRA, a powerful differentiation agent of CSCs) and chemotherapy agent doxorubicin together to make into a nano-drug, which can significantly enhance the concentration of drugs in tumor tissues and CSCs, greatly enhance the inhibitory effect on tumor growth, and cooperatively decrease the population of CSC. As a result, ATRA combined with doxorubicin can be used as a promising therapeutic approach to inhibit the development and relapse of cancer via targeting CSCs and non-CSCs.

18.8 Limitation, Barriers, and Controversy in the CSCs

There are some limitations in the treatment of targeted CSCs.

Firstly, it is crucial to select the appropriate CSC markers for these researches about CSCs, as misrecognition of CSC subpopulations will lead to the wrong conclusion. Nonetheless, how to identify and isolate CSCs has always been an obstacle in this field. Some CSC populations do not express the cellular markers found so far, while some non-CSC tumor cells also express these markers. At present, the isolation and identification of CSCs is mainly based on cell surface markers like CD133. Nevertheless, the credibility of this method remains to be answered. [155, 156] Novel techniques like live-cell RNA detection and single-cell DNA and RNA sequencing methods may help to identify CSCs, but these methods are still unlikely to identify a unique CSC marker [157].

Secondly, despite CSCs having been found in numerous dysfunctional signaling pathways, these pathways also express normal stem cells that are of great significance to normal physiological activity. Therefore, drugs targeting these signaling pathways can affect not only CSCs but also normal stem cells, leading to serious side effects. Considering this limitation, we must improve the specificity of targeting CSC drugs, through continuous optimization or in combination with other technologies (such as nanotechnology) in order to gain effective treatment and avoid serious side effects [158]. In addition, the cross talk between different pathways should also be considered. Therefore, the strategy of targeted CSC-related signaling pathway in the treatment of tumor still needs to be further explored before it is used clinically.

18.9 Conclusion and Future Perspectives

CSCs have been shown to be able to evade current cancer treatments including novel immunotherapies, thereby leading to tumor recurrence, metastasis, and resistance to radiotherapy and chemotherapy. Therefore, targeted CSC treatment for more thorough treatment of tumors to achieve a better prognosis is necessary. In summary, the application of CSCs is promising, opening a new era for cancer treatment and evaluation of prognosis. However, there remain several unresolved problems in CSCs. Further research is needed to find reliable and accurate markers to distinguish CSCs from normal stem cells for developing the treatment approaches with higher specificity and fewer side effects. In addition, before the clinical application of targeted CSCs in tumor therapy, it is necessary to clarify its effective dose and side effects and other important factors. After all, the current findings from in vitro or animal experiments may not achieve satisfactory results or even cause major accidents when applied to humans. Signaling pathway and tumor microenvironment as an approach of targeting CSCs have shown encouraging results. Nevertheless, it is worth noting that the signal pathway regulating CSCs does not operate separately and the combination of drugs may be a more effective targeting strategy in the future. Although nano-carriers were able to enhance the delivery and drug activity of CSC inhibitors, only a small number of nano-drugs could be approved for clinical treatment at present. With the further research of CSCs and nano-drugs, nano-drugs loaded with different therapeutic drugs may become the most effective drugs for tumors.

In the future, the combination of traditional antineoplastic drugs and targeted CSC drugs combined with nanotechnology may be expected to be an effective approach for the therapy of cancer.

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