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Emerging agents that target signaling pathways in cancer stem cells

Yue Yang[1†], Xiaoman Li[2†], Ting Wang[1], Qianqian Guo[3], Tao Xi[1*] and Lufeng Zheng[1*]

Abstract

Cancer stem cells (CSCs) contribute to the initiation, recurrence, and metastasis of cancer; however, there are still no drugs targeting CSCs in clinical application. There are several signaling pathways playing critical roles in CSC progression, such as the Wnt, Hedgehog, Notch, Hippo, and autophagy signaling pathways. Additionally, targeting the ferroptosis signaling pathway was recently shown to specifically kill CSCs. Therefore, targeting these pathways may suppress CSC progression. The structure of small-molecule drugs shows a good spatial dispersion, and its chemical properties determine its good druggability and pharmacokinetic properties. These characteristics make small-molecule drugs show a great advantage in drug development, which is increasingly popular in the market. Thus, in this review, we will summarize the current researches on the small-molecule compounds suppressing CSC progression, including inhibitors of Wnt, Notch, Hedgehog, and autophagy pathways, and activators of Hippo and ferroptosis pathways. These small-molecule compounds emphasize CSC importance in tumor progression and propose a new strategy to treat cancer in clinic via targeting CSCs.

Keywords: Cancer stem cells, Small-molecule compounds, Wnt, Hedgehog, Notch, Hippo, Autophagy, Ferroptosis

Background

Although traditional therapeutic methods can reduce tumor volume extensively, cancer recurrence and metastasis always occur [1]. CSCs constitute a small portion of cancer cells, but they show resistance to chemotherapy and radiotherapy. Most conventional treatment methods kill cells with a proliferative potential to shrink the tumor but have no effect on stationary CSCs. This action explains why the tumor volume is reduced but the patient survival rate is not improved [2]. Due to their self-renewal ability and therapeutic resistance, CSCs are considered to be the root of tumor growth, recurrence, metastasis, and drug resistance [3]. Notably, because of their plasticity, stationary CSCs may produce cycling CSCs, which contributes to the relapse of cancer [4].

Therefore, more specific therapies targeting CSCs may lead to better results, and combining them with the traditional therapeutic methods may even achieve healing [5]. Cancer is a disease caused by disordered cell growth due to genetic mutations. CSCs have the same genetic driver mutations as most cancer cells, but CSCs have developmental characteristics that differ from those of non-stem cells, including differences in epigenetic modifications and gene expression profiles [6]. Many changes in the signaling pathways in CSCs provide a preliminary basis for developing compounds targeting CSCs. Signaling pathways that regulate the self-renewal and differentiation of CSCs include Wnt, Hedgehog (Hh), Notch, and Hippo, and these signaling pathways have been extensively studied [7, 8]. In addition, the signaling pathways inhibiting the proliferation of CSCs, in newly discovered mechanisms, have also gradually gained attention, and many promising results have been obtained (Fig. 1) [9]. Targeting CSCs with traditional therapy (chemotherapy and radiotherapy) may yield better
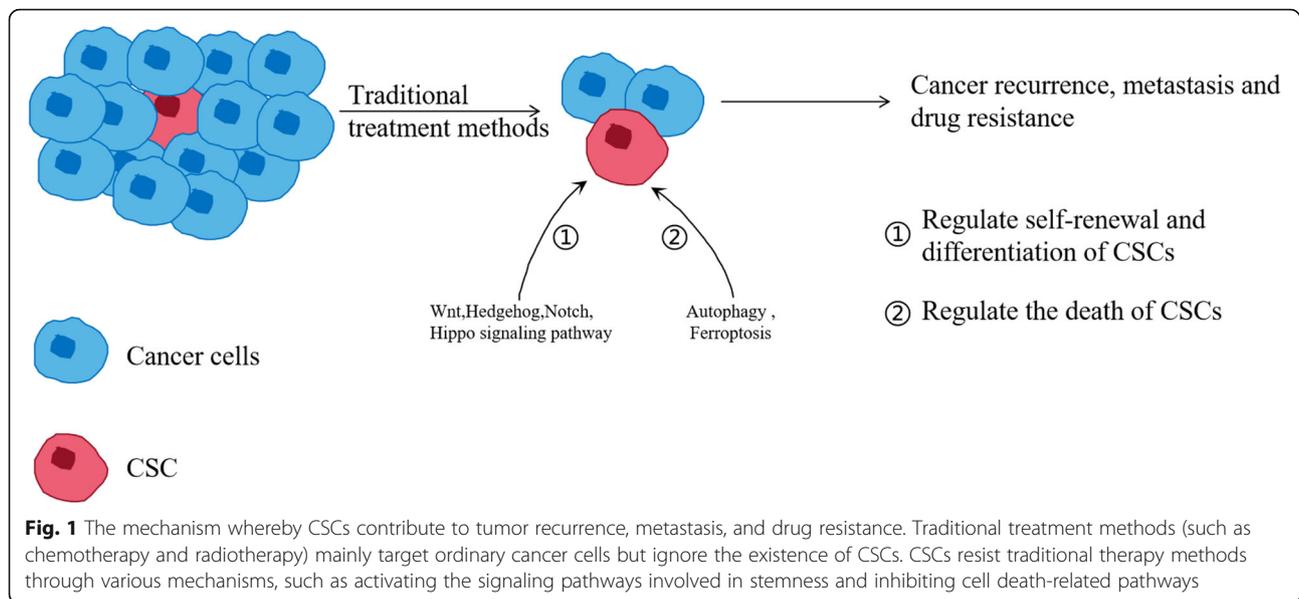
* Correspondence: xitao18@hotmail.com; zhlf@cpu.edu.cn
†Yue Yang and Xiaoman Li contributed equally to this work.
1School of Life Science and Technology, Jiangsu Key Laboratory of Carcinogenesis and Intervention, China Pharmaceutical University, 639 Longmian Road, Nanjing 211198, People's Republic of China
Full list of author information is available at the end of the article

Fig. 1 The mechanism whereby CSCs contribute to tumor recurrence, metastasis, and drug resistance. Traditional treatment methods (such as chemotherapy and radiotherapy) mainly target ordinary cancer cells but ignore the existence of CSCs. CSCs resist traditional therapy methods through various mechanisms, such as activating the signaling pathways involved in stemness and inhibiting cell death-related pathways

results in controlling tumor growth, preventing cancer recurrence and metastasis, and decreasing drug resistance. Small-molecule compounds targeting CSCs play important role in this attenuation process. This review summarizes the research status of small-molecule compounds that suppress CSC progression, including inhibitors of the Wnt, Notch, Hh, and autophagy signaling pathways and activators of Hippo and ferroptosis signaling pathways, which may provide a basis for a current treatment for cancer.

Signaling pathway regulators

CSCs display many characteristics of embryonic or tissue stem cells and often show continuous activation of one or more highly conserved signaling pathways related to development and tissue homeostasis. In these signaling pathways, the Wnt, Hh, Notch, and Hippo signaling pathways are associated with CSC self-renewal [10] and have been used to explore new drugs targeting CSCs.

Wnt signaling pathway inhibitors

The Wnt signaling pathway is highly conserved among species and is divided into the β-catenin-dependent pathway and noncanonical Wnt pathway. When Wnt ligands bind to the Frizzled protein and low-density lipoprotein receptor-related protein (LRP) coreceptors, they initiate the transduction of Wnt/β-catenin signaling, eventually leading to β-catenin stabilization, nuclear translocation, and activation of target genes [31]. Since the β-catenin-dependent pathway has been extensively characterized in mammals, regulates the pluripotency of stem cells, and plays a critical role in self-renewal and differentiation ability, it is thought that abnormal activation of the Wnt pathway promotes CSC progression and

thus leads to the deterioration and metastasis of cancer. Thus, the inhibition of CSCs can be mediated through this pathway [32, 33] (Table 1).

In clinical trials, several small-molecule compounds have been used to target CSCs through the Wnt/β-catenin signaling pathway. For example, LGK-974 (Wnt974) can target porcupine to inhibit the post-translational acetylation of Wnt, thereby inhibiting Wnt secretion, and it has been reported that Wnt974 can inhibit the proliferation of breast CSCs (BCSCs) [34, 35]. Notably, in a phase I study, Wnt974 was found to be safe and effective in treating triple negative breast cancer (TNBC) (NCT01351103). Niclosamide has been approved by the FDA as an antihelminthic, and as a Wnt/β-catenin pathway inhibitor, it has anticancer ability that has been established by various studies. Niclosamide has been shown to selectively target ovarian CSCs [36]. In addition, niclosamide can decrease the population of ALDH$^+$ cells by reducing the expression of LRP6 (a Wnt ligand receptor that plays a key role in the Wnt/β-catenin pathway) and β-catenin in basal-like breast cancer [37]. Notably, in a phase II trail, niclosamide was proved to safely and effectively treat colorectal cancer (CRC) [12]. Mechanistically, it can reduce the expression of many signaling components in the Wnt/β-catenin signaling pathway, the CSC population, and the self-renewal ability of CRC cells [38]. Additionally, ONC201, which is in a phase I/II study for patients with advanced cancer (NCT02038699), can inhibit CSC self-renewal and the expression of CSC-related genes in prostate and glioblastoma tumors through suppressing the Wnt signaling pathway [39].

Furthermore, there are many potential small-molecule compounds targeting CSCs through Wnt/β-catenin

Table 1 Small-molecule compounds inhibiting CSC progression through suppressing Wnt signaling pathway

Name	Target	Mechanism	Type of cancer	Phase	NCT number (starting time)/ publication date	Assessment
Wnt974	Wnt	Inhibits the proliferation of breast CSCs	Breast cancer	Phase I	NCT01351103(May 10, 2011)	Dysgeusia [11]
Niclosamide	Wnt/β-catenin	Selectively targets ovarian CSCs	Ovarian cancer	Preclinical	July, 2014	Without significant toxicity [12–14]
	LRP6, β-catenin	Decreases ALDH+ population cells	Basal-like breast cancer	Preclinical	April, 2014	
	Wnt/β-catenin	Suppresses CSC populations and self-renewal ability	Colorectal cancer	Phase II	NCT02519582 (August 11, 2015)	
ONC201	Wnt/β-catenin	Inhibits CSC self-renewal and deregulates CSC markers and CSC-related gene expression	Glioblastoma cancer	Phase I/II	NCT02038699 (January 16, 2014)	Well tolerated, Grade III neutropenia, Grade II allergic [15, 16]
			Prostate cancer	Preclinical	August 2, 2017	
XAV939	β-catenin	Attenuates CSC-mediated chemoresistance	Colon cancer	Preclinical	April, 2016	Induces cardiotoxicity and limited therapeutic window [17]
			HNSCC	Preclinical	October, 2019	
TFP	Wnt/β-catenin	Inhibits lung CSC spheroid formation and suppresses lung CSC marker expression (such as CD44/CD133)	Lung cancer	Preclinical	December 1, 2012	Induces little systemic toxicity, but grade 0–2 neurologic toxicity [18, 19]
Chelerythrine	β-catenin	Inhibits CSCs invasion, spheroid-forming ability, and the stem marker such as SOX2	NSCLC	Preclinical	January 6, 2020	Without systemic toxicity [20, 21]
FH535	Wnt/β-catenin	Deregulates pancreatic CSC marker CD24 and CD44 expression	Pancreatic cancer	Preclinical	July 26, 2016	Without side effects according to the current studies, still need experiments to prove [22]
Wnt-C59	Wnt	Decreases sphere formation of CSCs	NPC	Preclinical	June 10, 2015	Exhibits no apparent toxicity in mice; needs experiments to prove [23]
IWR-1	β-catenin	Impairs CSCs self-renewal and hampers the expression of key stem markers, and increases doxorubicin sensitivity	Osteosarcoma	Preclinical	February 1, 2018	Well tolerated in mice, but still needs to be thoroughly studied [24]
IC-2	Wnt	Reduces the population of CD44$^+$ (liver CSCs) and the ability of sphere-forming ability	HCC	Preclinical	July, 2017	Not reported, still needs experiments to prove
	Wnt	Reduces the expression of CSC marker and sphere formation ability	CRC	Preclinical	August, 2017	
JIB-04	β-catenin	Inhibits the metastasis of colorectal CSCs	Colorectal cancer	Preclinical	April 26, 2018	Without general toxicity in JIB-04-treated mice, but still needs experiments to prove [25]
DTX and SFN	β-catenin	Inhibits the self-renewal ability of breast CSCs	Breast cancer	Preclinical	July, 2016	DTX has the side effect of neurological toxicity, nausea, diarrhea, and alopecia, but SFN is without significant toxicity [26, 27]
PP	β-catenin	Inhibits the self-renewal ability of breast CSCs	Breast cancer	Preclinical	March, 2016	PP shows no obvious toxicity in mice. But the poor targeting of it made the dosage large. It would be better to improve dosage form and develop new derivatives [28]
OXT-328					October, 2012	Safety according to the recent researches [28]
AD and Ts	Wnt/β-catenin	Decreases CSC number and activity, and reduces CSC marker expression (such as SOX2, ALDH1, and NOS2)	Lung cancer	Preclinical	December 3, 2019	AD has hepatoxicity, and TS is without toxic side effects in nude mice [29, 30]

signaling pathway in preclinical experiments. For example, XAV939 can inhibit β-catenin signaling and thus attenuate CSC progression by interacting with the terminal anchor polymerase-binding domain (TBD) in Axin [40], which can abrogate CSC-mediated chemoresistance in head and neck squamous cell carcinoma (HNSCC) and colon cancer cells [41, 42]. Trifluoperazine (TFP) is used as an antipsychotic and an antiemetic, and it has been found to inhibit lung CSC spheroid formation ability and suppress lung CSC marker expression (such as CD44/CD133) by inhibiting Wnt/β-catenin signaling [43]. Chelerythrine chloride (Chelerythrine) can downregulate β-catenin and inhibit CSC invasion, spheroid-forming ability, and the expression of the stem marker SOX2 in non-small cell lung carcinoma (NSCLC) [44]. FH535 can suppress the expression of the pancreatic CSC marker CD24 and CD44 by inhibiting the Wnt/β-catenin signaling pathway [45]. Wnt-C59 (C59), an inhibitor of Wnt, can decrease the sphere formation ability of CSCs in a dose-dependent manner in nasopharyngeal carcinoma (NPC) [46]. IWR-1, a tankyrase inhibitor, can impair osteosarcoma CSC self-renewal, hamper the expression of key stem markers in osteosarcoma, and increase doxorubicin sensitivity in vivo by inhibiting β-catenin translocation [24]. IC-2, a novel small-molecule Wnt inhibitor, can reduce the population of CD44+ (liver CSCs) and the sphere-forming ability of hepatocellular carcinoma (HCC) cells [47]. It can also reduce the expression of CSC markers and the sphere formation ability in CRC. In addition, it can increase the sensitivity of 5-FU in the DLD-1 CRC cell line [48]. JIB-04, a selective inhibitor of histone demethylase, can inhibit the metastasis of colorectal CSCs by regulating the recruitment of β-catenin [49]. The combination of docetaxel (DTX) and sulforaphane (SFN), pyrvinium pamoate (PP), and phosphor-sulindac (OXT-328) can inhibit CSC self-renewal ability, the EMT (epithelial-mesenchymal transition), and drug resistance by decreasing β-catenin expression in BCSCs [50–52]. Additionally, actinomycin D (AD) and telmisartan (TS) can also decrease the CSC number and activity and reduce CSC marker expression (such as SOX2, ALDH1, and NOS2) in lung cancer by inhibiting the Wnt-β/catenin signaling pathway [53].

Notch signaling pathway inhibitors

The Notch signaling pathway is an evolutionarily conserved pathway that is closely related to all aspects of cancer biology including CSC progression, angiogenesis, and tumor immunity [54]. The Notch pathway mainly consists of Notch receptors (Notch 1–4) and Notch ligands (Jagged 1, Jagged 2, delta-like ligand (DLL) -1, DLL-3, and DLL-4). When the receptors bind to the ligands, the Notch intracellular domain (NICD) is released into the nucleus through three cleavage processes

mediated by γ-secretase, thereby activating the transcription of Notch target genes (Hes-1 and Hey-1) [55]. The activation of the Notch signaling pathway promotes tumor proliferation and metastasis; in contrast, inhibition of this pathway can eliminate CSCs and increase drug sensitivity. Therefore, genes in the Notch signaling pathway may represent potential cancer therapeutic targets [56]. Notch inhibitors can be used alone or in combination with chemotherapy agents to treat cancer and prevent recurrence [57].

Currently, inhibitors targeting the Notch signaling pathway mostly target γ-secretase or Notch ligands (Table 2). For example, MK-0752, a γ-secretase inhibitor, can decrease the population of CD44+/CD24− and ALDH+, reduce mammosphere-forming efficiency, and inhibit tumor regeneration in BCSCs [63]. In a phase I study, the combined use of MK-0752 with docetaxel increased MK-0752-induced anti-BCSC ability and improved the efficiency of docetaxel in treating breast cancer (NCT00645333). PF-03084014, another γ-secretase inhibitor, can inhibit CSC self-renewal and proliferation and induce CSC differentiation by targeting Notch signaling pathways in HCC [64]. PF-03084014 can also decrease the CD44+/CD24− and ALDH+ populations by suppressing N1ICD cleavage and the expression of Hes-1 and Hey-1 in pancreatic cancer [65]. In a phase II trail, PF-03084014 combined with gemcitabine and nab-paclitaxel increased the overall survival of patients with metastatic pancreatic adenocarcinoma (NCT02109445) compared to that of patients treated with PF-03084014. When combined with docetaxel, PF-03084014 can increase docetaxel efficiency against breast cancer; this combination is in a phase I study (NCT01876251). Mechanistically, PF-03084014 can diminish CD133+/CD44+ and ALDH+ subpopulations and eliminate BCSCs by targeting the Notch signaling pathway, thereby decreasing drug resistance [66].

Additionally, the γ-secretase inhibitor RO4929097 can significantly inhibit Notch target genes Hes1 and Hey1, and it is in a phase II clinical setting to treat breast cancer, ovarian cancer, and renal cell carcinoma [67]. The combination of RO4929097 and 5-FU can decrease the proportion of the CSC subgroup with insulinomas (INS) [68]. DAPT (GSI-IX) is a new type of Notch1 inhibitor that was initially used to treat Alzheimer's disease, and increasing number of studies have shown that it can inhibit CSCs [69]. Studies have demonstrated that DAPT can inhibit the proliferation and self-renewal ability of leukemia stem cells (LSCs) and ovarian CSCs [70, 71]. Additionally, quinomycin A can inhibit pancreatic cancer microsphere formation, stem marker expression, and CSC number by decreasing the expression of Notch ligands [72].

Table 2 Small-molecule compounds inhibiting CSC progression through suppressing Notch signaling pathway

Name	Target	Mechanism	Type of cancer	Phase	NCT number (starting time)/ publication date	Assessment
MK-0752	γ-secretase	Decreases the population of CD44$^+$/CD24$^-$ and ALDH$^+$, reduces mammosphere-forming efficiency, and inhibits tumor regeneration in BCSCs	Breast cancer	Phase I	NCT00645333 (March 27, 2008)	Well tolerated, but exists dose-limiting toxicity (DLT) [58]
PF-03084014	γ-secretase	Inhibits CSC self-renewal and proliferation, and induces CSCs differentiation	HCC	Preclinical	August, 2017	Induces gastrointestinal toxicity and exists DLT [59]
	N1ICD, Hes-1, and Hey-1	Decreases CD44+/CD24− and ALDH+ population	Pancreatic cancer	Phase II	NCT02109445(April 9, 2014)	
	Notch	Diminishes CD133$^+$/CD44$^+$ and ALDH$^+$ subpopulations and eliminates CSCs	Breast cancer	Phase I	NCT01876251(June 12, 2013)	
RO4929097	γ-secretase	Combined with 5-FU can decrease the proportion of CSC subgroup	INS	Preclinical	February, 2018	Fatigue is the most common toxicities, but it has DLT [60]
DAPT	Notch1	Inhibits the proliferation of LSCs and regulates LSC self-renewal	Leukemia	Preclinical	December, 2006	Induces low toxicity in cell and mice [61]
		Inhibits the self-renewal ability of ovarian CSCs and the expression of stem markers	Ovarian cancer	Preclinical	June, 2011	
Quinomycin A	Notch ligands	Inhibits pancreatic cancer microsphere formation, the stem marker and the number of CSCs	Pancreatic cancer	Preclinical	January 19, 2016	Induces gastrointestinal toxicity [62]

Hh signaling pathway inhibitors

The Classical Hh signaling pathway is critical for embryonic development. The Hh signaling pathway regulates the self-renewal of CSCs and tissue homeostasis in cancer [80]. When extracellular Hh ligands (SHh, IH, and DHh) bind to PTCH, the inhibition of PTCH on Smoothened (SMO) is decreased, thereby GlI is translocated to the nucleus and induces the transcription of target genes [81]. Abnormal activation of the Hh signaling pathway is a crucial driver of breast cancer, prostate cancer, NSCLC, gastric cancer, and hematopoietic malignancies [82]. Hh signaling pathway inhibitors have been proven to be effective in early clinical trials. In addition, the development of Hh inhibitors has drawn significant interest for anticancer drug development. Recently, it has been reported that inhibition of the Hh signaling pathway can inhibit the self-renewal and drug resistance of pancreatic and breast CSCs [83, 84] (Table 3).

For example, ciclesonide was approved by the FDA to treat asthma, and it was found that ciclesonide can inhibit the growth of lung CSCs through Hh signaling-mediated SOX2 regulation [85]. Sonidegib, an SMO antagonist, was approved by the FDA for the treatment of advanced basal cell carcinoma [86]. Recently, it was shown that sonidegib can downregulate the expression of CSC markers and increase the sensitivity of TNBC to paclitaxel, thus improving patient survival and reducing metastasis [87]. In addition, in a phase I study, sonidegib obtained a better result in advanced TNBC when it was combined with docetaxel (NCT02027376). Vismodegib (GDC-0449), another SMO inhibitor, was approved by the FDA to treat basal cell carcinoma [88]. Recently, many studies have found that vismodegib can inhibit BCSC self-renewal and mammosphere formation [89]. In a phase II trial, vismodegib was added to neoadjuvant chemotherapy for TNBC patients (NCT02694224). It can also suppress pancreatic CSC proliferation and survival by inhibiting Hh signaling pathways [90]. In a phase II trial, vismodegib combined with gemcitabine and nab-paclitaxel was used against untreated metastatic pancreatic cancer [91]. In another phase Ib/II trial, vismodegib plus gemcitabine was used to treat metastatic pancreatic cancer [92]. Additionally, vismodegib decreased the stem markers (such as CD44 and ALDH) of colon CSCs [93], and it was used to treat untreated metastatic CRC in a phase II trial [94]. These results suggest that vismodegib can target CSCs through the Hh signaling pathway. Furthermore, glasdegib (PF-04449913), an Hh signaling pathway inhibitor, was approved by the FDA to treat acute myeloid leukemia [73]. Glasdegib can attenuate the potential of leukemia-initiation and increase the sensitivity of LSCs to chemotherapy by inhibiting the Hh signaling pathway [95]. Cyclopamine is a natural compound that can specifically target SMO and inhibit the Hh signaling pathway [96], and it was reported that cyclopamine can inhibit bladder CSC self-renewal [97]. GANT61, another Hh inhibitor, can decrease the CSC population by downregulating the expression of GLI1 and GLI2 in ER (estrogen receptor)-positive breast cancer [98].

Table 3 Small-molecule compounds inhibiting CSC progression through suppressing Hh signalling pathway

Name	Target	Mechanism	Type of cancer	Phase	NCT number (starting time)/ publication date	Assessment
Glasdegib	Hh	Attenuates the potential of leukemia-initiation and increases the sensitivity of LSCs to chemotherapy	Leukemia	Approved	November 21, 2018	Induces common side effect of chemotherapy drugs such as fatigue, nausea, and febrile neutropenia, but also has embryo-fetal toxicity [73]
Sonidegib	SMO	Downregulates the expression of CSC markers and increases the sensitivity to paclitaxel	Breast cancer	Phase I	NCT02027376 (January 6, 2014)	Induces myalgia, fatigue, and abnormal hepatic function, and gastrointestinal toxicity and alopecia are related to the dose of Sonidegib [74, 75]
Vismodegib	SMO	Inhibits BCSC self-renewal and mammosphere formation	Breast cancer	Phase II	NCT02694224 (February 29, 2016)	DLT, hyperbilirubinemia [76]
		Suppresses pancreatic CSC proliferation and survival	Pancreatic cancer	Phase II	NCT01064622 (February 8, 2010)	
		Decreases the stem markers (such as CD44 and ALDH) of colon CSCs	Colorectal cancer	Phase II	NCT00636610 (March 14, 2008)	
Ciclesonide	Hh	Inhibits the growth of lung CSCs	Lung cancer	Preclinical	February 4, 2020	Well tolerated, but as corticosteroid, it may inhibit bone growth [77]
Cyclopamine	SMO	Inhibits bladder CSC self-renewal	Bladder cancer	Preclinical	March 1, 2016	Induces holoprosencephaly, dystonia, and lethargy in rodents [78]
GANT61	GLI1 and GLI2	Decreases the CSC population	Breast cancer	Preclinical	May, 2017	No side effects in the mice according to the current studies [79]

Hippo pathway activators

The Hippo signaling pathway plays an essential role in CSC self-renewal, the EMT, and drug resistance. Upon activation of the Hippo signaling pathway, MST1/2 phosphorylate and activate LATS1/2. Then, LATS1/2 inactivate YAP/TAZ, which was subsequently translocated into the cytoplasm, and thus inhibits the expression of TEAD (TEA domain family member)-mediated genes, thereby suppressing CSC progression [106]. In contrast, inhibition of the Hippo signaling pathway activates YAP/TAZ, conferring CSC-like characteristics to the cell and leading to tumorigenesis [107]. YAP/TAZ-TEAD acts as a tumor promoter in the Hippo signaling pathway, while the other members of the Hippo signaling pathway are mostly tumor suppressor genes. Thus, targeting YAP/TAZ may serve as a strategy to inhibit CSCs (Table 4).

For instance, verteporfin is a photosensitizer approved by the FDA, and it has garnered increasing interest for its anticancer role in gastric and esophageal cancer. Verteporfin can inhibit the transcriptional activity of YAP/TAZ-TEAD, reduce the expression of CSC markers, and suppress CSC proliferation [108, 109]. Evodiamine (Evo), which is isolated from the Chinese herb *Evodia rutaecarpa Benham*, can activate MST1/2-mediated phosphorylation of LATS1/2, which leads to YAP/TAZ phosphorylation and prevents YAP/TAZ translocation from the cytoplasm into the nucleus, and it has been shown that Evo can inhibit the proliferation of colon CSCs [110, 111]. Additionally, tanshinone IIA and limonin, which are extracted from

Chinese herbs, have been shown to attenuate the stemness of cervical carcinoma stem cells by inhibiting the cytoplasmic-nuclear translocation of YAP [112, 113]. Moreover, statins such as fluvastatin can reduce the expression of CD44 by accelerating YAP phosphorylation, thereby reducing the characteristics of malignant mesothelioma stem cells and drug resistance [114]. Atorvastatin, another stain, can target TAZ in breast cancer, and is in a phase II trial (NCT02416427). Notably, atorvastatin can decrease the stemness of MDA-MB 231 cells, as evident by the decrease in the CD44$^+$/CD24$^-$ subpopulation of cells by inducing LATS1 expression and downregulating the expression of YAP/TAZ [115].

Recently, a new type of YAP inhibitor, CA3, was screened from a chemical library and found to attenuate the transcriptional activity of YAP/TEAD, and CA3 shows an excellent ability to target CSCs and inhibit tumor growth, as evident by its role in suppressing tumor sphere formation and reducing the proportion of ALDH1$^+$ cells [104]. In addition, the antipsychotic drug chlorpromazine (CPZ) can kill breast cancer and BCSCs, which were characterized by inhibited tumor microsphere-formation and stem marker expression through promoting YAP degradation [116].

Selective inducers of signaling pathways that contribute to cell death

According to measurable biochemical characteristics and molecular mechanisms, signaling pathways contributing

Table 4 Small-molecule compounds inhibiting CSC progression through activating Hippo pathway

Name	Target	Mechanism	Type of cancer	Phase	NCT number (starting time)/ publication date	Assessment
Verteporfin	YAP/TAZ	Reduces the expression of CSC markers and suppresses CSC proliferation	Gastric and esophageal cancer	Preclinical	August 1, 2014	Without visible toxicity in the mice [99]
			gastric cancer	Preclinical	Apr 15, 2020	
Evodiamine	LATS1/2	Inhibits the proliferation of colon CSCs	Colon cancer	Preclinical	December 10, 2019	Induces low toxicity and still needs much experiments to prove [100]
Fluvastatin	YAP	Reduces the expression of CD44 and the characteristics of malignant mesothelioma stem cells	Malignant mesothelioma	Preclinical	January 28, 2017	Without any genotoxic, and relatively safe in patients [101, 102]
Atorvastatin	TAZ	Decreases MDA-MB 231 cells stemness-related features (such as the decrease of CD44+/CD24- subpopulation of cells)	Breast cancer	Phase II	NCT02416427(April 15, 2015)	Muscle loss [103]
CA3	YAP/TEAD	Suppresses tumor microsphere, formation and reduces the proportion of ALDH1+ cells	Esophageal adenocarcinoma	Preclinical	February, 2018	Without apparent toxicity in mice according to the current studies [104]
CPZ	YAP	Inhibits tumor microsphere-formation and stem marker expression	Breast cancer	Preclinical	April 1, 2019	Induces fatal hepatic failure [105]

to cell death mainly include apoptosis, autophagy, necroptosis, and ferroptosis. Cancer cells also undergo multiple forms of cell death during tumor development, including apoptosis, autophagy, and necrosis. Recently, ferroptosis has been shown to play a critical role in the development of cancer and may be a beneficial anticancer treatment strategy, which has gradually gained attention. Because classic apoptosis-inducing drugs have a poor effect on CSCs, current research is more intent on inhibiting CSCs by inducing autophagy and ferroptosis, which can effectively inhibit cancer recurrence and metastasis

Autophagy and CSCs

Autophagy is a self-digesting mechanism in which proteins, lipids, and damaged organelles (such as mitochondria) are sequestered into vesicles called autophagosomes for degradation and recycling. Under physiological conditions, autophagy is critical for maintaining cell homeostasis and controlling protein and organelle quality. High levels of autophagy often occur in CSCs. Autophagy can help CSCs maintain their diversity and overcome low nutrients and hypoxia in the tumor microenvironment; therefore, it promotes CSCs to metastasis, drug resistance, and immune surveillance evasion [122]. Recently, autophagy was associated with CSC progression in breast cancer, NSCLC, prostate cancer, leukemia, gastric cancer, and myeloma, and its dysfunction affects the self-renewal ability of CSCs. Therefore, autophagy can be used as a CSC target. Although promotion of autophagy in CSCs is specific, different types, periods, and microenvironments have been shown to inhibit CSC progression through

autophagy. For example, in some acute myeloid leukemias, many autophagy-related genes are mutated or downregulated in patients [123]. Therefore, currently, the compounds targeting autophagy mostly inhibit the autophagy of CSCs to suppress CSC progression (Table 5).

For example, chloroquine (CQ) is widely used in clinical antimalarial drugs and can be used in combination with anticancer drugs to treat cancer. CQ can effectively target CSCs by inhibiting autophagy, which causes the destruction of mitochondrial structures and double-stranded DNA, and the combination of CQ and carboplatin may be a useful adjuvant drug for treating TNBC [124]. CQ can inhibit the expression of stemness markers such as CD133 and decrease the proportions of CSCs, subsequently enhancing the efficacy of cisplatin against NSCLC by inhibiting autophagy [125]. In a phase II study, CQ inhibited breast cancer (NCT02333890). In addition, hydroxychloroquine (HCQ), a derivative of CQ, shows a stronger anticancer ability by targeting autophagy [126]. Additionally, the combination of HCQ and imatinib (IM) can effectively eliminate LSCs in chronic myeloid leukemia, and it is in a phase II trial [127]. A high dose of pantoprazole, a proton pump inhibitor (PPI), can affect docetaxel resistance in metastatic castration-resistant prostate cancer (mCRPC) by inhibiting autophagy, and it is in a phase II trial [128]. Moreover, pantoprazole can inhibit the chemoresistance of gastric CSCs [129]. Therefore, pantoprazole may target CSCs by inhibiting autophagy.

Moreover, concanamycin A is a selective inhibitor of V-ATPase that can inhibit the degradation of

Table 5 Small-molecule compounds targeting autophagy to inhibit CSCs

Name	Target	Mechanism	Type of cancer	Phase	NCT number (starting time)/publication date	Assessment
CQ	Autophagy	Targets CSCs by inhibiting autophagy	Breast cancer	Phase II	NCT02333890 (January 7, 2015)	Induces cardiotoxicity [117]
	Autophagy	Inhibits the stemness marker of CD133+ and decreases the CSC proportions	NSCLC	Preclinical	December, 2019	
HCQ	Autophagy	Eliminates LSCs	Leukemia	Phase II	NCT00771056 (October 10, 2008)	Induces retinal toxicity [118]
Pantoprazole	Autophagy	Inhibits autophagy	Prostate cancer	Phase II	NCT01748500 (December 12, 2012)	DLT, grade 3 to 4 [119]
	EMT/β-catenin	Inhibits the chemoresistance of gastric cancer stem cells	Gastric cancer	Preclinical	December, 2016	
3-MA	Autophagy	Reduces the resistance of mesenchymal stem cells	Myeloma	Preclinical	September, 2017	No significant side effect [120]
Rott	Autophagy	Induces autophagy leading to breast CSC death	Breast cancer	Preclinical	December 23, 2013	Without toxicity in the mice [121]

autolysosomes but has no effect on the formation of lysosomes; therefore, lysosomal degradation inhibitors may not reduce the rate of autophagosome-mediating chelation in destroying mitochondria. In this situation, some mitochondrial-dependent drugs may reduce the efficiency of inhibitors on CSCs; therefore, targeting early autophagy such as with VPS34 or ULK1 inhibitors may lead to better results. In addition, 3-methyladenine (3-MA) can prevent the formation of autolysosomes and reduce the resistance of mesenchymal stem cells; therefore, it can provide new treatment strategies for patients with multiple myeloma resistance [130]. Rottlerin (Rott), an active molecule that is isolated from *Mallotus philippensis*, is used in the treatment of allergies and helminthiasis and can induce autophagy, leading to BCSC death [131]. However, it is noteworthy that, although autophagy-targeting therapy has fewer side effects on normal cells than do conventional therapies, it also has some unknown effects that remain to be explored.

Ferroptosis and CSCs

Ferroptosis is a new type of programmed cell death that is different from apoptosis, necrosis, and autophagy at the morphological and biochemical levels. It is induced by the accumulation of iron and lipid peroxidation caused by reactive oxygen species (ROS). The destruction of iron homeostasis and uncontrolled lipid peroxidation are two key features of ferroptosis [132]. The regulation of intracellular iron homeostasis is mainly regulated by IRP2 (iron regulatory protein 2). IRP2 can bind to iron-responsive elements (IREs) in mRNA 5'- and 3'-untranslated regions (UTRs) to inhibit transcription or stabilize mRNA. In the absence of iron, IRP2 binds to 5'-end IREs in FPN1 and ferritin to decrease their mRNA stability, and it binds to 3'-end IREs in TFR1 and DMT1 to stabilize mRNA. When there is

excess iron in the cells, the synthesis of FPN1 and ferritin mRNA is inhibited and the degradation of TFR1 and DMT1 mRNA is enhanced (Fig. 2) [133].

Additionally, the selenoenzyme glutathione peroxidase 4 (GPX4), which can inhibit the peroxidation of phospholipids (PLs), is considered to be a regulator of ferroptosis, and inactivation of GPX4 will lead to an increase in lipid peroxides. In addition, the accumulation of iron produces lipid peroxides in a nonenzymatic or enzyme-dependent manner [134]. The nonenzymatic reaction of iron to produce lipid peroxides is the Fenton reaction, $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + (OH)^- + OH \cdot$ [135, 136] (Fig. 3). Another pathway for ferroptosis is mediated by the X_c^- system, formed by SLC3A2 and SLC7A11, which internalizes cystine cells and expels glutamic acid, and then cystine is reduced to cysteine. Cysteine participates in the synthesis of GSH. GSH acts as an electron donor to maintain the activity of GPX4, thereby preventing ferroptosis.

Relationship between ferroptosis and CSCs

The changes in iron homeostasis in CSCs are usually manifested by high intracellular iron content. In addition, abnormal iron metabolism is associated with accelerated tumor growth and a poor prognosis for cancer patients. Therefore, because of the dependence of cancer on iron, iron-dependent mechanisms such as ferroptosis can be used as targets for drug development [137]. A higher level of iron in a CSC may affect its redox state, which is manifested by the increase in peroxidation and OH ·[138, 139].

Recently, it was reported that the levels of TfR1 and its ligand transferrin expressed by glioblastoma stem cells are higher than those expressed by non-CSCs, and iron-tracking experiments using these CSCs have shown that the level of iron intake by CSCs is greater than that
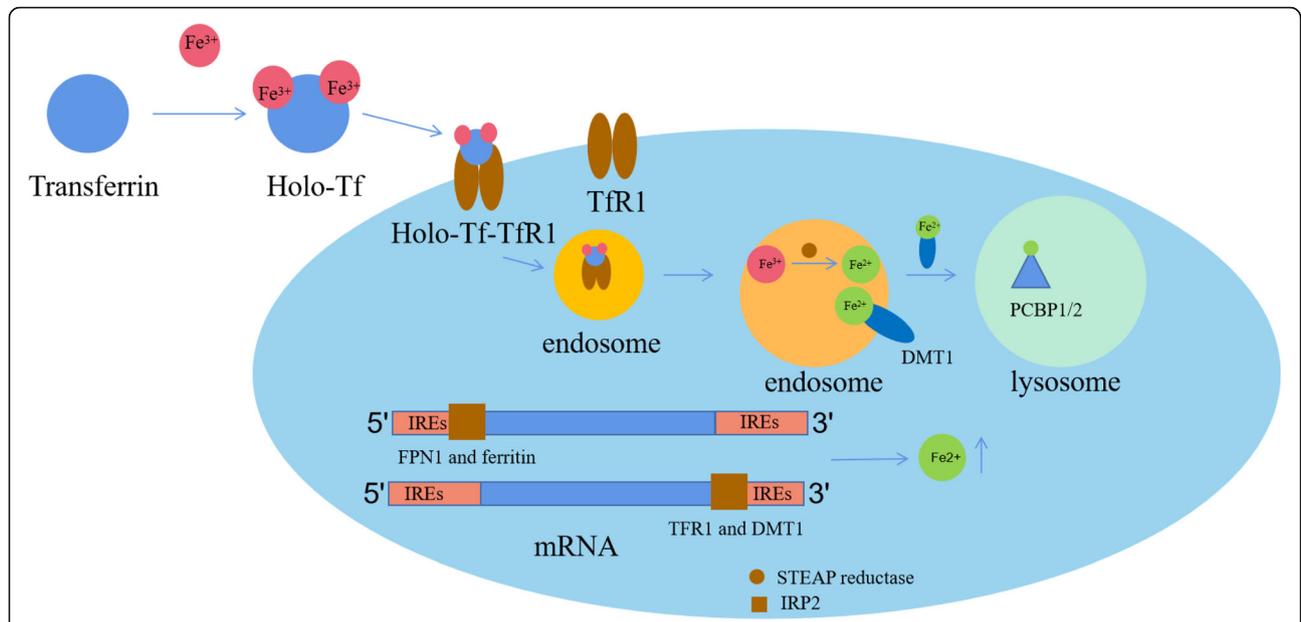
Fig. 2 The regulation of iron. Transferrin can combine with two Fe^{3+} ions and then bind to transferrin receptor 1 (TfR1), forming the holo-Tf-TfR1 complex. This complex is encased in endosomes. In the endosome, Fe^{3+} is converted to Fe^{2+}, which then binds to DMT1 (divalent metal transporter 1), forming the Fe^{2+}-DMT1 complex. This complex is transferred to the labile iron pool (LIP)/lysosome, and Fe^{2+} binds to PCPB1/2 (poly-(rC)-binding protein 1/2). IRP2 binds to the 5'-end IREs in FPN1 and ferritin to inhibit their transcription and 3'-end IREs in TFR1 and DMT1 to prevent their degradation; therefore, the iron level in the cell increases

internalized by non-CSCs [140], indicating that increased iron intake may also be a characteristic of CSCs. Additionally, ferritin is overexpressed in a variety of cancers, including breast cancer, pancreatic cancer, liver cancer, Hodgkin's lymphoma, and glioblastoma. Ferritin may protect CSCs, but the degradation of ferritin produces a source of iron leading to ferroptosis. Targeting the H and L subunits of ferritin with siRNA causes a significant reduction in the growth of CSCs in vivo and in vitro [140].

Ferroptosis and small-molecule compounds

Current studies on the activators of ferroptosis are relatively extensive, but there are few small-molecule compounds that can target CSCs by mediating ferroptosis (Fig. 4). Additionally, the role of GPX4 in ferroptosis is decisive. Some experiments have already confirmed that the dysfunction of GPX4 causes mesenchymal stem cell ferroptosis; therefore, small-molecule compounds targeting GPX4 may induce ferroptosis in CSCs, although experimental proof is currently lacking.
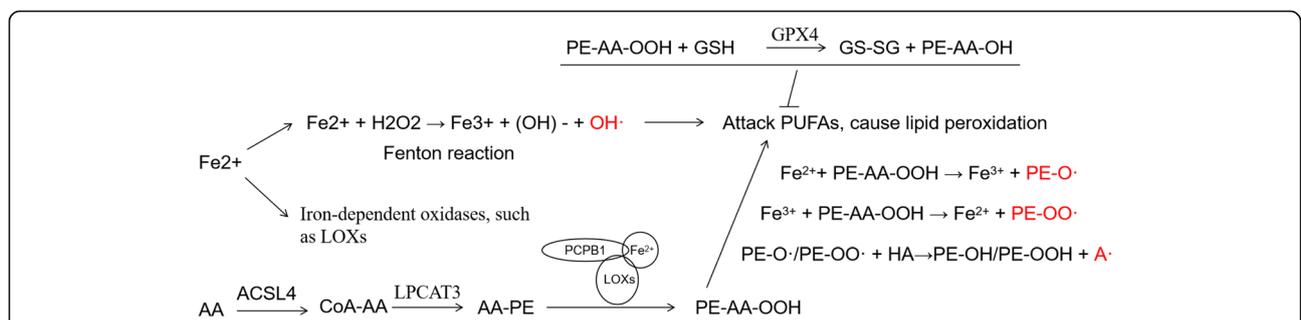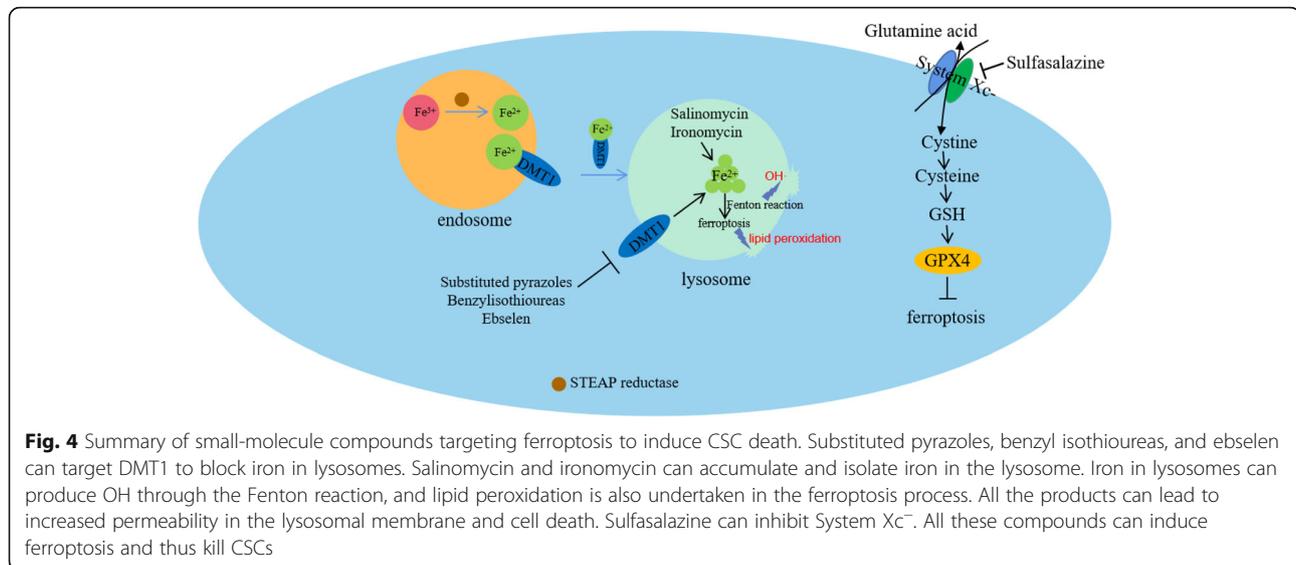

Fig. 3 The role of iron in lipid peroxidation. Through the Fenton reaction, Fe^{2+} and hydrogen peroxide produces OH, and AA (arachidonic acid) can produce PE-AA-OOH through ACSL4 (acyl-CoA synthetase long-chain family member 4), LPCAT3 (lysophosphatidylcholine acyltransferase 3), and the complex of PCPB1, Fe^{2+}, and LOXs (lipoxygenases). PE-AA-OOH, through Fe^{2+} + PE-AA-OOH → Fe^{3+} + PE-O, Fe^{3+} + PE-AA-OOH → Fe^{2+} + PE-OO, and PE-O/PE-OO + HA → PE-OH/PE-OOH + A produces PE-O, PE-OO, and A. OH, PE-O, PE-OO, and A can attack PUFAs (polyunsaturated fatty acids) to cause lipid peroxidation. In addition, GPX4 can inhibit the production of lipid peroxidation through PE-AA-OOH + GSH → GS-SG + PE-AA-OH

Fig. 4 Summary of small-molecule compounds targeting ferroptosis to induce CSC death. Substituted pyrazoles, benzyl isothioureas, and ebselen can target DMT1 to block iron in lysosomes. Salinomycin and ironomycin can accumulate and isolate iron in the lysosome. Iron in lysosomes can produce OH through the Fenton reaction, and lipid peroxidation is also undertaken in the ferroptosis process. All the products can lead to increased permeability in the lysosomal membrane and cell death. Sulfasalazine can inhibit System Xc^-. All these compounds can induce ferroptosis and thus kill CSCs

The following is a summary of some ferroptosis inducers. Erastin, sulfasalazine, and sorafenib can reduce the level of cystine by inhibiting the X_c^- system. Sulfasalazine can also inhibit the X_c^- system, thereby inhibiting the progression of CSCs overexpression of CD44 in gastrointestinal cancer [141]. (1S, 3R)-RSL3, altretamine and withaferin A can increase lipid peroxidation by inhibiting or silencing GPX4 function [142]. Notably, salinomycin can increase the production of lipid peroxidation by blocking iron transport and depleting ferritin, by which it can specifically kill CSCs [143]. Other studies indicate that a combination of salinomycin and docetaxel can effectively kill gastric CSCs [144]. Salinomycin can effectively destroy non-CSCs and CSCs, as well as cancer cells with a multi-drug resistance (MDR) phenotype; therefore, it shows strong antitumor activity against a variety of cancers [145]. Additionally, salinomycin-loaded gold nanoparticles show enhanced ability to target BCSCs [146]. Considering the effect of salinomycin in killing CSCs, many salinomycin derivatives have also been developed and exhibit higher activity and selectivity, such as ironomycin and the products of C20-amination, C1-esterification, C9-oxidation, and C28-dehydration. These derivatives of salinomycin can accumulate and isolate iron in the lysosome, and the accumulation of iron in the lysosome initiates the Fenton reaction, leading to increased permeability in the lysosomal membrane and cell death. They have been shown to be at least ten-fold more potent than salinomycin in vivo and in vitro. In addition, ironomycin can effectively reduce the number of CSCs in docetaxel-resistant xenograft models [143, 147]. Furthermore, ebselen, substituting pyrazole, and benzyl isothiourea, which are the inhibitors of DMT1, can selectively target BCSCs by blocking iron in lysosomes [148]. These results indicate

that increasing iron levels in lysosomes can initiate cell death in a manner similar to ferroptosis, and thus specifically and effectively kill CSCs. However, the concrete mechanisms by which the drugs target DMT1 are still unclear.

Notably, recent studies have shown that ovarian CSCs rely on iron for self-renewal and metastasis [149]. This finding reveals an opportunity to treat cancer by regulating iron balance. For example, after treatment with erastin, CSCs are more likely to induce ferroptosis than are non-CSCs [149]. The combination of temozolomide (TMZ) and CQ can cause glioblastoma stem cells (GSCs) to die in the form of ferroptosis, and specifically, this combination can reduce the self-renewal of GSCs, weaken the invasion of glioblastoma, and improve the therapeutic efficiency of chemotherapy and radiotherapy, but the specific target has not been identified [150].

Recently, an increasing number of studies have shown that artemisinin derivatives have anticancer ability. For example, dihydroartemisinin (DHA) can increase iron levels in the cell and inhibit the synthesis of ferritin through the IRP-IRE axis, which increases the concentration of intracellular iron, making cancer cells (such as lung, colorectal, and breast cancer cells) more sensitive to ferroptosis [151]. In addition, it was found that DHA can inhibit sphere formation and stem marker (CD133, SOX2, and nestin) expression in glioma CSCs [152]. Therefore, DHA may inhibit CSCs through ferroptosis. Through a platform of induced cancer stem-like cells (iCSCLs) used for high-throughput screening, artesunate can induce mitochondrial dysfunction in CSCs; therefore, it can inhibit the stemness of CSCs [153]. Artesunate can induce ferroptosis in pancreatic cancer [154]. All these findings indicate that artemisinin derivatives may target CSCs through ferroptosis. Ferumoxytol is a

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superparamagnetic iron oxide nanoparticle approved by the FDA, and its anticancer ability is associated with ferroptosis [155]. Magnetic hyperthermia is a new method by which to selectively kill CSCs (A549 and MDA-MB-231), and the key aspect of this technology is superparamagnetic iron oxide nanoparticles [156]. The report indicated that ferumoxytol was a new material for use in magnetic hyperthermia [157]. In addition, DOX@FMT-MC, a novel magnetic hydrogel complex consisting of ferumoxytol, doxorubicin, and chitosan, was applied in the clinic to treat colon carcinoma [158]. Therefore, ferumoxytol may target CSCs by ferroptosis (Table 6).

According to the current research, the relationship between ferroptosis and CSCs has received increasing attention. In contrast, there are few reports of GPX4 in CSCs. Therefore, to some extent, the role of iron in CSCs is seemingly more dependent on GPX4 dysfunction, which causes normal stem cells to die in an iron-dependent manner [171, 172].

Multiple-signaling pathway inhibitors targeting CSCs

As we stated above, there are many signaling pathways regulating CSC progression. Hence, the compounds that simultaneously target multiple signaling pathways critical for CSC progression may yield better results than single-target treatments in controlling tumor occurrence, recurrence, and drug resistance (Table 7). For example, Z-ajoene, a compound extracted from garlic, was confirmed to inhibit CSC sphere-forming ability in glioblastoma multiforme (GBM), and Notch-, Wnt-, and Hh-related genes ware changed after treatment with Z-ajoene. In addition, Z-ajoene induced no cytotoxicity in normal cells [173]. Poziotinib, a pan-human epidermal growth factor receptor (HER) inhibitor, can decrease ovarian CSC sphere formation ability by disrupting the Wnt, Notch, and Hh signaling pathways in epithelial ovarian cancer (EOC) [176]. According to clinical studies, patients may experience diarrhea and rash when treated with poziotinib [174]. However, considering the multiple targets and anti-CSC activity of poziotinib, more studies are needed in the future. Additionally, 6-shogaol reduces the number of CD44$^+$/CD24$^-$ cell subpopulation in BCSCs and inhibit their sphere-forming ability by inducing autophagy and inhibiting the Notch signaling pathway [177]. Importantly, 6-shogaol shows low toxicity induction in normal cells [175].

Conclusions

This review summarizes several well-characterized signaling pathways involved in CSCs, such as Wnt, Hh, Notch, Hippo, autophagy, and ferroptosis, and focuses on small-molecule compounds regulating these pathways (Fig. 5). Finally, these small-molecule compounds

have the potential to kill CSCs, which provides a basis for cancer treatment. It is noteworthy that there are many other pathways, such as PI3K/Akt, MAPK, JAK/Stat, and TGF-β [178], essential for CSC survival, but these pathways are also widely engaged in other biological processes, and targeting them would not be specific to CSCs; however, the Wnt, Hh, Notch, and Hippo pathways are the mainstream pathways in CSCs, which have already been well studied. Therefore, in this current review, we summarize the small-molecule compounds targeting the Wnt, Hh, Notch, and Hippo pathways to kill CSCs. Although targeting ferroptosis to kill CSCs has recently been invoked, the effects have been strongly established by many studies [143, 147, 148]. Recently, although biologics have been rapidly developed, small-molecule compounds are still needed for clinical application.

Compared with biologics (such as monoclonal antibodies and antibody-drug conjugates), small-molecule compounds are inexpensive, and the generic drugs made from them are relatively simple, which means that small-molecule compounds may have the potential to compete with biologics. In addition, we present an overall assessment of these small-molecule compounds in Tables 1, 2, 3, 4, 5, and 6. Most chemotherapy drugs induce toxicity to different degrees. Therefore, we need to find a balance between anticancer effects and side effects of compounds used to target CSCs. The process from drug development to clinical use is long. Although approved drugs always induce specific toxicities, they have been well studied, and drug repurposing may offer a shortcut to prevent financial loss and detoured efforts. Therefore, in this review, we list many drugs approved by FDA, such as Wnt inhibitors (niclosamide, TFP, DTX and SFN, PP, AD and Ts); Notch inhibitors (DAPT); Hh inhibitor (glasdegib, sonidegib, vismodegib, ciclesonide); Hippo inhibitors (verteporfin, fluvastatin, atorvastatin, CPZ); autophagy regulators (CQ, HCQ, pantoprazole); ferroptosis inducers (TMZ and CQ, artesunate, ferumoxytol, sulfasalazine). These drugs display anti-CSC abilities, and some have been entered into clinical studies. In particular, an Hh inhibitor (glasdegib) has been approved by the FDA for its efficient anti-CSC action, which is similar to that of the biologics ELZONRIS. In addition, there are many new small-molecule compounds that are well tolerated, according to the current studies, but conclusive outcomes still need to be proven by more studies, and the toxicities of the following compounds have not been reported, which may be the result of insufficient experiments: Wnt inhibitors (ONC201, TFP, chelerythrine, FH535, Wnt-C59, IWR-1, IC-2, JIB-04, PP, OXT-328, OXT-328), Notch inhibitors (MK-0752, DAPT), Hh inhibitors (ciclesonide, GANT61), Hippo inhibitors (verteporfin, evodiamine, fluvastatin,

Table 6 Small-molecule compounds that induce ferroptosis to inhibit CSCs

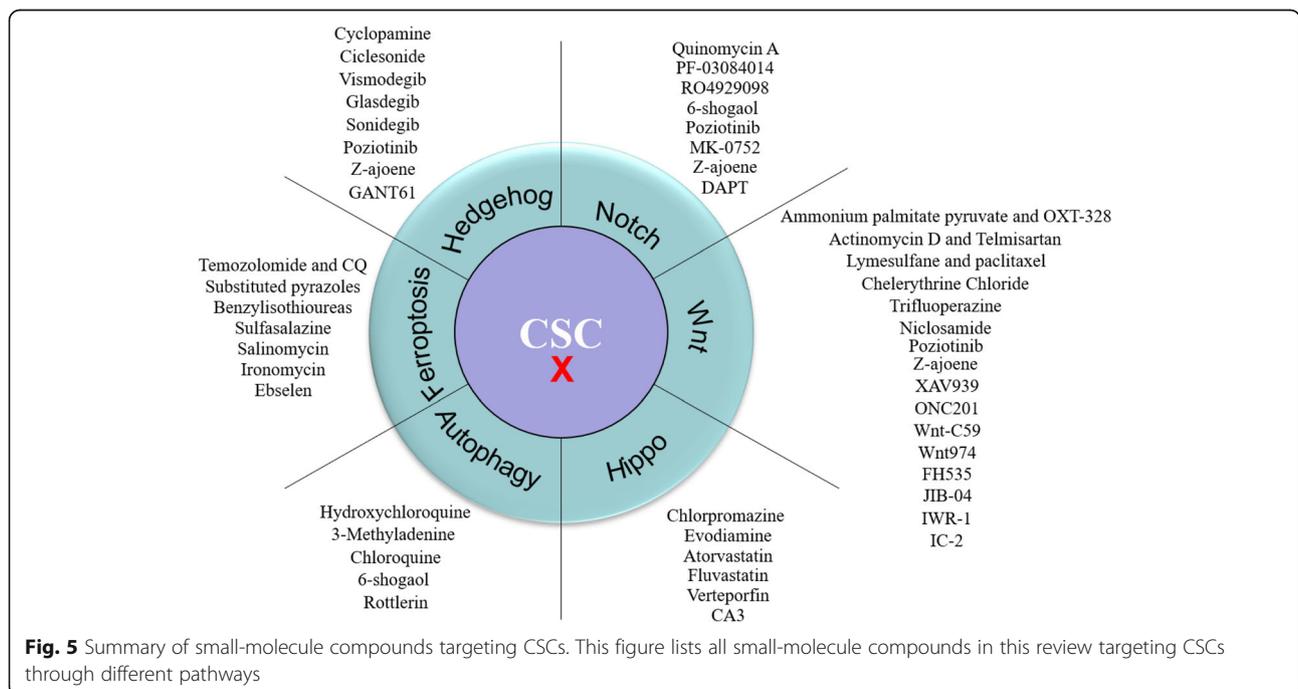| Name	Target	Mechanism	Type of cancer	Phase	NCT number (starting time)/ publication date	Assessment
Salinomycin	Ferroptosis	Increases the production of lipid peroxidation by blocking iron transport and depleting ferritin; these can specifically kill CSCs	Breast cancer	Preclinical	October, 2017	Induces neural and muscular toxicity; changing dosages and making chemical modifications may reduce toxicity [159]
	Ferroptosis	Combined with docetaxel can kill gastric CSCs	Gastric cancer	Preclinical	October, 2017	
Ironomycin	Ferroptosis	Reduces the number of CSCs in docetaxel-resistant xenograft models	Breast cancer	Preclinical	February 21, 2020	The potency against CSCs is ten-fold that of salinomycin; may cause nephrotoxicity and hepatotoxicity [160]
Ebselen	Ferroptosis	Targets BCSCs by blocking iron in lysosomes	Breast cancer	Preclinical	February 21, 2020	Induces low toxicity and shows good blood-brain barrier permeability and oral absorption [161]
Substituted pyrazoles					February 21, 2020	Not reported; need studies to prove
Benzylisothioureas					February 21, 2020	Hemoglobinopathy including thalassemia [162]
TMZ and CQ	Ferroptosis	Causes glioblastoma stem cells (GSCs) to die through a form of ferroptosis and reduce the self-renewal ability of GSCs	Glioblastoma	Preclinical	August 6, 2018	TMZ is well tolerated, but may induce hematological toxicity and infection; CQ shows cardiotoxicity [117, 163]
DHA	Ferroptosis	Ferroptosis	Lung, colorectal, and breast cancer cells	Preclinical	January, 2020	Induces neurotoxicity, cardiotoxicity and the toxicity in embryos [164]
	Apoptosis	Inhibits sphere formation and stem marker (CD133, SOX2, and nestin) expression in glioma CSCs	Gliomas	Preclinical	October, 2014	
Artesunate	Mitochondrial	Inhibits the stemness of CSCs	Not mentioned	Preclinical	September 2, 2016	Excellently tolerated, and with low adverse effects [165, 166]
	Ferroptosis	Induces cell death through ferroptosis	Pancreatic cancer	Preclinical	May 2, 2015	
Ferumoxytol	Ferroptosis	Selectively kills CSCs (A549 and MDA-MB-231 cells)	Lung cancer and breast cancer	Preclinical	August 26, 2013	Well tolerated, but intravenous may cause hypersensitivity, hypotension, and gastrointestinal side effects [167–169]
Sulfasalazine	System X_c^-	Inhibits the progression of CSCs overexpressing CD44	Gastrointestinal cancer	Preclinical	March 8, 2011	Induces gastrointestinal toxicity, and combed with other drugs, this side effect may be overcome [170]

Table 7 Small-molecule compounds regulating multi-signaling pathways to inhibit CSCs

Name	Target	Mechanism	Type of cancer	Phase	NCT number (starting time)/publication date	Assessment
Z-ajoene	Notch, Wnt, and Hh	Inhibits CSC sphere-forming ability	Glioblastoma multiforme	Preclinical	July, 2014	Without cytotoxic in normal cells [173]
Poziotinib	Wnt, Notch, and Hh	Decreases ovarian CSC sphere formation ability	Epithelial ovarian cancer	Preclinical	May 21, 2020	Diarrhea and rash [174]
6-Shogaol	Notch and autophagy	Inhibits the number of CD44+/CD24– cell subpopulation and decreases sphere-forming ability	Breast cancer	Preclinical	September 10, 2015	Induces low toxicity in normal cells [175]

CA3), autophagy regulators (3-MA, Rott), ferroptosis inducers (ebselen, substituted pyrazoles, TMZ, CQ, Artesunate, Ferumoxytol). Furthermore, we also listed some new CSC-targeting compounds that are in the preclinical or phase I/II stage: Wnt inhibitors (Wnt974, XAV939), Notch inhibitors (PF-03084014, RO4929097, quinomycin A), Hh inhibitor (cyclopamine), ferroptosis inducers (salinomycin, ironomycin, benzylisothioureas). These compounds also show anti-CSC activities, and the toxicities they induce may be reduced by changing the dosage, and developing new derivatives and combination therapies. To our surprise, some compounds are DLTs, especially the Notch inhibitors, and because of their side effect, additional research is needed to determine precise effective and safe dosage. In summary, according to the current studies, the compounds targeting CSCs are well tolerated in the mice or patients, and the compounds targeting multi-oncogenic signaling pathways may play important role in the clinic studies.

There are more detailed studies supporting the use of small-molecule compounds targeting Wnt, Hh, Notch, Hippo, and autophagy to inhibit CSCs, and many compounds are currently in clinical research. Although many small-molecule compounds activate ferroptosis, these studies have been conducted at the cell and mouse levels, and no clinical studies have been reported. However, based on the specificity and strong inhibitory ability of salinomycin and ironomycin and their derivatives on CSCs, these small-molecule compounds may have strong potential as target CSCs, and ferroptosis induced by iron in lysosomes may be a valuable prospect as a clinical target in CSCs to promote the development of anticancer drugs. However, it is noteworthy that there are different pathways in CSCs abnormally expressed; therefore, monotherapies that can target different

Fig. 5 Summary of small-molecule compounds targeting CSCs. This figure lists all small-molecule compounds in this review targeting CSCs through different pathways

pathways simultaneously or combined therapies may yield the best results in the future. Importantly, to our knowledge, targeting CSCs alone is sufficient for cancer therapy in the early stage of tumorigenesis, but it is not sufficient in the tumor development period, as many small-molecule compounds targeting CSCs cannot kill cancer cells, and the combined use of drugs targeting CSCs and chemotherapeutics may lead to better effects during tumor development period.

Abbreviations
CSCs: Cancer stem cells; Hh: Hedgehog; LRP: Lipoprotein receptor-related protein; TNBC: Triple negative breast cancer; TBD: Terminal anchor polymerase binding domain; HNSCC: Neck squamous cell carcinoma; CRC: Colorectal cancer; TFP: Trifluoperazine; NSCLC: Non-small cell lung carcinoma; NPC: Nasopharyngeal carcinoma; HCC: Hepatocellular carcinoma; DTX: Docetaxel; SFN: Sulforaphane; PP: Pyrvinium pamoate; EMT: Epithelial-mesenchymal transition; AD: Actinomycin D; TS: Telmisartan; DLL: Delta-like ligand; NICD: Notch intracellular domain; DLT: Dose-limiting toxicity; INS: Insulinomas; LSCs: Leukemia stem cells; SMO: Smoothened; ER: Estrogen receptor; BCSC: Breast cancer stem cell; TEAD: TEA domain family members; Evo: Evodiamine; CPZ: Chlorpromazine; CQ: Chloroquine; HCQ: Hydroxychloroquine; IM: Imatinib; mCRPC: Metastatic castration-resistant prostate cancer; PPI: Proton pump inhibitor; 3-MA: 3-Methyladenine; Rott: Rottlerin; ROS: Reactive oxygen species; TfR1: Transferrin receptor 1; DMT1: Divalent metal transporter 1; LIP: Labile iron pool; PCBP1/2: Poly (rC)-binding protein 1/2; IRP2: Iron regulatory proteins 2; IREs: Iron-responsive elements; UTRs: Untranslated regions; Gpx4: Glutathione peroxidase 4; PLs: Phospholipids; PUFAs: Polyunsaturated fatty acids; LOXs: Lipoxygenases; AA: Arachidonic acid; ACSL4: Acyl-CoA synthetase long-chain family member; LPCAT3: Lysophosphatidylcholine acyltransferase 3; MDR: Multi-drug resistance; TMZ: Temozolomide; GSCs: Glioblastoma stem cells; DHA: Dihydroartemisinin; iCSCL: Induced cancer stem-like cells; GBM: Glioblastoma multiforme; HER: Pan-human epidermal growth factor receptor; EOC: Epithelial ovarian cancer

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Author details
¹School of Life Science and Technology, Jiangsu Key Laboratory of Carcinogenesis and Intervention, China Pharmaceutical University, 639 Longmian Road, Nanjing 211198, People's Republic of China. ²Jiangsu Key Laboratory for Pharmacology and Safety Evaluation of Chinese Materia Medica, School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, People's Republic of China. ³Department of Pharmacy, Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, 127 Dongming Road, Zhengzhou 450003, People's Republic of China.

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