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MINIREVIEWS

Cancer stem cell impact on clinical oncology

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Abstract

Cancer is a widespread worldwide chronic disease. In most cases, the high mortality rate from cancer correlates with a lack of clear symptoms, which results in late diagnosis for patients, and consequently, advanced tumor disease with poor probabilities for cure, since many patients will show chemo- and radio-resistance. Several mechanisms have been studied to explain chemo- and radio-resistance to anti-tumor therapies, including cell signaling pathways, anti-apoptotic mechanisms, stemness, metabolism, and cellular phenotypes. Interestingly, the presence of cancer stem cells (CSCs), which are a subset of cells within the tumors, has been related to therapy resistance. In this review, we focus on evaluating the presence of CSCs in different tumors such as breast cancer, gastric cancer, lung cancer, and hematological neoplasias, highlighting studies where CSCs were identified in patient samples. It is evident that there has been a great drive to identify the cell surface phenotypes of CSCs so that they can be used as a tool for anti-tumor therapy treatment design. We also review the potential effect of nanoparticles, drugs, natural compounds, aldehyde dehydrogenase inhibitors, cell signaling inhibitors, and antibodies to treat CSCs from specific tumors. Taken together, we present an overview of the role of CSCs in tumorigenesis and how research is advancing to target these highly tumorigenic cells to improve oncology patient outcomes.

Key words: Cancer; Targeted therapy; Clinical outcome; Drug resistance; Cancer stem cells

Core tip: Tumor heterogeneity can explain the presence of cells that display high tumorigenic capacity along with chemo- and radio-resistance properties. These cells, identified as cancer stem cells (CSCs), are partially responsible for recurrence and tumor progression. Most tumors follow the CSC model, which indicates the existence of a subset of highly tumorigenic cells. This has been shown to be the case for several patients with several types of tumors. In this review, we focus on the phenotypes used for the study and identification of CSCs from human samples, as well as promising strategies to target CSCs.

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INTRODUCTION

Cancer stem cells (CSCs) comprise a cell population within a tumor that, among other factors, is responsible for cancer initiation, propagation, metastasis and recurrence. It is known that solid tumors are composed of heterogeneous cell populations[1-3] with different phenotypic characteristics at different stages of development, with variable abilities to proliferate. However, only the CSC population is clonogenic *in vitro* and *in vivo*, suggesting that these cells are the only ones with the highest tumorigenic potential[4,5].

The existence of a subset of cancer cells that possesses an extensive proliferative capacity was reported in leukemia and multiple myeloma in the 1970s[6,7]. In both cancer types, only a cell population derived from a tumor was able to grow in clonogenic assays, where they formed spherical colonies, and induce tumors in mice that recapitulated the original tumor. At that time, the most reliable criterion for CSC identification was the capacity of these cells to produce colonies[6].

The first CSCs were isolated from acute myeloid leukemia (AML) by transplantation into severe combined immune-deficient (SCID) mice. They were identified as $CD34^+CD38^-$ cells and named AML-initiating cells because of their ability to establish human leukemia in SCID mice. Since the identified $CD34^+CD38^-$ cells were less differentiated than colony-forming cells, a hierarchy or heterogeneity in AML was proposed[1]. Later, in 1997, the model was reproduced in non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID) mice, where $CD34^+CD38^-$ CSCs were capable of differentiating into leukemic blasts *in vivo*, supporting the existence of a hierarchy in leukemia[8].

Some years later, enriched CSC populations were obtained from human brain tumors[9], using cells with a $CD133^+$ phenotype that showed a higher capacity for proliferation, self-renewal, and differentiation. $CD133^+$ cells were xenotransplanted into NOD/SCID mice and formed tumors that, when serially transplanted, recapitulated the original human tumor[10,11]. Since then, CSCs from various solid tumors have been reported[5].

In recent years, several research groups have focused on the identification and isolation of these cells. Besides leukemia and multiple myeloma, CSCs from solid tumors have been identified and isolated through the use of surface and functional markers[12-15], their growing capacity as spheroids *in vitro*[16,17], the evaluation of CSC clonogenic capacity[18,19] and their *in vivo* tumorigenic capacity in xenotransplant experiments[16,17,20,21].

Due to the reported participation of CSCs in chemo- and radio-resistance[22-24], an increasing interest in implementing strategies against CSCs in patients to improve their clinical outcome has grown in recent years because conventional therapies are effective in controlling tumor growth at the beginning, but over time, relapse is a main problem due to remaining CSCs[22,25,26].

CSC GENERALITIES

A CSC is defined as a cell within a tumor that is able to produce an identical cell with the same properties to give rise heterogeneous differentiated progeny, and has the ability to modulate differentiation and self-renewal (homeostatic control). These CSCs possess the ability to propagate themselves, as well as recapitulate a tumor[2,3,27].

A major characteristic of CSCs relies on their ability to regulate stemness pathways such as Wnt/β-catenin, Sonic hedgehog (Shh), transforming growth factor beta (TGF-β), *etc*[28]. These pathways are dysregulated in CSCs, and targeting them has been proposed as a strategy to increase the effectiveness of cancer therapies.

The CSC model postulates that solid tumors and leukemia are hierarchically organized, with CSCs at the apex of this hierarchy, driving tumor growth, relapse, metastasis and drug resistance[5,29]. Cell heterogeneity is responsible for varying cell morphology, different proliferative index, genetic changes and therapeutic response[30]. For a successful therapy, all CSCs should be specifically eliminated to avoid relapse.

Typically, CSCs are defined as a small or a rare cell population[2,31] that forms tumors after being xenotransplanted into immunodeficient mice. However, recent reports have suggested that the percentage of CSCs within a tumor can vary from 0.02% to 25% depending on the tumor type, where higher CSC proportions are found in undifferentiated tumors[31-34]. Typically, higher CSC frequencies have been found in mouse models, leukemias and lymphomas, while lower frequencies are frequently found in solid tumors[35]. Based on this information, it has been suggested that not all cancers follow the CSC model[27]. Instead, a dynamic or plastic CSC model has been proposed, where CSCs and non-CSCs could alternate between two phenotypic states[36].

In this dynamic model, both cell types show varying levels of tumor-forming capacity, drug response and the ability to give rise to differentiated cells[29,35]. CSCs and non-CSCs can still be easily distinguished through surface and functional markers, but mainly by their self-renewal capacity.

It is very important to note that the CSC model is widely reported in several cancer types (Figure 1), although there are a few publications about cancers that do not follow a CSC model or a dynamic CSC model, specifically in lymphoma mice models[37] and melanoma[32], where the tumors are homogeneous. In 2007, Strasser and his group inoculated 10 to 10^5 pre-B/B lymphoma cells into recipient mice. All of the animals developed lymphoma within 35 d, regardless of the number of inoculated cells, differing only in tumor growth rate[37].

Although CSCs are able to self-renew and differentiate, they do not necessarily originate from the malignant transformation of stem cells[33]. The cell of origin refers only to the cell type that received the first genetic or epigenetic hit, which confers the ability for self-renewal or tumor growth[35]. Examples of these cells are: normal stem cells, restricted progenitor cells and more differentiated cells. All of them could have acquired or maintained self-renewal capacity, and some of them can even undergo epithelial to mesenchymal transition (EMT), giving rise to metastatic CSCs[36].

In conclusion, the variable phenotype of the CSC population in patients and tumor types proposed in the CSC dynamic model constitutes the main challenge for the possible use of anti-CSC therapy.

CSC CHARACTERISTICS WITH CLINICAL RELEVANCE

The CSC population possesses several characteristics that can be useful for cancer therapy development, primarily focusing on the elimination of these cells.

Usually, a distinctive profile of surface and functional markers characterizes the CSC population, and their identification and purification usually begins with the description of such markers[3,29]. Moreover, there is an increasing interest in identifying the role of each marker in CSCs, as well as targeting CSC-specific pathways, which could increase the radio- and chemo-sensitivity of CSCs.

To date, several CSC markers from distinct tumor types have been proposed and validated through different experimental models (Table 1 and Figure 1). Some of these markers are discussed below.

Surface markers

Nowadays, there are CSC markers that are widely used to identify several tumor types. Such markers have been reported in CSC-enrichment culture models from cell lines or primary cultures derived from patient samples and serial xenotransplantation of putative CSCs in mouse models, which must be able to recapitulate the original heterogeneous populations and be directly validated in human tumor samples. It is important to note that the use of a single marker to define a CSC population is not recommended. For this purpose, a phenotypic profile that combines various markers should be established, as well as carrying out self-renewal assays (Figure 1)[2,25].

CD133, also known as prominin-1, is a transmembrane cell surface glycoprotein traditionally used as a hematopoietic stem cell marker that is effective for detection of non-stem cells from various tumor and tissue samples. The Dirks laboratory used the CSC marker CD133 for brain CSC identification. The purified $CD133^+$ population from primary human brain tumors samples showed higher proliferation and self-renewal capacity in neurosphere formation assays than $CD133^-$ cells[10]. Moreover, the inoculation of only a few $CD133^+$ cells was sufficient to produce a tumor, which was then successfully transplanted[11]. In 2013, the Pelicci laboratory reported that CD133 was found in an interconvertible state in glioblastoma patient-derived neurospheres and that the use of short hairpin RNA (shRNA) against CD133 diminished their self-renewal and tumorigenicity potential[18]. Interestingly, some studies have proposed that CD133 could maintain CSC properties through the Wnt/β-catenin signaling pathway[38].

CD133 has also been tested in colorectal cancer cell lines and tumor tissue samples[39,40] through the use of various techniques, including flow cytometry and serial xenotransplantation in mice[41]. Additionally, $CD133^+$ CSCs have been reported in many other solid cancer models, including endometrial cancer[42], lung cancer[43], small cell lung cancer[44], laryngeal cancer[45,46], liver cancer[47], colorectal cancer[48], and gastric cancer[49].

CD133 has been found in samples that represent higher stage tumors and are predictors of poor prognosis. For this reason, CD133 is considered a promising therapeutic target. This year, a phase I trial for testing the efficacy of CD133-directed CAR-T cells showed that $CD133^+$ cells were successfully eliminated after CART-133 infusion[50].

CD44 is a multifunctional glycoprotein involved in cell adhesion, signaling, proliferation, migration, hematopoiesis, and lymphocyte activation[51]. It functions as a receptor for hyaluronan and other extracellular matrix components[52]. CD44 is widely used as a CSC marker, especially for tumors of epithelial origin, and it is used alone or in combination with CD24 for the identification of breast CSCs[5]. CD24 is a small surface protein that is found in many tumor types. However, reports from cancer cell lines show that there is a substantial variation in CD24 expression even among the same tumor types[53].

Though $CD24^-$ cells are commonly associated with CSC phenotypes, there are some cases in which $CD24^+$ has been found to be a marker for cell populations with CSC features. For example, in nasopharyngeal carcinoma (NPC) cell lines[54] and in HPV-16 SiHa cervical cancer

Table 1 Cancer stem cells markers in solid tumors

Cancer type	Phenotype	Model	References
Prostate cancer	$CD44^+$	PCa cell line and tumor xenograft in mice	[58]
Breast cancer	$CD44^+$ $CD24^{-/low}$	Patient-derived tumor xenograft in mice	[5]
Cervical cancer	$CD44^+$ $CD24^+$	SiHa cell line	[55]
Gastric cancer	$CD44^+$ $CD24^+$	AGS cell line and patient tissue samples	[56]
Nasopharyngeal carcinoma	$CD24^-$	NPC cell lnes, mice	[54]
Gastric adenocarcinoma	$CD44^+$ $CD133^+$	Patient tissue samples	[51]
Oral squamous cell carcinoma	$CD44^+$ ALDH1	Metastatic lymph nodes	[153]
Breast cancer	CD44v	Clinical samples	[154]
Prostate cancer	CD133	Primary prostate cancer cell lines	[155]
Endometrial cancer	CD133	Human endometrial cell lines	[42]
Liver cancer	CD133	Huh-7 cells and tumor xenograft in mice	[47]
Prostate cancer	CD133	Primary human prostate cancer cell lines	[155]
Cervical cancer	CD49f	SiHa and HeLa cell lines	[156]
Non-small cell lung cancer	CD49f	Patient-derived sphere-forming assays	[157]
Gastric cancer	CD49f	Gastric tumor tissues and tumor xenograft in mice	[75]
Colon cancer	CD49f	HT29 and Caco2 cell lines, clinical samples	[77]
Cervical cancer	ALDH	SiHa and HeLa cell lines, mice model	[85]
Colon cancer	ALDH1A3	HT29 cell line	[158]
Colon cancer	ALDH1A1	HT29 cell line and tumor xenograft in mice	[159]
Breast cancer	ALDH	Breast cancer tumor tissues	[160]

CSCs: Cancer stem cells; ALDH: Aldehyde dehydrogenase; NPC: Nasopharyngeal carcinoma.

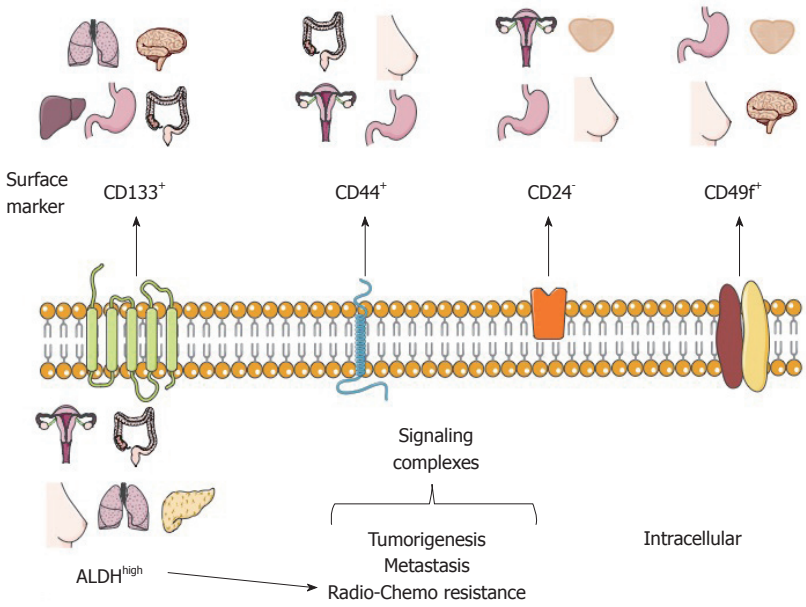

Figure 1 Schematic representation of common cancer stem cell markers. CD133, CD44, CD24 and CD49f are common phenotype markers used for the identification of cancer stem cells (CSCs) and their isolation from tissue samples from cancer patients, such as the stomach, lung, liver, ovary, breast, prostate and colon carcinoma. In addition, the metabolic and functional marker aldehyde dehydrogenase (ALDH) is represented in CSCs derived from ovarian carcinoma, colon carcinoma, breast, lung and liver cancer. The CSC markers shown have a specific and relevant function in the high tumorigenic capacity of CSCs, metastasis, and resistance to radio- and chemotherapy.

cells, isolated $CD44^+CD24^+$ cells were radioresistant and more tumorigenic than those negative for the same markers[55]. The same $CD44^+CD24^+$ phenotype was used to identify gastric CSCs[56].

A known classic publication demonstrated that only a small population isolated from breast tumors, defined as $CD44^+CD24^{-/low}$, has the capacity to sustain tumor growth in NOD/SCID mice and generate heterogeneous cell populations as the original breast tumor[5]. Later, in human prostate cancer samples, CSCs characterized through immunofluorescence with the $CD44^+/\beta_2\beta_1{}^{hi}/CD133^+$ phenotype were identified and characterized[57]. The next year, $CD44^+$ prostate cancer cell populations were obtained[58]. Also, CD44 and CD133 expression was evaluated in gastric adenocarcinoma tumors by immunohistochemistry, and it was found that both markers could be correlated with clinical and pathological parameters[51].

Although CD44 is widely reported as a CSC marker, it is very important to note that it is a ubiquitously expressed molecule derived from a gene with 18 exons. When all variable exons are spliced out, the standard form (CD44s) is expressed, and when alternative splicing occurs, variant forms (CD44v) are expressed[59]. In spite of this, there are only a few reports in which CD44 isoforms are considered when evaluating CSCs. In 2005, Mackenzie and his group demonstrated the existence of two CSC populations, both expressing $CD44^{high}$ (and $CD44^{+}$), derived from head and neck cutaneous squamous cell carcinoma. One was associated with EMT properties and the other one possessed an epithelial phenotype[60]. They demonstrated that the $CD44^{high}$ cells that undergo EMT preferably expressed the CD44s isoform; while the epithelial $CD44^{high}$ cells expressed the CD44v isoform. Using RNAseq, another group later confirmed these results. The CD44v6 isoform was identified as the predominant isoform in a prostate cancer epithelial cell line[61].

A very important contribution from the Mackenzie laboratory is that they demonstrated that the use of enzymes (for example, trypsin or collagenase) for cell extraction from tissues caused destruction of cell surface CD44v isoforms, leaving only the CD44s isoform[62]. Moreover, CD44-specific antibodies are not able to distinguish between all isoforms. Specifically, in breast cancer, CD44v was found to be associated with better prognosis while CD44s was related to poor prognosis[63]. As a consequence, CD44 is the most frequently found CSC marker[64,65]. Other examples are found in colorectal cancer, in which CD44 was found together with CD133[66,67], head and neck squamous cell carcinoma[68,69], ovarian CSCs[70], and gastric cancer using the specific isoform CD44v8-10[71].

CD49f or integrin α6, is a transmembrane glycoprotein that functions as the receptor for the extracellular matrix protein laminin[72,73]. CD49f is widely distributed in stem cells and in the brain[73]; because of its role in tumor cell proliferation, survival, self-renewal and tumor growth, it has been proposed that it could be used as a CSC marker[73].

In sphere colony forming cell culture using prostate cancer cells, CD49f was shown to be a better marker than CD133 and CD44[74]. In gastric cancer, $CD49^{high}$ cells displayed CSC characteristics, including resistance to doxorubicin, 5-fluorouracil and doxifluridine[75].This has also been reported in breast[76] and colon cancer[77]. Besides the examples mentioned above, there are other surface markers that have been proposed as CSC markers, such as CXCR4 and LGR5, among others.

Functional markers

Another strategy for CSC identification and purification is the use of functional or intracellular markers (Figure 1), which are considered to be more stable than surface markers. The principal functional CSC marker is aldehyde dehydrogenase or ALDH, part of an enzyme superfamily encoded by 19 genes that metabolize endogenous and exogenous aldehydes. It is present in practically all organisms, and its levels and isozymes vary depending on tissue and organ[78].

For ALDH identification, specific ALDH antibodies are available; nonetheless, we suggest that the most appropriate way for ALDH identification is the measurement of its activity using the commercial ALDH fluorescent substrate ALDEFLUOR® kit assay by Stem Cells Technologies, Inc. (Vancouver, BC, Canada). Cells that display high ALDH activity, (named $ALDH^{high}$ or $ALDH^{+}$ or $ALDH^{br}$), can be identified and isolated using flow cytometry[79]. Several works have shown that high ALDH activity is often associated with CSCs derived from solid tumor types[80]. These cells are generally characterized by a higher proliferation potential, colony-forming capacity, self-renewal, *in vivo* tumorigenic capacity, metastasis, and drug resistance. For instance, $ALDH^{high}$ CSCs have been identified in colon cancer[81,82], lung cancer[83], cervical cancer[14,84,85], breast cancer[86], pancreatic cancer[87,88], and melanoma[89,90], to mention some examples.

As for surface markers, ALDH is often reported in combination with other cell markers to increase the accuracy of CSC validation. In some cases, high ALDH activity is found together with high expression of markers like CD133. Some cases have been identified in ovarian cancer[91,92], invasive ductal breast carcinoma tumors[93], and lung cancer[94]. The combination $ALDH^{+}$/$CD44^{+}$ has been evaluated in various tumors such as breast cancer[95] and lung cancer[96].

CSCs AND THERAPY RESISTANCE

Several cancers acquire drug resistance during or after treatment, which is the case for cancers that possess cells that are more resistant than the rest of the tumor. Generally, resistant cells have proteins that remove drugs from cells[97]. One of the most studied mechanisms of drug resistance in CSCs is their ability to actively expel therapeutic drugs *via* transport proteins. Such proteins are a family known as ATP-binding cassette transporters. These proteins use ATP-dependent drug efflux pumps for drug elimination, mostly into the extracellular space, and they have been found to be overexpressed in CSCs using side population assays[41,98-100].

Additionally, high ALDH activity is directly related to a higher resistance to several drugs, for example, cyclophosphamide, temozolomide, irinotecan, paclitaxel, and doxorubicin[101-103]. Resistance conferred by ALDH has been observed in numerous cell lines and patient samples[97,104]. A well known case is the resistance to cyclophosphamide, where ALDH irreversibly oxidizes aldophosphamide, an active metabolite of cyclophosphamide, into an inert compound[105]. In breast cancer, the inhibition of ALDH activity in $ALDH^{high}$ $CD44^{+}$ cells leads to a reduction in chemoresistance to doxorubicin and paclitaxel[106]. This information suggests that the

inhibition of ALDH activity leads to cell sensitization to chemotherapeutics[99].

Besides higher resistance to conventional cancer treatments, evidence shows that highly metastatic tumors correlate with a higher percentage of CSCs[28].

CSCs IN PATIENTS: PHENOTYPE AND TYPE OF STUDIES

Most publications about the identification of CSCs have been performed in cell lines. However, in this section, we will discuss the cases in which CSCs were identified in patient samples.

CD133 was analyzed in a meta-analysis of 32 studies of non-small cell lung cancer, and a higher CD133 expression was associated with poor tumor differentiation and lymph node metastasis[107].

Gastric CSCs have been identified in tumor tissues and peripheral blood using the $CD44^+CD54^+$ phenotype[108]. Nevertheless, in another study, $CD133^+/CD44^+$ cells sorted from 44 patients who underwent gastrostomy failed to produce tumors in mice and did not show any CSC properties[109].

The presence of ALDH has been analyzed in normal mammary and breast cancer tissues[110]. The activity of ALDH1A3 is associated with metastasis in patient breast cancer samples by microarray analysis[86]. In another analysis of formalin-fixed paraffin-embedded tissue samples from primary stage Ⅳ breast cancer, ALDH and CD44/CD24 expression was correlated with response to endocrine therapy and clinical outcome but was not statistically significant[111].

CSC approaching therapy

Despite the broad variety of CSC publications in the last years, the discovery of effective therapies has remained elusive. However, some advances have been made in the field that could be getting us closer to direct CSC elimination. A brief outline of some of these strategies is showed in Figure 2.

Targeting deregulated pathways in CSCs aims at developing effective strategies against CSCs. In adult pancreas, the Hedgehog (Hh) signaling pathway is dormant, but it is upregulated in pancreatic ductal adenocarcinoma, specifically in $CD44^+/CD24^+/ESA^+$CSCs. In a phase Ⅰ study, 68 patients were treated with GDC-0449 or Vismodegib, a Hh pathway antagonist[112], alone or in combination with gemcitabine. GDC-0449 inhibited Hh signaling, but there was no correlation with survival or other parameters[113]. Other drugs that show promising results in inhibiting this pathway are PF-044449913[114] and thiostrepon, which attenuates $CD44^+/CD24^-$ triple-negative breast CSCs[115].

In addition, γ–secretase inhibitors target the Notch pathway and possess a stronger anti-neoplastic activity when combined with chemotherapeutic agents[116]. Nevertheless, adverse effects have been reported, as patients developed cutaneous rash in phase Ⅰ clinical trials[117,118].

Several drugs that aim to inhibit the Wnt/β-catenin signaling pathway are being developed. One such drug is Celecoxib, a non-steroidal anti-inflammatory drug that inhibits β-catenin signaling by cyclo-oxygenase (commonly known as COX)-dependent and COX-independent mechanisms[116]. This drug downregulates CD133 expression in colon cancer cells by inhibiting Wnt signaling[119] and intestinal cancer growth[120]. The Wnt inhibitor LGK-974 inhibits porcupine, an O-acyltransferase required for Wnt secretion. In liver cancer cells, LGK-974 blocks secretion of the Wnt3A protein, and as a consequence, cells become more sensitive to radiation[121]. A recent study showed that LGK-974 downregulates ALDH1A3 and reduces chemoresistance in glioblastoma cells[122].

Curcumin is an antioxidant derived from turmeric whose anti-cancer effect is well documented. Referring specifically to CSCs, curcumin has shown the potential to regulate the CSC self-renewal pathways, as well as specific microRNAs[123]. In $CD133^+$ lung CSCs, curcumin suppresses the activation of Wnt/β-catenin and Shh pathways, as well as other CSC traits[124]. It has been demonstrated that in bladder cancer, curcumin suppress the Shh pathway[125] and in laryngeal carcinoma treatment, curcumin enhances the effectiveness of cisplatin, reducing $CD133^+$ cells *in vitro*[46]. Additionally, a combination of curcumin and FOLFOX chemotherapy inhibits colorectal CSCs in *ex vivo* models[126].

An interesting strategy is to target CSCs using nanoparticles to reduce side effects on surrounding normal cells. In 2015, construction of glucose-coated gold nanoparticles (Glu-GNPs) that used glucose to facilitate GNP entry into leukemic stem cells overexpressing CD44 (TH1-P) was reported. Leukemic cells were cultured for one hour in the absence of glucose for better Glu-GNP uptake, and then X-ray irradiation tests were performed. Results showed that Glu-GNPs enhanced cell death compared to either irradiation or GNPs alone[127]. Formulated mangostin-encapsulated poly(lactic-co-glycolic acid) nanoparticles (Mang-NPs) successfully downregulated the known stemness genes c-Myc, Nanog and Oct4, two CSC markers, CD24 and CD133, and the Shh pathway[128]. Salinomycin and paclitaxel nanoparticles are also being used to eliminate breast cancer cells including CD44 breast CSCs[129].

Interestingly, CSCs have a strict dependence on mitochondrial biogenesis. Five classes of FDA-approved antibiotics that inhibit mitochondrial biogenesis were used on eight different cancer cell lines, and the results suggested that the observed therapeutic effects were infection-independent[130]. Clinical trials using doxycycline showed positive results in cancer patients[131]. Another drug that has been shown to specifically eliminate CSCs is metformin, and its effects are enhanced when it is used in combination with doxorubicin[132]. Moreover, it has been observed that metformin reduces metastasis by targeting both EMT

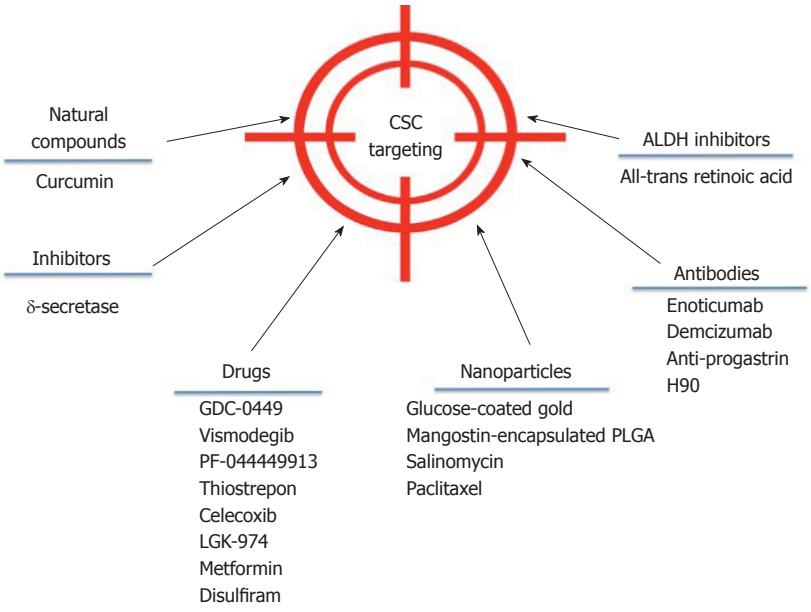

Figure 2 Drugs that may target cancer stem cells. Promising therapeutics to treat cancer patients. The flowchart highlights the new and more promising cancer therapies that can be directed toward cancer stem cells to eliminate them. CSC: Cancer stem cell.

and CSCs[133]. In the ovarian cancer cell line SKOV3, low doses of metformin diminished $CD44^+CD117^+$ CSCs in xenograft tissue and enhanced the effect of cisplatin[134]. In esophageal cancer, metformin reduced the number of ALDH+ cells, tumor growth *in vivo*[135], and in pancreatic cancer, it increased radiation sensitivity[136].

Using antibodies is another strategy to block CSC signaling pathways and reduce tumor activity in different models. For instance, the anti-DLL4 (Enoticumab) antibody that targets the dominant Notch ligand DLL4 has shown anti-tumor activity, especially in VEGF-resistant tumors in human phase Ⅰ studies[137]. Furthermore, another anti-DLL4 antibody (Demcizumab) is effective in decreasing tumor size but produces hypertension[138]. In colon cancer patients, increased progastrin levels in the blood have been observed, which is a tumor-promoting peptide that participates in colon CSC self-renewal and is also a direct target gene of β–catenin/Tcf4. Based on this information, specific anti-progastrin antibodies have been developed and tested in colon cancer cell lines and in mice. The antibodies, alone or in combination with chemotherapy, decreased self-renewal, migration and invasion. Moreover, they mitigated Wnt-driven intestinal neoplasia and induced tumor cell differentiation *in vivo*[139]. H90 is a mouse IgG1 mAb against human CD44 that directly targets CSCs to induce differentiation and proliferation in AML xenograft mouse models[140]. Additionally, anti-CD44s-specific antibodies are effective in eliminating pancreatic stem cells[141]. For more extensive information about antibodies against CSCs, we recommend reference[142].

ALDH is an important CSC marker that is overexpressed in several cancers. Specific ALDH inhibitors are effective in modulating cell growth, apoptosis and differentiation. Additionally, increased chemo- and radio-sensitivity is usually observed. All-trans retinoic acid (commonly known as ATRA) is a first generation systemic retinoid that promotes cell differentiation[143,144] and has been used in clinical trials[145]. ATRA has also been tested in breast cancer cells[106,146,147] and in gastric cancer, where it inhibited tumor growth[148], and in head and neck cancer, where it suppressed Wnt/β-catenin signaling[149]. In a phase Ⅰ/Ⅱ trial, advanced breast cancer patients did not show a significant improvement when treated with ATRA and tamoxifen compared with tamoxifen alone[150].

Disulfiram is a drug used for treating alcoholism, and it shows anti-cancer activity *in vitro* and *in vivo*, further potentiating the chemotherapeutic response. Its effectiveness has been demonstrated on paclitaxel-resistant triple-negative breast cancer cells[151], in non-small cell lung cancer cells[152], and glioblastoma.

CONCLUSION

CSCs are potential cancer therapy targets due to their tumorigenic capabilities, such as chemo- and radio-resistance, phenomena involved in tumor relapse in patients. Several efforts have been made to continue to identify the CSCs in several tumors to better understand the mechanisms related to tumor resistance in oncologic patients. It is known that de-regulated cell signaling pathways are partially responsible for maintaining CSC stemness. Consequently, Wnt, Notch and Hh signaling pathways have been studied to develop an efficient anti-CSC therapy. However, innovative anti-cancer treatments need to be developed to improve the lifespan and quality of life of cancer patients. Finally, we suggest that there cannot be a generalized CSC phenotype to design and promote new drugs, antibodies, nanoparticles, and cellular treatments to treat oncological patients. Taken together, we suggest

the full characterization of phenotypes and capabilities of CSCs in patients, a cellular component responsible for tumor progression, tumor relapse and metastasis.

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