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Toledo-Guzmán ME *et al*. CSC impact on clinical oncology

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**Abstract**

Cancer is a chronic disease widespread around the world. In most cases, the high rate of mortality 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 phenotype. Interestingly, the presence of cancer stem cells (CSCs), which is found as 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 among others, highlighting those 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, to be used as a tool for anti-tumoral therapy treatment design. We also review the potential effect of nanoparticles, drugs, natural compounds, ALDH 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 in order to improve oncologic patients’ outcome.

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

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**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 responsible, in part, for recurrence and tumor progression in patients. 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 fort 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) are 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 by heterogeneous cell populations[1-3], with different phenotypic characteristics at different stages of development, and posses variable ability to proliferate. However, only the CSC population is clonogenic *in vitro* and *in vivo*, and so, these cells are considered to be the only ones with 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 70s[6,7]. In both cancer types, only a cell population derived from a tumor was able to grow in clonogenic assays, forming spherical colonies, and induce tumors that recapitulated the original one in mice. 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 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, in order 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, relapsing is a main problem due to sparing of CSCs[22,25,26].

**CSC GENERALITIES**

A CSC is defined as a cell within a tumor that is able to produce an identical cell, or self-renew, give rise to heterogeneous differentiated progeny, and has the ability to modulate differentiation and self-renewal (homeostatic control). These CSCs posses 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 deregulated in CSCs and targeting them is proposed as a strategy for increasing the effectiveness of cancer therapies.

The CSC model postulates that solid tumors and leukemia are hierarchically organized, with CSCs in 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 lesions 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 mice 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, where CSCs and non-CSCs could alternate between two phenotypic states, has been proposed[36]. In this 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 although the CSC model is widely reported in several cancer types (Figure 1), there are also a few publications about cancers that do not follow a CSC model or a dynamic CSC model, specifically in leukemia and lymphoma mice models[37] and melanoma[32], where the tumors are homogeneous. In 2007, Strasser and his group inoculated 10 to 105 pre-B/B lymphoma cells into recipient mice. All 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 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 maintain 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 a 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, focusing mainly 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 on 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, 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 mice models, which must be able to recapitulate the original heterogeneous populations and must be validated directly 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 or 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. Dirks and his group used the CSC marker CD133 for brain CSC identification. The purified CD133+ population coming 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 a few CD133+ cells produced a tumor and was successfully transplanted[11]. In 2013, Pelicci and her group reported that CD133 is 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 authors propose that CD133 could maintain CSC properties through 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 like flow cytometry and serial xenotransplantation in mice[41]. Additionally, CD133+ CSCs have been reported in many other solid cancer models, for instance, 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], among others.

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, mainly for the identification of breast CSCs[5]. CD24 is a small surface protein that is found in many tumor types, however, cancer cell line reports suggest that there is a substantial variation in CD24 expression even among 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 cervical cancer, where cells with CSC characteristics were isolated from the HPV-16 SiHa cell line using the markers CD44+CD24+, which were, above all, radioresistant and more tumorigenic[55]. The same phenotype CD44+CD24+ 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 phenotype CD44+/α2β1hi/CD133+ were identified and characterized[57]. The next year, CD44+ tumor prostate cancer cell populations were obtained[58]. Also, the presence of CD44 and CD133 was evaluated in gastric adenocarcinoma tumors by immunohistochemistry and it found that both markers could be correlated with clinical and pathological parameters[51].

Although CD44 is widely reported as a CSC marker, we consider that it is very important to highlight that it is an ubiquitously expressed molecule that derives 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 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 CD44high (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 proved that the EMT CD44high cells preferably expressed the CD44s isoform; while the epithelial CD44high cells expressed the CD44v isoform. Another group using RNAseq 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 of Mackenzie’s group 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], among others.

CD49f or integrin α6, is a transmembrane glycoprotein that functions as the receptor for the extracellular matrix (ECM) 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, CD49high 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 CSCs 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’s 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 its activity measurement using the commercial ALDH fluorescent substrate ALDEFLUOR® kit assay by Stem Cells Technologies, Inc., Vancouver, BC, Canada. Cells that display high ALDH activity, (named ALDHhigh or ALDH+ or ALDHbr), can be identified and isolated using flow cytometry[79]. Several works have shown that a high ALDH activity is often associated to CSCs derived from solid tumor types[80]. These cells are generally characterized by a higher proliferation potential, colony-forming capacity, self-renewal, posses more tumorigenic capacity *in vivo*, metastasis, and drug resistance. For instance, ALDHhigh 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 in order 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 are found 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, such is the case of those that possess cells that are more resistant than the rest of the tumor cells. 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 cell drug elimination, mostly into the extracellular space, and they have been found overexpressed in CSCs, using side population (SP) assays[41,98–100].

Additionally, a high ALDH activity is directly related to a higher resistance to several drugs, for example, cyclophosphamide, temozolomide, irinotecan, paclitaxel, and doxorubicin[101–103]. The 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, ALDH activity inhibition leads to a reduction in chemo resistance to doxorubicin and paclitaxel in cells that are ALDHhigh CD44+[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 higher percentage of CSCs[28].

**CSC IDENTIFIED IN PATIENTS: PHENOTYPE AND TYPE OF STUDIES**

Most publications about CSC identification have been carried out in cell lines; however, in this section we will mention those where CSCs were identified in patient samples.

CD133 was analyzed in a meta-analysis using 32 studies about NSCLC 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 phenotype CD44+CD54+[108]. Nevertheless, in another study, sorted CD133+/CD44+ cells 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, expression of ALDH and CD44/CD24 CSC markers was correlated with response to endocrine therapy and clinical outcome but no statistically significant correlation was found[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 resume 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 in pancreatic ductal adenocarcinoma (PDA) it’s upregulated, specifically in PDA CD44+/CD24+/ESA+CSCs. In a phase I study, 68 patients were treated with GDC-0449 or Vismodegib, an antagonist that inhibits the Hh signaling pathway[112], alone or in combination with gemcitabine. GDC-0449 was able to inhibit 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, δ–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 of them is Celecoxib, a non-steroidal anti-inflammatory drug that inhibits β-catenin signaling by cyclo-oxygenase (COX)-dependent and COX-independent mechanisms[116]. This drug downregulates CD133 expression of colon cancer cells through inhibition of Wnt signaling[119] as well as 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, as a consequence, cells become more sensitive to radiation[121]. A recent work 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 with FOLFOX chemotherapy inhibits colorectal CSCs in *ex vivo* models[126].

An interesting strategy is to target CSCs using nanoparticles in order to reduce side effects on surrounding normal cells. In 2015, construction of glucose-coated gold nanoparticles (Glu-GNPs) that use glucose to facilitate GNP entry to leukemic stem cells that overexpress CD44 (TH1-P) was reported. Leukemic cells were cultured one hour without glucose for better Glu-GNP uptake and then X-ray irradiation tests were performed. Results showed that Glu-GNPs enhanced cell death when compared to irradiation and GNPs alone[127]. Formulated mangostin-encapsulated PLGA 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]. Nanoparticles of salinomycin and paclitaxel are also being used to eliminate CD44 breast CSCs together with breast cancer cells[129].

Interestingly, CSCs have a strict dependence on mitochondrial biogenesis. Five classes of FDA-approved antibiotics that inhibit mitochondrial biogenesis were used on 8 cell lines of different tumor types and the results suggested that 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 the effects are enhanced when in combination with doxorubicin[132]; moreover, it has been observed that metformin reduces metastasis by targeting both EMT 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 increases 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, specially in VEGF-resistant tumors in human phase I studies[137]. Furthermore, another anti-DLL4 (Demcizumab) is effective in decreasing tumor size but produces hypertension[138]. In patients with colon cancer, increased levels of blood progastrin 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/Tcd4. Based on this information, specific anti-progastrin antibodies were 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 differentiation of tumor cells *in vivo*[139]. H90 is a mouse IgG1 mAb anti-human CD44 that directly targets CSCs inducing differentiation and proliferation in AML xenograft mice 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 or 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], in gastric cancer inhibiting tumor growth[148] and in head and neck cancer suppressing Wnt/β-catenin[149]. In a phaseI/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 shows anti-cancer activity *in vitro* and *in vivo*, further potentiating 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 targets for cancer therapy, due to CSC 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 with tumor resistance in oncologic patients. It is known that the de-regulated cell signaling pathways are partially responsible for maintaining stemness of CSCs. In consequence, Wnt, Notch and Hh cell 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 patients with cancer. 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 promote the characterization of full phenotype and capabilities of real CSCs in patients, a cellular component responsible for tumor progression, tumor relapse and metastasis.

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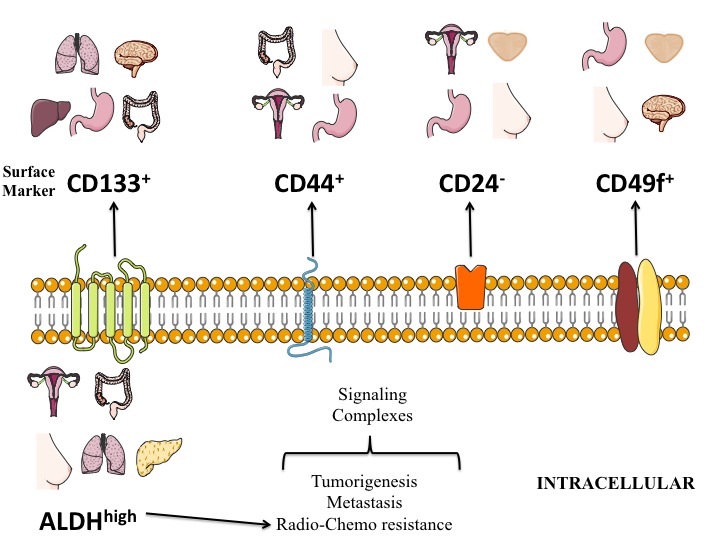
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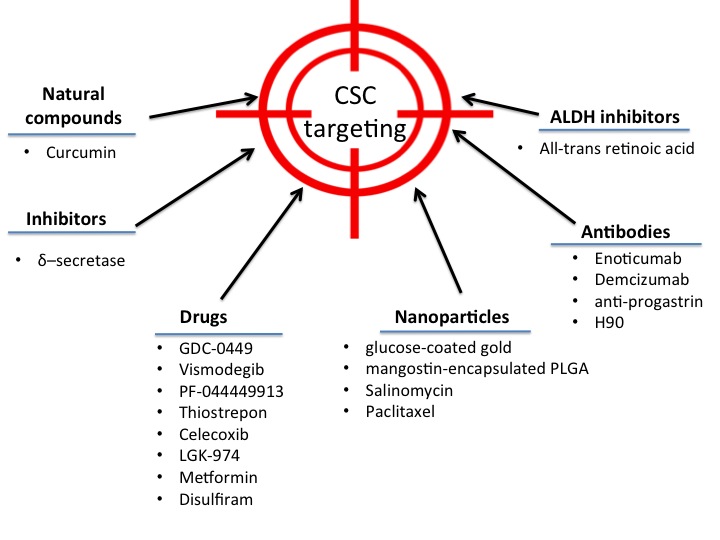
**Table 1 Cancer stem cells markers in solid tumors**

|  |  |  |  |
| --- | --- | --- | --- |
| **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 lineand tumor xenograft in mice | [159] |
| Breast cancer | ALDH | Breast cancer tumor tissues | [160] |

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

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**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, is represented in CSCs derived from ovarian carcinoma, colon carcinoma, breast, lung and liver cancer. The CSC markers shown (they are not the only ones) have a specific and relevant function in the high tumorigenic capacity of CSCs, metastasis, and resistance to radio and chemotherapy. CSCs: Cancer stem cells; ADLH: Aldehyde dehydrogenase.



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