**Name of Journal:** *World Journal of Stem Cells*

**Manuscript NO:** 42244

**Manuscript Type:** MINIREVIEWS

**Cancer stem cell impact on clinical oncology**

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

Mariel E Toledo-Guzmán, Gabriele D Bigoni-Ordóñez, Miguel Ibáñez-Hernández, Elizabeth Ortiz-Sánchez

**Mariel E Toledo-Guzmán, Gabriele D Bigoni-Ordóñez, Elizabeth Ortiz-Sánchez,** Subdirección de Investigación Básica, Instituto Nacional de Cancerología, Mexico City 14080, Mexico

**Mariel E Toledo-Guzmán, Miguel Ibáñez-Hernández,** Departamento de Bioquímica, Laboratorio de Terapia Génica, Escuela Nacional de Ciencias Biológicas, Posgrado de Biomedicina y Biotecnología Molecular, Instituto Politécnico Nacional, Mexico City 11340, Mexico

**ORCID number:** Mariel E Toledo-Guzmán (0000-0003-4992-8486); Gabriele D Bigoni-Ordóñez (0000-0003-2091-6107); Miguel Ibáñez-Hernández (0000-0003-4013-6888); Elizabeth Ortiz-Sánchez (0000-0001-9855-9491).

**Author contributions**:Toledo-Guzmán ME participated in the conception and writing of the manuscript; Bigoni-Ordóñez GD generated the figures; Ibáñez-Hernández M reviewed and suggested modifications to the content; Ortiz-Sánchez E designed the aim of the editorial, participated in the conception and contributed to the writing of the manuscript.

**Supported by** Institutional funding at Instituto Nacional de Cancerología.

**Conflict-of-interest statement:** The authors declare no conflict of interest.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Corresponding author: Elizabeth Ortiz-Sánchez, MD, PhD, Academic Research, Professor, Research Scientist, Senior Researcher,** Subdirección de Investigación Básica, Instituto Nacional de Cancerología, Av San Fernando 22, Colonia Sección XVI, Mexico City 14080, Mexico. elinfkb@yahoo.com.mx

**Telephone:** +52-55-54280400

**Received:** September 13, 2018

**Peer-review started:** September 13, 2018

**First decision:** October 5, 2018

**Revised:** October 15, 2018

**Accepted:** November 15, 2018

**Article in press:** November 16, 2018

**Published online:** December 26, 2018

**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; Cancer stem cells; Clinical outcome; Targeted therapy; Drug resistance

**© The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

**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.

**Citation**: Toledo-Guzmán ME, Bigoni-Ordóñez GD, Ibáñez-Hernández M, Ortiz-Sánchez E. Cancer stem cell impact on clinical oncology. *World J Stem Cells* 2018; 10(12): 183-195

**URL**: https://www.wjgnet.com/1948-0210/full/v10/i12/183.htm

**DOI**: https://dx.doi.org/10.4252/wjsc.v10.i12.183

**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 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 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, or self-renew, give rise to 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 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 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 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 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, 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 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+/β2β1hi/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 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 demonstrated that the CD44high cells that undergo EMT preferably expressed the CD44s isoform; while the epithelial CD44high 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, 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 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 ALDHhigh or ALDH+ or ALDHbr), 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, 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 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 ALDHhigh 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 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 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 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.

**ACKNOWLEDGEMENTS**

The authors thank Marco Antonio Meraz Rodríguez for his constructive suggestions and Dr. Elizabeth Langley McCarron for her editing and Instituto Politécnico Nacional, Posgrado de Biomedicina y Biotecnología Molecular.

**REFERENCES**

1 **Lapidot T**, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994; **367**: 645-648 [PMID: 7509044 DOI: 10.1038/367645a0]

2 **Clarke MF**, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL, Wahl GM. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res* 2006; **66**: 9339-9344 [PMID: 16990346 DOI: 10.1158/0008-5472.CAN-06-3126]

3 **Dalerba P**, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med* 2007; **58**: 267-284 [PMID: 17002552 DOI: 10.1146/annurev.med.58.062105.204854]

4 **Reya T**, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105-111 [PMID: 11689955 DOI: 10.1038/35102167]

5 **Al-Hajj M**, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003; **100**: 3983-3988 [PMID: 12629218 DOI: 10.1073/pnas.0530291100]

6 **Park CH**, Bergsagel DE, McCulloch EA. Mouse myeloma tumor stem cells: a primary cell culture assay. *J Natl Cancer Inst* 1971; **46**: 411-422 [PMID: 5115909]

7 **Hamburger AW**, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977; **197**: 461-463 [PMID: 560061]

8 **Bonnet D**, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; **3**: 730-737 [PMID: 9212098]

9 **Hemmati HD**, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci U S A* 2003; **100**: 15178-15183 [PMID: 14645703 DOI: 10.1073/pnas.2036535100]

10 **Singh SK**, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003; **63**: 5821-5828 [PMID: 14522905]

11 **Singh SK**, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004; **432**: 396-401 [PMID: 15549107 DOI: 10.1038/nature03128]

12 **Ginestier C**, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007; **1**: 555-567 [PMID: 18371393 DOI: 10.1016/j.stem.2007.08.014]

13 **Greve B**, Kelsch R, Spaniol K, Eich HT, Götte M. Flow cytometry in cancer stem cell analysis and separation. *Cytometry A* 2012; **81**: 284-293 [PMID: 22311742 DOI: 10.1002/cyto.a.22022]

14 **Rao QX**, Yao TT, Zhang BZ, Lin RC, Chen ZL, Zhou H, Wang LJ, Lu HW, Chen Q, Di N, Lin ZQ. Expression and functional role of ALDH1 in cervical carcinoma cells. *Asian Pac J Cancer Prev* 2012; **13**: 1325-1331 [PMID: 22799327]

15 **Shackleton M**. Normal stem cells and cancer stem cells: similar and different. *Semin Cancer Biol* 2010; **20**: 85-92 [PMID: 20435143 DOI: 10.1016/j.semcancer.2010.04.002]

16 **Yu SC**, Ping YF, Yi L, Zhou ZH, Chen JH, Yao XH, Gao L, Wang JM, Bian XW. Isolation and characterization of cancer stem cells from a human glioblastoma cell line U87. *Cancer Lett* 2008; **265**: 124-134 [PMID: 18343028 DOI: 10.1016/j.canlet.2008.02.010]

17 **Qiang L**, Yang Y, Ma YJ, Chen FH, Zhang LB, Liu W, Qi Q, Lu N, Tao L, Wang XT, You QD, Guo QL. Isolation and characterization of cancer stem like cells in human glioblastoma cell lines. *Cancer Lett* 2009; **279**: 13-21 [PMID: 19232461 DOI: 10.1016/j.canlet.2009.01.016]

18 **Brescia P**, Ortensi B, Fornasari L, Levi D, Broggi G, Pelicci G. CD133 is essential for glioblastoma stem cell maintenance. *Stem Cells* 2013; **31**: 857-869 [PMID: 23307586 DOI: 10.1002/stem.1317]

19 **Wang L**, Guo H, Lin C, Yang L, Wang X. Enrichment and characterization of cancer stem‑like cells from a cervical cancer cell line. *Mol Med Rep* 2014; **9**: 2117-2123 [PMID: 24676900 DOI: 10.3892/mmr.2014.2063]

20 **Fillmore CM**, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. *Breast Cancer Res* 2008; **10**: R25 [PMID: 18366788 DOI: 10.1186/bcr1982]

21 **Bertolini G**, Roz L, Perego P, Tortoreto M, Fontanella E, Gatti L, Pratesi G, Fabbri A, Andriani F, Tinelli S, Roz E, Caserini R, Lo Vullo S, Camerini T, Mariani L, Delia D, Calabrò E, Pastorino U, Sozzi G. Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. *Proc Natl Acad Sci U S A* 2009; **106**: 16281-16286 [PMID: 19805294 DOI: 10.1073/pnas.0905653106]

22 **Li X**, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, Hilsenbeck SG, Pavlick A, Zhang X, Chamness GC, Wong H, Rosen J, Chang JC. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst* 2008; **100**: 672-679 [PMID: 18445819 DOI: 10.1093/jnci/djn123]

23 **Liao J**, Qian F, Tchabo N, Mhawech-Fauceglia P, Beck A, Qian Z, Wang X, Huss WJ, Lele SB, Morrison CD, Odunsi K. Ovarian cancer spheroid cells with stem cell-like properties contribute to tumor generation, metastasis and chemotherapy resistance through hypoxia-resistant metabolism. *PLoS One* 2014; **9**: e84941 [PMID: 24409314 DOI: 10.1371/journal.pone.0084941]

24 **Bao S**, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006; **444**: 756-760 [PMID: 17051156 DOI: 10.1038/nature05236]

25 **Clarke MF**. A self-renewal assay for cancer stem cells. *Cancer Chemother Pharmacol* 2005; **56 Suppl 1**: 64-68 [PMID: 16273355 DOI: 10.1007/s00280-005-0097-1]

26 **Eyler CE**, Rich JN. Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis. *J Clin Oncol* 2008; **26**: 2839-2845 [PMID: 18539962 DOI: 10.1200/JCO.2007.15.1829]

27 **Gupta PB**, Chaffer CL, Weinberg RA. Cancer stem cells: mirage or reality? *Nat Med* 2009; **15**: 1010-1012 [PMID: 19734877 DOI: 10.1038/nm0909-1010]

28 **Ajani JA**, Song S, Hochster HS, Steinberg IB. Cancer stem cells: the promise and the potential. *Semin Oncol* 2015; **42 Suppl 1**: S3-17 [PMID: 25839664 DOI: 10.1053/j.seminoncol.2015.01.001]

29 **Vlashi E**, Pajonk F. Cancer stem cells, cancer cell plasticity and radiation therapy. *Semin Cancer Biol* 2015; **31**: 28-35 [PMID: 25025713 DOI: 10.1016/j.semcancer.2014.07.001]

30 **Visvader JE**. Cells of origin in cancer. *Nature* 2011; **469**: 314-322 [PMID: 21248838 DOI: 10.1038/nature09781]

31 **Ishizawa K**, Rasheed ZA, Karisch R, Wang Q, Kowalski J, Susky E, Pereira K, Karamboulas C, Moghal N, Rajeshkumar NV, Hidalgo M, Tsao M, Ailles L, Waddell TK, Maitra A, Neel BG, Matsui W. Tumor-initiating cells are rare in many human tumors. *Cell Stem Cell* 2010; **7**: 279-282 [PMID: 20804964 DOI: 10.1016/j.stem.2010.08.009]

32 **Quintana E**, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, Morrison SJ. Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer Cell* 2010; **18**: 510-523 [PMID: 21075313 DOI: 10.1016/j.ccr.2010.10.012]

33 **Visvader JE**, Lindeman GJ. Cancer stem cells: current status and evolving complexities. *Cell Stem Cell* 2012; **10**: 717-728 [PMID: 22704512 DOI: 10.1016/j.stem.2012.05.007]

34 **Eppert K**, Takenaka K, Lechman ER, Waldron L, Nilsson B, van Galen P, Metzeler KH, Poeppl A, Ling V, Beyene J, Canty AJ, Danska JS, Bohlander SK, Buske C, Minden MD, Golub TR, Jurisica I, Ebert BL, Dick JE. Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat Med* 2011; **17**: 1086-1093 [PMID: 21873988 DOI: 10.1038/nm.2415]

35 **Visvader JE**, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008; **8**: 755-768 [PMID: 18784658 DOI: 10.1038/nrc2499]

36 **Khan IN**, Al-Karim S, Bora RS, Chaudhary AG, Saini KS. Cancer stem cells: a challenging paradigm for designing targeted drug therapies. *Drug Discov Today* 2015; **20**: 1205-1216 [PMID: 26143148 DOI: 10.1016/j.drudis.2015.06.013]

37 **Kelly PN**, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. *Science* 2007; **317**: 337 [PMID: 17641192 DOI: 10.1126/science.1142596]

38 **Alvarado-Ortiz E**, Sarabia-Sanchez MA, Garcia-Carranca A. Molecular mechanisms underlying the functions of cellular markers associated with the phenotype of Cancer Stem Cells. *Curr Stem Cell Res Ther* 2018; [PMID: 30147013 DOI: 10.2174/1574888X13666180821154752]

39 **Ren F**, Sheng WQ, Du X. CD133: a cancer stem cells marker, is used in colorectal cancers. *World J Gastroenterol* 2013; **19**: 2603-2611 [PMID: 23674867 DOI: 10.3748/wjg.v19.i17.2603]

40 **Lin L**, Fuchs J, Li C, Olson V, Bekaii-Saab T, Lin J. STAT3 signaling pathway is necessary for cell survival and tumorsphere forming capacity in ALDH⁺/CD133⁺ stem cell-like human colon cancer cells. *Biochem Biophys Res Commun* 2011; **416**: 246-251 [PMID: 22074823 DOI: 10.1016/j.bbrc.2011.10.112]

41 **Catalano V**, Di Franco S, Iovino F, Dieli F, Stassi G, Todaro M. CD133 as a target for colon cancer. *Expert Opin Ther Targets* 2012; **16**: 259-267 [PMID: 22385077 DOI: 10.1517/14728222.2012.667404]

42 **Nakamura M**, Zhang X, Mizumoto Y, Maida Y, Bono Y, Takakura M, Kyo S. Molecular characterization of CD133+ cancer stem-like cells in endometrial cancer. *Int J Oncol* 2014; **44**: 669-677 [PMID: 24366104 DOI: 10.3892/ijo.2013.2230]

43 **Wang S**, Xu ZY, Wang LF, Su W. CD133+ cancer stem cells in lung cancer. *Front Biosci (Landmark Ed)* 2013; **18**: 447-453 [PMID: 23276935]

44 **Sarvi S**, Mackinnon AC, Avlonitis N, Bradley M, Rintoul RC, Rassl DM, Wang W, Forbes SJ, Gregory CD, Sethi T. CD133+ cancer stem-like cells in small cell lung cancer are highly tumorigenic and chemoresistant but sensitive to a novel neuropeptide antagonist. *Cancer Res* 2014; **74**: 1554-1565 [PMID: 24436149 DOI: 10.1158/0008-5472.CAN-13-1541]

45 **Wu CP**, Du HD, Gong HL, Li DW, Tao L, Tian J, Zhou L. Hypoxia promotes stem-like properties of laryngeal cancer cell lines by increasing the CD133+ stem cell fraction. *Int J Oncol* 2014; **44**: 1652-1660 [PMID: 24573690 DOI: 10.3892/ijo.2014.2307]

46 **Zhang H**, Yu T, Wen L, Wang H, Fei D, Jin C. Curcumin enhances the effectiveness of cisplatin by suppressing CD133+ cancer stem cells in laryngeal carcinoma treatment. *Exp Ther Med* 2013; **6**: 1317-1321 [PMID: 24223665 DOI: 10.3892/etm.2013.1297]

47 **Piao LS**, Hur W, Kim TK, Hong SW, Kim SW, Choi JE, Sung PS, Song MJ, Lee BC, Hwang D, Yoon SK. CD133+ liver cancer stem cells modulate radioresistance in human hepatocellular carcinoma. *Cancer Lett* 2012; **315**: 129-137 [PMID: 22079466 DOI: 10.1016/j.canlet.2011.10.012]

48 **Abbasian M**, Mousavi E, Arab-Bafrani Z, Sahebkar A. The most reliable surface marker for the identification of colorectal cancer stem-like cells: A systematic review and meta-analysis. *J Cell Physiol* 2018; [PMID: 30317669 DOI: 10.1002/jcp.27619]

49 **Lee HH**, Seo KJ, An CH, Kim JS, Jeon HM. CD133 expression is correlated with chemoresistance and early recurrence of gastric cancer. *J Surg Oncol* 2012; **106**: 999-1004 [PMID: 22674531 DOI: 10.1002/jso.23178]

50 **Wang Y**, Chen M, Wu Z, Tong C, Dai H, Guo Y, Liu Y, Huang J, Lv H, Luo C, Feng KC, Yang QM, Li XL, Han W. CD133-directed CAR T cells for advanced metastasis malignancies: A phase I trial. *Oncoimmunology* 2018; **7**: e1440169 [PMID: 29900044 DOI: 10.1080/2162402X.2018.1440169]

51 **Nosrati A**, Naghshvar F, Khanari S. Cancer Stem Cell Markers CD44, CD133 in Primary Gastric Adenocarcinoma. *Int J Mol Cell Med* 2014; **3**: 279-286 [PMID: 25635255]

52 **Yan Y**, Zuo X, Wei D. Concise Review: Emerging Role of CD44 in Cancer Stem Cells: A Promising Biomarker and Therapeutic Target. *Stem Cells Transl Med* 2015; **4**: 1033-1043 [PMID: 26136504 DOI: 10.5966/sctm.2015-0048]

53 **Jaggupilli A**, Elkord E. Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity. *Clin Dev Immunol* 2012; **2012**: 708036 [PMID: 22693526 DOI: 10.1155/2012/708036]

54 **Yang CH**, Wang HL, Lin YS, Kumar KP, Lin HC, Chang CJ, Lu CC, Huang TT, Martel J, Ojcius DM, Chang YS, Young JD, Lai HC. Identification of CD24 as a cancer stem cell marker in human nasopharyngeal carcinoma. *PLoS One* 2014; **9**: e99412 [PMID: 24955581 DOI: 10.1371/journal.pone.0099412]

55 **Liu H**, Wang YJ, Bian L, Fang ZH, Zhang QY, Cheng JX. CD44+/CD24+ cervical cancer cells resist radiotherapy and exhibit properties of cancer stem cells. *Eur Rev Med Pharmacol Sci* 2016; **20**: 1745-1754 [PMID: 27212166]

56 **Zhang C**, Li C, He F, Cai Y, Yang H. Identification of CD44+CD24+ gastric cancer stem cells. *J Cancer Res Clin Oncol* 2011; **137**: 1679-1686 [PMID: 21882047 DOI: 10.1007/s00432-011-1038-5]

57 **Collins AT**, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; **65**: 10946-10951 [PMID: 16322242 DOI: 10.1158/0008-5472.CAN-05-2018]

58 **Patrawala L**, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, Reilly JG, Chandra D, Zhou J, Claypool K, Coghlan L, Tang DG. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* 2006; **25**: 1696-1708 [PMID: 16449977 DOI: 10.1038/sj.onc.1209327]

59 **Prochazka L**, Tesarik R, Turanek J. Regulation of alternative splicing of CD44 in cancer. *Cell Signal* 2014; **26**: 2234-2239 [PMID: 25025570 DOI: 10.1016/j.cellsig.2014.07.011]

60 **Biddle A**, Liang X, Gammon L, Fazil B, Harper LJ, Emich H, Costea DE, Mackenzie IC. Cancer stem cells in squamous cell carcinoma switch between two distinct phenotypes that are preferentially migratory or proliferative. *Cancer Res* 2011; **71**: 5317-5326 [PMID: 21685475 DOI: 10.1158/0008-5472.CAN-11-1059]

61 **Hernandez JR**, Kim JJ, Verdone JE, Liu X, Torga G, Pienta KJ, Mooney SM. Alternative CD44 splicing identifies epithelial prostate cancer cells from the mesenchymal counterparts. *Med Oncol* 2015; **32**: 159 [PMID: 25850653 DOI: 10.1007/s12032-015-0593-z]

62 **Biddle A**, Gammon L, Fazil B, Mackenzie IC. CD44 staining of cancer stem-like cells is influenced by down-regulation of CD44 variant isoforms and up-regulation of the standard CD44 isoform in the population of cells that have undergone epithelial-to-mesenchymal transition. *PLoS One* 2013; **8**: e57314 [PMID: 23437366 DOI: 10.1371/journal.pone.0057314]

63 **Inoue K**, Fry EA. Aberrant Splicing of Estrogen Receptor, HER2, and CD44 Genes in Breast Cancer. *Genet Epigenet* 2015; **7**: 19-32 [PMID: 26692764 DOI: 10.4137/GEG.S35500]

64 **Zöller M**. CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat Rev Cancer* 2011; **11**: 254-267 [PMID: 21390059 DOI: 10.1038/nrc3023]

65 **Lobo NA**, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. *Annu Rev Cell Dev Biol* 2007; **23**: 675-699 [PMID: 17645413 DOI: 10.1146/annurev.cellbio.22.010305.104154]

66 **Zhang L**, Zheng W, Wang Y, Wang Y, Huang H. Human bone marrow mesenchymal stem cells support the derivation and propagation of human induced pluripotent stem cells in culture. *Cell Reprogram* 2013; **15**: 216-223 [PMID: 23713432 DOI: 10.1089/cell.2012.0064]

67 **Jing F**, Kim HJ, Kim CH, Kim YJ, Lee JH, Kim HR. Colon cancer stem cell markers CD44 and CD133 in patients with colorectal cancer and synchronous hepatic metastases. *Int J Oncol* 2015; **46**: 1582-1588 [PMID: 25625240 DOI: 10.3892/ijo.2015.2844]

68 **Joshua B**, Kaplan MJ, Doweck I, Pai R, Weissman IL, Prince ME, Ailles LE. Frequency of cells expressing CD44, a head and neck cancer stem cell marker: correlation with tumor aggressiveness. *Head Neck* 2012; **34**: 42-49 [PMID: 21322081 DOI: 10.1002/hed.21699]

69 **Faber A**, Barth C, Hörmann K, Kassner S, Schultz JD, Sommer U, Stern-Straeter J, Thorn C, Goessler UR. CD44 as a stem cell marker in head and neck squamous cell carcinoma. *Oncol Rep* 2011; **26**: 321-326 [PMID: 21617876 DOI: 10.3892/or.2011.1322]

70 **Meng E**, Long B, Sullivan P, McClellan S, Finan MA, Reed E, Shevde L, Rocconi RP. CD44+/CD24- ovarian cancer cells demonstrate cancer stem cell properties and correlate to survival. *Clin Exp Metastasis* 2012; **29**: 939-948 [PMID: 22610780 DOI: 10.1007/s10585-012-9482-4]

71 **Lau WM**, Teng E, Chong HS, Lopez KA, Tay AY, Salto-Tellez M, Shabbir A, So JB, Chan SL. CD44v8-10 is a cancer-specific marker for gastric cancer stem cells. *Cancer Res* 2014; **74**: 2630-2641 [PMID: 24618343 DOI: 10.1158/0008-5472.CAN-13-2309]

72 **Watt FM**. Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J* 2002; **21**: 3919-3926 [PMID: 12145193 DOI: 10.1093/emboj/cdf399]

73 **Lathia JD**, Gallagher J, Heddleston JM, Wang J, Eyler CE, Macswords J, Wu Q, Vasanji A, McLendon RE, Hjelmeland AB, Rich JN. Integrin alpha 6 regulates glioblastoma stem cells. *Cell Stem Cell* 2010; **6**: 421-432 [PMID: 20452317 DOI: 10.1016/j.stem.2010.02.018]

74 **Yamamoto H**, Masters JR, Dasgupta P, Chandra A, Popert R, Freeman A, Ahmed A. CD49f is an efficient marker of monolayer- and spheroid colony-forming cells of the benign and malignant human prostate. *PLoS One* 2012; **7**: e46979 [PMID: 23071686 DOI: 10.1371/journal.pone.0046979]

75 **Fukamachi H**, Seol HS, Shimada S, Funasaka C, Baba K, Kim JH, Park YS, Kim MJ, Kato K, Inokuchi M, Kawachi H, Yook JH, Eishi Y, Kojima K, Kim WH, Jang SJ, Yuasa Y. CD49f(high) cells retain sphere-forming and tumor-initiating activities in human gastric tumors. *PLoS One* 2013; **8**: e72438 [PMID: 24015244 DOI: 10.1371/journal.pone.0072438]

76 **Ye F**, Zhong X, Qiu Y, Yang L, Wei B, Zhang Z, Bu H. CD49f Can Act as a Biomarker for Local or Distant Recurrence in Breast Cancer. *J Breast Cancer* 2017; **20**: 142-149 [PMID: 28690650 DOI: 10.4048/jbc.2017.20.2.142]

77 **Haraguchi N**, Ishii H, Mimori K, Ohta K, Uemura M, Nishimura J, Hata T, Takemasa I, Mizushima T, Yamamoto H, Doki Y, Mori M. CD49f-positive cell population efficiently enriches colon cancer-initiating cells. *Int J Oncol* 2013; **43**: 425-430 [PMID: 23708747 DOI: 10.3892/ijo.2013.1955]

78 **Sládek NE**. Human aldehyde dehydrogenases: potential pathological, pharmacological, and toxicological impact. *J Biochem Mol Toxicol* 2003; **17**: 7-23 [PMID: 12616643 DOI: 10.1002/jbt.10057]

79 **Storms RW**, Trujillo AP, Springer JB, Shah L, Colvin OM, Ludeman SM, Smith C. Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. *Proc Natl Acad Sci U S A* 1999; **96**: 9118-9123 [PMID: 10430905]

80 **Toledo-Guzmán ME**, Ibañez Hernández M, Gomez-Gallegos AA, Ortiz-Sánchez E. ALDH as a Stem Cell marker in solid tumors. *Curr Stem Cell Res Ther* 2018; [PMID: 30095061 DOI: 10.2174/1574888X13666180810120012]

81 **Huang EH**, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, Fields JZ, Wicha MS, Boman BM. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res* 2009; **69**: 3382-3389 [PMID: 19336570 DOI: 10.1158/0008-5472.CAN-08-4418]

82 **Shenoy A**, Butterworth E, Huang EH. ALDH as a marker for enriching tumorigenic human colonic stem cells. *Methods Mol Biol* 2012; **916**: 373-385 [PMID: 22914954 DOI: 10.1007/978-1-61779-980-8\_27]

83 **Jiang F**, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, Wang H, Liu Z, Su Y, Stass SA, Katz RL. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Mol Cancer Res* 2009; **7**: 330-338 [PMID: 19276181 DOI: 10.1158/1541-7786.MCR-08-0393]

84 **Liu SY**, Zheng PS. High aldehyde dehydrogenase activity identifies cancer stem cells in human cervical cancer. *Oncotarget* 2013; **4**: 2462-2475 [PMID: 24318570 DOI: 10.18632/oncotarget.1578]

85 **Ortiz-Sánchez E**, Santiago-López L, Cruz-Domínguez VB, Toledo-Guzmán ME, Hernández-Cueto D, Muñiz-Hernández S, Garrido E, Cantú De León D, García-Carrancá A. Characterization of cervical cancer stem cell-like cells: phenotyping, stemness, and human papilloma virus co-receptor expression. *Oncotarget* 2016; **7**: 31943-31954 [PMID: 27008711 DOI: 10.18632/oncotarget.8218]

86 **Marcato P**, Dean CA, Pan D, Araslanova R, Gillis M, Joshi M, Helyer L, Pan L, Leidal A, Gujar S, Giacomantonio CA, Lee PW. Aldehyde dehydrogenase activity of breast cancer stem cells is primarily due to isoform ALDH1A3 and its expression is predictive of metastasis. *Stem Cells* 2011; **29**: 32-45 [PMID: 21280157 DOI: 10.1002/stem.563]

87 **Kim SK**, Kim H, Lee DH, Kim TS, Kim T, Chung C, Koh GY, Kim H, Lim DS. Reversing the intractable nature of pancreatic cancer by selectively targeting ALDH-high, therapy-resistant cancer cells. *PLoS One* 2013; **8**: e78130 [PMID: 24194908 DOI: 10.1371/journal.pone.0078130]

88 **Hoshino Y**, Nishida J, Katsuno Y, Koinuma D, Aoki T, Kokudo N, Miyazono K, Ehata S. Smad4 Decreases the Population of Pancreatic Cancer-Initiating Cells through Transcriptional Repression of ALDH1A1. *Am J Pathol* 2015; **185**: 1457-1470 [PMID: 25769430 DOI: 10.1016/j.ajpath.2015.01.011]

89 **Luo Y**, Nguyen N, Fujita M. Isolation of human melanoma stem cells using ALDH as a marker. *Curr Protoc Stem Cell Biol* 2013; **26**: Unit 3.8. [PMID: 24510792 DOI: 10.1002/9780470151808.sc0308s26]

90 **Luo Y**, Dallaglio K, Chen Y, Robinson WA, Robinson SE, McCarter MD, Wang J, Gonzalez R, Thompson DC, Norris DA, Roop DR, Vasiliou V, Fujita M. ALDH1A isozymes are markers of human melanoma stem cells and potential therapeutic targets. *Stem Cells* 2012; **30**: 2100-2113 [PMID: 22887839 DOI: 10.1002/stem.1193]

91 **Kryczek I**, Liu S, Roh M, Vatan L, Szeliga W, Wei S, Banerjee M, Mao Y, Kotarski J, Wicha MS, Liu R, Zou W. Expression of aldehyde dehydrogenase and CD133 defines ovarian cancer stem cells. *Int J Cancer* 2012; **130**: 29-39 [PMID: 21480217 DOI: 10.1002/ijc.25967]

92 **Silva IA**, Bai S, McLean K, Yang K, Griffith K, Thomas D, Ginestier C, Johnston C, Kueck A, Reynolds RK, Wicha MS, Buckanovich RJ. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. *Cancer Res* 2011; **71**: 3991-4001 [PMID: 21498635 DOI: 10.1158/0008-5472.CAN-10-3175]

93 **Mansour SF**, Atwa MM. Clinicopathological Significance of CD133 and ALDH1 Cancer Stem Cell Marker Expression in Invasive Ductal Breast Carcinoma. *Asian Pac J Cancer Prev* 2015; **16**: 7491-7496 [PMID: 26625750]

94 **Roudi R**, Korourian A, Shariftabrizi A, Madjd Z. Differential Expression of Cancer Stem Cell Markers ALDH1 and CD133 in Various Lung Cancer Subtypes. *Cancer Invest* 2015; **33**: 294-302 [PMID: 26046383 DOI: 10.3109/07357907.2015.1034869]

95 **Qiu Y**, Pu T, Guo P, Wei B, Zhang Z, Zhang H, Zhong X, Zheng H, Chen L, Bu H, Ye F. ALDH(+)/CD44(+) cells in breast cancer are associated with worse prognosis and poor clinical outcome. *Exp Mol Pathol* 2016; **100**: 145-150 [PMID: 26687806 DOI: 10.1016/j.yexmp.2015.11.032]

96 **Liu J**, Xiao Z, Wong SK, Tin VP, Ho KY, Wang J, Sham MH, Wong MP. Lung cancer tumorigenicity and drug resistance are maintained through ALDH(hi)CD44(hi) tumor initiating cells. *Oncotarget* 2013; **4**: 1698-1711 [PMID: 24091605 DOI: 10.18632/oncotarget.1246]

97 **Januchowski R**, Wojtowicz K, Zabel M. The role of aldehyde dehydrogenase (ALDH) in cancer drug resistance. *Biomed Pharmacother* 2013; **67**: 669-680 [PMID: 23721823 DOI: 10.1016/j.biopha.2013.04.005]

98 **Leonard GD**, Fojo T, Bates SE. The role of ABC transporters in clinical practice. *Oncologist* 2003; **8**: 411-424 [PMID: 14530494]

99 **Abdullah LN**, Chow EK. Mechanisms of chemoresistance in cancer stem cells. *Clin Transl Med* 2013; **2**: 3 [PMID: 23369605 DOI: 10.1186/2001-1326-2-3]

100 **Eyre R**, Harvey I, Stemke-Hale K, Lennard TW, Tyson-Capper A, Meeson AP. Reversing paclitaxel resistance in ovarian cancer cells via inhibition of the ABCB1 expressing side population. *Tumour Biol* 2014; **35**: 9879-9892 [PMID: 24993095 DOI: 10.1007/s13277-014-2277-2]

101 **Zhao J**. Cancer stem cells and chemoresistance: The smartest survives the raid. *Pharmacol Ther* 2016; **160**: 145-158 [PMID: 26899500 DOI: 10.1016/j.pharmthera.2016.02.008]

102 **Pearce DJ**, Taussig D, Simpson C, Allen K, Rohatiner AZ, Lister TA, Bonnet D. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. *Stem Cells* 2005; **23**: 752-760 [PMID: 15917471 DOI: 10.1634/stemcells.2004-0292]

103 **Tanei T**, Morimoto K, Shimazu K, Kim SJ, Tanji Y, Taguchi T, Tamaki Y, Noguchi S. Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential Paclitaxel and epirubicin-based chemotherapy for breast cancers. *Clin Cancer Res* 2009; **15**: 4234-4241 [PMID: 19509181 DOI: 10.1158/1078-0432.CCR-08-1479]

104 **Sreerama L**, Sladek NE. Cellular levels of class 1 and class 3 aldehyde dehydrogenases and certain other drug-metabolizing enzymes in human breast malignancies. *Clin Cancer Res* 1997; **3**: 1901-1914 [PMID: 9815579]

105 **Vasiliou V**, Pappa A, Estey T. Role of human aldehyde dehydrogenases in endobiotic and xenobiotic metabolism. *Drug Metab Rev* 2004; **36**: 279-299 [PMID: 15237855 DOI: 10.1081/DMR-120034001]

106 **Croker AK**, Allan AL. Inhibition of aldehyde dehydrogenase (ALDH) activity reduces chemotherapy and radiation resistance of stem-like ALDHhiCD44⁺ human breast cancer cells. *Breast Cancer Res Treat* 2012; **133**: 75-87 [PMID: 21818590 DOI: 10.1007/s10549-011-1692-y]

107 **Chen E**, Zeng Z, Bai B, Zhu J, Song Z. The prognostic value of CSCs biomarker CD133 in NSCLC: a meta-analysis. *Oncotarget* 2016; **7**: 56526-56539 [PMID: 27489355 DOI: 10.18632/oncotarget.10964]

108 **Chen T**, Yang K, Yu J, Meng W, Yuan D, Bi F, Liu F, Liu J, Dai B, Chen X, Wang F, Zeng F, Xu H, Hu J, Mo X. Identification and expansion of cancer stem cells in tumor tissues and peripheral blood derived from gastric adenocarcinoma patients. *Cell Res* 2012; **22**: 248-258 [PMID: 21727908 DOI: 10.1038/cr.2011.109]

109 **Rocco A**, Liguori E, Pirozzi G, Tirino V, Compare D, Franco R, Tatangelo F, Palaia R, D'Armiento FP, Pollastrone G, Affuso A, Bottazzi EC, Masone S, Persico G, Nardone G. CD133 and CD44 cell surface markers do not identify cancer stem cells in primary human gastric tumors. *J Cell Physiol* 2012; **227**: 2686-2693 [PMID: 21898409 DOI: 10.1002/jcp.23013]

110 **Singer CF**, Zabkova P, Rappaport C, Muhr D, Pfeiler G, Gschwantler-Kaulich D, Fink-Retter A, Staudigl C, Walter I, Hudelist G, Spiess AC, Kubista E. Presence of intratumoral stem cells in breast cancer patients with or without BRCA germline mutations. *Curr Cancer Drug Targets* 2012; **12**: 44-50 [PMID: 22111833]

111 **Hashimoto K**, Shimizu C, Tsuda H, Saji S, Osaki A, Shigekawa T, Aogi K. Immunohistochemical detection of breast cancer stem cells in hormone receptor-positive breast cancer and their role in response to endocrine therapy and clinical outcome. *Oncology* 2012; **82**: 168-174 [PMID: 22433454 DOI: 10.1159/000336078]

112 **Singh BN**, Fu J, Srivastava RK, Shankar S. Hedgehog signaling antagonist GDC-0449 (Vismodegib) inhibits pancreatic cancer stem cell characteristics: molecular mechanisms. *PLoS One* 2011; **6**: e27306 [PMID: 22087285 DOI: 10.1371/journal.pone.0027306]

113 **Kim EJ**, Sahai V, Abel EV, Griffith KA, Greenson JK, Takebe N, Khan GN, Blau JL, Craig R, Balis UG, Zalupski MM, Simeone DM. Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma. *Clin Cancer Res* 2014; **20**: 5937-5945 [PMID: 25278454 DOI: 10.1158/1078-0432.CCR-14-1269]

114 **Sadarangani A**, Pineda G, Lennon KM, Chun HJ, Shih A, Schairer AE, Court AC, Goff DJ, Prashad SL, Geron I, Wall R, McPherson JD, Moore RA, Pu M, Bao L, Jackson-Fisher A, Munchhof M, VanArsdale T, Reya T, Morris SR, Minden MD, Messer K, Mikkola HK, Marra MA, Hudson TJ, Jamieson CH. GLI2 inhibition abrogates human leukemia stem cell dormancy. *J Transl Med* 2015; **13**: 98 [PMID: 25889765 DOI: 10.1186/s12967-015-0453-9]

115 **Yang N**, Zhou TC, Lei XX, Wang C, Yan M, Wang ZF, Liu W, Wang J, Ming KH, Wang BC, Xu BL, Liu Q. Inhibition of Sonic Hedgehog Signaling Pathway by Thiazole Antibiotic Thiostrepton Attenuates the CD44+/CD24-Stem-Like Population and Sphere-Forming Capacity in Triple-Negative Breast Cancer. *Cell Physiol Biochem* 2016; **38**: 1157-1170 [PMID: 26963129 DOI: 10.1159/000443066]

116 **Takebe N**, Miele L, Harris PJ, Jeong W, Bando H, Kahn M, Yang SX, Ivy SP. Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat Rev Clin Oncol* 2015; **12**: 445-464 [PMID: 25850553 DOI: 10.1038/nrclinonc.2015.61]

117 **Krop I**, Demuth T, Guthrie T, Wen PY, Mason WP, Chinnaiyan P, Butowski N, Groves MD, Kesari S, Freedman SJ, Blackman S, Watters J, Loboda A, Podtelezhnikov A, Lunceford J, Chen C, Giannotti M, Hing J, Beckman R, Lorusso P. Phase I pharmacologic and pharmacodynamic study of the gamma secretase (Notch) inhibitor MK-0752 in adult patients with advanced solid tumors. *J Clin Oncol* 2012; **30**: 2307-2313 [PMID: 22547604 DOI: 10.1200/JCO.2011.39.1540]

118 **Messersmith WA**, Shapiro GI, Cleary JM, Jimeno A, Dasari A, Huang B, Shaik MN, Cesari R, Zheng X, Reynolds JM, English PA, McLachlan KR, Kern KA, LoRusso PM. A Phase I, dose-finding study in patients with advanced solid malignancies of the oral γ-secretase inhibitor PF-03084014. *Clin Cancer Res* 2015; **21**: 60-67 [PMID: 25231399 DOI: 10.1158/1078-0432.CCR-14-0607]

119 **Deng Y**, Su Q, Mo J, Fu X, Zhang Y, Lin EH. Celecoxib downregulates CD133 expression through inhibition of the Wnt signaling pathway in colon cancer cells. *Cancer Invest* 2013; **31**: 97-102 [PMID: 23245395 DOI: 10.3109/07357907.2012.754458]

120 **Egashira I**, Takahashi-Yanaga F, Nishida R, Arioka M, Igawa K, Tomooka K, Nakatsu Y, Tsuzuki T, Nakabeppu Y, Kitazono T, Sasaguri T. Celecoxib and 2,5-dimethylcelecoxib inhibit intestinal cancer growth by suppressing the Wnt/β-catenin signaling pathway. *Cancer Sci* 2017; **108**: 108-115 [PMID: 27761963 DOI: 10.1111/cas.13106]

121 **Tian D**, Shi Y, Chen D, Liu Q, Fan F. The Wnt inhibitor LGK-974 enhances radiosensitivity of HepG2 cells by modulating Nrf2 signaling. *Int J Oncol* 2017; **51**: 545-554 [PMID: 28627706 DOI: 10.3892/ijo.2017.4042]

122 **Suwala AK**, Koch K, Rios DH, Aretz P, Uhlmann C, Ogorek I, Felsberg J, Reifenberger G, Köhrer K, Deenen R, Steiger HJ, Kahlert UD, Maciaczyk J. Inhibition of Wnt/beta-catenin signaling downregulates expression of aldehyde dehydrogenase isoform 3A1 (ALDH3A1) to reduce resistance against temozolomide in glioblastoma in vitro. *Oncotarget* 2018; **9**: 22703-22716 [PMID: 29854309 DOI: 10.18632/oncotarget.25210]

123 **Li Y**, Zhang T. Targeting cancer stem cells by curcumin and clinical applications. *Cancer Lett* 2014; **346**: 197-205 [PMID: 24463298 DOI: 10.1016/j.canlet.2014.01.012]

124 **Zhu JY**, Yang X, Chen Y, Jiang Y, Wang SJ, Li Y, Wang XQ, Meng Y, Zhu MM, Ma X, Huang C, Wu R, Xie CF, Li XT, Geng SS, Wu JS, Zhong CY, Han HY. Curcumin Suppresses Lung Cancer Stem Cells via Inhibiting Wnt/β-catenin and Sonic Hedgehog Pathways. *Phytother Res* 2017; **31**: 680-688 [PMID: 28198062 DOI: 10.1002/ptr.5791]

125 **Wang D**, Kong X, Li Y, Qian W, Ma J, Wang D, Yu D, Zhong C. Curcumin inhibits bladder cancer stem cells by suppressing Sonic Hedgehog pathway. *Biochem Biophys Res Commun* 2017; **493**: 521-527 [PMID: 28870814 DOI: 10.1016/j.bbrc.2017.08.158]

126 **James MI**, Iwuji C, Irving G, Karmokar A, Higgins JA, Griffin-Teal N, Thomas A, Greaves P, Cai H, Patel SR, Morgan B, Dennison A, Metcalfe M, Garcea G, Lloyd DM, Berry DP, Steward WP, Howells LM, Brown K. Curcumin inhibits cancer stem cell phenotypes in ex vivo models of colorectal liver metastases, and is clinically safe and tolerable in combination with FOLFOX chemotherapy. *Cancer Lett* 2015; **364**: 135-141 [PMID: 25979230 DOI: 10.1016/j.canlet.2015.05.005]

127 **Hu C**, Niestroj M, Yuan D, Chang S, Chen J. Treating cancer stem cells and cancer metastasis using glucose-coated gold nanoparticles. *Int J Nanomedicine* 2015; **10**: 2065-2077 [PMID: 25844037 DOI: 10.2147/IJN.S72144]

128 **Verma RK**, Yu W, Shrivastava A, Shankar S, Srivastava RK. α-Mangostin-encapsulated PLGA nanoparticles inhibit pancreatic carcinogenesis by targeting cancer stem cells in human, and transgenic (Kras(G12D), and Kras(G12D)/tp53R270H) mice. *Sci Rep* 2016; **6**: 32743 [PMID: 27624879 DOI: 10.1038/srep32743]

129 **Muntimadugu E**, Kumar R, Saladi S, Rafeeqi TA, Khan W. CD44 targeted chemotherapy for co-eradication of breast cancer stem cells and cancer cells using polymeric nanoparticles of salinomycin and paclitaxel. *Colloids Surf B Biointerfaces* 2016; **143**: 532-546 [PMID: 27045981 DOI: 10.1016/j.colsurfb.2016.03.075]

130 **Lamb R**, Ozsvari B, Lisanti CL, Tanowitz HB, Howell A, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Antibiotics that target mitochondria effectively eradicate cancer stem cells, across multiple tumor types: treating cancer like an infectious disease. *Oncotarget* 2015; **6**: 4569-4584 [PMID: 25625193 DOI: 10.18632/oncotarget.3174]

131 **Lamb R**, Fiorillo M, Chadwick A, Ozsvari B, Reeves KJ, Smith DL, Clarke RB, Howell SJ, Cappello AR, Martinez-Outschoorn UE, Peiris-Pagès M, Sotgia F, Lisanti MP. Doxycycline down-regulates DNA-PK and radiosensitizes tumor initiating cells: Implications for more effective radiation therapy. *Oncotarget* 2015; **6**: 14005-14025 [PMID: 26087309 DOI: 10.18632/oncotarget.4159]

132 **Hirsch HA**, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res* 2009; **69**: 7507-7511 [PMID: 19752085 DOI: 10.1158/0008-5472.CAN-09-2994]

133 **Rattan R**, Ali Fehmi R, Munkarah A. Metformin: an emerging new therapeutic option for targeting cancer stem cells and metastasis. *J Oncol* 2012; **2012**: 928127 [PMID: 22701483 DOI: 10.1155/2012/928127]

134 **Zhang R**, Zhang P, Wang H, Hou D, Li W, Xiao G, Li C. Inhibitory effects of metformin at low concentration on epithelial-mesenchymal transition of CD44(+)CD117(+) ovarian cancer stem cells. *Stem Cell Res Ther* 2015; **6**: 262 [PMID: 26718286 DOI: 10.1186/s13287-015-0249-0]

135 **Honjo S**, Ajani JA, Scott AW, Chen Q, Skinner HD, Stroehlein J, Johnson RL, Song S. Metformin sensitizes chemotherapy by targeting cancer stem cells and the mTOR pathway in esophageal cancer. *Int J Oncol* 2014; **45**: 567-574 [PMID: 24859412 DOI: 10.3892/ijo.2014.2450]

136 **Fasih A**, Elbaz HA, Hüttemann M, Konski AA, Zielske SP. Radiosensitization of pancreatic cancer cells by metformin through the AMPK pathway. *Radiat Res* 2014; **182**: 50-59 [PMID: 24909911 DOI: 10.1667/RR13568.1]

137 **Chiorean EG**, LoRusso P, Strother RM, Diamond JR, Younger A, Messersmith WA, Adriaens L, Liu L, Kao RJ, DiCioccio AT, Kostic A, Leek R, Harris A, Jimeno A. A Phase I First-in-Human Study of Enoticumab (REGN421), a Fully Human Delta-like Ligand 4 (Dll4) Monoclonal Antibody in Patients with Advanced Solid Tumors. *Clin Cancer Res* 2015; **21**: 2695-2703 [PMID: 25724527 DOI: 10.1158/1078-0432.CCR-14-2797]

138 **Smith DC**, Eisenberg PD, Manikhas G, Chugh R, Gubens MA, Stagg RJ, Kapoun AM, Xu L, Dupont J, Sikic B. A phase I dose escalation and expansion study of the anticancer stem cell agent demcizumab (anti-DLL4) in patients with previously treated solid tumors. *Clin Cancer Res* 2014; **20**: 6295-6303 [PMID: 25324140 DOI: 10.1158/1078-0432.CCR-14-1373]

139 **Prieur A**, Cappellini M, Habif G, Lefranc MP, Mazard T, Morency E, Pascussi JM, Flacelière M, Cahuzac N, Vire B, Dubuc B, Durochat A, Liaud P, Ollier J, Pfeiffer C, Poupeau S, Saywell V, Planque C, Assenat E, Bibeau F, Bourgaux JF, Pujol P, Sézeur A, Ychou M, Joubert D. Targeting the Wnt Pathway and Cancer Stem Cells with Anti-progastrin Humanized Antibodies as a Potential Treatment for K-RAS-Mutated Colorectal Cancer. *Clin Cancer Res* 2017; **23**: 5267-5280 [PMID: 28600477 DOI: 10.1158/1078-0432.CCR-17-0533]

140 **Jin L**, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med* 2006; **12**: 1167-1174 [PMID: 16998484 DOI: 10.1038/nm1483]

141 **Li L**, Hao X, Qin J, Tang W, He F, Smith A, Zhang M, Simeone DM, Qiao XT, Chen ZN, Lawrence TS, Xu L. Antibody against CD44s inhibits pancreatic tumor initiation and postradiation recurrence in mice. *Gastroenterology* 2014; **146**: 1108-1118 [PMID: 24397969 DOI: 10.1053/j.gastro.2013.12.035]

142 **Naujokat C**. Monoclonal antibodies against human cancer stem cells. *Immunotherapy* 2014; **6**: 290-308 [PMID: 24762074 DOI: 10.2217/imt.14.4]

143 **Pérez-Alea M**, McGrail K, Sánchez-Redondo S, Ferrer B, Fournet G, Cortés J, Muñoz E, Hernandez-Losa J, Tenbaum S, Martin G, Costello R, Ceylan I, Garcia-Patos V, Recio JA. ALDH1A3 is epigenetically regulated during melanocyte transformation and is a target for melanoma treatment. *Oncogene* 2017; **36**: 5695-5708 [PMID: 28581514 DOI: 10.1038/onc.2017.160]

144 **Gudas LJ**, Wagner JA. Retinoids regulate stem cell differentiation. *J Cell Physiol* 2011; **226**: 322-330 [PMID: 20836077 DOI: 10.1002/jcp.22417]

145 **Petrie K**, Zelent A, Waxman S. Differentiation therapy of acute myeloid leukemia: past, present and future. *Curr Opin Hematol* 2009; **16**: 84-91 [PMID: 19468269 DOI: 10.1097/MOH.0b013e3283257aee]

146 **Ginestier C**, Wicinski J, Cervera N, Monville F, Finetti P, Bertucci F, Wicha MS, Birnbaum D, Charafe-Jauffret E. Retinoid signaling regulates breast cancer stem cell differentiation. *Cell Cycle* 2009; **8**: 3297-3302 [PMID: 19806016 DOI: 10.4161/cc.8.20.9761]

147 **Yan Y**, Li Z, Xu X, Chen C, Wei W, Fan M, Chen X, Li JJ, Wang Y, Huang J. All-trans retinoic acids induce differentiation and sensitize a radioresistant breast cancer cells to chemotherapy. *BMC Complement Altern Med* 2016; **16**: 113 [PMID: 27036550 DOI: 10.1186/s12906-016-1088-y]

148 **Nguyen PH**, Giraud J, Staedel C, Chambonnier L, Dubus P, Chevret E, Bœuf H, Gauthereau X, Rousseau B, Fevre M, Soubeyran I, Belleannée G, Evrard S, Collet D, Mégraud F, Varon C. All-trans retinoic acid targets gastric cancer stem cells and inhibits patient-derived gastric carcinoma tumor growth. *Oncogene* 2016; **35**: 5619-5628 [PMID: 27157616 DOI: 10.1038/onc.2016.87]

149 **Lim YC**, Kang HJ, Kim YS, Choi EC. All-trans-retinoic acid inhibits growth of head and neck cancer stem cells by suppression of Wnt/β-catenin pathway. *Eur J Cancer* 2012; **48**: 3310-3318 [PMID: 22640830 DOI: 10.1016/j.ejca.2012.04.013]

150 **Budd GT**, Adamson PC, Gupta M, Homayoun P, Sandstrom SK, Murphy RF, McLain D, Tuason L, Peereboom D, Bukowski RM, Ganapathi R. Phase I/II trial of all-trans retinoic acid and tamoxifen in patients with advanced breast cancer. *Clin Cancer Res* 1998; **4**: 635-642 [PMID: 9533531]

151 **Liu P**, Kumar IS, Brown S, Kannappan V, Tawari PE, Tang JZ, Jiang W, Armesilla AL, Darling JL, Wang W. Disulfiram targets cancer stem-like cells and reverses resistance and cross-resistance in acquired paclitaxel-resistant triple-negative breast cancer cells. *Br J Cancer* 2013; **109**: 1876-1885 [PMID: 24008666 DOI: 10.1038/bjc.2013.534]

152 **Duan L**, Shen H, Zhao G, Yang R, Cai X, Zhang L, Jin C, Huang Y. Inhibitory effect of Disulfiram/copper complex on non-small cell lung cancer cells. *Biochem Biophys Res Commun* 2014; **446**: 1010-1016 [PMID: 24657266 DOI: 10.1016/j.bbrc.2014.03.047]

153 **Ortiz RC**, Lopes NM, Amôr NG, Ponce JB, Schmerling CK, Lara VS, Moyses RA, Rodini CO. CD44 and ALDH1 immunoexpression as prognostic indicators of invasion and metastasis in oral squamous cell carcinoma. *J Oral Pathol Med* 2018; **47**: 740-747 [PMID: 29791975 DOI: 10.1111/jop.12734]

154 **Hu J**, Li G, Zhang P, Zhuang X, Hu G. A CD44v+ subpopulation of breast cancer stem-like cells with enhanced lung metastasis capacity. *Cell Death Dis* 2017; **8**: e2679 [PMID: 28300837 DOI: 10.1038/cddis.2017.72]

155 **Kanwal R**, Shukla S, Walker E, Gupta S. Acquisition of tumorigenic potential and therapeutic resistance in CD133+ subpopulation of prostate cancer cells exhibiting stem-cell like characteristics. *Cancer Lett* 2018; **430**: 25-33 [PMID: 29775627 DOI: 10.1016/j.canlet.2018.05.014]

156 **Bigoni-Ordóñez GD**, Ortiz-Sánchez E, Rosendo-Chalma P, Valencia-González HA, Aceves C, García-Carrancá A. Molecular iodine inhibits the expression of stemness markers on cancer stem-like cells of established cell lines derived from cervical cancer. *BMC Cancer* 2018; **18**: 928 [PMID: 30257666 DOI: 10.1186/s12885-018-4824-5]

157 **Zhang Y**, Xu W, Guo H, Zhang Y, He Y, Lee SH, Song X, Li X, Guo Y, Zhao Y, Ding C, Ning F, Ma Y, Lei QY, Hu X, Li S, Guo W. NOTCH1 Signaling Regulates Self-Renewal and Platinum Chemoresistance of Cancer Stem-like Cells in Human Non-Small Cell Lung Cancer. *Cancer Res* 2017; **77**: 3082-3091 [PMID: 28416482 DOI: 10.1158/0008-5472.CAN-16-1633]

158 **Durinikova E**, Kozovska Z, Poturnajova M, Plava J, Cierna Z, Babelova A, Bohovic R, Schmidtova S, Tomas M, Kucerova L, Matuskova M. ALDH1A3 upregulation and spontaneous metastasis formation is associated with acquired chemoresistance in colorectal cancer cells. *BMC Cancer* 2018; **18**: 848 [PMID: 30143021 DOI: 10.1186/s12885-018-4758-y]

159 **Kozovska Z**, Patsalias A, Bajzik V, Durinikova E, Demkova L, Jargasova S, Smolkova B, Plava J, Kucerova L, Matuskova M. ALDH1A inhibition sensitizes colon cancer cells to chemotherapy. *BMC Cancer* 2018; **18**: 656 [PMID: 29902974 DOI: 10.1186/s12885-018-4572-6]

160 **Pal D**, Kolluru V, Chandrasekaran B, Baby BV, Aman M, Suman S, Sirimulla S, Sanders MA, Alatassi H, Ankem MK, Damodaran C. Targeting aberrant expression of Notch-1 in ALDH+ cancer stem cells in breast cancer. *Mol Carcinog* 2017; **56**: 1127-1136 [PMID: 27753148 DOI: 10.1002/mc.22579]

**P-Reviewer:** Scuteri A, He XH, Haider KH, Zheng YW **S-Editor:** Dou Y **L-Editor:** Filipodia **E-Editor:** Song H

**Specialty type:** Cell and tissue engineering

**Country of origin:** Mexico

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B, B, B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**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.



**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.