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Cancer Stem Cell Biomarkers: Critical Roles, Challenges, Clinical Application, and Perspectives in Cancer Therapy

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ABSTRACT

Progress in cancer stem cells (CSCs) has opened up a new window to develop better cancer treatment methods. Several preclinical and clinical trial studies use CSCs targeting via surface markers method and inhibiting stem cell pathway to eradicate cancer. Investigations disclose that CSCs are more resistant to chemo- and radiotherapy than non-CSCs. If CSCs are destroyed with treatment, cancer cells will be deleted. This feature is mainly related to their surface biomarkers and thus, detection and Isolation of CSCs are so critical. This study introduced the most important cell surface markers of CSCs such as CD133, CD44, CD24, and CD90 and evaluated these biomarkers. Today, more than 60 ongoing trials have been evaluated, few of which are used in clinical trials to determine if a new drug is effective. Then, we discuss the challenges of several therapies.

Keywords: Cancer Stem Cells (CSCs); Cancer Therapy; Prognostic Markers

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Introduction of Cancer stem cells (CSCs)

CSCs are small cell populations in tumors with self-renewal capacity, making cancer treatments ineffective [1]. CSCs are pluripotent cells and can differentiate into tumor cells with different phenotypes. They can create new tumors or cause cancer to grow as well [1]. CSCs can also show drug resistance, invasion, migration, and metastasis. Therefore, these cells can be the leading cause of unsuccessful treatment and drug resistance. Thus, CSC should be targeted to destroy cancer [2]. CSCs are different from other differentiated cancer cells because they are silent and in their niche regulate self-renewal [3]. Recent therapies are based on changes in stem cell niche and surface markers, although some are under clinical and preclinical evaluations [1].

Many studies have been conducted on CSCs. For the first time in Clarke laboratory, CSCs were detected in breast cancer (solid tumors) using CD44+ CD24- lineage marker phenotype [5]. CSCs are also found in other tumors such as hematopoietic malignancies and various solid tumors [6]. The development of CSCs occurs due to the following events: 1) changes in the microenvironment or niche of CSCs; 2) changes in epigenetic condition, cellular metabolism, cell cycle control, and signaling pathways. 3) proliferation of cells with modification of molecular phenotype that cause primary tumors and metastasis [7].

CSCs make cancer resistant to treatment, so it is essential to understand this property. When the cell cycle of CSCs is arrested, cancer resists the drug that targets proliferated cells. Also, overexpression of genes involved in membrane transport (such as the ABC superfamily) leads to chemical drug resistance. In addition, radical scavengers in these cells remove reactive oxygen species (ROS) produced by radiography [8,9]. If CSCs are destroyed with treatment, cancer cells will be deleted. This is shown in Fig 1. Thus, CSCs are the best target for cancer treatment. These cells are detected with markers such as CD133, CD44, CD24, EpCAM, THY1, ATP-binding cassette (ABCB5), and CD200. Still, some studies suggest that CSCs are highly tissue-specific, and establishing a universal CSC marker is questionable [9]. Therefore, it

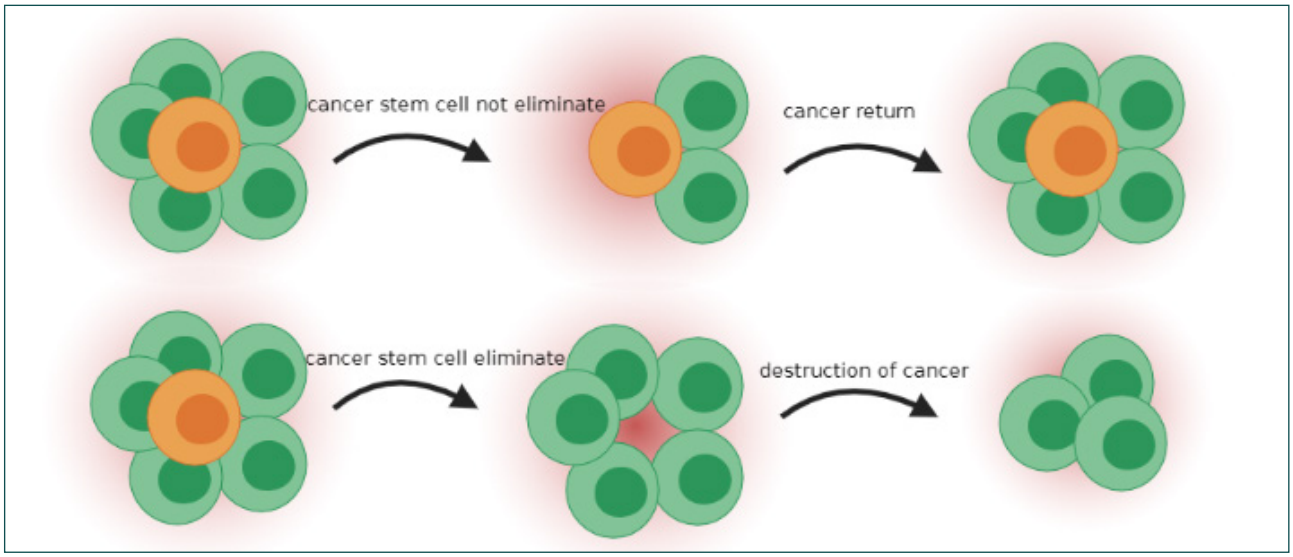

Figure.1. If CSCs are eliminated, cancer will destruct, and if CSCs remain, cancer cells will survive.

is crucial to have a comprehensive understanding of the regulatory network and transcriptional mechanisms of CSCs to have a successful treatment.

We aimed to broaden the interpretation of the potential mechanism of CSC biomarkers and develop new therapeutic targets. This review declares several important CSC biomarkers and provides evidence for their potential roles as portable new diagnostic markers, prognostic biomarkers, or therapeutic goals. We discussed current challenges and future ways to lead to the best use of CSCs for clinical applications.

The Origin of CSCs

Study by Abarrategi et al. in 2016 disclose that CSCs usually root from the cell-of-origin during cancer development. Normal cells sustain the first cancer mutation [4]. Considering all cancers originate from a single cell, there are two hypotheses about CSCs: the "stem cell hypothesis" and "Re-differentiation hypothesis". Studies suggest that there are two types of CSCs. Some of them lack expression of connexins or gap junction, or these cells expressed connexins, but these are dysfunctional. These types of cells express Oct4. Other CSCs do not express Oct4A genes but dysfunctional connexin genes [6-8].

Some researchers point out that CSCs originate from normal stem cells in cancer tissue (because both have self-renewal properties) [9].

Molecular mechanism of CSCs

Carcinogenesis occurs when a single normal cell is not able to gain terminal differentiation, but this cell can proliferate. This first step of carcinogenesis followed by mutagenesis process and is irreversible. The mistake in DNA repair or replication lead to mutation in cancer-related genes [10]. CSCs look like normal stem cells [11]. Investigations show that Wnt signaling pathway is the same as Bmil pathway in CSCs and probably have similar effects in stem cells' common pathways [12]. Evaluations show that CSCs and normal stem cells have a common genotype, but they have a different epigenetic profile that produce various signaling pathways [13]. Identification of the molecular mechanism of CSCs will help detect crucial targets for future cancer therapies [11].

The structure of CSCs markers

Because of the role of CSCs in cancer disease incidence, identification of these cells are significant. The molecular structure of CSC markers has been disclosed in the literature. So we explain the critical roles of the critical CSC markers in cancer and refer to their signaling pathways.

Epithelial cell adhesion molecule (EpCAM)

Denzel et al. show that in cancer cells, the intracellular domain of EpCAM translocates to the cytoplasm and interacts with β-catenin after activation. Then it regulates the expression of c-myc, cyclin A, and cyclin E genes [10-11].

The Wnt/β-catenin signaling pathway regulates EpCAM expression. This signaling pathway contributes to proliferation in normal cells. Blocking EpCAM may suppress c-Myc signaling and cellular invasion. Thus, the tumorigenicity of EpCAM+ HCC cells could be prevented [14]. It is demonstrated that the inhibition of either β-catenin or EpCAM gene expression causes a reduction in cell tumorigenesis.

Numerous studies demonstrate that the blockage of EpCAM inhibits cancer development and metastasis. Various research groups use multiple monoclonal antibodies for the identification of EpCAM. Sears et al. showed that EpCAM monoclonal antibody 17-A protein could be used for gastrointestinal cancer therapy [13].

Yamashita et al., 2008 introduced a new classification method that worked in the absence and presence of EpCAM and alpha-fetoprotein (AFP) in hepatocellular carcinoma (HCC) [14]. They showed that EpCAM+ AFP+ HCC has a poor prognosis, while EpCAM+ AFP+ HCC cells have an excellent prognosis. Terries et al. affirmed that EpCAM+ AFP+ HCC has shorter survival and a higher rate of portal vein invasion than EpCAM- AFP- cells [15].

In 2009, European Commission approved catamaxob (first EpCAM antibody) for cancer therapy.

In 2010, Schmidt et al. showed that in EpCAM+ patients with metastatic breast cancer (MBC), Adecatumumb

has dose- and target-dependent effects [16]. Münz and colleagues demonstrated that blockage of EpCAM decreases proliferation and metastasis [17].

Sighede et al. produced an aptameric RNA that could bind to colorectal and breast cancer cells (with EpCAM) while could not attach to normal cells (that do not express EpCAM) [18].

An investigation on EpCAM expression in prostate cancer (CaP) in 2013 by Ni, J, et al. demonstrated that EpCAM has a critical role in proliferation, invasion, sphere formation, chemo-/radiosensitivity. It also roots in E-cadherin, p-Akt, p-mTOR, p-4EBP1, and p-S6K expression in CaP cells by activating the PI3K/Akt/mTOR signaling pathway [15].

In 2015, Laio et al. showed that EpAb2-6 (a novel monoclonal antibody against EpCAM) induces apoptosis in the mouse model of metastatic pancreatic cancer and human colon cancer xenografts in mice [16].

The findings of EpCAM signaling and its involvement in various cellular pathways provide a strong therapeutic potential for EpCAM and require further studies to understand better its potential prognostic and therapeutic value in epithelial cancer patients.

CD133 (Prominin 1)

Numerous studies have shown that CD-133 cells can be regenerated in brain tumors. So prominin 1 has a role in tumorigenesis and stemness of tumors [21,22]. This macromolecule formed the topology of the cell membrane [23]. It has been proposed that CD133 [cholesterol-binding protein] is involved in plasma membrane protrusions remodeling. CD133 is expressed continuously in specific types of stem and progenitor cells during tissue evolution, and its expression seems to be regulated by region and developmental stages.

Studies show that CD133+ cells in pancreatic cancer cells upregulate N-cadherin. In these cells, SRC [a classical non-receptor tyrosine kinase] binds to the cytosolic domain of CD133 that activates the PI3K/Akt signaling pathway. This pathway regulates self-renewal and tumorigenesis. The Activation of ERK and SRC can induce CD133 gene expression and lead to CD133/ERK/SRC complex formation. So, it causes N-cadherin expression and EMT (epithelial-mesenchymal transition) program [24].

Kemper et al. in 2012 showed a mutation in K-Ras and B-Raf genes related to CD133 expression. This group reported that Ras-Raf-Mek-Erk pathways are CD133 regulators [11].

Hypoxia, Iron, transcriptional factors (SOX17, Af4, and ETS), and low mitochondrial activity are essential regulators of Prominin 1 gene expression [8].

The Ras/ERK/ETS pathway affects two E26 transformation-specific (ETS) binding sites in a PROM1 promoter and regulates gene expression. It has been demonstrated that Ras inhibition cause radiosensitization in cancer cells. Thus, Ras controls the radioresistance of the cells [17].

ERK, activated in Ras/MapK pathway, phosphorylate and stabilize HIF-1a. HIF-1a is a transcriptional factor that plays multiple roles in CSC specifications, such as the PROM1a expression [26,27].

Wang et al. demonstrated that the blockage of the Akt and Erk signaling pathways decreases CD133+ survival and their tumorigenesis. Other studies have shown that Erk and Akt siRNA (that downregulate these gene expressions) decrease CD133+ cell colony-forming [25]. More recently, studies have shown that Silibinin (a chemo-protective agent) affects several cancers. This compound acts by inhibiting the PP2Ac/Akt/mTOR pathway, associated with reducing CD133 expression in CRC spheroid cultures [18].

Researchers have found the simultaneous presence of CD44, notably as a reliable marker of colon CSCs, with other cell surface markers besides CD133, or CD44 presence without CD133 to identify colon CSCs. Overall, these topics raise doubt about the role of CD133 as a CSC marker in colon cancer. So we can say that there are several types of colon cancer cells. Even CSCs may express several markers at the cell surface that change growth features and interact with a changeable cell nearby the microenvironment. Another study demonstrated that membrane expression of CD133 significantly decreased during colon differentiation. The decrease was probably

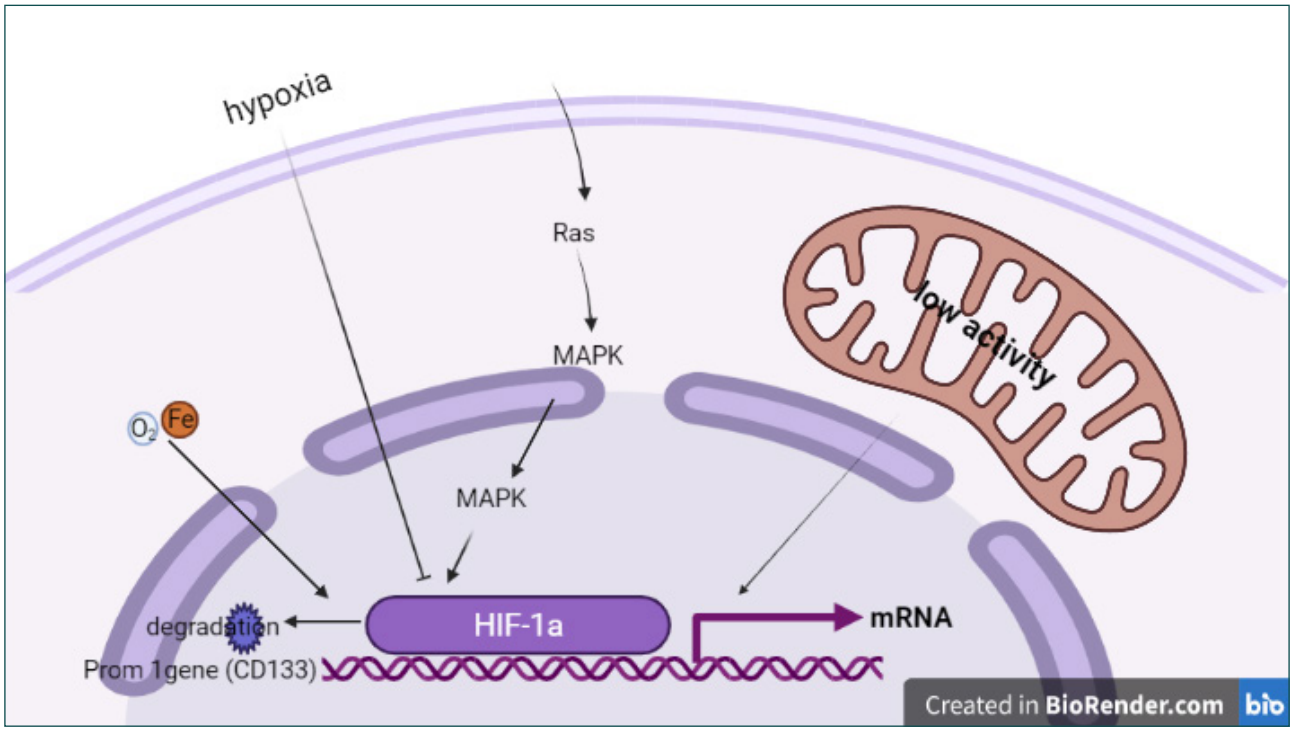

Figure.1. HIF-1a is the transcription factor that regulates CD133 gene expression and causes the low activity of mitochondria, hypoxia, O2, and Fe. HIF-1: hypoxia-inducible factor

due to posttranslational mechanisms; because there were no changes in the methylation status of the CD133 gene promoter and its mRNA expression level [19]. However, the precise functional significance of these observations remains unclear.

Although treatment with 5-azacytidine (5-AC) [an unmethylated drug that activates tumor suppressor genes] restores PROM1 gene expression [20], DNA methylation might have different effects on CSCs and even the other cancerous cells. So other studies on gene hypomethylation mechanisms are required to expand our knowledge of cancer stemness.

Many studies have suggested that CD133 is closely associated with the size of the tumor, a worse prognosis, higher rates of lymph node metastasis, and persistence to assistant therapies. However, other studies have reported contradictory results [19]. The use of CD133 as a CSC marker is challenging due to opposite discoveries. Recently, it was shown by Feng et al. that the CD133+ and CD133- SW620 colon cancer cells could convert to each other., conducive to conflicting data [32]. Moreover, Hsu et al.'s findings [22] resulted in that disposal to environmental pressure, hypoxia, and cell-adhesion-free condition increased switching of SW620 CD133- cells to SW620 CD13+ cells while exposure to ECM components promotes switching of SW620 CD133+ to SW620CD133- cells [19]. This conversation in tumor colonization may disclosure adapting to the microenvironment.

It should be considered that detection limits are contributed to controversial concerns with CD133 as a CSC marker. Notwithstanding, some lack of detection may be due to using inappropriate identification tools and procedures. Different types of antibodies have different epitopes and different results [19]. For instance, prior studies did not recognize CD133 expression in glioblastoma U87 cells, which showed CSCs properties. But Recently, Wang and colleagues generated new anti-human CD133 monoclonal antibodies (mAbs) to detect CD133 expression in glioblastoma U87 cells. This novel antibody has two extracellular domains recombinant from human CD133 (CD133 ectodomain 1 (amino acids 171-420) and CD133 ectodomain 2 (amino acids 507-716).

High expression levels of CD133 protein in glioblastoma U87 cells are assessed by an antibody opposite CD133 ectodomain 2, C2E1. Moreover, C2E1 can bind to the full-length glycosylated CD133 on the cell surface and inhibit tumor cell reproduction [23]. In previous studies, various standard anti-CD133, composed of AC133, 293C3, or W6B3C1, had been used. There is an incompatibility in immunolabeling and various protein sizes in different studies because different CD133 antibodies do not detect all the connection variants. Over 28 alternatively spliced CD133 variants have been assessed. The inconsistency of CSCs immunolabeling is probably correlated with the glycosylation status and several types of the CD133 glycoprotein. So, all the CD133 variants must be analyzed to achieve more consistent results [23].

Studies have demonstrated that CD133+ CSCs induce tumorigenesis. Ricci-Vitianiet al. and OO'Brienet al. (2007) reported this in stem cells of colorectal cancer [19]; Eramo et al. showed this in small and non-small cells of lung cancer [24]; and Hori Y reported this in human pancreatic CSCs [25]. CD133 is one of the widely used markers in solid cancers [16, 26, 27].

Drugs conjugated with antibodies (against CD133) target cancer cells and affect CD133 expression. Nanoparticles conjugated with CD133 aptamers and miRNA can be used for cancer therapy. For example, Smith et al., in 2008, demonstrated that when AC133 is conjugated to the monomethyl auristatin F (MMAF, a potent cytotoxic drug), it induces apoptosis in Hep3B hepatocellular and KATO III gastric cancer cells [28]. Celecoxib is a COX-2 inhibitor and an anti-inflammatory drug (NSAID) used to induce differentiation in chemoresistant CD133-positive colon CSCs.

Celecoxib can inhibit Wnt signaling pathways in HT29 and DLD1 cells, causing downregulation of CD133 expression [29]. Another compound, 5-fluorouracil [5-FU], is one of the conventional chemotherapy drugs that can upregulate Wnt activity of CD133+ colon CSCs (like CSLCs) and cause 5-FU resistant CSLCS formation as a subsequent incidence of modified Wnt signaling pathway [30].

In 2011, Lim et al. showed that malignant brain tumors with polymeric nanoparticles (formulated from curcumin) efficiently decrease the growth of the CD133+ stem-like population. Schraivogel et al. demonstrate that CD133+ cells in glioblastoma cell lines overexpressed miR-9, miR-9* (miR-9/9*), miR-17, and miR-106b. The overexpression of miR-9/9* or miR-17 reduces differentiation and downregulate calmodulin-binding transcription activator 1 (CAMTA1) and cardiac hormone natriuretic peptide A (NPPA), which is a survival factor [31].

In 2015, Ni et al. reported that salinomycin-loaded PEGylated nanoparticle (SAL-NP) conjugated with CD133 aptamers specifically inhibit CD133+ osteosarcoma in vitro and in vivo [32]. Finally, efforts are required to understand CD133 regulation networks better and to use a specified antibody as a critical instrument to study CD133 (a CSC marker). It may be remarkable in further cancer treatments [23].

Aldehyde Dehydrogenase

Over two decades ago, it was initially observed that ALDH is active in hematopoietic and leukemic stem cells and causes these cells to be highly resistant to cyclophosphamide, an alkylating agent [33]. Although the mechanisms of ALDHs are not precisely understood, we know that this cytosolic enzyme oxidizes aldehydes to carboxylic acids. This enzyme also has a remarkable role in the oxidation of cyclophosphamide, alcohols, and vitamin A (retinal) and the detoxification of cells from ROS (reactive oxygen species) [34]. Some of these features are related to CSCs, which increase the number of ALDH+ drug-resistant CSCs after chemotherapy. Chemotherapy drugs create aldehyde that is oxidized with ALDH-1. Also, oxidation of Retinal to retinoic acid (RA) leads to cellular differentiation, stem cell self-protection, and drug resistance [35].

The promoter of ALDH1A1 has an enhancer region (-91 to +53) that contains a CCAAT box. When the concentration of the RA is low, RA receptors bind to the RA response element (RARE), and the CCAAT/enhancer-binding protein-/enhancer-binding protein-β (C/EBPβ) binds to the CCAAT box. Then, it activates the Alldh1 promoter, and transcription is done. ALDH1

increases RA synthesis and cellular protein against the cytotoxic drugs. RA binds to RAR and induces differentiation of breast CSCs. So, the similar role of ALDH1A1 to RA is related to the stemness of stem cells and CSCs [44]. In 2012, Zhao, D. et al. showed that acetylation inhibits ALDH1A1 activity. This group indicated that low acetylation of ALDH1A1 inhibits the self-renewal of stem cells in breast cancer. NOTCH signaling induces deacetylase sirtuin 2 (SIRT2) and activates ALDH1A1 by deacetylation, and thus, they develop breast cancer cells [36]. In 2013, Kim et al. showed that diethylaminobenzaldehyde (DEAB) inhibits ALDH and prevents 4t1 (synergic mouse model) metastasis to the lung. Studies demonstrate a relation between hypoxia and ALDH in breast cancer. In response to hypoxia, ALDH increases the 2α factor (Hypoxia-inducible factor 2α, HIF-2α). DEAB by HIF-2α reduction inhibits in vitro self-renewal capacity and in vivo tumor beginning in ALDH+ 4T1 cells [37]. Some points should be considered to verify and use ALDH1A1 as a CSC marker. Several recent studies have depicted that ALDH1A1 is differentially expressed in normal tissues. The expression of ALDH1A1 is inhibited (i.e., in breast, lung, and esophagus), relatively low ALDH1A1 (i.e., in colon and stomach epithelium), or high (i.e., in liver and pancreas) [38-42]. So, ALDH1 can be considered a CSC marker at some tissues [that do not express ALDH1 in high levels, for example, breast, lung, colon, and stomach epithelium], but not in other tissue (liver and pancreas).

There are 19 known human ALDH enzymes, but just a small number of them have been distinguished biochemically. Different ALDH isozymes have specific substrates that sometimes overlap, making it challenging to identify isozyme-specific effects precisely. Pharmacological inhibition studies have been done for three isozymes of ALDH, including ALDH1A1, ALDH2, and ALDH3A1. These enzymes participate in Alcohol and anticancer oxazaphosphorine drugs metabolism [33]. Antagonists (specific inhibitors) of the different ALDH isozymes have not been detected. The lack of specificity of antagonists as an anticancer agent has caused an impermissible side-effect profile in the clinical trial. Indeed, the ALDH targeting requires special consideration to deliver and prevent out-of-aim toxicities.

Thus, using inhibitors of SC-signaling pathways or antibody-based therapy is preferred in cancer treatment. More studies are required to identify additional isotypes and the critical regulatory signaling pathways associated with ALDH1A1 to maximize the efficacy of therapeutics.

CD90 (THY1)

Studies show that CD90 increases disease promotion, invasive capacity, metastasis, and drug resistance. However, CD90 is expressed in specific normal cells. Still, this marker is known as CSC marker in numerous cancers, like hepatocellular carcinoma, esophageal cancer, glioma, breast and lung cancer, and so used for stem cell isolation [43]. Studies show that CD90 has an essential role in cellular adhesion and migration [55]. So, overexpression of CD90 increases tumorigenicity.

Epithelial-mesenchymal transition (EMT) is related to the establishment of CSCs. EMT upregulates CD90, and so, targeting EMT or CD90 in insulinoma (INS) in clinical trials would be valuable [54].

Lobba et al. assessed several stem cell markers in human breast cancer cell lines and revealed that, due to more than 90% of the Hs578-T cell line being CD90+ cells, CD90 could be a possible marker in breast CSCs [44]. Based on obtained results by Zhu et al. (2014), in CD90+ CAFs (cancer-associated fibroblasts) it was determined that growth factor and cytokine might immediately stimulate pancreatic adenocarcinoma (PDAC) proliferation, and also CD90 expression on vascular endothelium shows that CD90 probably play a role in PDAC angiogenesis. Buishand et al. also reported that CAFs induce INS proliferation, and CD90 has a role in INS angiogenesis [43]. Anti-CD90 monoclonal target INS cells or INS microenvironment and thus is a novel anticancer therapy. In vitro anti-proliferative anti-CD90 mAb was introduced in T-cell and B-cell lymphoma cell lines [45]. It was demonstrated that anti-CD90 mAb stimulated apoptosis in murine T-lymphoma cells and cell cycle arrest in B-cells [46]. Buishand and colleagues showed that anti-CD90 mAb decreased cancer cell viability in an in vivo

model. However, the mechanism of this effect should be further studied.

Thy-1 seems to have an opposite effect on other tumor types. Thy-1 inhibits tumor growth through an unknown mechanism in ovarian and nasopharyngeal cancer and metastasis. It will be interesting to determine whether Thy-1 affects transendothelial cell migration or induces apoptosis of ovarian and nasopharyngeal carcinoma. The role of Thy-1 in diseases could be further investigated [47].

CD44 (PGP1)

CD44 promotes carcinogenesis molecular pathways such as the Rho GTPases that promote cytoskeletal altering and the PI3K/AKT and Ras-MAPK pathways, which develop growth, survival, and invasion [48]. It is a crucial tumor-promotion agent in transformed tumor cells with loss of the function of p53. CD44 expression in the presence of mutated p53 is essential for the survival of immortalized, premalignant cells [49].

CD44 is the receptor for hyaluronic acid (HA) and other extracellular matrix (ECM) components. With this receptor, cells feel environmental changes and regulate the CSCs status.

When osteopontin attaches to CD44, it triggers the Nanog-stat3, Oct4-Sox2-Nanog pathway and regulates survival, self-renewal, maintenance, and chemoresistance. In hypoxia in solid tumors such as glioma, osteopontin binding to CD44 adjust aggressive glioma growth and stemness with HIF-2α gene expression.

CD44 acts as a coreceptor and interacts with many growth factors and cytokines such as EGF, FGF, HGF, VEGF, TGF-β, MMPs, promoting CSCs self-renewal and metastasis [61].

Also, in hypoxia conditions, upregulation of HIF-1α promotes angiogenesis and plays a key role in surviving cancer stemness [61-63].

It is established that the expression of Twist in breast cancer and cervical cancer cells induces EMT. Cancer cells obtain stem cell-like traits in EMT, such as tumorsphere formation. Other modifications under the Twist effect include Overexpression of ALDH1 and CD44 and activation of β-catenin and Akt pathways [50]. Also, HA interacting with CD44 stimulates EMT, whereas inhibiting HA synthesis decreases EMT and metastasis. So, CD44-specific antibodies inhibit invasion of breast cancer [51].

Glycolysis in cancer cells is ATP production repositories [because of low local oxygen concentration] that produce the reduced form of NADPH. NADPH was protecting cells versus reactive oxygen species (ROS). CD44 [v isoform] triggers glycolysis and then protects cells against ROS, too. Also, CD44v isoform stabilizes one subunit of cystine-glutamate transporter (CT) and allows cystine uptake. Thus CD44 and CD44v isoforms have a protective role for CSCs.During EMT, a crucial leading in the metastatic process and acquiring stemness in cancer cells, CD44s isoforms switch to CD44v. It has been reported isoforms CD44v2, CD44v3, CD44v5, CD44v6, CD44v9, CD44v10, and CD44v8–10 in various cancers have prognostic value [51].

In 2009, Afify et al. demonstrated that 45% of cell invasion and metastasis could be inhibited by pre-incubation of Matrigel with anti-CD44s [52]. So, it can be affirmed that interaction between HA and CD44 is substantial in this process. In 2010, a study by Liu C et al. showed that miR-34a represses CD44 inhibits prostate CSCs and metastasis. They demonstrated expression of miR-34a in CD44+ prostate cancer cells blocked tumor regeneration and metastasis. Conversely, the use of miR-34a antagomirs in CD44- prostate cancer cells promote cancer progression and metastasis [53]. Cheng, W et al. in 2012 reported miR-199a probably stop tumorigenesis in human ovarian cancer (which have CD44+/CD117+ stem cells, also known as cancer-initiating cells (CICs) by targeting CD44 in the 3′-UTR [54].

Although CD44 was known as a cancer promoter in most studies, numerous reports have shown CD44 can also have a role as a tumor suppressor. Evidence indicates the connection between CD44, hyaluronic acid, and the PI3K–Akt system. PI3K–Akt is a survival pathway. Hyaluronan oligomers suppress this pathway, leading to reduced phosphorylation of BAD and FKHR, enhanced PTEN expression, and caspase-3 activity as pro-apoptotic events. This evidence demonstrates that these effects

are typically due to disruption of hyaluronan CD44 interactions [55]. The role of CD44 in the inhibition of angiogenesis is related toHMW hyaluronan engagement. HMW hyaluronan can prevent migration of cultured bovine aortic endothelial cells by inhibiting c-fos and c-jun gene expression as early response genes [48].

Since in different cancer cells CD44 expression increased, it is crucial to realize the molecular mechanisms of its transcriptional regulation. It was detected that p53 suppresses CD44 expression and tumor progression [60,70]. Also, it was found CD44 expression is regulated positively by SWI/SNF chromatin remodeling complex. BRG-1 and BRM are two subunits ofSWI/SNF that promote CD44 expression and inhibit Cyclin A expression [48]. These principal findings may elucidate why CD44 is overexpressed in cancer cells. A new technique named RNA-targeting CRISPR/Cas9 complex (RCas9) seemed useful for studying CD44 alternative splicing pathway[51].

Due to most of our information about molecular structures, various isoforms of CD44 and its role obtained from normal stem cells. There are still many challenges. However, the predictive cost of CD44s and CD44v isoforms seems different by cancer types; one study showed that the inhibition of CD44s expression in cancer cells in the lesion depth was a good marker for predicting potential metastasis to the other tissue. The differences in prognostic cost between CD44s and CD44v isoforms among different types of cancer are unclear.

Up to now, different methods did for CD44 targeting therapy; these strategies showed that inhibition of CD44 and CD44v isoform have meaningful antitumor effects [61].

One strategy was the application of monoclonal antibodies such as H90. This antibody can reduce leukemia in immune-deficient mice transplanted with human myelogenous. These antibodies target terminal differentiation and self-renewal [71].

P245 was another CD44 monoclonal antibody was used for xenograft mice with human triple-negative breast cancer. This antibody reduced tumor growth [72].

Bivatuzumab is a CD44v6 monoclonal antibody used for clinical trials and had a good antitumor effect. Still, due to its immunogenic effect on non-tumor tissue, the use of this antibody is forbidden [73].

Recently, RO5429083, a humanized CD44 antibody developed by Roche (Indianapolis, IN, http://www.roche.com) that targets CD44, and blocks the binding of HA to CD44, was started in clinical trials. Recently, several new humanized anti-CD44 or anti-CD44v antibodies are under preclinical assessment for anti-CSC therapy [51].

Another strategy is intervention in HA and CD44 interaction. In this strategy, soluble CD44 ectodomain is used as a competitor with an antitumor effect [74]. Several studies show that peptide binds to CD44 also has an antitumor effect. A5G27 (RLVSYNGIIFFLK), A6 [an 8-amino acid peptide (acetylKPSSPPEE-amino, derived from human urokinase plasminogen activator), a new peptide from pro matrix metalloproteinase-9 hemopexin [PEX9] can bind to CD44 and compete with HA. So, these compounds have therapeutic and diagnostic values and are considered significant advances [61].

According to HHA's high affinity to CD44, it could be a suitable carrier for drug delivery to cancer cells. The anticancer agent could be conjugated to HA or entrapment in HA-binding nanoparticles that complete therapeutic efficiency. Even CD44v-xCT targeting compound could be used as a vaccine [61].

Anyway, there are a few things to keep in mind: a) CD44 is also present on the normal cell surface, b) expression level of CD44 on different types of cancer cells, c) CD44 is similar to another molecule such as lyve 1 [75].

Generally, further research is necessary to identify novel CD44v isoforms and their critical role in CSCs, which will help disclose their prognostic, diagnostic, and practical therapeutic targeting potential in cancers, particularly in CSCs.

CD24 (HSA)

In 2009 in the study by Yang, X.R et al., CD24 expression was investigated in Hepatocellular Carcinoma [HCC] after surgery. They showed CD24 overexpression in highly metastatic HCC cell lines and recurrence of HCC tissue tumors. They established thatCD24 expression was significantly related to cytoplasmic and nuclear reposition of β-catenin and activation of the

Wnt/β-catenin pathway [56].

In 2010 Wang et al. showed activation of ERK1/2, Raf-1, and p38 MAPK that induce CD24 in colorectal cancer cells and then simulate proliferation. Using CD24 siRNA showed that CD24 has a role in the growth pathway. The correlation between CD24 and mitogen-activated protein kinase pathway as a regulator of mitosis and proliferation rate of tumor cells suggested CD24 could be a new goal to prevent and treat colon cancer cells [57].

Also, it has been demonstrated that CD24 can activate Src, induce the activation of c-Jun and c-Fos, and suppress Pdcd4 and PTEN expression through induction of miR-21 promoter activation and expression miR-21. MiR-21 is upregulated by Src in addition to CD24. As studies by Muppala, S et al. in 2013, it has been demonstrated that miR-34a post-transcriptionally downregulates CD24 and Src expression, then deactivate c-Jun that reduced expression of c-Jun and c-Fos, inhibition of miR-21, and upregulation of Pdcd4 and PTEN. Finally, inhibition of Src expression reduced migration and invasion of colorectal cancer cells [58].

PI3K-AKT pathway is a downstream effector of CD24. It has been established that trastuzumab, as an anti-HER2 drug, eliminates HER2-positive breast cancer cells by suppressing phosphatidylinositol 3-kinase (PI3K)-Akt and MAPK pathways. HER2 overexpression leads to CD24 overexpression. Knockdown of CD24 in breast cancer cells suppresses the phosphorylation of Akt and reduces HER2 expression. Promotion of cell survival occurs as a consequence event of this function. For this reason, CD24 targeting therapy can provide better results in the elimination of HER2-positive breast cancer [59].

Although the role of CD24 as a CSCs marker is still debatable, many studies confirmed its role in tumorigenesis. As a study in 2015 by Rostoker and colleagues accomplished on the gene profiles of both CD24+ and CD24- mammary cancer cells, CD24+ cells transcripts have aggressive and invasive phenotypes and show high tumorigenic capacity. Because of recently known elevated expression of ECM (extracellular matrix) transcripts promotes tumorigenesis and metastasis in different types of circulating cancer cells. According to this finding, CD24+ cells contain the high expression of ECM genes, similar to Ting DT et al.'s results [60]. This gene profile may show new pathways in tumorigenesis and can be the start point for further research with a significant clinical implication for metastatic tumors.

CD44/CD24

In 2010, Meyer et al. demonstrated that epithelial-like CD44+ CD24+ cells could be converted to invasive mesenchymal CD44+ Cd24- in vitro and in vivo conditions. This process has been done with Activin/Nodal signaling. For this reason, arresting of Activin/Nodal signaling may be required in combination with targeting CD44+ CD24- cells as a Treatment process [58].CD44+CD24– cells with stem cell-like specification inverse differentiated like CD44–CD24+ breast cancer cells. Since the IL-6/JAK2/Stat3 pathway is active in CD44+CD24– breast cancer cells, targeting JAK2 and Stat3 can be a more effective therapeutic approach [61]. Hedgehog (Hh) signaling pathway might represent a new candidate for breast cancer therapy. Tanaka, H et al., in 2009, reported that Hedgehog (Hh) signaling pathway strongly expresses in the CD44+CD24- populations of breast cancer. Inhibition of this pathway prevents the proliferation of CD44+CD24- cell population [62]. (1N,12N)bis(ethyl)-cis-6,7-dehydrospermine (PG11047) reduces the CD44+CD24- subpopulation, decrease self-renewal capability of the CSCs population, slow down cell motility, and induces mesenchymal to epithelial transition and inhibits malignancy and resistance to trastuzumab by epidermal growth factor receptor 2 (EGF2) suppression [63].

In 2009, Mine, T et al. showed that Numb-1 peptide-activated T cells could remove CD44+ CD24- breast cancer cells [64]. Moreover, Ju, J.H., in 2011, reported that CD24 could induce apoptosis against DNA damage by suppressing anti-apoptotic NF-κB signaling in CD44-expressing cells [65].

Chen, J. et al., in 2015, reported that the PI3K/Akt/mTOR signaling pathway was highly activated in colon CSCs (that have CD133, CD44, and CD24 markers). They affirmed that inhibition of this pathway by the inhibitor BEZ235 suppresses colon cancer stem cell proliferation.

Tumors originating from EpCAM(high)/CD44+ cells retained a differentiated phenotype and rehabilitated their parental lesions' full morphologic and phenotypic heterogeneity. EpCAM, CD44v6, claudin-7, and ALDH1 involve in the growth of the aggressive phenotype of anaplastic thyroid carcinoma [66]. Furthermore, EpCAM, CD44v6 expression was upregulated in colon cancer and liver metastasis [67, 68]. CD24+CD44+EpCAM high cells compose a small population of extrahepatic cholangiocarcinoma (ECCs), exhibiting CSC properties [69]. In 2011, Bao B, et al. showed Over-expression of Notch-1 induces pancreatospheres formation expression of CSC surface markers such as CD44 and EpCAM in pancreatic cancer. A known natural antitumor agent, genistein, inhibits many malignancy characters in pancreatic cancer such as cell growth, clonogenicity, migration, invasion, EMT, formation of pancreatospheres, and expression of CD44 and EpCAM, by targeting Notch-1 signaling [70].

Prostate stem cell antigen (PSCA)

Metastatic prostate cancer cells (PCa) overexpressed prostate stem cell antigen (PSCA). This marker is also said in the bladder, placenta, colon, kidney, and stomach and has low expression in normal tissues [71].

Zhang, L.Y et al. studied retinoblastoma 1-inducible coiled-coil 1 (RB1CC1) in esophageal squamous cell carcinoma (ESCC). RB1CC1 is a crucial signaling compound that regulates cellular proliferation and differentiation. It interacted specifically with PSCA in ESCC cells. The binding of PSCA and RB1CC1 in the cytoplasm helps transfer RB1CC1 to the nucleus. The differentiation results from RB1CC1 presence in the nucleus [72]. Morgenroth, A et al. in 2007 genetically modified cytotoxic T-cells to generate a chimeric T-cell receptor (TCR] that recognizes PSCA. Chimeric alpha-PSCA-beta2/CD3zeta-TCR obtained from fusing anti-PSCA scFv 7F5 into the beta2 conserve region derived from the beta-chain of a TCR and CD3zeta-signaling domain. After being transduced to the T-cell line, this compound leads to cytotoxicity activation against PSCA+ cells [93]. In Earlier studies, PSCA was targeted in prostate cancer xenografts using monoclonal antibodies [73].

Ahmad, Sm et al. performed another study in 2009 and targeted PSCA as a therapeutic approach. In this study, a vaccine plasmid (pmPSCA) was produced, and this vaccine was delivered by intramuscular electroporation (EP). pmPSCA inhibited tumor growth and metastasis and was effective in survival rate. Moreover, activation of Th-1 type immunity against PSCA was seen [74].

By investigating Kim et al., they suggest that PSCA is a helpful tissue marker for predicting BCR in patients with high-risk PC receiving NHT and radical [75]. Also in another study by Kim et al. showed the quantified level of circulating mRNA antigen in prostate stem cells relative to GAPDH level is a good marker for predicting biochemical relapse in prostate Cancer Patients after Radical Prostatectomy. They confirmed the successful quantification of PSCA with its significance for BCR-related risk factors; however, further studies are needed to affirm this [76].

Youssef et al. concluded that PSCA expression levels increase from benign prostatic hyperplasia (BPH) through low-grade prostatic intraepithelial neoplasia (LGPIN) and High-grade prostatic intraepithelial neoplasia (HGPIN) to prostatic carcinoma (Pca). Thus, PSCA might represent an excellent indicator to distinguish between malignant and benign glands. In addition, it may possess prognostic advantages and be targeted for the treatment and diagnosis of prostatic adenocarcinoma [77].

CD200 (OX2)

According to Dorfman, D.M et al. studies in 2010, CD200 is Expressed in B Cell-Derived Neoplasms. Moreover, chronic lymphocytic leukemia (CLL]/small lymphocytic lymphoma (SLL) includes the number of B-cell lymphoproliferative disorders, including hairy cell leukemia and B-lymphoblastic leukemia/lymphoma. The broad range of CD200 expressed neoplasms confirm this subject that anti-CD200 can be used as an immunotherapeutic agent [78].

In 2014, Moertel et al. reported that interaction between CD200 and CD200R develop myeloid-derived suppressor cell (MDSC), so blockage of CD200 increases the influence of immunotherapy. Even inhibitors of the

CD200R pathway can be used as supplements of immunotherapy [79].

Downstream of Tyrosine Kinase 1 and 2 pathways Play opposing roles in CD200 Receptor Signaling, meaning that the CD200 receptor (CD200R) directly interacts with the adaptor

proteins downstream of tyrosine kinase 2 (Dok2). Activation of Ras GTPase-activating protein (RasGAP) is a consequential event of this pathway. Ligand engagement of CD200R also results in phosphorylation of Dok1. Phosphorylation of Dok1 results in CT10 sarcoma oncogene cellular homolog-like (CrkL) recruitment. The Dok1-CrkL complex appears to initiate a negative feedback loop in this receptor's signaling pathway. Although Dok2-mediated RasGAP activation is required for the inhibitory function of the CD200R [80].

Previous studies have confirmed the diagnostic value of CD200 in differentiating chronic lymphocytic leukemia (CLL) from other B-cell chronic lymphoproliferative disorders, especially mantle cell lymphoma. Still, the prognostic significance of CD200 in CLL needs more investigations. Recently, in a study accompanied by Miao Y et al. in 2016, CD200 MFI was identified as a potential prognostic factor in CLL. Because using flow cytometry delineated patients with lower CD200 mean fluorescence intensity (MFI) (< 189.5) had a significantly shorter time-to-treatment (TTT) than those with higher CD200 MFI [81]. Also, Li et al. confirmed this issue in cutaneous squamous cell carcinoma (CSCC), and CD200expression level was associated with tumor differentiation grade (P=0.041) and clinical stage. So indicated that CD200 could be an independent marker for the prognosis of CSCC [82].

Anti-CD200 Ab administration to mice bearing CD200-expressing tumors resulted in nearly complete tumor growth inhibition even in the context of established receptor-ligand interactions. Evaluation of an anti-CD200 Ab with abrogated effector function provided evidence that blocking the receptor-ligand interaction was sufficient to control CD200-mediated immune modulation and tumor growth inhibition in this model [83].

Obtained data of Atfy M et al. indicate that expression of CD200 high in the blast of Acute Myeloid Leukemia (AML) patients are usually accompanied by a bad prognosis and increased risk of relapse. This suggests that utilization of CD200 blocking antibody in treatment strategies as a novel therapy may be effective in remission, especially if there was a prescription that lymphocyte populations began to repopulate.

CD200 Suppresses the Natural Killer Cells and Decreased its Activity in Acute Myeloid Leukemia Patients [84].

The role of CSC in cancer progression and treatment

Cancer cells affect their microenvironment, and it causes changes in the other cells' phenotype and tumorigenesis potential. So cancer cells become metastatic, and tumors

Table 1. Overall cancer stem cell markers expressed on the cell surface

Cancer stem cell marker	Type of cancers
Epithelial cell adhesion molecule (EpCAM)	Pancreatic cancer in the mouse, human colon cancer, and hepatocellular carcinoma
CD133 (Prominin 1)	Brain tumors and colon cancer
Aldehyde Dehydrogenase	Breast cancer
CD90 (THY1)	Hepatocellular carcinoma, esophageal, glioma, breast, and lung cancer
CD44 (PGP1)	Prostate cancer
CD24 (HSA)	Hepatocellular carcinoma
CD44/CD24	Colon cancer
Prostate stem cell antigen (PSCA)	Prostate cancer
CD200 (OX2)	Acute Myeloid Leukemia

re-initiate [9]. Studies showed that CSCs have a progression effect on tumorigenesis, with adding CSCs to mice can cause repopulating tumor cells [85]. CSCs are regulated by the tumor microenvironment and their components, such as cancer-associated fibroblast (CAFs).

One of the most critical properties of CSCs they are resistant to therapies. This feature causes that after treatment, the residual cells are leading to tumor relapses and so metastasis, show the crucial role of CSC in cancer re-initiation and progression even in metastasis. These cells accumulate carcinogenesis conditions, mutagenic inducers, for example, inflammation and oxidative stress. So, CSCs as potential therapeutic targets will be important in developing therapies that control cancer and improve patients' clinical responses [85].

The application of CSC in therapeutic cancers

Several preclinical and clinical trial studies use CSCs targeting via surface markers method and inhibiting stem cell pathway to eradicate cancer [86]. Although, some important question was unclear about CSCs origin and molecular mechanism of self-renewal, the structure of CSCs markers, etc. It will be essential to know self-renewal in normal stem cells and CSCs to recognize the best molecular targets for treatment.

However, recently biological drugs such as anti-VEGF and anti-EGFR (monoclonal antibody) have been used together with chemotherapy, but clinical trials slightly developed over the past decade. Alternative therapeutic methods of targeting CSCs are currently in progression. One of these aims is to destroy necessary signaling pathways such as Wnt, Hedgehog, and Notch. In addition, epigenetic manipulation also can activate tumor suppressor genes. Another unique approach is immunotherapy targeting CSCs. Foster et al. produced cytotoxic lymphocytes against a stem cell-like SP of cells in chronic lymphocytic leukemia. The same group similarly assessed the cytotoxic ability of cytotoxic lymphocytes against SP cells in Hodgkin lymphoma [87, 88].

In recent several years, researchers have made various efforts to develop new therapeutic methods, such as the introduction of nanomedicine. These therapies can be used to exclusively target cell-surface indicators, moieties within a stem cell niche, or different signaling pathways and used for tumor checking. The capability of the nanoparticle to carry multiple compounds and drugs helps it be applicable against numerous members of a heterogeneous tumor cell population, such as tumor cells and CSCs. Applying engineered nanoparticles carrying multiple profluorophores would permit a physician to analyze whether the particle has been absorbed by a CSC, normal stem cell, or tumor cell. Such imaging would greatly facilitate the novel application of cell-specific population density tumor assessing. Additionally, photothermal properties could be used to obliterate remaining tumor cells. However, intelligent nanomedicine needs to be more investigated about cancer cell biology, CSCs, specifically surface markers, genes, anatomical location, and the reliability of their interactions with their microenvironment [86].

Applying novel methods and the combination of targeted therapies may lead to synergistic strategies and cancer therapy.

The challenges of using CSC in the clinic

Research shows that CSCs are less sensitive to chemo- and radiotherapy than non-CSCs. So, clinical treats targeted CSCs with surface markers, inhibition of cellular pathways, or elimination of CSCs niches [89]. It so causes CSCs targeted therapy more difficult.

Wicha, an OncoMed co-founder and consultant to many companies targeting CSCs, says more than 60 underway trials that a few of them are used in clinical trials whether new drugs are effective [90].

Harvard University cancer biologist William Kaelin says that It is false reasoning to mention that "If you kill the CSCs, your work is done".

This section shows several examples of CSCs usage in clinical trials.

Tarextumab, a drug produced by Oncomed, works not by killing CSCs but trigger them to differentiate into bulk tumor cells that these are sensitive to chemotherapy[90].

Repairing, a drug initially developed by the Italian company Dompé to conflict transplant rejection, appears to

block a receptor that operates their growth in response to inflammation and kills CSCs [90].

Conclusion

CSCs have pluripotency and self-renewal characteristics essential for tumor proliferation, metastasis, and recurrence. Studies show that CSCs are more resistant to chemo- and radiotherapy than non-CSCs. If CSCs are eliminated with treatment, cancer cells will be deleted. So, detection and isolation of CSCs are critical. This characterization is mainly based on their surface biomarkers. More than 60 trials are underway to evaluate the effect of new drugs. In recent years, CSCs have targeted the design of new therapies. Evidence suggests that comprehensive strategies for characterizing CSCs may improve cancer treatment. The CSC hypothesis has widened the horizons and offered scientists new therapies to eradicate malignant tumors. CSC-related biomarkers are essential for tumor diagnostics and staging tumors, as well as treatment choosing (Table 1). However, almost all markers of CSCs were also found on normal stem cells, lead to the development of CSC-specific drugs a challenging task due to potential toxic side effects on the normal stem cell portion [92]. However, more researches are needed to explore the CSC features and the related signaling pathways.

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