J Human Gen Genom. 2018 July; 2(1):e65253. doi: 10.5812/jhgg.65253.

Published online 2017 July 27. **Review Article**

# The Most Well - known Markers of CSCs and Their Role in Growth of Tumors, Drug Resistance and Metastasis

Mohammad Javad Dehghan Esmatabadi,[1,*] Fatemeh Movahedi Motlagh,[2,3] Azadeh Etemadzadeh,[4] and Samira Hajigholami[5]

[1]Malek - Ashtar University of Technology, Tehran, Iran
[2]Cellular and Molecular Research Center, Faculty of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran
[3]Department of Medical Genetics, Tehran University of Medical Sciences, Tehran, Iran
[4]Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
[5]Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

[*]*Corresponding author*: Mohammad Javad Dehghan Esmatabadi, Fatemeh Movahedi Motlagh, Malek - Ashtar University of Technology, Tehran, Iran. E-mail: dnaresearchcenter@gmail.com

**Received** 2017 May 27; **Revised** 2017 June 20; **Accepted** 2017 July 25.

**Abstract**

The capability of cancer cells including tumor initiation, maintenance, and extension is associated with cancer stem cells. The substantial features of CSCs are self - renewal and pluripotency. Therapeutic resistance of CSCs can result in cancer recurrence and failure of cancer treatment. Isolation and characterization of CSCs are required for targeted cancer therapies. CSCs can be distinguished and separated by surface markers. In this review, after definition of CSC hypothesis, different markers of CSC are introduced. The information about CSC markers provides promise for better isolation and more effective CSC-targeting therapeutic in future.

*Keywords:* Cancer, Stem Cells, Markers

## 1. Context

In 1937, researchers found that every cell in tumors doesn't have the ability of tumor generation, exception of cancer stem cells. Based on CSC hypothesis, the small population of tumors, cancer stem cells, is a cause of generation and propagation of tumors, cancer recurrence and drug resistance. CSCs are immortal and pluripotent cells with capability of tumor initiation. They can be generated from transformation of stem cells, progenitor cells or differentiated cells by mutation.

One of the general characteristics of cancer stem cells is the ability to self-renewal: this ability is uncontrollable, unlike normal stem cell. The cancer stem cells division, like normal stem cells, is asymmetric and one CSC can produce one cancer stem cell and one differentiated cell, differentiated cells encompass the bulk of tumor cells.

Other CSCs characteristic is slow proliferation or inactive state. These states make the cancer stem cells less responsive to treatment. Thus, the re-activation of inactive cancer stem cells can cause cancer recurrence even decades after complete treatment.

CSCs have key roles in tumor genesis, invasion, drug resistance and cancer relapse. In this regard, for eradication of these cells, CSCs targeted therapy along with chemotherapy can be useful. Isolation of CSCs is vital prerequisite for this aim. Now the most common method to detect CSCs is the technique of fluorescence - activated cell sorting (FACS) based on identification of specific markers. Several specific surface biomarkers distinguishing CSCs from other tumor cells or normal stem cells are detected. In addition to its role in cell interactions, these markers donate unique characteristics to CSCs.

Another method of isolation is Magnetic Cell Sorting (MACS), a standard method for cell separation. According to this method, cells are separated based on expressing specific markers, like CD133 marker for stem cells isolation. At first, cellular markers are labeled using specific monoclonal antibody or microbead. After labeling of the cells magnetic separation is performed. The best way for separating targeted cells from the cell suspension is positive selection (1).

Despite the numerous methods of separation, there are many limitations in using them. So it is vital to develop methods for separating CSCs (2). In this review, some of surface markers used to identify CSCs are introduced and their role in growth of tumors, drug resistance and metastasis are explained.

## 2. Results

### 2.1. Epithelial Cell Adhesion Molecule (EpCAM) or Epithelial Specific Antigen (ESA) or TROP1

Epithelial cell adhesion molecule (EpCAM) is expressed on some normal and most neoplastic epithelial cells. This molecule is a 39 - 42 kDa transmembrane glycoprotein with 314 amino acids, a hemophilic cell - cell adhesion molecule. It was found in cells initiating colon, breast, pancreas and prostate carcinomas (3, 4). EpCAM first described in 1979 as a dominant surface antigen on human colon carcinoma. EpCAM usually overexpress in cancers of epithelial origin but not in non-epithelial tumors (e.g. melanomas, sarcomas, lymphomas). Furthermore, this $Ca^{2+}$ - independent transmembrane glycoprotein plays different roles in cell signaling, migration, proliferation and differentiation tumorigenesis and metastasis of carcinomas (5). The EpCAM has different domains including extracellular domain, an epidermal growth factor (EGF) - like domain, and a thyroglobulin (TY) domain, and a short cytoplasmic tail (6) (Figure 1).

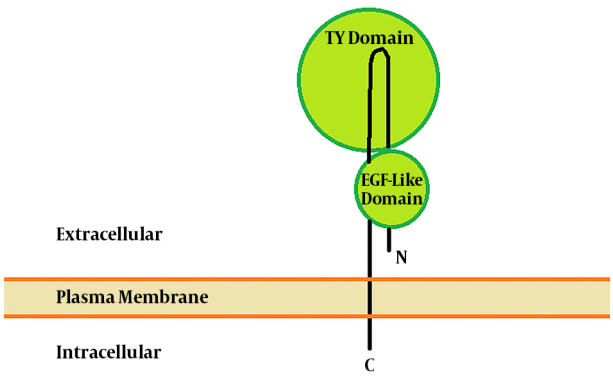


**Figure 1.** Diagram of EpCAM Protein at the Cell Membrane with the Extracellular N-terminus (N) and the Intracellular C-terminus (C) (6)

It was demonstrated that anti - EpCAM antibodies like EBA-1, AUA-1, and 9C4, can be used to detect and isolate circulating tumor cells (CTCs) (7).

Carcinogenic role of EPCAM is demonstrated by EpCAM upregulation that associate with the co-expression of EPCAM with proliferation markers such as Ki-67 and increasing dysplastic grade of carcinoma (5). Adhesions induced by classical cadherins are affected by the negative regulation of adhesion formation mediated by Ep-CAM. Growth and differentiation of epithelial cells is affected by classic cadherins. Expression of Ep-CAM is correlated with decreasing of cell differentiation and Increasing of epithelial proliferation (8).

Moreover, it has been suggested that a deletion existence in the 3'part of Ep-CAM gene can be cause of promoter region hypermethylation and epigenetic inactivation of MSH2 gene, MSH2 defect could lead to Lynch syndrome (9).

### 2.2. CD133 (Prominin 1)

PROM1 encode a cholesterol - interacting pentaspan (5-TM) transmembrane glycoprotein called CD133 (prominin-1). This glycoprotein has 842 (for mouse) or 865 (for human) amino acid residues with variable molecular weights depending on the length of splice variants and their glycosylation levels and it was described separately by three groups in 1997 (10).

CD133 was preliminary identified as a hematopoietic stem cell (11), progenitor cells and bone marrow - derived circulating endothelial progenitors surface protein marker (3). It was identified in various human normal tissues as well as on CSCs of solid tumors. In differentiated cells the expression of CD133 was down-regulated ,however, CD133+ CSCs has been found in melanoma prostate cancer, osteosarcoma, laryngeal carcinoma (12), osteosarcoma, ovarian cancer, pancreatic cancer, breast cancer hepatocellular carcinoma and osteosarcoma. A study by Singh et al. Showed that only cells isolated from the CD133+ brain tumor are able to start the tumor in NOD - SCID (non-obese diabetic, severe combined immunodeficient) mouse brains by using a xenograft assay (13). Primary glioblastomas in contrast with secondary tumors contain a significant subpopulation of CD133+ cells. In that study, primary glioblastomas cells that were Cultured demonstrated a significant proliferation and differentiation to neural cells (14).

Different studies have shown that this macromolecule plays a role in tumorigenesis and stemness of tumor. However, several studies had suggested that CD133- cells could regenerate heterogeneous brain tumors in vivo (15).

In 2007, Ricci - Vitiani et al. and O'Brien et al. recognized CD133 on stem cells of colorectal cancer. They showed CD133+ CSCs can induce tumorigenesis more stronger than unsorted cancer cell populations in tumor xenografts which showed histologically original tumor features (3).

Immunocompromised mice that were injected by lung cancer CD133+ cells generate tumor identical to the original one. This study suggests that small population of undifferentiated cells that express CD133, are tumorigenic cells (16).

### 2.3. Aldehyde Dehydrogenase

Interestingly, it has been reported that ALDH1A1 may serve as an SC marker in somatic SCs but recent studies

have reported that other ALDHs (i.e., ALDH1A2, ALDH1A3, ALDH1A7, ALDH2*2, ALDH3A1, ALDH4A1, ALDH5A1, ALDH6A1, and ALDH9A1 (17) have also activity and characteristic feature of primitive human hematopoietic stem cells.

A high ALDH activity is reported in human hematopoietic and neural stem and progenitor cells (18). Furthermore, in multiple myeloma and acute myeloid leukemia (AML), the activity of stem cell populations have increased (19). For detecting and isolating malignant and normal mammary stem cells, as well as predicting for hardly detectable human breast cancer, the expression of ALDH can be contemplated as a strong marker (20).

The study by Jiang et al. in 2009, indicated a positive correlation between expression of ALDH1 and the stage or grade of lung tumor (21). In addition, in a study by Chen et al., immunocompromised mice was injected by head and neck squamous cancer (HNSCC) cells that characterized as ALDH1+ cells and represent a capability to produce a new tumor composed of result of HNSCC cells (22). Additionally, in multiple human solid cancers including prostate, pancreatic and bladder cancers, ALDH1 positive tumor cells may be considered as a new prognostic marker. Regarding this, ALDH1 activity may be appropriate to be used as a common marker for malignant stem cell populations (23).

### 2.4. CD90 (THY1)

A 25 - 37 kDa glycophosphatidylinositol (GPI) is called Thymocyte differentiation antigen 1 (THY1) or CD90 (Cluster of Differentiation 90). Many cell types including T cells, thymocytes, neurons, endothelial cells, fibroblasts as well as mesenchymal and hepatic stem cells (24) express a single V-like immunoglobulin domain that capable to anchored into cell surface. Thy-1 has important roles in nerve regeneration, metastasis, inflammation, and fibrosis and can regulate cell - cell and cell - matrix interactions (25). Besides its expression on certain normal cell types, CD90 has been described as a CSC marker in various malignancies more commonly used to isolate and characterize stem cells (26).

Bone marrow - derived mesenchymal stem cells (24) as well as hematopoietic stem cells could be identified by Thy-1 as a marker. In a study by Yang et al., cancer stem cells (CSCs) were characterized in tumor specimens, blood samples, and cell lines of hepatocellular carcinoma (HCC) (27). A study by Yamazaki et al. showed that for the isolation of CSCs in some cases of T-acute lymphoblastic leukemia, CD90/CD110 are helpful positive-selection markers (28). He et al. suggested that in a GBM - derived stem - like neurosphere line the level of expression for CD90 is higher than in three traditional adherent GBM cell lines (29).

In their study an increased capability to initiate tumorigenesis in vivo and the ability to re-establish the cellular hierarchy of tumors from single - cell implantation, appeared in CD90+ cells owing to their self-renewal properties. Jintang et al. by using tissue microarrays corroborated that CD90 could be consider as a high specific and sensitive marker, with medium or high level of expression in all the high - grade glioma tissues but hardly detectable in normal brains and low - grade gliomas. Thus, it seemed that CD90 could be considered as a hopeful prognostic marker for gliomas. A study in 2011, illuminate the role of CD90 as a specific factor for early detection of lung diseases as well as lung neoplastic and/or cancer stem cell marker. In another study, ten different markers of stem cell in human breast cancer cell lines were analyzed by Lobba et al. and suggested CD90 as a potential marker for breast CSCs, by cause of more than 90% of the Hs578-T cell line was CD90+ cells and represent a direct correlation with high tumorigenicity and metastatic potential of them (30).

Contrarily, although in various studies on different tumor cells CD90 was known as a cancer promoter, there are evidences that show CD90 can act as a tumor suppressor in ovarian cancer and nasopharyngeal carcinoma (NPC). Abeysinghe, H.R study showed that in an ovarian cancer cell line TSP-1 and fibronectin levels up-regulated by Thy-1 expression. This up-regulation prevents tumor angiogenesis and metastasis (31). Thy-1 expression plays a critical role in tumor suppression in nasopharyngeal carcinoma and ovarian cancer because it may inhibit proliferation (32).

In a study by Yamashita et al. in 2013, CD90 suggested as a mesenchymal and vascular endothelial metastatic CSC marker that occurence along with chemosensitivity to imatinib mesylate and expression of c-Kit (33). In addition, their study demonstrated that a risk for distant metastasis in liver cancer is abundant CD90+ cells in a tumor (26).

### 2.5. CD44 (PGP1)

The CD44 antigen is a cell - surface glycoprotein and involved in various cell mechanisms including cell proliferation, cell - cell interactions, cell differentiation, cell migration and adhesion, angiogenesis, inflammation, wound healing, bone metabolism, presentation of chemokines, cytokines, and growth factors corresponding with their receptors, and signaling for cell survival as well as docking of proteases at the cell membrane (34). Protein size diversity ranging from 80 to 200 kDa is mainly due to alternative splicing, post-transcriptional regulations and protein modifications. Alternative splicing during cancer progression can be regulated by oncogenic pathways like the Ras-MAPK cascade and Environment - specific factors (35). Hyaluronic acid (HA) is the principal ligand of CD44, a major component of the ECM (extracellular matrix), and a coreceptor for many growth factors and cytokines. Other

CD44 ligands include the ECM components such as collagen, laminin, fibronectin and chondroitin sulfate. Cell growth, differentiation and survival influenced by changes in the extracellular matrix that monitored by CD44 and its associated partner proteins (36).

CD44 has many various isoforms, up to 1000, due to splicing of 10 alternative exons and various post - translational modifications (36).

During the responses against invading microbes, naive T lymphocytes activate and upregulate CD44. Protection against re-infection mediated by elevated levels of CD44 that remains on the surface of memory T cells, thus selectively regulates the survival of the Th1 subset of CD4 T cells (37). CD44 organized signaling cascades due to association with the actin cytoskeleton and established specific transmembrane complexes by participates in signal transduction processes (36). CD44 individually has been used for the isolation of CSCs in oral, ovary, prostate, pancreatic, small intestine, colon, liver, stomach, head and neck, bladder and breast malignancies. In addition, it has been used in combination with other marker to identify these malignancies (7).

A correlation between poor prognosis and high expression of CD44 was affirmed. In laryngeal tumors, high expression of CD44 appears to correlate more strongly with a poor prognosis (38). Prince et al. showed that in a head and neck squamous cell carcinoma tumor, only less than ten present of CD44+ cancer cells, are capable to rise to new tumors in vivo, although, the CD44- cancer cells express the markers of differentiation (39). Furthermore, Takaishi et al. applied CD44 to identified gastric cancer - initiating cells. They demonstrated that CD44+ gastric cancer cells are able to self - renew and have the ability for establishment of differentiated progeny as the stem cell properties (7).

### *2.6. Prostate Stem Cell Antigen (PSCA)*

Prostate stem cell antigen (PSCA) is a cell membrane glycoprotein. PSCA is expressed in the bladder, stomach, kidney, colon and placenta. It showed confined expression in normal tissues, although, it highly expressed on the surface of primary and metastatic Prostate Cancer (PCa) cells (40). The gene of PSCA includes a polymorphism that results in an upstream start codon in some individuals.

In 2000, Gu et al. by usage of monoclonal antibodies, characterized the PSCA protein expression (41). They demonstrated that PSCA mRNA is overexpressed in a subset of prostate cancers and PSCA protein may be appear in basal pancreatic cells as well as secretory cells. They approved that PSCA protein have higher expression in bone metastases and have correlated with tumor stage. In 2001, PSCA overexpression in about two-thirds of the pancreatic cancers was approved by Argani et al. using RT-PCR, immunohistochemical and online SAGE database analysis (42). In a study by Amara et al. intense PSCA staining was seen in transitional cell tumors that were poorly differentiated and represented squamous features. Their study suggested the association between squamous differentiation and PSCA expression (43). They suggested the usage of PSCA detection on the surface of bone marrow and blood circulating cells in micrometastases identification for some cases of bladder cancer. In 2004, Zhigang et al. showed that the increasing level of expression in PSCA, occurrence along with high Gleason grade (44). Moreover, in situ hybridization and immunohistochemical analysis represented a high degree of correlation between mRNA upregulated transcription and PSCA protein. Wu et al. conducted a GWAS study and found a missense variant (rs2294008) in the PSCA gene associated with bladder cancer in European and US populations (45). This SNP change the start codon, therefore, it can produce truncated primary PSCA translation product.

### *2.7. CD200 (OX2)*

CD200 (Cluster of Differentiation 200) also named OX2 is a memberane glycoprotein. It has two immunoglobulin - like domains and is a member of the immunoglobulins superfamily. Animal studies have demonstrated that myeloid activity may be regulated by CD200. In diverse tissues, this protein can be considered as an inhibitory component for the macrophage lineage (46). CD200 expression was detected in several cancer tissues or cell lines, including head and neck carcinoma, ovarian, testicular, melanoma, malignant mesothelioma, renal cell carcinoma, neuroblastoma, chronic lymphocytic leukemia, colon, breast and prostate cancers. In acute myeloid leukemia (AML) and multiple myeloma (MM) CD200 has been considered as a prognostic factor. For a large array of malignancies CD200 is a potential therapeutic target. Kawasaki et al. recognized that the cancer stem cells avoid the detection by the host immune system through a potential mechanism that conducted by co-expression of CD200 on a subpopulation of cancer stem cell (47).

Seeking for molecular mechanisms related to CD200 have been showed that MAPK/ERK (Ras/Raf/Mek/Erk) signaling pathway regulated the CD200 mRNA and protein. In most metastatic melanomas (MM), mutation of B-RAF or N-RAS was recognized, so overexpression of CD200 is the outcome of this occurrence. On the other hand, expression of CD200 mRNA correlates with repression of primary T cell activation by dendritic cells (DCs) and melanoma progression, however, when CD200 gene was knocked down by shRNA, this immunosuppressive effect was abrogated (48). Furthermore, p53 plays a role in CD200 regulation. CD200

participate in apoptosis - associated immune tolerance, because in response to self - antigens, expression of CD200 on apoptotic DCs decrease pro-inflammatory cytokine production (49).

### 2.8. CD24 (HSA)

CD24 is a 28 - 75 kDa protein that has a core consists of 27 highly glycosylated amino acids (50). In mice, CD24 was found as a heat-stable antigen (HAS) (51). Several studies have demonstrated that CD24 was expressed on a variety of cell types such as pre B lymphocytes, neutrophils, and certain epithelial cells but not on normal T cells, monocytes, and cells outside the blood system. It was used as a biomarker for distinguishing B-cells from T-cells (52). In addition, the expression of CD24 was recognized on erythroleukemia, B-cell lymphomas and several solid tumors including esophageal squamous cell carcinoma, gliomas, hepatocellular carcinoma, pancreatic adenocarcinoma, cholangiocarcinoma, urothelial carcinoma, breast cancer, ovarian cancer, prostate carcinomas, primary neuroendocrine carcinomas, small cell lung cancer, rhabdomyosarcoma, neuroblastoma (53) and renal cell cancer.

In 2010, Lee et al. considered CD24 as a Metastasis - associated protein and a new stem cell marker in the human neoplasm. The expression of CD24 in NSCLC is along with adenocarcinoma histology and disease progression as well as cancer - related death. CD24 play a role in cell adhesion and metastatic tumor spread. In addition, poor prognosis in human neoplasm has association with increased level of CD24 expression. CD24 has recently been considered as a new interested marker for cancer stem cells. CD24 often associated with adhesion and/or migration molecules including CD29, CD44 and CD31 (platelet - derived/endothelial cell adhesion molecule) (54).

### 2.9. CD24/CD44

CD24, CD44 and ALDH1 are the most utilized biomarkers for identification and characterization of breast cancer stem cell (CSC) phenotype (51). Breast cancer stem - like cells showed the expression of CD44 (a cell surface protein) while they did not express CD24. CD44+ CD24- cells play a critical role in tumor formation in prostate. pancreatic cancer cells that expressed both CD44 and CD24 (CD44+ CD24+), showed cancer stem cell characters including self - renewal, enhancement of sonic hedgehog expression and production of differentiated progeny (55). The tumors organized by these cells were able to produce various cell types as well as all stem cells (56).

## 3. Conclusions

Uncontrolled growth of CSCs with self - renewal ability can lead to tumors. Studies show the importance of these cells in maintaining tumors (2). CSCs are small population of cancer cells with specific features that distinguish them from other tumor cells (57). CSCs are distinguished from other tumor cells by expressing surface proteins and some cellular activity, like the release of Hoechst dye or activity of aldehyde dehydrogenase. These markers are inducible by exposure to anti - cancer substances. The findings not only show the mobility of CSCs but also indicate the functional roles of them (58). These markers are related to CSCs features, including drug resistance, proliferation, self - renewal, and metastases. In this article we mentioned examples of the role and function of these markers. The therapeutic resistance of CSCs may be the result of increasing aldehyde dehydrogenase enzyme that destroys toxic intermediates. Therefore, these markers can be useful in the diagnosis of invasive resistant population. As a result, identifying signaling pathways and cellular functions of these markers help in finding better treatments (58).

In each cancer cell type, CSCs markers are special and unique. Noted that CSCs markers still don't fully understood, all of CSCs don't express these markers, and some of other cells; non-CSCs may express these markers. That's why these markers can be used to detect CSCs rich population. However, all CSCs can't be distinguished from other cells (2).

Scientific studies related to cancer stem cells have explosively increased over the current years. For example, radiotherapy is indicated as a successful anti-cancer approach. Krause et al. consider CSCs as strategies to optimize anti - cancer treatments by their evaluation as markers for patient classification to specific treatment strategies as well as developing treatment approaches that specifically target tumor cells expressing presumed CSC markers (59).

## Footnote

**Authors' Contribution:** The authors contributed equally to this work.

## References

1. Mansouri A, Abbaszadegan MR, Gholamin M. A Review on the Biology of Cancer Stem Cells. *Stem Cell Discov*. 2014;**4**(4):83–9. doi: 10.4236/scd.2014.44009.
2. Ojha R, Bhattacharyya S, Singh SK. Autophagy in Cancer Stem Cells: A Potential Link Between Chemoresistance, Recurrence, and Metastasis. *Biores Open Access*. 2015;**4**(1):97–108. doi: 10.1089/biores.2014.0035. [PubMed: 26309786]. [PubMed Central: PMC4497670].

3. Fanali C, Lucchetti D, Farina M, Corbi M, Cufino V, Cittadini A, et al. Cancer stem cells in colorectal cancer from pathogenesis to therapy: controversies and perspectives. *World J Gastroenterol*. 2014;**20**(4):923–42. doi: 10.3748/wjg.v20.i4.923. [PubMed: 24574766]. [PubMed Central: PMC3921545].
4. Litvinov SV, Velders MP, Bakker HA, Fleuren GJ, Warnaar SO. Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. *J Cell Biol*. 1994;**125**(2):437–46. [PubMed: 8163559]. [PubMed Central: PMC2120036].
5. Munz M, Kieu C, Mack B, Schmitt B, Zeidler R, Gires O. The carcinoma-associated antigen EpCAM upregulates c-myc and induces cell proliferation. *Oncogene*. 2004;**23**(34):5748–58. doi: 10.1038/sj.onc.1207610. [PubMed: 15195135].
6. Baeuerle PA, Gires O. EpCAM (CD326) finding its role in cancer. *Br J Cancer*. 2007;**96**(3):417–23. doi: 10.1038/sj.bjc.6603494. [PubMed: 17211480]. [PubMed Central: PMC2360029].
7. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med*. 2011;**17**(2):211–5. doi: 10.1038/nm.2284. [PubMed: 21240262]. [PubMed Central: PMC3076220].
8. Armstrong A, Eck SL. EpCAM: A New Therapeutic Target for an Old Cancer Antigen. *Canc Biol Ther*. 2014;**2**(4):320–5. doi: 10.4161/cbt.2.4.451.
9. Tomita N, Yamano T, Matsubara N, Tamura K. [A novel genetic disorder of Lynch syndrome - EPCAM gene deletion]. *Gan To Kagaku Ryoho*. 2013;**40**(2):143–7. [PubMed: 23411950].
10. Tabu K, Bizen N, Taga T, Tanaka S. Gene Regulation of Prominin-1 (CD133) in Normal and Cancerous Tissues. *Adv Exp Med Biol*. 2013;**777**:73–85. doi: 10.1007/978-1-4614-5894-4_5. [PubMed: 23161076].
11. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood*. 1997;**90**(12):5002–12. [PubMed: 9389720].
12. Zhou L, Wei X, Cheng L, Tian J, Jiang JJ. CD133, one of the markers of cancer stem cells in Hep-2 cell line. *Laryngoscope*. 2007;**117**(3):455–60. doi: 10.1097/01.mlg.0000251586.15299.35. [PubMed: 17334305].
13. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. *Nature*. 2004;**432**(7015):396–401. doi: 10.1038/nature03128. [PubMed: 15549107].
14. Beier D, Hau P, Proescholdt M, Lohmeier A, Wischhusen J, Oefner PJ, et al. CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res*. 2007;**67**(9):4010–5. doi: 10.1158/0008-5472.CAN-06-4180. [PubMed: 17483311].
15. Ogden AT, Waziri AE, Lochhead RA, Fusco D, Lopez K, Ellis JA, et al. Identification of A2B5+CD133- tumor-initiating cells in adult human gliomas. *Neurosurgery*. 2008;**62**(2):505–14. discussion 514-5. doi: 10.1227/01.neu.0000316019.28421.95. [PubMed: 18382330].
16. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ*. 2008;**15**(3):504–14. doi: 10.1038/sj.cdd.4402283. [PubMed: 18049477].
17. Chiba T, Zheng YW, Kita K, Yokosuka O, Saisho H, Onodera M, et al. Enhanced self-renewal capability in hepatic stem/progenitor cells drives cancer initiation. *Gastroenterology*. 2007;**133**(3):937–50. doi: 10.1053/j.gastro.2007.06.016. [PubMed: 17673212].
18. Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanhehco Y, et al. Characterization of clonogenic multiple myeloma cells. *Blood*. 2004;**103**(6):2332–6. doi: 10.1182/blood-2003-09-3064. [PubMed: 14630803]. [PubMed Central: PMC3311914].
19. Pearce DJ, Taussig D, Simpson C, Allen K, Rohatiner AZ, Lister TA, et al. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. *Stem Cells*. 2005;**23**(6):752–60. doi: 10.1634/stemcells.2004-0292. [PubMed: 15917471].
20. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell*. 2007;**1**(5):555–67. doi: 10.1016/j.stem.2007.08.014. [PubMed: 18371393]. [PubMed Central: PMC2423808].
21. Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, et al. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Mol Cancer Res*. 2009;**7**(3):330–8. doi: 10.1158/1541-7786.MCR-08-0393. [PubMed: 19276181]. [PubMed Central: PMC4255559].
22. Chen YC, Chen YW, Hsu HS, Tseng LM, Huang PI, Lu KH, et al. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. *Biochem Biophys Res Commun*. 2009;**385**(3):307–13. doi: 10.1016/j.bbrc.2009.05.048. [PubMed: 19450560].
23. Moreb J. Aldehyde Dehydrogenase as a Marker for Stem Cells. *Curr Stem Cell Res Ther*. 2008;**3**(4):237–46. doi: 10.2174/157488808786734006.
24. Ades EW, Zwerner RK, Acton RT, Balch CM. Isolation and partial characterization of the human homologue of Thy-1. *J Exp Med*. 1980;**151**(2):400–6. [PubMed: 6153212]. [PubMed Central: PMC2185777].
25. Rege TA, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. *FASEB J*. 2006;**20**(8):1045–54. doi: 10.1096/fj.05-5460rev. [PubMed: 16770003].
26. Buishand FO, Arkesteijn GJ, Feenstra LR, Oorsprong CW, Mestemaker M, Starke A, et al. Identification of CD90 as Putative Cancer Stem Cell Marker and Therapeutic Target in Insulinomas. *Stem Cells Dev*. 2016;**25**(11):826–35. doi: 10.1089/scd.2016.0032. [PubMed: 27049037]. [PubMed Central: PMC4892226].
27. Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, et al. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell*. 2008;**13**(2):153–66. doi: 10.1016/j.ccr.2008.01.013. [PubMed: 18242515].
28. Yamazaki H, Nishida H, Iwata S, Dang NH, Morimoto C. CD90 and CD110 correlate with cancer stem cell potentials in human T-acute lymphoblastic leukemia cells. *Biochem Biophys Res Commun*. 2009;**383**(2):172–7. doi: 10.1016/j.bbrc.2009.03.127. [PubMed: 19341705].
29. He J, Liu Y, Xie X, Zhu T, Soules M, DiMeco F, et al. Identification of cell surface glycoprotein markers for glioblastoma-derived stem-like cells using a lectin microarray and LC-MS/MS approach. *J Proteome Res*. 2010;**9**(5):2565–72. doi: 10.1021/pr100012p. [PubMed: 20235609]. [PubMed Central: PMC2866009].
30. Lobba AR, Forni MF, Carreira AC, Sogayar MC. Differential expression of CD90 and CD14 stem cell markers in malignant breast cancer cell lines. *Cytometry A*. 2012;**81**(12):1084–91. doi: 10.1002/cyto.a.22220. [PubMed: 23090904].
31. Abeysinghe HR, Li LQ, Guckert NL, Reeder J, Wang N. THY-1 induction is associated with up-regulation of fibronectin and thrombospondin-1 in human ovarian cancer. *Cancer Genet Cytogenet*. 2005;**161**(2):151–8. doi: 10.1016/j.cancergencyto.2005.02.014. [PubMed: 16102586].
32. Rege TA, Hagood JS. Thy-1, a versatile modulator of signaling affecting cellular adhesion, proliferation, survival, and cytokine/growth factor responses. *Biochim Biophys Acta*. 2006;**1763**(10):991–9. doi: 10.1016/j.bbamcr.2006.08.008. [PubMed: 16996153]. [PubMed Central: PMC1781924].
33. Yamashita T, Honda M, Nakamoto Y, Baba M, Nio K, Hara Y, et al. Discrete nature of EpCAM+ and CD90+ cancer stem cells in human hepatocellular carcinoma. *Hepatology*. 2013;**57**(4):1484–97. doi: 10.1002/hep.26168. [PubMed: 23174907].
34. Spring FA, Dalchau R, Daniels GL, Mallinson G, Judson PA, Parsons SF, et al. The Ina and Inb blood group antigens are located on a glycoprotein of 80,000 MW (the CDw44 glycoprotein) whose expression is influenced by the In(Lu) gene. *Immunology*. 1988;**64**(1):37–43. [PubMed: 2454887]. [PubMed Central: PMC1385183].
35. Louderbough JM, Schroeder JA. Understanding the dual nature of CD44 in breast cancer progression. *Mol Cancer Res*. 2011;**9**(12):1573–86. doi: 10.1158/1541-7786.MCR-11-0156. [PubMed: 21970856].
36. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules

to signalling regulators. *Nat Rev Mol Cell Biol*. 2003;**4**(1):33–45. doi: 10.1038/nrm1004. [PubMed: 12511867].
37. Baaten BJ, Li CR, Bradley LM. Multifaceted regulation of T cells by CD44. *Commun Integr Biol*. 2010;**3**(6):508–12. doi: 10.4161/cib.3.6.13495. [PubMed: 21331226]. [PubMed Central: PMC3038050].
38. Trapasso S, Allegra E. Role of CD44 as a marker of cancer stem cells in head and neck cancer. *Biologics*. 2012;**6**:379–83. doi: 10.2147/BTT.S37906. [PubMed: 23152667]. [PubMed Central: PMC3496191].
39. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A*. 2007;**104**(3):973–8. doi: 10.1073/pnas.0610117104. [PubMed: 17210912]. [PubMed Central: PMC1783424].
40. Reiter RE, Gu Z, Watabe T, Thomas G, Szigeti K, Davis E, et al. Prostate stem cell antigen: A cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci Unit States Am*. 1998;**95**(4):1735–40. doi: 10.1073/pnas.95.4.1735.
41. Gu Z, Thomas G, Yamashiro J, Shintaku IP, Dorey F, Raitano A, et al. Prostate stem cell antigen (PSCA) expression increases with high gleason score, advanced stage and bone metastasis in prostate cancer. *Oncogene*. 2000;**19**(10):1288–96. doi: 10.1038/sj.onc.1203426.
42. Argani P, Rosty C, Reiter RE, Wilentz RE, Murugesan SR, Leach SD, et al. Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. *Cancer Res*. 2001;**61**(11):4320–4. [PubMed: 11389052].
43. Amara N, Palapattu GS, Schrage M, Gu Z, Thomas GV, Dorey F, et al. Prostate stem cell antigen is overexpressed in human transitional cell carcinoma. *Cancer Res*. 2001;**61**(12):4660–5. [PubMed: 11406532].
44. Zhigang Z, Wenlv S. Prostate stem cell antigen (PSCA) expression in human prostate cancer tissues and its potential role in prostate carcinogenesis and progression of prostate cancer. *World J Surg Oncol*. 2004;**2**:13. doi: 10.1186/1477-7819-2-13. [PubMed: 15132743]. [PubMed Central: PMC420493].
45. Wu X, Ye Y, Kiemeney LA, Sulem P, Rafnar T, Matullo G, et al. Genetic variation in the prostate stem cell antigen gene PSCA confers susceptibility to urinary bladder cancer. *Nat Genet*. 2009;**41**(9):991–5. doi: 10.1038/ng.421. [PubMed: 19648920]. [PubMed Central: PMC3313685].
46. Douds JJ, Long DJ, Kim AS, Li S. Diagnostic and prognostic significance of CD200 expression and its stability in plasma cell myeloma. *J Clin Pathol*. 2014;**67**(9):792–6. doi: 10.1136/jclinpath-2014-202421. [PubMed: 24958785].
47. Kawasaki BT, Mistree T, Hurt EM, Kalathur M, Farrar WL. Co-expression of the toleragenic glycoprotein, CD200, with markers for cancer stem cells. *Biochem Biophys Res Commun*. 2007;**364**(4):778–82. doi: 10.1016/j.bbrc.2007.10.067. [PubMed: 17964286]. [PubMed Central: PMC2168034].
48. Petermann KB, Rozenberg GI, Zedek D, Groben P, McKinnon K, Buehler C, et al. CD200 is induced by ERK and is a potential therapeutic target in melanoma. *J Clin Invest*. 2007;**117**(12):3922–9. doi: 10.1172/JCI32163. [PubMed: 18008004]. [PubMed Central: PMC2075477].
49. Rosenblum MD, Olasz E, Woodliff JE, Johnson BD, Konkol MC, Gerber KA, et al. CD200 is a novel p53-target gene involved in apoptosis-associated immune tolerance. *Blood*. 2004;**103**(7):2691–8. doi: 10.1182/blood-2003-09-3184. [PubMed: 14644999].
50. Kraus S, Shapira S, Kazanov D, Naumov I, Moshkowitz M, Santo E, et al. Predictive Levels of CD24 in Peripheral Blood Leukocytes for the Early Detection of Colorectal Adenomas and Adenocarcinomas. *Dis Markers*. 2015;**2015**:916098. doi: 10.1155/2015/916098. [PubMed: 26078485]. [PubMed Central: PMC4442284].
51. Jaggupilli A, Elkord E. Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity. *Clin Dev Immunol*. 2012;**2012**:708036. doi: 10.1155/2012/708036. [PubMed: 22693526]. [PubMed Central: PMC3369436].
52. Li O, Zheng P, Liu Y. CD24 expression on T cells is required for optimal T cell proliferation in lymphopenic host. *J Exp Med*. 2004;**200**(8):1083–9. doi: 10.1084/jem.20040779. [PubMed: 15477346]. [PubMed Central: PMC2211842].
53. Lim SC, Oh SH. The role of CD24 in various human epithelial neoplasias. *Pathol Res Pract*. 2005;**201**(7):479–86. doi: 10.1016/j.prp.2005.05.004. [PubMed: 16164042].
54. Lee HJ, Choe G, Jheon S, Sung SW, Lee CT, Chung JH. CD24, a novel cancer biomarker, predicting disease-free survival of non-small cell lung carcinomas: a retrospective study of prognostic factor analysis from the viewpoint of forthcoming (seventh) new TNM classification. *J Thorac Oncol*. 2010;**5**(5):649–57. doi: 10.1097/JTO.0b013e3181d5e554. [PubMed: 20354454].
55. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. *Cancer Res*. 2007;**67**(3):1030–7. doi: 10.1158/0008-5472.CAN-06-2030. [PubMed: 17283135].
56. 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**(2). e57314. doi: 10.1371/journal.pone.0057314. [PubMed: 23437366]. [PubMed Central: PMC3577706].
57. Chen W, Dong J, Haiech J, Kilhoffer MC, Zeniou M. Cancer Stem Cell Quiescence and Plasticity as Major Challenges in Cancer Therapy. *Stem Cells Int*. 2016;**2016**:1740936. doi: 10.1155/2016/1740936. [PubMed: 27418931]. [PubMed Central: PMC4932171].
58. Keysar SB, Jimeno A. More than markers: biological significance of cancer stem cell-defining molecules. *Mol Cancer Ther*. 2010;**9**(9):2450–7. doi: 10.1158/1535-7163.MCT-10-0530. [PubMed: 20716638]. [PubMed Central: PMC3618879].
59. Krause M, Dubrovska A, Linge A, Baumann M. Cancer stem cells: Radioresistance, prediction of radiotherapy outcome and specific targets for combined treatments. *Adv Drug Deliv Rev*. 2017;**109**:63–73. doi: 10.1016/j.addr.2016.02.002. [PubMed: 26877102].