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Review Article

Cancer stem cell markers

Maryam Adelipour[1], Foad Abdollahpour[1], Abdolamir Allameh[1]*

1. Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Abstract

Cancer stem cells (CSC) are the tumor-associated cells existed within tumors or hematological cancers which share characteristics similar to normal stem cells. The common characteristics of a normal stem cell and a CSC are their differentiation capacity and self-renewal in tumors. The expression pattern of CSC markers differs depending on the type and location of cancers. CD molecules are probably the most common biomarkers for CSCs. CD molecules such as CD133, CD24, CD44, CD138 and similar CD molecules are well known markers for identification of CSCs. In addition, ATP-Binding Cassette (ABC) transporters such as ABCG2 and ABCB5 as well as EpCAM, ALDH1 and CXCR4 have been used to identify certain CSCs. Therefore these markers may be considered specific for better identification and diagnosis of a specific tumor. Currently studies are in progress to find new cell surface markers which can distinguish specific markers from other markers for isolation and characterization of CSCs. The future of this area of research is promising in developing novel prognostic assays and therapeutic approaches based on cellular and signaling functions of these markers.

Keywords: Cancer stem cell, Biomarker, Tumor

Introduction

Cancer stem cell (CSC) is basically discovered in hematological malignancies and developed to solid tumors. The cells called CSCs or tumor stem cells (TSCs) are a group of tumor cells responsible for initiating and maintaining the tumor, allowing the spread of tumor cells to distant sites and resistant to standard drug chemotherapy (Keysar SB and Jimeno A, 2010). There are three hypotheses about the source of CSCs. According to these hypotheses CSCs may arise from stem cells, progenitor cells or differentiated cells that undergo one or more mutations (Rapp UR, 2008; Yu J, 2007; Hope KJ, 2004).

CSCs and normal stem cells share similar properties such as self-renewal capacity, the ability to differentiate into other lineages, active anti-apoptotic signalling pathways, increased activity of membrane transporters and migration/metastatic capacity (Wicha MS, 2006).

According to the "CSC hypothesis" cancers originate from a small population of tumor-initiating cells (TIC) or cancers initiating cells (CIC). TICs or CICs can form heterogenous

***Corresponding author. Abdolamir Allameh, PhD**
Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; P.O. Box: 14115-111
Tel. +9821-82883877
Email: allameha@modares.ac.ir

cancer cell population within tumors which are similar to that formed in solid tumors. The heterogeneity of tumors is the result of genetic and epigenetic changes in the cells occur during the carcinogenesis. Transformation of normal cells occurs through accumulation of different mutations and generation of tumors with different sub-populations of cells (Clarke MF, 2006; Reya T, 2001; Croker AK and Allan AL, 2008).

Studies show that established cancer cell lines grown in culture are straightforward to identify CSCs and their characteristic. However, patient's tumor sample is the gold standard for identification of the CSC-related markers. Isolation of CSCs is a major difficulty to be obtained on a regular basis. In addition, a limited number of CSCs in tumor tissue, technical difficulties in maintaining the CSCs in every culture, and unusually strong resistance to drugs are other challenging issues to work with CSCs (Keysar SB and Jimeno A, 2010; Dou J and Gu N, 2010).

Identification of CSCs based on the cellular and signaling functions can be used to distinguish potential and specific tumor markers from other unspecific biomarkers. Identification of such specific markers can also help to discriminate biomarkers of tumors depending on cancer type and location. This article has been reviewed the current documents introducing common CSC markers and discussed the potential implication of them for cancer targeted therapy and diagnosis.

Biomarkers of CSCs

Variety of CSC markers with different patterns can be seen in various cancers. These markers are required for isolation and analysis of biological characteristics of CSCs to target them for therapeutic purposes. The identification of CSC-specific markers remains a challenge. Some CSC markers in common cancers are shown in Table 1.

Table 1 Some examples of CSC markers in common cancers

Cancer	Cancer stem cell marker(s)	References
Breast	ALDH1, CD44, CD24, CD138, ABCG2	(Ginestier C, 2007), (Draffin JE, 2004), (Ginestier C, 2007), (Ibrahim SA, 2013)
Colon	CD44, CD133, EpCAM, CXCR4	(Du L, 2008), (Schneider M, 2012), (Milner B, 2015), (Dessein A-F, 2010)
Brain	CD133, CD44	(Brescia P, 2013), (Anido J, 2010)
Lung	CD133, ALDH1	(Wen L, 2013), (Jiang et al., 2009)
Melanoma	ABCB5, CD133	(Chen KG, 2009), (Sharma BK, 2010)
Ovarian	CD133	(Osman WM, 2016), (Baba T, 2009)
Liver	CD133, EpCAM, ABCG2	(Suetsugu A, 2006; Yao J, 2009), (Yamashita T, 2009), (Zhang G, 2013)
Pancreas	CD133, CD24	(Moriyama T, 2010; Hermann PC, 2007), (Li C, 2007)
Gastric	CD133, CD44, ABCG2	(Ishigami S, 2010), (Takaishi S, 2009), (Jiang Y, 2012)
Prostate	CD44, CD24, ABCG2	(Draffin JE, 2004), (Cremers N, 2016), (Guzel E, 2014)

This table summarizes the most important CSC markers that refer in the text of this article.

CD molecules

Cluster of Differentiation (abbreviated as CD) is a term used to immuno-identification and study of cell surface molecules. They act as receptors or ligands and play their role in cell signaling, cell adhesion and some other functions including cellular identification (Keysar SB and Jimeno A, 2010; Zola H, 2007).

1-CD133

CD133 protein (a pentaspan membrane protein) is regarded as a global marker for normal hematopoietic stem cells and organ-specific stem cells. It has been recognized as a CSC surface marker in solid primary tumors (Grosse-Gehling P, 2013). Some of studies in vitro showed CD133 as a CSC marker in some cancer including medulloblastomas (Morrison LC, 2013), mouse melanoma (Dou J, 2007), colon cancer (Schneider M, 2012) and hepatocellular carcinoma (HCC) (Suetsugu A, 2006; Yao J, 2009). Also, some studies showed CD133 as CSC marker in some human cancer including glioblastomas (Brescia P, 2013), ovarian cancer (Baba T, 2009), pancreatic cancer (Moriyama T, 2010; Hermann PC, 2007), gastric cancer (Ishigami S, 2010) and melanoma (Sharma BK, 2010). In this connection, Qu et al. reviewed the correlation between CD133 expression as a CSC marker and clinicopathological features of non-small cell lung cancer (NSCLC) patients. They showed that CD133 expression was associated with worse prognosis and common clinical parameters of NSCLC, such as tumor differentiation and lymph node metastasis (Wen L, 2013). Therefore, CD133 expression can be used as a diagnosis and prognosis marker in various cancers.

2-CD44

CD44 is one of the most studied stem-like cells surface markers, which is expressed by almost every tumor cell. CD44 is a transmembrane glycoprotein with 85 to 90 kDa molecular weight and has many roles in proliferation, motility, cell adhesion, drug resistance, cell survival, cell migration, wound healing, and the growth and metastasis of cancer cells (Marhaba R and Zöller M, 2004; Afify A, 2009; Ponta H, 2003). CD44 is a major receptor for hyaluronan (HA) -a primary ingredient of the extracellular matrix (ECM)- and their interaction plays an important role in cancer cell signaling. HA has been shown to be rich in stem cell niche and plays a major role in the manner of CD44 in CSCs (Keysar SB and Jimeno A, 2010). According to some studies CD44 is a CSCs surface marker in some solid tumors (Miletti-González KE, 2005) including human glioblastoma (Anido J, 2010), primary cell line of head and neck cancer (Prince M, 2007), prostate and breast

cancer cell lines (Draffin JE, 2004) and gastric cancer cell lines (Takaishi S, 2009). In this line, Du et al. showed that CD44 is a robust colorectal CSC marker which has important role in colorectal cancer initiation (Du L, 2008). Based on this information, it can be suggested that CD44 and its related signalling pathway can be used for diagnosis and treatment of some cancers.

3-CD24

CD24 (single chain protein with 27aa) is a very small glycosylated cell surface protein linked to cell membrane by glycosylphosphatidylinositol anchor. Variable glycosylation on CD24 responsible for distinct functions in different cells is still unclear. This molecule is regarded as a global ligand in different cells such as malignant tumor cells. CD24 is a heat stable antigen, first discovered in mice which is involved in cell adhesion, proliferation, and migration (Aigner S, 1997; Baumann P, 2005).

Overexpression of CD24 in various cancer types implies that this marker is a good candidate marker for cancer prognosis and diagnosis. For instance expression of CD24 as a CSC marker in human pancreatic adenocarcinomas (Li C, 2007) and human esophageal squamous cell carcinoma (Sano A, 2009) has been reported.

According to Chio et al. the expression of CD24 correlates with the degree of differentiation in colorectal adenocarcinoma patients but there was not significant correlation between survival and CD24 expression (Choi D, 2009). Also, Yang et al. introduced the CD24 as a novel CSC biomarker for nasopharyngeal carcinoma. They showed that CD24+ cells isolated from human nasopharyngeal cell lines have stem cell properties with the ability to initiate tumors following their injection into immunodeficient mice (Yang C-H, 2014). However, the part played by CD24 in tumor initiation and progression is not well known. Very recently, Cremers et al. reported that although CD24 is expressed during tumor development but it is not essential for tumor initiation and development in mouse model of breast and prostate cancers (Cremers N, 2016).

4-CD138

CD138, also called Syndecan-1, is a proteoglycan and regarded as a biochemical marker in epithelial-mesenchymal transition (EMT) and is considered as principal marker for cell-cell and cell-matrix interactions during development and carcinogenesis. This proteoglycan acts as a co-receptor for chemokines and growth factors (Palaiologou M, 2014).

CD138 (syndecan-1) is expressed by multiple myeloma (MM) cells which were obtained from most of MM cell lines and patient

specimens. Expression of CD138 has been used to detect MM clinical specimens and cell lines. The expression of CD138 is considered to be specific for differentiated plasma cells during normal B-cell development. CD138 is the most specific marker for normal and MM plasma cells (Bayer-Garner IB, 2001; Matsui W, 2004). It has been demonstrated that anti-CD138-targeted interferon is an effective novel therapeutic factor against MM (Yoo EM, 2015). Furthermore, according to Ibrahim et al. suppression of CD138 using RNA interference reduces CSC phenotype in breast cancer cell lines through regulation of the Wnt and IL-6/STAT3 signaling pathways (Ibrahim SA, 2013). Hence, CD138 emerges as a target for cancer therapeutic approaches.

5-Other CD Molecules

The tumor cell lines have complex CSC markers pattern which is associated with the type of tumor. In this section several CSC markers associated with tumor is briefly introduced. CD90 (THY1) is a glycoprotein with cell membrane glycosylphosphatidyl-inositol anchorage that involved in signal transduction. It may also mediate adhesion between thymocytes and thymic stroma. It is shown that CD90 can be as a CSC marker in insulinoma human cell line and therapeutic target in mouse model of insulinomas (Buishand FO, 2016). CD200 (OX-2) is a type 1 membrane glycoprotein, which delivers an inhibitory signal to immune cells including T cells, NK cells and macrophages. CD200 was related to CSC features in head and neck squamous cell carcinomas and might be a potential therapeutic for cancer(Jung YS, 2015). Putative CSC markers are CD15, CD24, CD44, CD133, CD166 and CD326 which expressed widely across the 60 cell lines. Clearly more studies to determine the complexity and specificity of CSCs markers is required (Stuelten CH, 2010).

ATP-Binding Cassette Transporters

Keeping the body's stem cells away from damage due to xenobiotics is crucial for all organisms. One of the protecting mechanisms is the expression of the ATP-binding cassette (ABC) transporter family. These transporters have a role in multidrug resistance of tumor cells which make CSCs resistant to many standard therapies and let them to survive in cytotoxic conditions and lead to tumor regrowth or relapse. More than fifty ABC transporters have been discovered in mammalian cells, including multi-drug resistance protein 1, (MDR1, ABCB1 or P-glycoprotein), ABCB5, ABCC1 and breast cancer resistance protein (BCRP/ABCG2) etc. Basically, ABC transporter operation enables cancer stem cells to escape the effects of cytotoxic chemotherapeutics which kills most

cells in a tumor (Schinkel AH and Jonker JW, 2003; Kim M, 2002; Dean M, 2005).

1-ABCG2

Based on the arrangement of component domains, human ABC transporters are divided into seven subfamilies (from A to G). Human ABCG2 is the second member of the G subfamily of ABC transporters. ABCG2 was first cloned from doxorubicin-resistant human MCF-7 breast cancer cells and named as breast cancer resistance protein (BCRP) (Doyle LA, 1998). ABCG2 is a half-transporter that requiring dimerization to become functionally active, which can pump many endogenous and exogenous compounds out of cells (Rocchi E, 2000). ABCG2 expressed in almost all stem cells, and is recognized as a universal marker of stem cells. ABCG2 plays an important role in promoting stem cell proliferation (Ding X-w, 2010). Very recently it has been reported that over-expression of ABCG2 is significantly correlated with prognosis and progression of human eyelid sebaceous gland carcinoma (Kim N, 2015). According to Zhang and colleagues (2013), ABCG2 expression is correlated with malignant behaviour including proliferation, drug resistance, migration, and invasion in hepatocellular carcinoma tissues and cell line. Furthermore, it has been demonstrated that knockdown of ABCG2 using RNA interference can modulate these malignant behaviors (Zhang G, 2013). Also according to Jiang et al. (2012), increased expression of ABCG2 is associated with poorly differentiated gastric cancer in human gastric adenocarcinomas and in the cancer cell lines. More recently it was shown that the expression of ABCG2 on recurrent prostate cancer samples might be associated with drug resistant (Guzel E, 2014). Likewise, association of ABCG2 expression with invasion and recurrence in tongue cancer of human tissue has been reported (Yanamoto S, 2014). Hence, it appears that the ABCG2 has potential applications in tumor prognosis and therapy.

2-ABCB5

ABCB5 also known as P-glycoprotein is a membrane-spanning molecule which expressed in skin and human malignant melanoma. ABCB5 is over-expressed on circulating melanoma tumor cells which is a chemotherapeutic drug resistance protein of CSCs in human malignant melanoma. The expression of ABCB5 observed in CSC tumor formation and metastasis. Because of the major mechanisms for protecting putative cancer stem cells is made by expression of efflux transporters such as ABCB5 which may help the cells to survive and help tumor relapse (Chen KG, 2009). Expression of ABCB5 might be associated with tumor formation and

metastasis in human tissues and cell lines of oral squamous cell carcinomas (Grimm M, 2012). Therefore, our knowledge on expression profile of ABC transporters can help therapeutic optimization for cancer patients.

Other markers

1-EpCAM

Epithelial cell adhesion molecule (EpCAM/ CD326) is a Ca^{2+}-independent adhesion molecule that firstly was considered as a dominant antigen in human colon cancer tissues (Cohen SJ, 2006). EpCAM with molecular weight 30- 40 kDa is a type- I membrane protein of 314 amino acids that comprise the extracellular domain of epidermal growth factor (EGF) and thyroglobulin like repeat domain, a single transmembrane domain, and an intracellular domain of 26 amino acid. It has been shown that the EpCAM is expressed in various human epithelial tissue, carcinoma, and stem cells. Hence, it is used as a therapeutic target for antibody-based approaches (Munz M, 2009). Yamashita et al. (2009), has reported that a subset of $EpCAM^{+}$ cells with stem/progenitor features can induce hepatocellular carcinoma, with the ability of initiating invasive hepatocellular carcinoma in NOD/SCID mice. Also, it has been shown that the expression of EpCAM as a CSC marker in colon cancer is associated with tumor stage and aggression (Milner B, 2015). Therefore the EpCAM can be considered as a potential CSC marker for prognosis of several cancers.

2-ALDH1

Aldehyde dehydrogenase-1 (ALDH1), the enzyme responsible for the oxidation of intracellular aldehydes has been shown to be a marker for tumor stem cells in lung cancer patients that correlated with the stage, grade and prognosis of lung (Jiang F, 2009). Su et al. (2010) has reported that ALDH1 as a marker for monitoring the progression of bladder tumor highly expressed in 26% human bladder tumor specimens, and this was correlated with the stage, grade, recurrence, progression, and metastasis of bladder cancer(Su Y, 2010). Increased activity of ALDH has also been shown in normal and cancer human mammary epithelial cells with stem/progenitor properties that have high capability of engraftment into NOD-SCID mice (Ginestier C, 2007).

3-CXCR4

CXCR4, also called fusin, is an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF-1). This receptor is known to be required for HIV isolates to infect $CD4^{+}$ T cells. Chemokine receptors are a family of seven transmembrane G protein-coupled cell surface receptors (GPCR) which have been classified into four groups (CXC,

CC, C, and CX3C) based on the position of the first two cysteines (Desurmont T, 2015). CXCR4 is one of the best studied chemokine receptors and is a 352-amino acid rhodopsin-like GPCR that selectively binds the CXC chemokine SDF-1, also known as CXCL12 (Zlotnik A and Yoshie O, 2000). CXCR4 is expressed on normal stem cells of various organs and tissues (Jazayeri M, 2008). Interestingly, while CXCR4 is expressed in a variety of cancers, its expression in adjacent normal tissue is minimal or absent, which may suggest that malignant cells may be derived from CXCR4-expressing normal stem cells (Müller A, 2001; Balkwill F, 2004). According to Dessein et al. invasive and metastatic phenotypes of colon cancer cell was induced by MIF-CXCR4 Axis (Dessein A-F, 2010). Also, according to Desurmont et al. the CXCL7/CXCR2 signalling pathways in samples of liver metastasis from colon cancer is correlated to shorter disease-free and overall survival (Desurmont T, 2015). A growing body of evidence shows that CXCR4 has a role not only in cancer metastasis but also in regulating CSCs and can be used as a CSCs marker.

4-Correlation of CSC markers and angiogenesis markers

There are also unspecific markers such as angiogenesis-related markers which may indirectly contribute to CSC activity and promotion of cancer. Angiogenesis is an important event in the progression of tumors. There are common markers in tumor development and abnormal angiogenesis. Markers expressed by endothelial cells such as, VEGFR2, CD31, vascular endothelial cadherin (VE-cadherin), vascular cell adhesion protein-1(VCAM-1) (Jazayeri et al., 2008) may be linked to other CSC markers. According to Osman et al. Nestin can be used as an angiogenesis and CSC marker in epithelial ovarian cancer and may be a novel therapeutic target for tumor associated angiogenesis. In addition, detection of nestin can be used as predictor marker of disease (Osman WM, 2016). Also, Zhang et al. showed that the correlation of CSC markers (CD133 and CD44) and vasculogenic mimicry are associated with prognosis in renal cell carcinoma (Zhang Y, 2013). The correlation between CSC specific markers with the angiogenesis-related markers may suggest the involvement of such CSCs in tumor progression. In addition, markers from both the origins possess prognosis and therapeutic applications.

D-Characterization of CSCs using a panel of markers

Because of the heterogeneity and complex nature of CSCs biology, it is rather difficult to

find a specific universal marker for detection of these cells in every cancer type. A number of studies used more than one maker for characterization of CSCs. For instance, report by Liu et al showed that CSCs with high tumorigenic capacity in breast tumor identified by CD44 expression but low level of CD24 ($CD44^+CD24^-$/low) (Liu R, 2007). Contrasting this, Ahmed et al. reported that the, the $CD44^-$/$CD24^+$ phenotype correlates with poor prognosis in early invasive breast cancer samples while $CD44^+$/$CD24^-$ phenotype has best prognosis (Ahmed MA, 2012). Han et al have shown a small population of $CD24^+$/$CD44^+$ cells with CSC properties that presented in human head and neck squamous cell carcinoma (Han J, 2014). Bonnet & Dick (1997) showed $CD34^+$/$CD38^-$ cell population with CSC properties that can initiate leukemia in NOD-SCID mice (Dick D, 1997). According to Milner et al. the co-expression of CD133/EpCAM as CSC-specific markers may be associated with tumor stage and aggression in human colon adenocarcinoma cell lines (Milner B, 2015). In another research on colorectal cancer, prognostic impact of the expression of CD133, CD166, CD44s, EpCAM, and ALDH1 as CSC markers was investigated. It has been shown that the loss of membranous CD44s, CD166, and EpCAM is linked to tumor progression (Lugli A, 2010). Also , Wilson et al reported currently available markers are not specific for liver CSCs and suggested that a range of markers should be examined for isolation and characterization of CSCs in each hepatocellular carcinoma phenotype (Wilson GS, 2013).

Some studies showed that CSC markers do not co-localize in the same region of cancer tissue. For example Du et al. showed lack of colocalization of CD44 and CD133 in of colorectal cancer tissues. They showed that a CD44-positive or CD133-positive single cell can form a sphere in vitro which can initiate a xenograft tumor resembling the properties of primary tumors although knockdown of CD44, but not CD133, strongly prevents the initiation of a tumor in vivo (Du L, 2008).

Future prospects

It is now clear that CSCs has an important role in initial formation of tumors, although there is no distinct method for their discrimination. Development of specific, sensitive and reliable methods for detection of CSCs as biomarkers is promising for early detection of cancer, metastasis and recurrences of malignant tumors. CSCs may also be useful in development of novel therapeutic approaches for targeting specific tumors.

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