Recent Research Progress on The Expression of Cancer Stem Cell Biomarkers

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Abstract. Cancer is the biggest cause of human death after ischemic heart disease, which places a huge burden on patients and society. Morever, scholars have to spend a lot of energy on research on early cancer screening and post diagnosis treatment. Malignant tumors can escape the monitoring of the immune system and develop metastasis and drug resistance. The research on cancer metastasis, drug resistance and recurrence has made some progress and is full of challenges. Also, cancer stem cell (CSC) has the ability of self replication. CSCs are considered to mediate the tumor progression, including but not limited to the above malignant manifestations. Because the stemness of CSCs has been confirmed by many experiments, people gradually began to pay attention to the correlation between the CSCs and other human natural stem cells, especially cell surface biomarkers. In fact, many cell biomarkers which are not expressed in normal tissues but are expressed in adult stem cells or human embryonic stem cells have been found, which provides a new possibility for cancer treatment against CSCs. In this article, we will discuss and summarize the similarities and differences in the expression of surface markers of human cancer stem cells, embryonic stem cells and adult stem cells. Morever, combined with the further research of existing CSCs, such as regulating tumor proliferation and reducing CSCs drug resistance through targeted markers, this review analyzes the value and challenges of tumor stem cells in this research field. We hope that this review will provide fertile ground for early screening and treatment of cancer.

Keywords: Cancer stem cell (CSC), Surface markers, Adult stem cell, Human embryonic stem cell (hESC), CD133, Mesenchymal stem cell (MSC).

1. Introduction

Due to the high mortality and intractability of cancer, scholars have shown increasing interest and research enthusiasm in the early diagnosis of cancer and treatment of cancer. Among them, exploring the mechanism of cancer occurrence, exploring the weak links in the proliferation and development of malignant tumor cells, and developing specific drugs for each stage of cancer progression are several hot ideas in the current research. Cancer stem cells (CSCs) are regarded as the "seeds" of cancer. A series of evidences show that CSC mediates the metastasis of malignant tumors. According to statistics, the 5-year relative survival rate of stage IV colorectal cancer with distant metastasis is only 14%[1]. CSC also shows adaptability to the body environment and resistance to traditional treatment. Although local resection is possible at the appropriate time after cancer recurrence, the later incidence rate and mortality rate during perioperative period are still remarkable. Therefore, when overcoming the metastasis, recurrence and drug resistance of malignant tumors has become the main difficulty in the treatment of cancer, seeking a breakthrough in CSC is the best choice after comprehensively analyzing the current research situation. However, identification, isolation and acquisition of CSC are the premise and difficulty of further study of CSC. Based on the fact that CSC is a kind of stem cells, this review takes the comparison of the expression of CSC and other normal human stem cell surface markers as the starting point to classify and elaborate the recognized CSC markers. It focuses on the expression and tissue specificity of more than 25 markers on human embryonic stem cells, human adult stem cells and CSCs, hoping to give hints on the origination of CSCs and their relationship with the heathy stem cells in human body.

2. Profile CSC

2.1. Nomal stem cell in human body

Human embryonic stem cells (hESCs) and various adult stem cells are included in the range of human normal stem cells. For example, the ematopoietic stem cells (HPCs), bone marrow mesenchymal stem cells (MSCs), and neural stem cells (NSCs) are included in the later.

Research on various stem cell markers has been widely carried out. Markers of human embryonic stem cells (hESCs) are SSEA-4, SSEA-3, teratoma derived growth factor 1 (TDGF1), growth differentiation factor 3 (GDF3), TRA antigen, Oct4, Thy1 and Nanog. CD markers such as CD24, CD133 and CD29 are often detected and studied. CD90 and CD117 were also expressed in hESCs.

Mesenchymal stem cells have considerable self proliferation and renewal ability, as well as the ability to differentiate into other cells. They also participate in immune regulation. The cell surface markers used for MSC screening are SH2, SH3, CK8, CK18, HLA-I, HLA-II, CD29, CD49e, CD90, CD105 and so on [2]. Most of them can not determine the identity of MSC alone, and they usually need to be jointly screened by multiple markers to obtain some MSC with high purity.

The surface markers of neural stem cells (NSCs) include sialic acid neural cell adhesion molecule, SSEA-1, nestin and vimentin expressed in neural precursor cells. Musashi1 and Musashi2 are expressed in neural stem cells as well as epithelial stem cells and stem cells from breast or intestinal tissue. Unfortunately, the specificity of these markers is not high, and the cells expressing these molecules are not necessarily neural stem cells.

At present, the research on most kinds of normal stem cells is relatively mature. The reason may be that most cells function normally and the expression of various markers is stable, which shows the relative conservatism of normal stem cells in the case of benign proliferation. At the same time, this provides a good and stable theoretical support for exploring the likeness and differences between the mean two kinds of stem cells.

2.2. CSC

Cancer stem cells (CSCs) are also named tumor proliferating cells, tumor progenitor cells (TPCS) and cancer initiating cells (CICS).

In the current biological research of tumor, the hypothesis of tumor stem cell has always been a research hot field. This hypothesis holds that only a few cells in a heterogeneous tumor are capable of replicating, renewing and differentiating into different sorts of cells, which is regarded as the source of malignancy.

In 1994, it was first found that the development of myeloid leukemia (AML) was caused by a known leukemia cell subgroup (CD38-, CD34+) alone. People have thus accepted the existence of stem cells in blood system tumors. Later, Al Hajj and his colleagues found that only hundreds of cells with a specific phenotype (CD44+, CD24-) can cause tumor in mice. At the same time, more than thousands of cells with different phenotypes can not simulate the formation of tumor spheres, which provides evidence for the existence of tumor stem cells in solid tumors.

CSC is called "the seed of tumor". Tumor stem cells have the characteristics of tumorigenicity, heterogeneity, drug resistance, metastasis and large fluctuation in the number of tumor masses. It is these characteristics that make it difficult for the tumor to be cured, cause tumor recurrence and predict the poor prognosis of patients with cancer metastasis.

The tumor stem cell hypothesis is supported by two models. Tumor stem cell model and clonal evolution model. The tumor stem cell model believes that tumor stem cells are located at the highest hierarchy of the tumor cells system, emphasizing the similar features with normal tissue stem cells, such as ability to achieve self-renewal and differentiate into other cells through homogeneous or heterogeneous division. However, these stem cell like characteristics of CSC are malignant manifestations out of normal orbit. The clonal evolution model believes that tumor cells have only those mutations that obtain growth advantage in random mutation, and these dominant tumor cell subsets have the potential of tumor regeneration. The two classic models are not mutually exclusive

and may exist in the course of carcinogenesis. Just like the performance of leukemia stem cells, CSC can clone and evolve to produce many tumor stem cells with different genetic backgrounds. On the other hand, when mutations give tumor cells stronger ability to invade and proliferate, new CSCs will be produced.

Based on these two models, scholars have speculated on the origin of tumor stem cells. There are three mainstream hypotheses of CSC origin. Firstly, CSC is derived from normal stem cells (NSC). Then NSC mutates, making it transformed into malignant CSC. The second probability is that the progenitor cells mutate in the process of differentiation, lose the ability of differentiation and obtain the ability of self-renewal, and transform into CSC. The third possibility is that the mature terminally differentiated cells may accidentally obtain unlimited proliferation and differentiation after gene mutation. These changes make them become CSCs. Among them, there is the most evidence supporting the origin of CSC from NSC. Scholars explored and found that CSC shares many similar signal pathways with NSC, and expresses the same markers on the cell surface.

At present, there are many methods to complete the identification and sorting of CSCs, including flow cytometry (FCM) or magnetic cell sorting (MACS) based on surface markers, sorting method based on side population cell (SP cell) capable of efflux nucleic acid dye, screening clonogenic stem cells with culture medium, orthotopic transplantation (the gold standard for CSC identification), non-adherent balloon analysis and so on.

3. Common surface markers of CSCs and human normal stem cells

Based on the biological source illustrated that CSC may come from human healthy stem cells, this review shows the horizontal and vertical comparison of their expression in Table 1. At the same time, I further divide stem cells into two categories: human embryonic stem cells and human adult stem cells for convenience of elaboration.

Table 1. Expression of certain CSC surface biomarkers on different types of cells [3].

CSCs marker	Expression on hESC	Expression on Adult stem cell	Expression on normal tissue	Expression on specific CSCs
SSEA3	Yes	Mesenchymal	Rare	Breast
CD117	Yes	Mesenchymal, cardiac	Mesenchymal Cardiac	Ovary
SSEA4	Yes	Mesenchymal, cardiac	Rare	Breast
PODXL-1	Yes	Mesenchymal Hematopoietic	Rare (podocyte)	Leukemia, breast, pancreas and lung
CD90	Yes	Mesenchymal, cardiac	Rare (T-cell,neuron)	Brain, liver
CD146	Yes	Mesenchyma	Mesenchymal	Rhabdoid tumor, sarcoma
SSEA1	Yes（mouse）	Cardiac	Rare	Teratocarcinoma, renal and lung
CD26	Yes	Hematopoietic	Hematopoietic	Colorectal, leukemia
CXCR4	No	Neural	Rare	Breast, brain and pancreas
CXCR1/2	Not available	Mesenchymal	Rare	Breast
CD271	No	Mesenchymal	Rare (neural crest)	Melanoma
TRA-1-60	Yes	Not available	Rare	Teratocarcinoma,
CD56	No	Mesenchyma	Rare	Lung
CD326	Yes	No	Rare (epithelial cell)	Colon, pancreas and liver
CD10	Yes	Mesenchyma	Mesenchymal	Breast, head and neck
TRA-1-81	Yes	Not available	Rare	Teratocarcinoma, breast
TDGF1	Yes	Not available	Rare (pancreas, hippocampus)	colon
CD34	No	Hematopoietic	Rare (lymphoid)	Leukemia, cell carcinoma
ABCG2	Yes	Hematopoietic Muscle,Neura	Rare (myogenic)	Lung, breast and brain
CD49f	Yes	Hematopoietic	Rare	Glioma
Notch2	Yes	Neural	Rare (subset in large intestine)	Pancreas, lung
CD133	Yes	Hematopoietic Neural, Prostate	Rare (proliferative cell)	Breast, prostate, colon, glioma, liver, ovary and lung
CD13	No	Mesenchymal	myeloid	Liver
CD105	No	Mesenchymal	endothelial	Renal
LGR5	No	Intestinal, nephric, gastric, Follicle of hair	Rare	Intestine, colorectum
CD24	Yes	Intestinal	Rare	Breast, gastric, pancreas

3.1. CSC markers have been detected on the surface of hESCs

Many cell surface markers are similarly expressed in hESCs of human and CSCs. However, they are hardly detected in normal tissue cells.

Stage specific embryonic antigen (SSEA) is named for its crucial function in the specific embryonic development stage of mammals. The widespread application of this antigen in the isolation and identification of embryonic stem cells has attracted extensive attention. People named them SSEA-1, SSEA-3 and SSEA-4 according to the order in which they were found. In their early studies, SSEA-4 was detected on adult human mesenchymal stem cells (MSCs). In recent decades, most studies about marker SSEA-3 or marker SSEA-4 focused on the breast tumor. Lv Huiyan's study showed that SSEA-3 and SSEA-4 were expressed in benign and malignant breast lesions, but not in normal breast tissues. This indicates that the up regulation of the two-expression level is related to the occurrence of breast cancer. Later, Sarah K. C. Cheung's [4] study showed that SSEA-3+ breast cancer CSC had higher tumorigenicity in vivo and in vitro.

A molecule called SSEA-1 is expressed on neural stem cells. SSEA-1has also been observed to be expressed in hematopoietic stem cells. SSEA-1 is carbohydrate eptopes and well characterized effective antibodies and is less commonly expressed in tissues and cells which are differentiated. SSEA-1+ cells derived from brain tumor tissue have the features of brain tumor stem cells. In the study of AML by Ben et al., the up-regulated expression of CSC marker SSEA-1 showed a significant correlation with poor prognosis. A 2020 study on high grade serous carcinoma (HGSC) suggests that SSEA1 could become a biomarker of the more serious clinical stage in metastatic HGSC. Based on Cox multivariate analysis, the publisher of the article believes that SSEA-1 is not an independent prognostic factor, nevertheless.

Antigen TRA-1-60 and antigen TRA-1-81 should have been expressed on pluripotent stem cells of human (hPSCs). Exceptionally, they over-expressed in human embryonic carcinoma cell lines, which are on behalf of malignant pluripotent stem cells. These two antigens have been shown to be highly related to the proliferation and migration of prostate malignant tumor, breast cancer and gastric cancer. Also, they have been applied to detect cancer cells with special stem cells. For example, TRA1-60 positive cells were detected in blood of prostate cancer patients, especially who has metastatic disease. The research results of Rajasekhar [5] et al. shown that prostate CSCs co-expressed by TRA-1-60, CD166 and CD151 can reproduce parental prostate tumors in mouse xenotransplantation model.

Prominin-1 (CD133) is a type of protein with five transmembrane structures, which become the most widely studied surface marker of CSCs in a wide range of cancers. Interestingly, CD133 has almost become a benchmark for other cancer stem cell markers. This marker has identified CSC cell groups in many common and uncommon cancers. For example, cancer in prostate, ovarian, liver and so on. We can find CD133 on the surface of hESCs and NSCs. Morever, the expression of it is down regulated during hESCs differentiation, indicating that the expression of CD133 is limited to undifferentiated hESCs. Lack of CD133 did not affect the pluripotency of hESC or its differentiation into 3 germ layers in vivo, but markedly inhibited the proliferation of cells. Now we know that CD133 can interact with many signaling pathways. For example, PI3K Akt signaling pathway, canonical Wnt pathway and their downstream branches. CD133 could also up regulate the transcription level of FLICE like inhibitory protein in CD133 positive cells, which is conducive to the delaying apoptosis. Interestingly, CD133 can also increase angiogenesis, which may be of great value for tumor volume expansion. The mechanism is relevant to the excitation of Wnt pathway and the increased expression of vascular endothelial growth factor-A (VEGF-A) and IL-8. Hence, the marker called CD133 can be regarded as the fatal weakness of CSCs and provide strategies for the detecting and targeting therapy of CSCs.

CD90 is a membrane GPI-anchored protein with an IgV-type superfamily domain originally observed in mouse T cells. CD90 is expressed in bone marrow MSCs and undifferentiated hESCs, but is not absolutely specific. For example, CD90 is also well expressed on the surface of fibroblasts. When studying the internal regulatory mechanism of human gastric cancer cell lines using several

presumed CSC markers including CD133, CD44, CD90, and EpCAM, Xiong et al. found that cells with independent co-expression of CD90 and CD44 could show the capacity to form tumor spheres in vitro and in mice lacking immune function. The paper published by the research team indicates that CD44+ and CD90+ cells have high metastasis rate and high proliferation rate, respectively, which further shows that we can identify metastatic CSC markers and tumorigenic CSC markers by observing the ability to regulate cell cycle and intervene the epithelial to mesenchymal transition (EMT) process.

Among CSC biomarkers, EpCAM (epithelial cell adhesion molecule, CD326) is a type I transmembrane glycoprotein, which plays a role as a adhesion molecule of cells from homophilic system. Morever, it is calcium independent. As early as 2010, EpCAM has been defined as one of the markers of undifferentiated hESC. Extensive studies on tumor characteristics have shown that EpCAM is involved in the EMT process of tumors. EpCAM+ hepatocellular carcinoma and pancreatic cancer cells are considered as tumor initiating cells with stem cell characteristics. In a paper published by Dong et al. in 2020, it was clarified that CSCs in liver which are highly expressed EpCAM could resist natural killer cell-mediated cytotoxicity by up regulating the express level of CEACAM1.

Similarly, it is associated with undifferentiated hESCs, especially the cell surface marker Cripto-1 (Teratocarcinoma-derived growth factor 1, TDGF1), which contributes to early embryogenesis, has also been proved to be related to cancer progression. Cripto-1 is identified as a CSC marker because it is highly expressed in a range of solid tumors and can be extracted and enriched from tumor cells with features of stem cells. In clinical esophageal squamous cell carcinoma samples that are clinically abbreviated as ESCC samples, the detected level of expression about Cripto-1 in cancer affected organs has positive correlation with the stage classified by tumor node metastasis classification. Sometimes it has positive correlation with infiltration degree and lymph node metastasis as well. The cox region analysis showed that the cripto-1 was an indicator that can give a disease prognosis independently [6]. Recently, scientific research in this area has been further developed. Cripto-1 is gradually considered as a great target which may have unexpected therapeutic effects for CSCs. Qiang et al. discussed the molecular mechanism of Cripto-1 in CSCs, and discussed the potential of Cripto-1 as a target of CSCs immunological therapy.

PODXL-1 (podocalyxin like protein 1) is a glycoprotein on cell surface found in glomerular epithelial cells for the initial time. In addition, it is rarely found in other normal human tissue. It affects cell migration by regulating actin, which explains the significance of its expression in undifferentiated hESCs and hematopoietic precursor cells. At the same time, the characteristics affecting cell migration also determine that once the expression of PODXL-1 is dysregulated in normal stem cells, it may cause the emergence and metastasis of some malignant tumors that is difficult to control. Early researches have demonstrated that PODXL-1 is expressed in leukemia, and the over expression of PODXL-1 is also linked with non-ideal prognosis of brain, pancreatic and breast cancer. Wen Ying's study further confirmed the rationality of PODXL-1 as a marker of CSC. PODXL-1 has a bearing on the stemness of tumor in colon cancer HCT15 cells. Inhibiting PODXL-1 can inhibit TAZ signaling pathway and further down regulate the genes downstream of Hippo.

ABCG2 is a member of ATP binding cassette sub-family G. ABCG2is generally considered to be related to cancer drug resistance. ABCG2 expressed in stem cells can enable cells to resist toxins and avoid stress. Through the experiment of over-expression of ABCG2 in HUES9 human embryonic stem cells, it was found that stable over-expression of ABCG2 could enhance the resistance of cells to toxins without affecting the stemness of cells and their differentiation into certain tissue cells. ABCG2 was previously detected in stem cell-rich lung tissue. ABCG2 has been used for flow cytometry sorting of pancreatic CSCs. Noriko Kawai and his collegues demonstrated that cell lines of gastrointestinal cancer with high levels of ABCG2 expression were rich in CSCs. However, knockout or inhibition of ABCG2 did not inhibit the formation of tumor spheres. Knockout or inhibition of ABCG2 can improve the staining level of 5-aminovulinic acid (5-ALA), which is conducive to photodynamic diagnosis or therapy (PDD/ PDT) of cancer [7]. This shows that the study

of ABCG2 is of great importance in the auxiliary diagnosis of malignant tumor, although the molecule may not have ideal effect as a therapeutic target.

CD24 is less expressed in normal tissues except neutrophils, neurons and other cells. CD24 was highly expressed in undifferentiated hESCs. Li et al. proved that the number of cells with positive CD44, EpCAM and CD24 on the surface of cell membrane accounted for only 0.2-0.8% of tumor cells, but the tumorigenic ability of the former was more than 100 times higher than that of the cells with negative three markers. Although Shackleton and his collegues have proved that CD24+ is a thermostable antigen expressed on human breast tumors in 2006, CD44+CD24- cells usually have mesenchymal or myodermoid phenotype and strong tumorigenic ability in breast cancer. Moreover, CD44+CD24- cells are located at the edge of the tumor, suggesting that the self replication and the EMT of breast malignant tumor are involved as well.

CD49f (integrinα6) is one of the proteins detected in stem cell lines and somatic cells. For example, we can see the appearance of CD49f in keratinocytes, platelets, epithelial cells and corneal basal cells. CD49f has been widely detected in more than 30 different stem cell lines. Integrinα6 has been identified as the only gene expressed in stem cell signals of stem cells in hemopoietic system (HSC), in hESCs and even in embryonic neuronal stem cells (NSC). It was highly expressed in hESCs and decreased in the formation of embryoid body. Except hESCs, CD49f was only weakly expressed in normal tissues. CD49f may fulfil a conservative function in stem cell physiology. Integrinα6 acts in controling tumorigenicity and self renewal in glioblastoma CSC (GSC). The CD49f+ or both CD49f and CD133 expressing cells screened from glioblastoma cancer tissue were proved to be highly enriched and can form tumorsphere GSCs in vitro. At the same time, the presence of integrinα6 in four different cervical cancer cells has also been proved to promote tumor proliferation. Cells extracted from breast cancer milk also enriched CD49f+/EpCAM- CSC-like cells.

Like most other CD molecules, CD146 belongs to immunoglobulin. It is one of the most studied markers from bone marrow MSCs surface in human body. It is also expressed in hESCs. The expression of CD146 is quite limited in normal tissues and organs of human. As a studied MSC biomarker, CD146 has already been used to purify pluripotent mesenchymal stem cell groups. CD146 is considerably homologous to neurocyte adhering molecule (NCAM). This phenomenon also occurs between melanoma cell adhering molecule (MCAM) and CD146. Morever, the expression level of MCAM is very high in metastatic malignant melanoma, suggesting the potential of CD146 as a marker and therapeutic target of melanoma. CD146 is expressed in a range of malignant tumors, such as malignant rhabdoid tumor and primary sarcoma. Among these cancers, CD146 promotes tumors to display a malignant phenotype, which involves metastasis and tumorigenicity. A relatively new study shows that CD146 can regulate the cell cycle in glioma stem cells with CD146+ CD133+[8].

CD10 is a membrane binding protein, which often needs to rely on zinc. This endopeptidase appears in hESCs that are undifferentiated and then down regulated in the period of nerve differentiating of human ESCs. CD10 can be observed in bone marrow from human as well as mesenchymal stem cells. CD10+ hematopoietic progenitor cells can differentiate and become B cell, T cell or NK cell. CD10 appaers in normal tissue of intestine. However, in healthy colon, CD10 cannot be detected. CD10 is abnormally expressed in some special types of colorectal cancer, which is related to liver metastasis and poor prognosis. People have long found the exists of CD10 in squamous cell carcinoma of head or neck and breast carcinomas. CD10 has been proved as a biomarker of stemness among cells suspected of being stem cells, too. Carcinoma associated fibroblast (CAF) is adequate and karyotype mixed stromal cell located in the neoplastic micro-environment. This micro-environment is very crucial in malignant tumor expanding. Shicheng et al. demonstrated that both CD10 and GPR77 positive CAFs can stimulate cancer formation and the resistance to chemotherapy by giving CSCs a viable micro-environment. Based on rich research results, CD10 has been approved as one of the medical targets by an administration regulating federal foods and drugs (FDA).

CD117 and CD26 have also been approved as drug targeting molecules. CD177 is hardly expressed in adult normal tissues, but it is observed on the plasma membrane of HSCs.

Myelodysplastic syndrome (MDS) is considered to be highly likely to develop into leukemia. Allogeneic hematopoietic cell transplantation (HCT) is often incomplete for the ablation of abnormal hematopoietic stem cells, and targeting CD177 on abnormal proliferative hematopoietic stem cells may provide great help for the treatment of MDS. That is because CD117 is functionally linked to tyrosine kinase receptors that interfere with normal stem cell physiology and cancer expansion. In the mouse model, anti-CD177 monoclonal antibody can eradicate invasive MDS cells and enhance the effect of HCT. Similarly, it is believed that the signal axis named SCF/CD117 has the opportunity to rule cancer progressing by regulating stem cell viability and resistance to tyrosine kinase inhibitors[9].

Dipeptidyl peptidase-4 (DPP4/CD26) can be deceted in MSCs and HSCs. Also, CD26 is highly expressed on the surface of human normal kidney as well as normal small intestine tissues. In addition, CD26 appears on endothelial cells, epidermal cell, and some immune related cells. Other normal tissue cells hardly express CD26. In human body, DPP4 controls the fountion of incretins, chemokines and some other types of polypeptides. In order to complete the above work, CD24 cuts the dipeptides from the peptide with Pro or Ala (two kinds of amino acidsin) on the second to last position of the N-terminal. Because of those influences as well as cross talking with other cellular molecules, the level of CD26 expression determines the development speed of tumor to a certain extent. And because of the solubility and easy availability of CD26, it is generally considered to be a CSC marker. In recent years, CSC research focusing on CD26 is mostly based on colon cancer. Alvin et al. quantitatively analyzed CD26 positive cancer tissue cells in more than 10 idiopathic tumor specimens by FCM. Then they found that there was a relatively high level of CD26+ in tumors diagnosed or suspected of metastasis in these samples. After culturing the selected CD26 negative tumor precursor cells for a period of time, masses of tumor cells containing CD26 positive cells appeared. This series of researches reveal the initial occurrence stage of metastatic colorectal CSCs. Two years later, Lorena's study confirmed this fact again.

Notch2 is a transmembrane receptor, which plays a series of important roles in the embryonic process by interacting with ligands. Notch2 is expressed on undifferentiated hESCs, especially in the process of neural differentiation of hESCs. Notch 2 stimulates tumor production by abnormally activating Notch signaling pathway. Up regulation of Notch 2 and abnormal activity of Notch signaling pathway can be observed in a wide range of tumors. For instance, the above phenomena are found in breast cancer, non-small cell and lung cancer. Blood system cancer, like diffuse large B-cell lympham as well as T-cell acute lymphoblastic leukemia. As early as 2015, Notch2 was used as a CSC marker for lung cancer and pancreatic cancer. Targeting Notch2 has become a hot research direction to eradicate CSCs and then cure cancer. In 2018, Xiaoyuan et al. found that miR-181b expression inhibited the characteristics of non-small cell lung cancer (NSCLC) tumor stem cells by directly inhibiting Notch2 and heightening the sensibility to cisplatin therapy [10].

3.2. CSC markers have been observed on the surface of adult stem cells

In addition, many cell surface markers are similarly expressed in adult stem cells as well as CSCs. These markers are seldom detected in natural differentiated cells.

Chemokines is a family of structurally related cytokines. The family contains more than 47 chemokines and more than 20 chemokine receptors. CXC motif chemokine receptor type 4 (CXCR4) is a G protein-coupled receptor. Most of the existing studies focus on the recruitment effect of CXCR4 on stem cells. CXCR4 is expressed in adult stem cells. For example, SDF-1/CXCR4 pathway mediates the migration of neural stem cells. CXCR4 also takes part in the homing and chemotaxis process of hematopoietic cells and immune cells [11]. A CXCR4 antagonist called Plerixafor has been authorized by the U.S. Food and Drug Administration (FDA) to cure a range of diseases, especially cancer. A study published in 2021 shows that miR-139 over-expression and down regulation of CXCR4/p-Akt axis can attenuate the metastasis of human breast cancer cells and their infiltration of other healthy tissues.

The membrane proteins called chemokine receptor 1 and chemokine receptor 2 (CXCR1, CXCR2) specifically bind and act with cytokines from the CXC chemokine group. Plenty of researches on

CXCR1/2, tumor and inflammation have been published, and most of the existing studies focus on an axis named CXCL8-CXCR1/2. This axis can mediate the development of multiple cancers such as cancer involving the prostate, breast, lung, colon and skin. CXCR1/2 exists on the MSCs surface. A study on ovarian tumors shows that when the genes related to tumor resistance begin to express in tumor cells, MSC will be transformed into cancer associated mesenchymal stromal cells (CA-MSCs), and excessive CXCR1/2 will be secreted at the same time. This leads to the medicine tolerance of CSCs and inhibits immune response of immune cells to ovarian tumor cells. CXCR1/2 are also markers of breast CSCs.

Human chromosome 1 undertakes the mission of encoding CD34. Two protein products of the gene located on the cell surface make the cell show the characteristics of progenitor cells. So now the transmembrane phosphoglycoprotein CD34 is widely recognized as one of the markers of hematopoietic progenitor cells. In the study of AML CSCs, CD34+ CD38- cell subsets were identified as CSCs. CD34 has been detected in many cancers at the transcriptional level, but the expression of CD34 at the protein level is not as extensive as expected. Although CD34 has been widely studied in hematological diseases, CD34 has also been accepted as a biomarker of tumor angiogenesis in solid tumors in recent years. It is worth noting that in the past few years, CD34 has also been detected to be appeared in non-hematopoietic cells. For example, it is expressed on the stromal cells, endothelial cells, muscle derived stem cells, regenerative stem cells, corneal keratinocytes and a variety of CSCs. The research results published by Mieun Lee-Theilen et al in 2021 show that CD34 can be used to identify CSCs of hepatoblastoma [12].

The receptor CD271 (NGFR) is expressed on bone marrow MSCs. Previously, CD271 was considered as a potential marker of melanoma CSC, but because both CD271+ and CD271- melanoma cells can metastasize in NOD/SCID IL2R null mice, using CD271 to identify and isolate melanoma CSC may not be the most efficient method. However, in a study targeting osteosarcoma stem cells, CD271 was identified as a marker of osteosarcoma CSC. This study also showed that biomimetic nanoparticles coupled with CD271 monoclonal antibody can be contacted and absorbed by osteosarcoma cells. Compared with non-targeted polyethylene glycol, hollow gold nanospheres (HGNs) coupled with CD271 monoclonal antibody and polyethylene glycol were observed to have more inhibitory effect on cell growth. In addition, cells extracted from breast cancer milk were also observed to be enriched for CD271+ CSC-like cells.

CD13 (alanine aminopeptidase) is a biomarker of MSCs, which is expressed on periodontal stem cells. CD13 is infrequently appear in healthy cells except renal tubules, gallbladder and prostate. In B-cell malignancies, the expression of CD13 symbolizes the differentiation of B cells into plasma cells. CD13 is of great significance in the diagnosing of Waldenström Macroglobulinaemia as well as lymphoplasmacytic lymphoma, which suggests the significance of CD13 as a CSC marker of B-cell malignant tumors. CD13 may be considered as a CSC marker of the liver in the early stage. As expected, CD13 was identified as a new marker of HCC CSC. CD13 can also mediate the resistance of hepatocellular carcinoma to sorafenib, although the mechanism is not clear.

Neural cell adhesion molecule (NCAM, CD56) is an immunoglobulin superfamily cell adhesion molecule. In normal tissues, CD56 appears only on the surface of neurons, NK cells and skeletal muscle cells. CD56 has been found to be expressed or over-expressed in neuroblastoma, glioblastoma, melanin, hepatocellular carcinoma, hepatoblastoma, thoracic pulmonary blastoma and some ovarian tumors. CD56 is a CSC marker of nephroblastoma and small cell lung cancer. Neuroblastoma tumors also showed a high expression level of NCAM. The screening and identification of CSCs by NCAM expression is still immature.

CD105 (endoglin) is a type I transmembrane protein that can activate endothelial cells. It can also interact with TGF-β receptors I or/and II interact to regulate the TGF-β signal path. CD105 is a typical mesenchymal stem cell marker. Researchers are particularly interested in studing the extent to which it is expressed in MSCs derived from bone marrow. CD105 enables hematopoietic stem cells to keep the features of stem cell. The expression of CD105 in solid tumor vessels and AML blasts symbolizes a poor prognosis. Some scholars believe that CD105 is a marker of cancer initiating cells and

maintains self-renewal, chemotherapy resistance and cancer stemness in clear cell renal cell carcinoma (ccRCC) [13]. At the same time, CD105 can also induce cells to show EMT state through stem cell cytokine MYC. Interestingly, in general cognition, this is associated with cancer metastasis. However, in this experimental study, CD105+ cells did not show the increase of metastatic potential as expected.

Leucine rich repeat containing G-protein coupled receptor (LGR5) is a G-protein coupled receptor. LGR5 is composed of 18 leucine rich repeat units and seven-transmembrane regions. LGR5 is a recognized marker of colon stem cells. It is found to be expressed in colon, the base of small intestinal mucosal recess and hair follicle stem cells. Under normal circumstances, LGR5 is a regulator to down regulate the Wnt signaling pathway, while LGR5 is highly expressed in colon cancer. The abnormally excessive expressing of LGR5 mRNA is an important explanation for many malignant phenotypes such as the development and drug resistance of colon cancer. Therefore, LGR5 and its RNA products are widely recognized as markers of colonic CSC.

4. Conclusions

Our research on cancer stem cells is less than 40 years, and the research on cancer stem cell (CSC) markers is in the early stage. However, there have been many effective evidences, such as the culture experiment of CSC marker positive cell population in vitro and the transplantation experiment in mice, which can prove the existence of a variety of CSCs with certain markers. Therefore, we can further explore the role of CSC in cancer development and cancer treatment. As described in this review, CSC mediates tumor proliferation, metastasis and drug resistance, and the research on tumor stem cells is of great significance for cancer diagnosis, targeted drug research and the comparison of the same type but different sub-types of cancer.

Many of the CSC markers found in humans are related to the abnormal expression of surface markers of hESCs and human normal stem cells in human body, which may provide help for us to explore the pathogenesis and early treatment of cancer. We can trace the expression of cell surface markers to explore their corresponding gene mutations and RNA transcription abnormalities. Morever, when we know the difference between CSCs and human normal stem cells expressing the identical marker, it will be greatly beneficial to reduce the side effects of molecular targeted drugs, that is, the toxicity of drugs to normal tissues. It is gratifying that a large number of cancer types have their own specific CSC markers and experimental studies on these markers.

The study of CSC markers, it should be noted, is not so ideal. There are still some problems to be improved in the existing research. Firstly, the CSC markers that have been found are not all independent prognostic factors corresponding to cancer, which limits the research value of markers to a certain extent. For example, scripto-1 and SSEA-1 are independent and non-independent prognostic indicators of esophageal square cell carcinoma and high-grade serial carcinoma, respectively. Secondly, sometimes the positive expression of a single marker is not enough to identify a certain cancer, and it often needs the assistance of other markers. For example, CD34+CD38− cell subsets were identified as acute myeloid leukemia CSC, while CD44+CD24− was used to identify breast cancer CSC. Finally, the role of each CSC marker is not exhaustive. For example, ABCG2 is useful for the auxiliary diagnosis of gastrointestinal cancer, but if it is used as a target of molecular targeted drugs, it may not achieve satisfactory results.

In general, the research results in the field of tumor markers are not very complete. In recent years, the increasing interest in this research field will undoubtedly contribute to an in-depth understanding of the function of known CSC markers in the cure of cancer and the discovery of novel CSC markers.

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