Cancer Stem Cells and Their Drug Resistance Mechanisms

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Abstract. Today, the prevalence rate of cancer continues to rise, and the mortality rate remains the first. In the face of tumor growth and deterioration, medical treatment methods are constantly updated, and drug resistance in the later stage of tumor is a thorny problem facing today. Cancer stem cells (CSCs) are an important factor leading to tumor drug resistance. The biological characteristics and drug resistance of tumor cells are important factors that determine tumor drug resistance. CSCs have unique biological characteristics, but they are similar to stem cells. It can self-proliferate and differentiate, have strong repair ability, can promote infinite growth of tumor, and have unique biological markers. The ABC protein drug pump function of CSCs can cope with the invasion of chemotherapy drugs. Its own microenvironment, strong inhibition of apoptosis and long-term stationary phase are the unique protective mechanism of CSCs. Aiming at the mechanism of cancer stem cells, the research on their properties is particularly important.

Keywords: Cancer Stem Cells, Resistance Mechanism, ABC Transporter, Niche.

1. Introduction

Cancer is the biggest problem in the world, and its mortality is extremely high, in many cancer cases, drug resistance is among the direct occasion of death. Drug resistance in tumors is closely related to the biological characteristics and drug resistance mechanism of cancer stem cell (CSCs). CSCs and stem cells have similar proliferative differentiation and repair abilities to maintain their biological activity. At the same time, CSCs have migration ability and can facilitate tumor invasive development, which is the basis of tumor invasion and metastasis [1]. In clinical practice, biomarkers of cancer stem cell are usually used to identify tumor types. Therefore, different types of CSCs have different biological markers, and biological markers are effective tools for tumor identification. The resistance of CSCs is a major cause of tumor resistance. Tumor cells have both innate and acquired resistance and are therefore resistant to chemotherapy. The ABC transporter protein in the cerebrospinal fluid pulls the drug out of the cell, preventing its toxicity [2]. CSCs possess a unique inhibitor of apoptosis gene, which can prolong the life of CSCs, have longer activity and produce more effects. Changes in the local microenvironment (niche) around CSCs can affect biological behaviors, play a barrier protection role, activate signaling pathways, and increase tumor invasiveness. At present, most chemotherapy drugs on the market act on continuously differentiated cells, and CSCs are in the stationary phase for a long time, which can escape the effect of chemotherapy drugs, and make the tumor continue to metastasize and grow while seemingly shrinking. The mechanism of CSCs makes the generation of cancer drug resistance clearer, and also provides new ideas and means for clinical treatment.

2. Biological characteristics of cancer stem cells

Cancer stem cells are specialized cells whose potential for self-regeneration and continuous differentiation is an important factor in tumor growth and resistance to chemical drugs. Other cells undergo normal differentiation and eventually apoptosis. Although cancer stem cell constitutes only 1%-4% of tumor cells, they are important factors in tumor formation and growth.

2.1. Strong proliferation, differentiation and repair ability

Cancer stem cells, like stem cells, have the functions of self-renewal, proliferation and differentiation, and have similar growth regulation mechanisms. CSCs are caused by genetic variation

and uncontrolled proliferation and differentiation in normal stem cells. Through heterogeneous division, CSCs can produce progeny cells with exactly the same phenotype as the previous generation and tumor cells with different phenotypes to form new lesions in vivo. Lessard established two groups of leukemia models: one group was introduced into normal mouse fetal hepatocytes with oncogene, and the other group was introduced into mouse fetal hepatocytes with Bmi-1 gene knockout. After irradiation, leukemia was formed in both groups. When the bone marrow of leukemia mice from these two groups was introduced into the other two groups of leukemia models, the normal mice still developed leukemia, while only a few or no leukemia cells were found in the blood of Bmi-1 knockout mice [3]. Experiments have shown that the presence of Bim-1 gene in CSC enables CSC DNA to repair itself after damage, producing strong repair ability and stabilizing biological traits.

2.2. Promoting tumor growth

CSC can grow invasively, which is the basis of continuous malignant transformation of tumors and an important substance in the continuous development of tumors. As early as 1997, leukemic blasts were extracted from immunodeficient mice with acute myeloid leukemia, and it was found that only leukemic blasts with phenotype of CD34+ CD38-, an important biomarker of CSCs, could cause the disease in immunodeficient mice. Thus, CSCs were found to have the ability to promote tumor growth (Figure 1) [4].

Clinically, it is found that tumors still grow after chemotherapy, and one of the factors is that the growth rate of tumors doubles after stimulation. In animal experiments, Lagade et al. found that breast cancer cells would transform into iBCSC after irradiation, and once transformed into breast cancer cells, the transformation capacity of breast cancer cells was more than 30 times that of universal cancer cells [5]. The capacity of CSCs to transform into tumor cells was exponentially accelerated after chemotherapy. Another factor promoting tumor growth is that CSC can promote the growth of tumor blood vessels. Researchers have found that CSC can overexpress angiogenic factors, and formylated peptide receptor and chemokine receptor XCR4 are important mediators of CSC angiogenesis [6]. CSCs can directly participate in the generation of blood vessels, or directly participate in the non-endothelial vascular mimicry of tumor microcirculation [7].

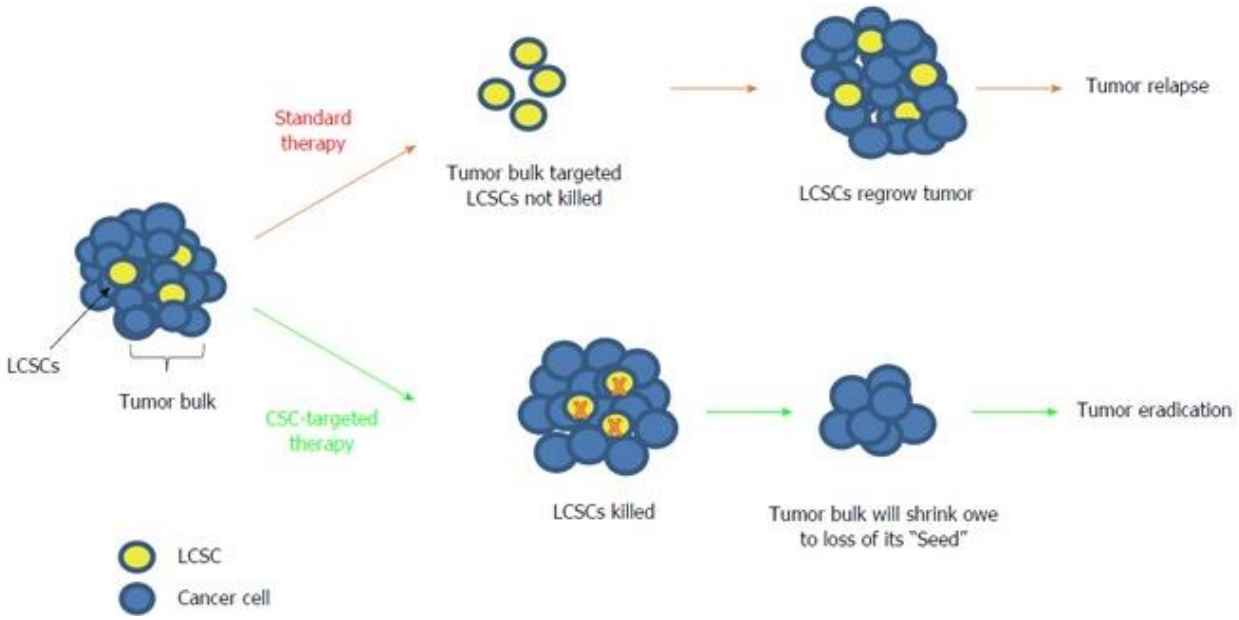

Figure 1. For cancer therapies against cancer stem cells resistance are resection, radiotherapy and chemotherapy [8].

2.3. Cancer stem cell biomarkers

The biological markers of various kinds of CSCs are not exactly the same. Even for the same type of tumor, there are differences in biological markers due to different cell lines. The presence of

specific up-regulated, down-regulated, mutated or missing proteins or glycoproteins in CSCs is an important marker for the detection and localization of CSCs. Some of these proteins and glycoproteins are cell surface antigens and some are found in the cytoplasm. Aldehyde dehydrogenase 1 is a soluble protein present in the cytoplasm, and its activity is bound up with the biological properties of CSCs [9]. Low specificity of CSCs biomarkers is the biggest problem at present. Therefore, two or more biomarkers are often used to identify CSCs in clinical practice. At present, some commonly used cell surface marker technologies have been applied to the expression of CD44, CD24, CD133, etc.; CD29, CD166, CD133; EpCAM on colorectal cancer.

Regulatory molecules related to signaling pathways are also CSCS-specific biomarkers. Six known signaling pathways can regulate the stemness of cells, such as Heidegger, JAK/STAT, Nanog, Notch, etc. (Figure 2); The PI3K/AKT and Wnt/β-catenin pathways are often discussed first. These signaling pathways exist during the embryonic period, and they enable CSCs to have stronger proliferation and migration abilities. In adulthood, CSCS are in a silent state in the human body, and when they are abnormally activated or deregulated, they cause primary or secondary growth of tumors.

In recent years, the appearance of cancer stem cells is closely related to the prognosis of patients. Thanks to Ginesteel et al. High-level expression of ALDH1 in breast CSCs is an important marker [10]. In this paper, 216 cases of bladder cancer patients with ALDH1 gene expression and clinical indicators were analyzed. The results showed that he expression of ALDH1 gene had a certain relationship with pathological type, clinical stage and prognosis. Patients with worse prognostic effect had higher expression of ALDH1, and high expression of ALDH1 predicted decreased overall survival [11, 12]. Therefore, timely detection of cancer stem cells can enable early diagnosis and metastasis of tumors, thereby improving the survival rate of patients.

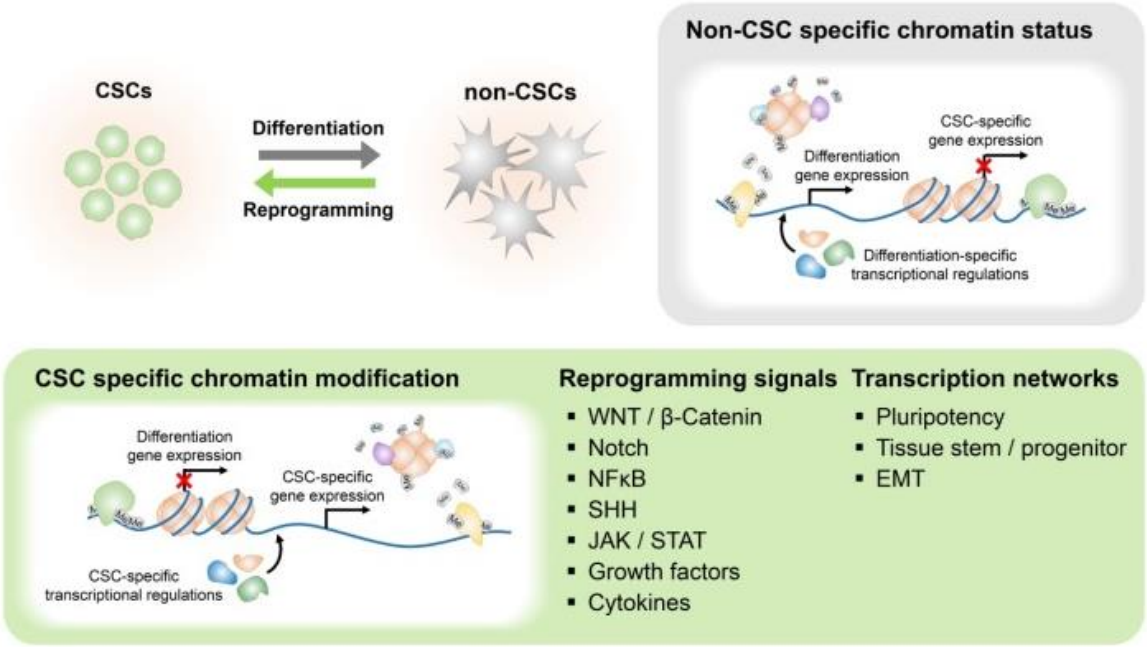

Figure 2. Core signaling pathway of CSCs [13]

3. Mechanism of drug resistance of CSCs

Multidrug resistance is a significance cause of chemotherapy failure. CSCs have tolerance to various chemotherapy drugs. The main drug resistance mechanisms can be explained from four aspects: drug pump effect of ABC transporter, inhibition of apoptosis by CSCs, niche microenvironment and long-term G0 phase of CSCs.

3.1. Cancer cell drug resistance

Multidrug resistance (MDR) of tumor cells refers to the fact that after exposure to a certain chemotherapy drug, Tumor cells become resistant not only to drugs, but also to other drugs with different structures and functions. The formation mechanism of MDR is very complex, which is related to many membrane proteins such as multidrug resistance related proteins and P-glycoproteins on the cell membrane, as well as DNA topoisomerase and protein kinase C in the cytoplasm. The mechanism can be roughly divided into two types: The first is congenital drug resistance. CSC is generally in the G0 phase and has a strong ability of self-repair and proliferation. ABC transporters can pull drugs out of cells, creating resistance. The second is acquired drug resistance. After long-term exposure to carcinogens, cancer stem cells and their similar progeny cells undergo genetic mutation and develop new drug resistance [14].

3.2. Drug pump effect of ABC transporter

ABC transporters is a kind of transmembrane protein, is an important member multi-resistant family, is located at the junction of the cell membrane of ATP box of protein, is one of the largest known gene families, an expression of the gene family is to protect the main mechanism of stem cells. The body's widely dispersed ABC transporters are capable of transporting endogenous peptide, lipid, nucleotide, and metabolic medicines, enzymes, etc. p-glycoprotein (P-gp/ABCB1), multidrug resistance-related protein (MRP/ABCC1), anti-breast cancer (BCRP/ABCG2) and anti-tumor protein (BCRP/ABCG2) are the three major anti-drug proteins. source. These three genes are not fundamental for the subsist of stem cells, but the development of drug resistance depends on their interaction. ABC transporters mainly protect cells from toxins. The ABC transporter family members to be transported nonselective hydrophobic and hydrophilic compounds. Its drug resistance mechanism is to utilize the drug through the hydrolysis of ATP and the reverse concentration gradient, so as to avoid the effect of the drug and cause drug resistance and the carrier protein in many physiological barriers between is also play a considerable part in the transport, such as the blood brain barrier.

ABCG2, a newly discovered ABC transporter, is a specific efflux pump with an extensive extent of substrate effects and can recognize molecular, organic ion and sulfate complexes. The ABCG2 structure expressed by ABCG2 gene is different from other ABC proteins in that it has only one transmembrane protein and one ATP domain. ATP binds to the associated region of ABCG2 and can be used as a drug pump, and imatinib is the first targeted drug for CML. Houghton et al. found that imatinib could effectively reverse ABCG2-mediated topotecan and SN-38 resistance and meaningfully increase the cumulation of topotecan in Saos2 cells with high ABCG2 expression [15]. And reduced the efflux effect of ABCG2.However, this small-molecule tinib is a high concentration of ABCG2 inhibitor and a substrate of ABCG2, which can bind to ABCG2 and be pumped out of cells. After binding to ABCG2, imatinib and ABCG2 will affect ABCG2's exclusion of other chemotherapy drugs, so as to reverse tumor resistance to some chemotherapy drugs and make tumors resistant to drugs [16].

The expression level of ABCG2 mRNA was high and decreased during cell differentiation. It has been found that ABCG2 is implicated in the formation of the side population (SP) cell phenotype. The results showed that in head and neck squamous cell carcinoma, the number of SP cells was 2.7%, and the expression levels of MRP1 and ABCG2 were significantly higher than those in non-lateral squamous cell carcinoma, while the verapamil group could reduce the proportion of SP cells to 0.7% [17]. In the Sp cells isolated from colorectal cancer by Xie et al., the expression of ABCG2 protein was significantly enhanced. ABCG2 is also a major ABC transporter in Sp cells, and ABCG2 is highly expressed in SP cells from different sources, which is considered to be an important component of multidrug resistance in tumor cells [18].

3.3. Inhibition of apoptosis

The mechanism of action of many chemotherapeutic drugs is to induce apoptosis, and if apoptosis is inhibited, resistance will develop. The results showed that Bcl-2 gene, NF-kB gene, p53 gene, C-

myc gene and other genes were closely related to the drug resistance of tumors, and some of them had higher anti-apoptotic genes. Bcl-2 is a novel tumor gene, which can inhibit or promote apoptosis, and its biological effects depend on the interaction between its molecules. The overexpression of Bcl-2 gene can convert the antitumor activity of chemotherapeutic drugs into antibacterial activity against tumor cells. According to their structure and function, they can be divided into three subcategories: (1) Anti-apoptotic protein subfamily (subfamily 1), including Bcl-2, Bcl-x1, etc. (2) Pro-apoptotic protein subfamily containing multiple regions (subfamily 2), including Bax, Bak, Bok (3) pro-apoptotic protein subfamily containing only BH3 region (subfamily 3), including Bik, Bid, etc.

Eisele et al. treated 14 patients with acute myeloid leukemia with cytarabine and anthracycline, and found that 7 patients had complete remission and 7 patients had persistent disease [19]. The results showed that there was valid diversity in apoptosis-related indices entre the complete remission group and the prolonged disease course. The amount of CD34++ was twice as high in the complete remission group than in patients who continued treatment. Leukemia stem cells have higher apoptosis and lower expression in tumor cells, resulting in resistance to chemotherapeutic drugs.

In addition to the high expression of Bcl-2, the high expression of other anti-apoptotic genes can also inhibit the apoptosis of CSCs, and many classic tumor-related signaling pathways can also regulate the occurrence of CSCs. normal stem cells. For example, transgenic technology significantly improved long-term hematopoietic stem cell numbers in mice with high expression of the bcl-2 apoptotic gene. At the same time, it can raise the tolerance of hematopoietic stem cells to lethal radiation dose KLF4 (Kruppel-like factor4) is a kind of transcriptional element binding protein widely existed in eukaryotes [20]. The expression of interleukin-4 was up-regulated in CD133+ colon cancer CSCs, resulting in reduced apoptosis [21]. A recent gastric cancer study explained that the promoter methylation of the DAPK gene is hard connected with the occurrence of gastric cancer [6].

3.4. Cancer stem cell niche

The study of CSCs has focused on the change of the cell's ecological niche as an important factor leading to the inability of stem cells to differentiate. In 1978, Schofield first proposed directed hematopoietic stem cells differentiated from spleen cells. This phenomenon was attributed to the loss of microenvironment supporting stem cell activity. Studies have shown that somatic support cells in nematodes and Drosophila melanogaster are capable of producing factors necessary to maintain stem cells in the reproductive line, thus demonstrating the existence of a microenvironment [22].

A stem cell niche is a specific microenvironment that enables stem cells to grow, transform, and maintain their stability (Figure 3) [23]. The hypoxic microenvironment of Osas induces changes in biological behavior and accelerates the malignant transformation of tumors. Endothelial progenitor cells, differentiated tumor cells, myofibroblasts, extracellular matrix, etc. surround the hypoxic niche environment of cancer stem cells. The extracellular matrix and three-dimensional niche structure act as barriers to block cancer stem cells' access to chemotherapeutic agents, and improve the drug avoidance effect of cancer cells, thereby increasing drug resistance. Radiation-induced DNA damage in radiotherapy requires oxygen, and cancer stem cells exist in a hypoxic microenvironment, so CSCs will not be affected in radiotherapy. Based on the current study, it can be inferred that CSCs may exist in two different microenvironments, namely, the hypoxic microenvironment far away from functional blood vessels and the hypoxic or non-hypoxic microenvironment around blood vessels.

Hypoxic microenvironment can cause changes in intracellular signal transduction pathways. HIF-1 plays a very important role in intracellular growth, differentiation and migration, and in angiogenesis and energy metabolism [24]. Carmeliet et al. demonstrated in their study that mouse embryonic stem cells with negative expression of HIF-1a protein showed accelerated growth characteristics, which may be due to apoptosis inhibited by hypoxia. Studies have shown that hypoxia can directly stimulate the growth of glioma stem cells, and this effect depends on HIF-1a and HIF-2a [25]. HIF-2a is a specialized pathway that regulates cellular signaling. Compared with HIF-1a, HIF-2a specifically expresses and accumulates a wider range of oxygen content, and has higher oxygen

content and a wider range of cell types. When HIF-1a can only be temporarily regulated in chronic hypoxia, HIF-2a can still be highly expressed [26].

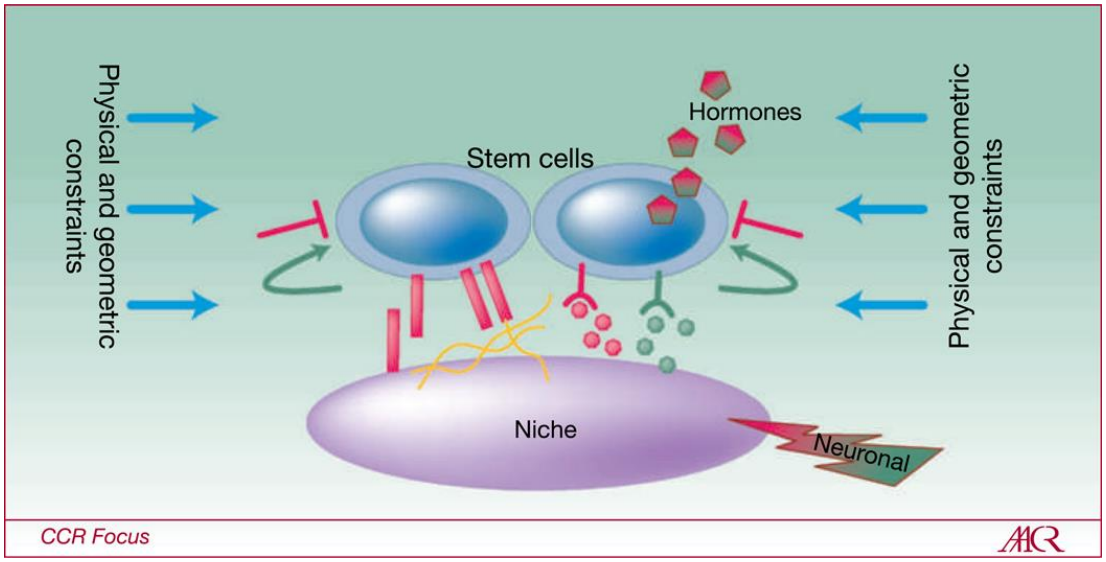

Figure 3. The basic concept of a stem cell niche [27]

3.5. Cancer stem cell are in G0 phase for a long time

Cancer stem cell are in the quiescent phase (G0) and are resistant to drugs themselves that act on the cell cycle or rapidly differentiating cells. Drugs known as cell cycle specific agents (CCSAs) are selectively reactive to cells in a certain stage of the proliferative cycle and are not reactive to cells in the G0 phase. For example, the antimetabolic drug fluorouracil, 5-fluorouracil (5-Fu) is commonly used to target tumor cells in the active cell cycle. The study of CSCs showed that Cyclin-dependent protein kinase inhibitors P21 and P53 inhibited the expression of CyclinD1, and the cell cycle in G0/G1 phase was arrested. Insensitive to general chemotherapy, often causing tumor recurrence [28]. Cancer stem cell are often in the quiescent phase, rarely undergo division and proliferation, and are insensitive to many anti-tumor drugs. Meng et AL found that in ovarian cancer CSCs, the sensitivity of ALDHI positive CSCs to chemotherapy drugs was significantly reduced [29]. Al-Dhia regulates KLF4 and P21 proteins and is a factor that regulates the cell cycle, and the cells were arrested in the G0/G1 phase, which was insensitive to chemotherapy drugs. Thus, al-Dhia can resist the fulling effect of drugs The sensitivity of Go cancer stem cell to chemotherapy drugs is different from that of other tumor cells in vivo functional assays showed that about 96% of leukemia patients with LSC in G0 phase did not divide and were not retained by chemotherapy drugs. Once properly stimulated. CSC would re-enter the cell division cycle, proliferate and differentiate into progeny cells, resulting in tumor recurrence which is a tricky problem in current cancer treatment.

4. Conclusion

In the process of tumor development, the proliferation, differentiation and regeneration ability of CSCs provide a better environment for tumor metastasis. CSCs are the underlying cause of tumor resistance during therapy. In cancer treatment, the inability to completely clear or identify all CSCs is the biggest challenge. The theory of CSCs provides a new idea for the treatment of cancer, which is of positive significance, but it is still in the basic stage. The unique biological characteristics of CSCs are the root causes of drug resistance. In the study of drug resistance, scientists can pay more attention to the biological characteristics of CSCs, and use the biological characteristics to control the development of tumor and drug resistance. ln future studies, most of the ideas can first be focused on CSCs biomarkers, which can not only identify tumor types faster, but also improve the prognosis of patients according to the characteristics of their high expression. Secondly, the DNA sequence can

be adjusted to promote CSCs apoptosis, reduce the generation of progenitor cells, and prevent tumor metastasis.

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