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| RESEARCH ARTICLE

An Analysis of Cancer Stem Cells' Effectiveness in Curing Tumor and Possible Therapies

Zibin Huang
Guangzhou Dublin International College of Life Sciences and Technology, South China Agricultural University, Guangzhou, China
Corresponding Author: Zibin Huang, **E-mail**: 2033828815@qq.com

| ABSTRACT
This review focuses on the role of cancer stem cells in tumor development and summarizes multiple therapeutic approaches based on them. Cancer research has been significant in medical research as it is increasingly recognised as a worldwide public health concern. Although modern medical treatment can be done utilizing chemotherapy or radiotherapy, tumors' high mortality and recurrence rate remain a critical issue. However, in recent years, based on a large number of studies on cancer, scientists have put forward the theory of cancer stem cells, aiming to provide prospective treatment theories and methods for cancer therapies. These innovative treatments have brought hope to a complete cure for cancer. This paper first reviews the current status of cancer development, the hot theory of cancer stem cells, and the role of cancer stem cells in curing cancer. Then, three popular cancer stem cell treatments are reviewed, briefly discussed, and analyzed. Finally, the prospect of a treatment system is examined based on cancer stem cells.

| KEYWORDS

Cancer, cancer stem cells, cell signaling pathway inhibition, nano-carriers, cancer vaccines

| ARTICLE INFORMATION

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1. Introduction

As a disease with high mortality and recurrence rate, cancer urgently needs effective treatment. Otherwise, it may continue to bedevil modern medicine. As the second most common cause of death worldwide, cancer is a persistent global public health challenge. It is expected to be the most crucial obstacle to life expectancy growth in the 21st century (Dekker et al., 2019). Compared with other major human diseases, such as heart disease and cerebral thrombosis, the cancer mortality rate remains high (Kolata, 2009), and cancer incidence is rising globally (Torre et al., 2016). Therefore, it is urgent to find effective ways to reduce cancer mortality. In February 2020, cancer was the first or second leading cause of premature death (i.e., death between 30 and 69 years of age) in 134 countries, according to a systematic summary of the latest World Cancer Report released by the International Center for Research on Cancer. It ranked third or fourth in another 45 countries (Wild et al., 2020). However, there are evident differences in cancer incidence and mortality across countries, closely related to inherent gaps in medical technology and health infrastructure (Bray et al., 2018). For example, cancer incidence and mortality rates differ significantly between developed countries and developing countries. Owing to effective prevention, early detection, and timely treatment, cancer mortality rates are declining in most countries with a high human development index. In contrast, cancer mortality rates are still increasing or stabilizing in transition countries (cf. Barta et al., 2019; Dekker et al., 2019; DeSantis et al., 2019). In recent years, the incidence and mortality of tumors have decreased, and 5-year survival rates have improved for all types of cancers (Allemani et al., 2018). However, cancer incidence and mortality rates remain high in most low-income countries. In other words, the priority of modern cancer research is to find suitable cancer treatments and promote them globally to reduce the cancer mortality rate.

Therefore, searching for new anti-cancer drugs has become a hot research topic in medicine. The current cancer treatment is based on the theory that all tumor cells can proliferate indefinitely. The existing concept is to reduce the number of tumor cells and curb tumor growth by traditional treatment methods such as surgical resection, radiotherapy, and chemotherapy as much as possible

to achieve a therapeutic effect (Clevers, 2011). However, many chemotherapy drugs have poor water solubility, short circulation time in vivo, and non-specific distribution, which dramatically limits the clinical therapeutic effect of chemotherapy, and the prognosis is abysmal. Most tumors may even relapse. While radiotherapy and chemotherapy have improved the survival rate of cancer patients to a certain extent, the drug toxicity and resistance of cancer cells to drugs also limit the clinical application of drugs. The main reason for tumor recurrence is the residual tumor cells that are not completely removed or cannot be removed during the treatment process and the formation of new tumors. Bonnet & Dick (1997) also pointed out that the main reason why malignant tumors are complex to cure is that tumor tissue is composed of heterogeneous cell populations. Those malignant tumor cells with solid tumorigenicity, high invasiveness, and strong metastatic ability are "permanent," meaning they have stem cell properties (Al-Hajj et al., 2003; Al-Hajj & Clarke, 2004; Chambers & Smith, 2004). These cells are few but are more likely than normal tumor cells to develop resistance and evade the killing of chemotherapy drugs. Without eliminating these malignant cells from the body, it is almost impossible to cure. Therefore, the new concept of tumor stem cells (TSCs) has been proposed by scientists because of the self-renewal ability of tumor cells, the heterogeneity of uncertain potential, and the strong tumorigenicity, high invasiveness, and solid metastatic ability of a few tumor cells (Meacham & Morrison, 2013).

2. The cancer stem cell hypothesis and cancer stem cells

2.1 Cancer stem cell hypothesis

2.1.1 Emergence of the cancer stem cell hypothesis

Cancer stem cells are currently considered the few cells with self-renewal and differentiation potential in cancer cells. The traditional idea that somatic mutations form tumors and that each tumor cell can grow without limitation does not explain the seemingly infinite vitality of tumor cells and that not all tumor cells can grow without restriction. Stem cells were linked to tumors as early as the 19th century (Cohnheim, 1875), but it was not until 1983 that Mackillop et al. (1983) first proposed the tumor stem cell hypothesis. The hypothesis states that a small number of cells with stem cell-like functions exist in tumors.

Like normal stem cells, cancer stem cells also can self-renew. The first is symmetric division, meaning tumor stem cells can self-renew basically (Al-Hajj & Clarke, 2004). The other is asymmetric division, which means that the uneven distribution of differentiation regulating proteins in the cytoplasm leads one of the two daughter cells to terminal differentiation and becomes a functionally specialized differentiated cell. While the other maintains its parental characteristics and remains a stem cell, this division method can produce many differentiated cells to form the entire tumor tissue. In addition, stem cells can migrate, and cancer stem cells can metastasize, which are very similar (Chambers & Smith, 2004). Thus, the hypothesis of tumor stem cells within a tumor was established. Nevertheless, compared with normal stem cells, tumor stem cells cannot differentiate into fully mature cells and tend to accumulate replication errors. Through continuous self-renewal and differentiation, tumor stem cells eventually generate many tumor cells, which maintain the growth and heterogeneity of the tumor.

However, the origin of cancer stem cells has been controversial, and there are three main origin hypotheses of cancer stem cells (Bjerkvig et al., 2005). The first hypothesis suggests that cancer stem cells are derived from adult stem cells (Collins et al., 2005). Normal stem cells mutate and eventually overactive their standard regulatory mechanisms of renewal and differentiation, transforming into malignant tumor stem cells. There is a hypothesis that normal stem cells are exposed to an oncogenic microenvironment for a long time. In that case, DNA damage repair cannot be effectively completed, which leads to abnormal activation or inactivation of crucial genes and dysregulation of signaling pathways (Kucia et al., 2009). All of these consequences may lead to the generation of tumor stem cells. The second hypothesis suggests that progenitor cells mutate during differentiation and acquire the ability to self-renew and terminate differentiation, transforming into cancer stem cells (Clevers, 2011). At the same time, there is much evidence that cancer stem cells are derived from poorly differentiated progenitor cells. For example, oligodendrocyte progenitor cells can acquire stem cell-like properties after being induced by extracellular signals. This process can cause chromatin reorganization and SOX2 reactivation, transforming into cancer stem cells. The last hypothesis is that cancer stem cells are derived from mature terminally differentiated cells (Najafi et al., 2019). That is, some mature cells can acquire the ability of self-renewal and differentiation through mutation and then form cancer stem cells. In 2006, the discovery of induced pluripotent stem cells (iPS cells) enabled scientists to realize that it is possible to transform from mature terminally differentiated cells into stem cells. Furthermore, it suggested that converting mature terminally differentiated cells into tumor stem cells is possible. These three hypotheses and theories are supported by corresponding experimental evidence, among which the most evidence supports that tumor stem cells are derived from normal stem cells. However, the other possibilities cannot be denied. More recent studies have shown that tumor cells can be converted into stem cells during epithelial-mesenchymal transition (EMT) (Chaffer et al., 2011; Adorno-Cruz et al., 2015).

The existence of cancer stem cells has also been confirmed in various subsequent studies. For example, Bonnet & Dick (1997) first isolated CSC from CD34 + human acute myeloid leukemia cells with stem cell markers and successfully proliferated it in immunodeficient mice. Subsequently, CSC subsets with stem cell phenotype were also found in breast, lung, prostate, and other cancers. These studies successfully confirmed that cancer stem cells were no longer a hypothesis. Examples in breast cancer (e.g.,

Al-Hajj et al., 2003), central nervous system neoplasms (e.g., Hemmati et al., 2003; Singh et al., 2003; Singh et al., 2004; Singh et al., 2004), prostate cancer (e.g., Richardson et al., 2004; Collins et al., 2005), pancreatic cancer (e.g., Li et al., 2005), liver cancer (e.g., Fiegel et al., 2004), lung cancer (e.g., Kim et al., 2005), colon cancer (e.g., Dalerba et al., 2007; Ricci-Vitiani et al., 2007; O' Brien et al., 2007) and melanoma (e.g., Frank et al., 2005; Monzani et al., 2007) and other solid tumors identified the existence of tumor stem cells, and gradually established the tumor stem cell model. Subsequently, Nguyen et al. (2012) proposed that tumor stem cells refer to the cell population with malignant proliferation ability and can promote tumor growth. Such cells must be eliminated to cure the tumor completely. In 2012, a mouse cell marker tracking experiment was reported, demonstrating the origin and existence of tumor stem cells (Schepers et al., 2012). In conclusion, cancer stem cells are no longer a hypothesis but a growing research hot spot in the field of cancer.

2.1.2 Influence of cancer stem cells in the process of curing cancer

Pan et al.'s (2018) research indicate that cancer stem cells (CSC) can induce tumorigenesis and play a decisive role in subsequent metastasis and treatment (radiotherapy or chemotherapy). It has been suggested that CSCs may be the leading cause of cancer development into invasive and metastatic disease (Najafi et al., 2019). Moreover, heterogeneity, a characteristic of malignancy, has been suggested to be associated with CSCs (Mayer et al., 2015; Pan et al., 2018; Tsao et al., 2018; Leão et al., 2017). As early as the 1970s, biologists found that cancer tissues contain cell fractions with different clonogenic abilities through in vitro experiments on cell clonogenic ability (Hamburger & Salmon, 1997). In addition, various components in the tissue have different sensitivities to cancer treatment drugs (Salmon et al., 1978), which is the heterogeneity of the tumor. CSCs have the ability of self-renewal, infinite proliferation, and continuous differentiation of normal stem cells, as well as the ability to generate heterogeneous lineages of cancer cells constituting tumors and can stimulate tumor growth (Nguyen et al., 2012; Tang, 2015; Leão et al., 2017). More evidence (e.g., Morgan et al., 2012) suggests CSCs play an essential role in breast cancer' s initiation, progression, metastasis, and drug resistance. Although the number of these cells is limited, they are more likely than normal tumor cells to develop drug resistance and escape the killing of chemotherapy drugs in cancer treatment. Najafi et al. (2019) also pointed out that cancer cells differentiated by CSCs also can dedifferentiate under certain conditions, that is, to transform from normal cancer cells back to CSCs, thus replenishing the proliferative parts of the tumor. Therefore, in theory, if there is still one cancer cell, it can develop into a complete tumor.

Therefore, based on this theory, subsequent cell and animal experiments related to cancer stem cells have proved that it is feasible to eliminate cancer stem cells and thereby eliminate cancer. In Schmidt et al.'s (2011) experiment, less than 2% of melanoma cells expressed both melanoma cell antigen and CD20 surface antigen. After obtaining this population of cells by cell sorting, it was found that the cells expressing both antigens contained melanoma tumor stem cells. In subsequent experiments, T cell activation was explicitly induced by constructing surface-specific antigens to eliminate tumor stem cells expressing both melanoma cell antigens and CD20 surface antigens, thereby eliminating melanoma (Schmid et al., 2011). However, according to the tumor stem cell hypothesis, the traditional methods cannot kill all tumor stem cells, so cancer still has the potential to relapse.

In recent years, the cancer stem cell hypothesis has attracted more and more attention, and many scholars have begun to support that cancer treatment should mainly target cancer stem cells. It has been widely hypothesized that tumor insensitivity to radiotherapy and chemotherapy may be due to the presence of tumor stem cells (cf. Nduom et al., 2012; Dittmer et al., 2013; Hassan et al., 2013). Therefore, cancer therapy is supposed to focus on killing tumor stem cells (e.g., Wang et al., 2011; Wang et al., 2013; Dittmer et al., 2013; Hassan et al., 2013; Wu et al., 2013). Scholars believe that after killing tumor stem cells, the ability of tumor proliferation and recurrence will be significantly reduced (Groth & Fortini, 2012; Espinoza & Miele, 2013). Therefore, it is crucial to focus on the biological function of cancer stem cells as a breakthrough to observe the influence of cancer stem cells in curing cancer. The focus of research should be shifted to therapies targeting cancer stem cells to reduce cancer mortality and recurrence rate and even cure cancer completely.

2.2. Biological characteristics of cancer stem cells

2.2.1 Plasticity of cancer stem cells

Plasticity refers to the ability of cancer stem cells to transform into another cell type (Najafi et al., 2019). First, cancer stem cells can self-renew, in which a cell divides into two cells, with one of the progenies remaining in the same undifferentiated state as the parental cell. On the other hand, the other progeny differentiated in a directed manner, a division also known as asymmetric division (Al-Hajj et al., 2003; Al-Hajj et al., 2004; Chambers & Smith, 2004). Moreover, CSCs, like normal stem cells, can differentiate and promote tumor formation and metastasis. It is generally believed that tumor stem cells maintain the continuous growth of tumors through self-renewal. Moreover, tumor stem cells accumulate genetic mutations of cancer, which lead to excessive proliferation of tumor cells, and even metastasis and spread. Therefore, the self-renewal characteristic of tumor stem cells is the main reason for tumor recurrence, metastasis, and poor prognosis (Kucia et al., 2005).

In addition, cancer stem cells can dedifferentiate. It has been found that non-stem cell cancer cells can be transformed into cancer stem cells (Chiu et al., 2011) because there is a balance between breast cancer stem cells and non-stem cell breast cancer cells. Najafi et al. (2019) also found that CSCs can dedifferentiate, transforming from non-stem cell cancer cells back into tumor stem cells, thereby complementing their source of infinite proliferation.

Therefore, the plasticity of cancer stem cells plays an essential role in the clinical treatment of cancer, mainly manifested by the difficulty of killing all cancer cells with ordinary chemotherapy or radiotherapy. However, the surviving cancer cells have plasticity, constantly replenishing the number of cancer cells, eventually leading to cancer recurrence.

2.2.2 Multidrug resistance of cancer stem cells

Multi-drug resistance is another important feature of cancer stem cells that makes it more difficult to cure cancer. Drug resistance of cancer stem cells is a multi-step regulatory process (Rosen & Jordan, 2009). Among them, the ABC transporter family (ATP-binding cassette) mediates the multi-drug resistance of cancer stem cells. These transporters protect themselves from cytotoxic drugs by actively pumping out intracellular drugs using the energy generated by ATP breakdown. Meanwhile, tumor stem cells highly expressing these molecules can also pump out chemotherapy drugs, reducing intracellular drug concentrations and thereby developing resistance (Leslie et al., 2005).

Second, the drug resistance process of cancer stem cells is in the quiescent phase of the cell cycle. Most cancer stem cells were in G0 (Leslie et al., 2005). The commonly used chemotherapy drugs in clinical practice mainly target tumor cells in the active cell cycle. In contrast, quiescent tumor stem cells do not divide, so they can remain in chemotherapy (Rosen & Jordan, 2009). Once the drug is discontinued, and these tumor stem cells are stimulated, they will enter the cell division cycle and form tumors through self-renewal and differentiation, resulting in tumor recurrence. This process is also a mechanism of drug resistance in cancer stem cells (Houghton et al., 2004). Another necessary means of drug resistance in cancer stem cells is that they can change the balance between intracellular oxidation and antioxidants, accelerate cellular oxidation, and lead to redox dysregulation in vivo, thereby inducing cell drug resistance (Houghton et al., 2004).

2.2.3 Cancer stem cells can lead to tumor metastasis

Cancer stem cells are also the key to tumor metastasis. Most tumor cells that enter the metastasis cycle eventually die. Only about 0.02% of the cells reach the metastasis site to form clinically significant metastases, but this proportion is tiny. To accomplish this, the tumor cells entering the circulation must be able to self-renew and proliferate in large numbers. The plasticity of tumor stem cells determines that they are the best metastasis vectors.

Meanwhile, it has been found that epithelial-to-mesenchymal transition (EMT) is closely related to tumor metastasis. EMT is a process in which epithelial cells with polarity transform into mesenchymal cells with migration ability and acquire invasion and migration ability. In conclusion, various characteristics of cancer stem cells indicate that they play an essential role in cancer recurrence, drug resistance, and metastasis. Thus, a new perspective for modern cancer treatment is to study cancer stem cells' relevant clinical treatment measures.

3. Treatment measures based on cancer stem cells

3.1 Targeted cell pathway therapy

Tumor stem cells are the root cause of cancer pathogenesis. The emergence of tumor stem cell theory reveals the mechanism of tumor genesis and brings revolutionary changes to cancer treatment. Under the consensus that it is possible to completely cure cancer only by eliminating all CSCs, the therapeutic methods targeting cancer stem cells have become the hot spot of the times (Dittmer et al., 2013). Because there are three main signaling pathways in cancer stem cells: Wnt, Sonic hedgehog, and Notch, and they are closely related to the occurrence of various tumors, the research of targeted drugs to treat cancer by killing cancer stem cells mainly focuses on these three signaling pathways. Studies have found that some tumor-related signaling pathways, such as Notch, Hedgehog, and Wnt signaling pathways, regulate normal stem cells simultaneously. However, these pathways are abnormally expressed in tumor stem cells (Nefedova et al., 2008). For example, Notch is overexpressed in various cancer stem cells (Sell, 2007), and inhibition of Notch expression can inhibit tumor proliferation. The Notch signaling pathway can be used as a new channel for cancer treatment. Other signaling pathways can also be used as breakthroughs in cancer treatment.

The notch signaling pathway is a very conserved signaling pathway, which can regulate stem cell self-renewal, mainly by affecting cell-cell signal communication, thereby regulating cell differentiation, proliferation, and apoptosis (Sell, 2007; Xu et al., 2012). When activated, the Notch pathway can promote tumor proliferation and metastasis, while blocking this pathway can cause tumor stem cells to apoptosis and increase their sensitivity to drugs, indicating that genes in the Notch signaling pathway can become potential cancer therapeutic targets (Nefedova et al., 2008). Hence, the role of Notch in cancer has recently received close attention. It has been shown that the Notch signaling pathway is overexpressed in the development and progression of pancreatic cancer. Notch1

induces pancreatic cancer cell proliferation, cloning, and migration (Lachej et al., 2012). In animal experiments, when Notch2 is knocked down in mouse pancreatic cancer, the survival rate of mice significantly increases (Mazur et al., 2010). It has been reported that the Notch signaling channel is also abnormally expressed or activated in breast cancer, uterine fibroids, colon cancer, renal cancer, acute myeloid Hodgkin's lymphoid disease, etc. (cf. Patrawala et al., 2006; Pannuti et al., 2010). Much evidence has demonstrated that overexpression of the Notch signaling pathway promotes tumor growth while blocking the Notch signaling pathway inhibits tumor proliferation (e.g., Chiu et al., 2011). Thus, the Notch signaling pathway is a research hot spot in cancer stem cell targeted therapy.

Based on this theory, inhibitors of the Notch signaling pathway are the principal means to inhibit cancer proliferation. Inhibitors of the Notch signaling pathway can be divided into four categories (Takebe et al., 2010). The first class is that soluble decoy receptors of Notch trick Notch ligands into binding to them to block Notch signaling. Class 2 acts on Y-secretase to inhibit tumors by inhibiting or altering the structure of Y-secretase so that it cannot cleat Notch protein. The third class is MAML1, which inhibits the Notch signaling pathway by inhibiting the expression of Notch target genes. Class 4 inhibitors are the most direct, using small interfering RNAs to directly inhibit Notch target genes' expression.

Similarly, various small molecules have been found to inhibit the Wnt signaling pathway in tumors. Chen et al. (2009) found two series of small molecules that can inhibit the Wnt signaling pathway. The first one acts on acyltransferase, which is essential in forming Wnt. However, the second one can stabilize Axin, thus stabilizing the "APC-axin-GSKβ complex". Other monoclonal antibodies, such as Wnt, soluble Wnt receptor antibody (decoy receptor), and Fz receptor antibody, have shown some potential. Abnormal activation, mutation, and dysregulation of Hedgehog (Hh) signaling are also associated with the development of various tumors. Hh signaling also plays an essential role in maintaining CML tumor stem cells. Cyclopamine, an inhibitor of the Hh signaling pathway, can inhibit EMT and metastasis of pancreatic cancer. Drugs targeting Hh signaling in tumor stem cells have been marketed (Takebe et al., 2010), mainly for treating basal cell carcinoma.

3.2 Combining nanocarriers for the treatment

As cancer stem cells are multi-drug resistant, they are usually inside the tumor as well as in the quiescent phase of cell division normally. In other words, ordinary chemotherapy and radiotherapy are challenging to respond to. As a result, tumor stem cells have a specific resistance to conventional chemotherapy and radiotherapy, which increases the difficulty and complexity of tumor treatment (Angeloni et al., 2015; Brandolini et al., 2015). Thus, effective tumor stem cell growth inhibition is critical for cancer treatment.

With the development of nanotechnology, various nano-drug carriers have been applied in the treatment of cancer (cf. Peer et al., 2007; Lammers et al., 2011; Wang et al., 2012). For example, some environmental stimulation-responsive nano-carriers can control the release of drugs at tumor sites and increase the drug content at tumor sites through environmental changes, thereby enhancing the therapeutic effect of drugs (Wang et al., 2010; Hu et al., 2012; Zhang et al., 2013; Jing et al., 2015; Xia et al., 2017). Using nano-drug carriers for tumor chemotherapy can efficiently concentrate on the tumor site through solid tumors' high permeability and retention effect (EPR effect) and improve the solubility and stability of hydrophobic drugs under physiological conditions and prolong the duration of drug action. Thus, the therapeutic effect can be enhanced, and the side effects of drugs can be alleviated (Fang et al., 2010; Yoo et al., 2011; Karageorgis et al., 2016). In addition, it is easy to connect various ligands on the surface of nanoparticles, which can improve the ability of nanoparticles to enter cells and enhance the therapeutic effect of chemotherapy by actively targeting tumor stem cells (Davis et al., 2010; Hrkach et al., 2012).

The specific practice methods are mainly targeted therapy of cancer stem cells with nano-carriers combined with drugs. For example, traditional drugs combined with nano-carriers can improve the killing effect on CSCs. Doxorubicin (DOX), a commonly used chemotherapy drug, mainly inhibits the growth of tumor cells but does not have an excellent inhibitory effect on tumor stem cells. On the other hand, Sun et al. (2013) linked DOX and surface-modified polyethylene glycol (PEG) gold nanoparticles by hydrazone bond. The composite nanoparticles formed could avoid the drug being pumped out of the body by P-glycoprotein, effectively delivering the drug to breast cancer stem cells. Furthermore, it inhibited the growth of tumor microspheres and the tumorigenic potential of breast cancer stem cells.

For example, nano-carrier loading of dual drugs can also improve the killing effect of cancer stem cells. Due to the heterogeneity of tumor tissue, using a variety of drugs with different mechanisms of action while inhibiting the growth of tumor stem cells and tumor cells is an effective means to improve the effectiveness of cancer treatment (Yuan et al., 2018). For example, salinomycin (SAL), a carboxylate polyether antibiotic, can effectively inhibit the growth of tumor stem cells, but its poor water solubility limits its application. The combination of SAL and common chemotherapeutic agents can significantly improve the efficacy of nanoparticles for tumor treatment (Li et al., 2017). Notably, Gong et al. (2016) found that the synergistic effect of the two drugs

was best when the drug ratio SAL: DOX=1:1 in the dual-drug liposomes. Thus, exploring the optimal balance of two drugs is a vital issue to consider in preparing dual-drug-loaded nanoparticles.

However, the emergence of vector-free nanoparticles targeting tumor stem cells can better avoid the uncertain bio-safety issues of inorganic and organic nanomaterials. Specific practices include not introducing foreign materials, using therapeutic drugs as carriers to prepare nanomaterials, or forming nanoparticles through drug self-assembly, to improve biosafety significantly based on effective cancer treatment. For example, ceramide is an important bioactive lipid regulating cell death, migration, stress response, and inflammatory response in cancer cells. Increased ceramide means chemotherapeutic agents are more effective against cancer, so simultaneous delivery of ceramide and chemotherapeutic agents can improve the efficacy of chemotherapy against multi-drug resistance. Using PEG-ceramide as a carrier and loaded with SAL, the formation of nanomicelle SCM can target both lung cancer cells and cancer stem cells.

3.3 Cancer Vaccines

The CSCs theory also provides a theoretical basis for applying the CSCs vaccine in the prevention and treatment of cancer. CSCs express molecular markers related to normal, tumor, and stem cells. For example, CSCs for colon cancer can express specific molecular markers such as CD44, CD133, and CD166. The colon cancer vaccine is taken as an example to illustrate. The colon cancer CSCs vaccine is the treatment of isolated colon cancer CSCs with physical, chemical, and biological factors to alter or eliminate their carcinogenicity and preserve their immunogenicity. It is a new type of vaccine that can induce a specific anti-CSCs immune response in the body by combining with adjuvants. Some researchers have prepared a CSCs vaccine for colon cancer by reducing the carcinogenicity of CSCs and improving their immunogenicity through gene modification. The colon cancer CSCs vaccine application in mice has significantly inhibited tumor growth and recurrence (Gerger et al., 2011; Giampieri et al., 2013).

Based on the specific surface antigens CD44 (Todaro et al., 2014), CD133 (Bostad et al., 2013), CD166 (Levin et al., 2011), CD44 (Todaro et al., 2014), CD133 (Bostad et al., 2013), CD166 (Levin et al., 2011). 2010), acetaldehyde dehydrogenase 1 (ALDH1) (Hou et al., 2013), and LGR5 (Wu et al., 2013), etc., the leading research focuses on vaccines targeting CSCs themselves currently. CSCs can be purified in vitro by flow cytometry or immunomagnetic bead sorting and treated by physical, chemical, and biological means (virus infection, gene transfer, etc.). In this way, the immunogenicity of the indicated antigens can be preserved so that the prepared CSCs vaccine can enhance the induction of specific immune responses against CSCs.

At the same time, there are also vaccines targeting the regulatory genes of CSCs in colon cancer. Vaccines that target the regulatory genes of CSCs in colon cancer can induce specific immune responses against the regulatory genes of CSCs. Furthermore, the regulation of CSCs by one or more genes can be blocked, thereby inhibiting or blocking the proliferation, differentiation, migration, invasion, and other pathological processes of CSCs. Through research, Kreso et al. (2014) found that the BMI-1 gene exists in colon cancer cells, which can regulate the self-renewal, proliferation, and survival of CSCs. Experiments have shown that BMI-1 gene knockdown can effectively reduce the growth of colon cancer, BMI-1 gene silencing can weaken the self-renewal ability of CSCs, and BMI-1 inhibitors can cause irreversible damage to CSCs. In addition, previous studies (Kim et al., 2004) have confirmed that 65% of colon cancer patients have a positive expression of BMI-1. Applying the relevant concept to CSCs regulatory gene preparation of CSCs vaccine will help to develop a more individualized anti-CSCs vaccine.

Cancer stem cells possess a variety of specific surface antigens, and vaccines targeting their surface antigens have also been designed. Because CSCs contain many specific surface antigens, such as CD44 (Todaro et al., 2014), CD133 (Bostad et al., 2013), CD166 (Levin et al., 2011), ALDH1 (Hou et al., 2013), and LGR5 (Levin et al., 2011), they can enhance the proliferation ability of colon cancer cells in a new environment. For example, CD44 is an important molecule to maintain the stemness of colon cancer CSCs. If the CD44 gene is knocked down, colon cancer CSCs will lose clonogenic ability in vitro and tumorigenic ability in vivo.
The follow-up vaccine identification method can be determined by referring to the preparation process of the vaccine. If the vaccine is a genetically modified CSCs vaccine, RT-PCR should be performed to identify the transcription of the target gene of the tumor vaccine. For CSCs vaccines with targeted proteins, immunofluorescence should be performed to determine the expression of target proteins. The anti-tumor effect of the colon cancer CSCs vaccine was achieved by detecting the proportion of immunized animals without tumor formation, tumor size, survival curve, and the balance of immunized animals without tumor formation after being challenged with a tumor vaccine. Various studies have shown that the surface markers of CSCS have also been isolated from other tumor sites. Therefore, the above design theory and the idea of a vaccine against colon cancer stem cells can be applied to the vaccine research of different tumor sites to develop other CSCs vaccines.

4. Recommendations and prospects for cancer stem cell targeted therapy

Although the above treatment methods for cancer stem cells can target and kill cancer stem cells, in theory, there are still some risks in the actual clinical treatment. For example, the signaling pathway inhibition of cancer stem cells, although only some specific antagonists need to be used to inhibit the main signaling pathway of cancer stem cells, is relatively easy to use in clinical practice.

However, Pattabiraman & Weinberg (2014) found that inhibition of the main signaling pathway of cancer stem cells may affect normal stem cells. Thus affecting the normal process of cell proliferation and differentiation in the human body. Some studies (Wang et al., 2009; Yao & Mishra, 2009) reported that Notch1 plays a vital role in the suppression of liver tumors, and overexpression of Notch1 can inhibit tumor proliferation and induce tumor cell death in vivo and in vitro. This also suggests that subtypes must be considered in developing drugs targeting signaling pathways.

Although using nano-drug delivery systems in tumor therapy has many advantages, its existing problems have not been solved. For example, it can improve the solubility and stability of hydrophobic anti-cancer drugs and therapeutic molecules under physiological conditions and enhance the therapeutic effect. Moreover, it can prevent the drug or molecule from being cleared by the blood during transportation and prolong the circulation time of the drug. Secondly, the nano-drug delivery system can control the targeted release of therapeutic molecules in tumor tissues and reduce the toxic and side effects on normal tissues. It can also deliver various medicinal drugs or diagnostic reagents and can be combined with other therapeutic methods to improve the therapeutic effect (Hrkach et al., 2012; Chen et al., 2016; Karageorgis et al., 2016). However, tumor stem cells share many signaling pathways and surface markers with normal stem cells. Hence, targeting tumor stem cells may cause damage to normal cells. In addition, tumor stem cells are heterogeneous. Different surface markers often represent different types of tumor stem cells due to the various sources of tumor stem cells. Finally, the toxicity of nano-drug vectors targeting tumor stem cells in the human body still needs further exploration. Safety is the top priority in whether nano-drug vectors can be applied in clinical practice. With the development of metabolomics technology, gas chromatography-mass spectrometry (GC-MS) and other methods can be used to analyze nanoparticles to evaluate their safety more comprehensively.

Although cancer stem cell vaccines also have many advantages, more experiments are needed to ensure that they can be applied to human cancer treatment before they can be used in empirical therapy. Since CSCs have more potent immunogenicity and are more effective as antigens, the antibodies elicited by CSCs can target CSCs and confer anti-tumor immunity. To further verify that the CSCs vaccine is more effective in inducing anti-tumor immunity and that CSCs can be targeted and killed by T cells, Ning et al. (2012) found that there are some highly expressed surface molecules in CSCs. If a CSCs vaccine is made, it can stimulate the body to produce specific immune responses against these highly expressed surface molecules. In this way, patients' immune systems can effectively inhibit or kill CSCs, thereby reducing the possibility of tumor resistance and recurrence. The limitation of this study is that colon cancer CSCs isolated from multiple passages of cell lines or tumors of vaccinated animals may be immunocompetent, which may not be the same as CSCs obtained directly from spontaneous tumors of patients.

As a result, the lack of a CSCs vaccine prepared from spontaneous tumors remains one of the limiting factors for the lack of objective outcome evaluation in this study. In addition, CSCs have similar biological characteristics to standard stem cells (SCs). Whether the CSCs vaccine can induce an immune response to standard SC remains unknown. There is no guarantee that the CSCs vaccine will not cause malignant transformation or even lead to tumor metastasis in vivo. Secondly, the specific antigen molecules on the surface of colonic CSCs have not been recognized. And the complex protein composition of CSCs has not been clarified, which may lead to adverse immune reactions and even severe side effects if the vaccine is used carelessly. Furthermore, the CSCs vaccine for colon cancer is still in the stage of preclinical trials in mouse colon cancer models. However, the immune systems of humans and mice are not identical, so whether the vaccine can be used to treat human colon cancer still needs scientific demonstration. Altogether, Table 1 summarizes the advantages and disadvantages of the three CSCS-targeted therapies.

Table 1: Advantages and disadvantages of the three CSCS-targeted therapies

	Signaling Pathway Inhibition	Nano-drug delivery system	Cancer stem cell Vaccines
Advantages	Its specific usage is simple.	Nanocarriers can improve the solubility and stability of drugs, achieve targeted release, and combine with other therapeutic methods.	Cancer stem cells are more immunogenic, and the resulting vaccines are more targeted.
Disadvantages (Potential risks)	The inhibitory signal may affect normal stem cells, and its subtype should be considered for drug development.	Nanocarriers may affect normal stem cells, and their toxicity to humans has not been determined.	The safety, efficacy, specificity, and applicability of cancer stem cell vaccines have not been recognized.

5. Conclusion

Cancer research has always been a hot spot and focuses on the field of medicine, and many research advances are published in influential journals. There is a consensus that cancer stem cells play an essential role in the occurrence and development of cancer, metastasis and recurrence, and prognosis. The stem cell theory provides a new framework for the academic community to understand and study tumors. At present, the research on cancer stem cells is changing rapidly. Some specific molecular markers of cancer stem cells have been identified based on proving the existence of cancer stem cells. The research and development of new drugs targeting anti-cancer stem cells are still needed. In the future, the main research directions might include: a) defining the tumor stem cell model and identifying its surface markers, b) studying the regulation mechanism of tumor intracellular signal, c) exploring the role of tumor stem cells in tumor genesis and development, and d) targeting their application in cancer therapy. Tumor stem cells provide a new direction for current cancer therapy, which will profoundly impact tumor prevention, early diagnosis, effective drug therapy, recurrence prevention, and prognosis judgment.

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