

Review

Cancer stem cells: A new target for cancer therapy

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Summary. The introduction of the theory of cancer stem cells (CSCs) has provided a new direction and perspective for our understanding of the nature and origin of tumors. Cancer stem cells are believed to be responsible for the treatment failure, drug tolerance, metastasis, and recurrence of tumors. However, it remains a challenge to identify or isolate tumor stem cells and determine their regulatory mechanisms. Therefore, further understanding of the biological characteristics and functions of CSCs is of great practical significance and value to develop new methods of tumor diagnosis and treatment, and may bring new hope patients with cancer.

Key words: Cancer stem cells, Identification and isolation, Regulatory pathway, Targeted therapy

Introduction

Malignancies are a lethal threat to human health. Traditional methods of tumor elimination include surgery, chemotherapy, and radiotherapy. However, a significant proportion of patients continue to experience recurrence and metastasis after adjuvant treatment, which makes tumors refractory. The mortality rate of patients with malignant tumor patients is high, making tumor therapy one of mankind's biggest challenges. It is

important to significantly improve the survival of patients with malignant cancer. Although our understanding of the biological theory of tumors is becoming deeper and biological technologies are improving, the root causes of tumor development, recurrence, and distant metastasis remain ambiguous. In recent years, the theory of cancer stem cells (CSCs) has become a hotspot of cancer research, which has suggested new ideas to treat cancer. CSCs are a subpopulation of tumor cells that have the potential for self-renewal and multidirectional differentiation, thus are capable of initiating and constructing tumor histological phenotypes. Although they are few in number, they play a decisive role in maintaining the proliferation, invasion, recurrence, and metastasis of tumors (Shuang and Qin, 2014). Based on the current research on CSCs at home and abroad, this article will give an overview of the following aspects: The theory of the origin of CSCs and their evolution, the biological characteristics of CSCs, how to isolate or purify them, and the possible mechanisms of CSCs self-renewal.

The origin of the CSC theory and its evolution

CSCs account for 0.01~0.10% of the total number of tumor cells, with the potential of unlimited proliferation. Makino (1959) found that tumor cells may originate from CSCs, and proposed the CSC hypothesis for the first time. The CSC hypothesis argues that different precursor cells or mature tumor cells are formed by the unidirectional differentiation of CSCs, and then further develop into malignant tumors. The concept of cancer stem cells was first proposed and defined in malignant

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leukemia. Nowell (1989) isolated a small amount of special cells from leukemia tissues. The *in vitro* and *in vivo* proliferation and differentiation abilities of these cells were very strong, and trace amounts of cells could form clones. Therefore, these cells were named as “Leukemia stem cells”, and the concept of cancer stem cells was proposed. Al-Hajj et al. (2003), for the first time, isolated cancer cell populations with stem cell-like features from the solid tumor (breast cancer), which were shown to display a CD44+CD24–/low Lineage-surface marker phenotype. Since then, it has been demonstrated that CSCs are present in most solid tumors.

In 2006, the American Association for Cancer Research defined a CSC as “a cell within a tumor that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor” (Clarke et al., 2006). That is, there are a small number of cancer cells in tumor tissue that act as stem cells in the process of tumor formation and have the potential of self-renewal, proliferation, and differentiation, playing an important role in tumorigenesis, development, recurrence, and metastasis. These cells were named as cancer stem cells because of their multitude of properties and similarities to stem cells.

The origin of cancer stem cells

There are two hypotheses about the origin of CSCs: One view is that CSCs are transformed from normal stem or progenitor cells (Fulawka et al., 2014), a process that results from genetic changes caused by various endogenous or exogenous stimuli, leading to the formation of a malignant phenotype; another view is that CSCs are tumor cells that have been reversed on the basis of oncogene-induced plasticity (Rapp et al., 2008). Most investigations and studies have focused on the first hypothesis. In liver cancer, mutation of isocitrate dehydrogenase 2 (*IDH2*) in hepatic progenitor cells prevents the cells from differentiating into hepatocytes. If there exists a second attack, such as mutation of K-Ras, this will cause a tumor. This suggests that CSCs in cholangiocarcinoma may originate from hepatic progenitor cells (Saha et al., 2014). Such studies have been carried out, for example, in colon cancer, prostate cancer, and lung cancer (Sutherland et al., 2011). That is, in progenitor cells with a particular marker, upon the activation of the proto-oncogene or the inactivation of tumor suppressor gene, the eventual change in cell type can be determined exactly, followed by lineage-tracing experiments in transgenic mice.

The biological characteristics of cancer stem cells

Self-renewal capacity

Similar to adult stem cells, CSCs have the capacity to self-renew. Tumors are generally considered as

diseases caused by the loss of control of self-renewal. The self-renewal of CSCs is the major cause of tumor recurrence, metastasis, and poor prognosis (Abetov et al., 2015).

High tumorigenicity

The number of CSCs is very small, however, their tumorigenicity is several hundred times higher than that of normal tumor cells. Only 100–1000 CSCs can form tumors in immunodeficient mice. Therefore, CSCs are the basis for maintaining the occurrence and development of tumors. Recent studies showed that CSCs play a key role in the carcinogenesis of hepatocellular carcinoma (Cheung et al., 2016), ovarian cancer (Vochem et al., 2014), and colorectal cancer (Asfaha et al., 2015).

Multi-directional differentiation potential

CSCs are capable of multidirectional differentiation both *in vitro* and *in vivo*, and can produce differentiated progeny tumor cells. It is because of the multi-directional differentiation potential of CSCs that tumor tissue heterogeneity is formed, mainly manifested by the degree and direction of differentiation.

The regulatory mechanisms of cancer stem cells

Currently, the molecular regulation of tumor stem cells is not fully understood. Research on the regulatory mechanisms of cancer stem cells has mainly focused on the regulation of signaling pathways, the tumor microenvironment, and microRNAs.

The signaling pathway abnormalities in CSCs

The Wnt pathway

The Wnt/ β -catenin signal transduction pathway consists of adenomatous polyposis coli protein (APC), Axin, transcriptional factor 4 (tcf4), and others. β -catenin plays a central role in this signaling pathway. In the past few decades, extensive research has proved that the Wnt/ β -catenin signaling pathway is involved in the formation and maintenance of CSCs in a variety of human malignancies. Byun et al. (2005) found that the self regulation of gastrointestinal stem cells was caused by regulation of the Wnt signaling pathway by Wnt antagonists, such as frizzled related protein (FrzB), Wnt inhibitor factor 1 (wif1), and Dickkopf Wnt signaling pathway inhibitor (DKK). It has been reported (Binda et al., 2017) that Wnt5a, a member of the Wnt family involved in the non-canonical Wnt signaling pathway, is an indispensable factor in promoting glioblastoma invasion. The authors found that Wnt5a was overexpressed in the most aggressive gliomas, which indicated poor patient prognosis. At the same time, mesenchymal glioblastoma, with strong infiltration, can

be distinguished from the proneural glioblastoma and classical glioblastoma. Overexpression of Wnt5a is associated with the tumor stem cell-like features of mesenchymal glioblastoma. These studies showed that the Wnt signaling pathway is crucial for the occurrence and fate of CSCs.

The Notch pathway

The Notch signaling pathway was first identified in genetic studies of *Drosophila melanogaster*, because some Notch alleles induce the formation of notched wings. The Notch signaling pathway mainly consists of Notch ligands (delta like canonical Notch ligand (Dll)1, Dll3, Dll4, Jag1, and Jag2), Notch receptors (Notch1–4), transcription factors, and downstream effectors. The Notch signaling pathway mainly regulates the proliferation, differentiation, apoptosis, and intercellular communication of normal stem cells. The mechanism by which the Notch signaling pathway maintains stem cell-like characteristics of cancer stem cells has been most thoroughly studied and elucidated in breast cancer and glioblastoma (GBM). Neutralizing antibodies to Notch4 markedly reduced the microsphere formation rate (MSFE) of ductal carcinoma *in situ*, reflecting the role of Notch in breast cancer stem cells (Grudzien et al., 2010). Gamma secretase inhibitors (GSIs) block GBM neurosphere growth and colony formation *in vitro* by preventing inhibiting Notch, whereas Notch activation promotes tumor growth (Kristoffersen et al., 2013). These data suggested that the Notch signaling pathway plays a significant role in the maintenance of stem cell-like features of some solid tumor stem cells, and that further regulation of the Notch pathway might serve as a therapeutic target for tumor therapy.

The Hedgehog pathway

The Hedgehog (Hh) signaling pathway regulates embryonic development, and self-renewal or proliferation, of adult stem cells in many tissues. The Hedgehog signaling pathway was discovered by genetic analysis in *Drosophila* and includes two transmembrane proteins patched homolog 1 (PTCH) and smoothed homolog (SMOH), and transcription factors such as fused homolog (Fu), suppressor of fused homolog (Su(Fu)) and Cubitus interruptus (CI)/glioblastoma protein (GLI) (Polizio et al., 2011). SMOH and transcription factor Fu play a positive regulatory role, while transmembrane protein PTCH, transcription factor Su(Fu), DISP (dispatched), and cos2 mainly play a negative regulatory role. Hedgehog signaling is a relatively classical pathway of stem cell regulation and is essential for the self-renewal of various stem cells. Sonic hedgehog (Shh) receptor activation can promote human epidermal stem cell proliferation, and Shh inhibitors can suppress stem cell proliferation (Zhou et al., 2006). In the nervous system, knockout of *SHH* can cause damage

to neurospheres (Palma and Ruiz i Altaba, 2004), while constitutive activation of Shh and C-myc can promote the proliferation of neural progenitor cells, leading to the formation of medulloblastomas. It is hypothesized that the Hh pathway plays an important role in the self-renewal and quantity maintenance of tumor stem cells.

Tumor stem cell microenvironment

In 1978, Schofield proposed the "niche" hypothesis to describe the physiological microenvironment that supports stem cell growth. The tumor microenvironment is the internal environment in which the tumor cells are produced and live, and includes the tumor cells themselves, the surrounding fibroblasts, various cells (such as immune and inflammatory cells, and glial cells), as well as the intercellular substances, microvessels, and biomolecules infiltrating them. These microenvironments are interrelated and mutually regulate the relevant tumor stem cells. Bao et al. demonstrated that the CSC microenvironment renders GBM CSCs resistant to conventional chemotherapy (Bao et al., 2006; Gilbertson and Rich, 2007). Subsequent studies murine medulloblastoma models have demonstrated that tumor cells located in the perivascular area are resistant to radiation treatment, mediated via the Akt signaling pathway (Hambardzumyan et al., 2008). Shinjima et al. (2013) reported that in the glioma tumor microenvironment, transforming growth factor- β (TGF- β) mediated mesenchymal stem cell migration to tumor stem cells, and that mesenchymal stem cells surrounding tumor stem cells can regulate tumor stem cell proliferation. Tumor-associated macrophages can also interact with tumor stem cells. For example, in pancreatic cancer tumors, the reduction in the expression of tumor-associated macrophages can significantly decrease the proportion of tumor stem cells in the tumor (Mitchem et al., 2013); tumor-associated macrophages promote epithelial-mesenchymal transition of hepatocellular carcinoma stem cells by secreting TGF- β , thereby enhancing tumor invasiveness (Fan et al., 2014). Hypoxia can increase the expression of stem cell-related genes such as octamer-binding protein 4 (OCT4), Nanog and C-myc in glioma cells, which can not only maintain the stem cell-like properties of CSCs, but also dedifferentiate the differentiated cells and increase the clonogenic capacity and migration ability of tumor cells (Li et al., 2013).

MicroRNAs

MicroRNAs (or miRNAs) are endogenous, non-coding, single-stranded RNAs comprising 21 to 25 nucleotides. MiRNAs inhibit gene expression post-transcriptionally and are involved in the regulation of many biological functions. Recent studies have found that miRNAs regulate the biological characteristics of cancer stem cells, such as differentiation and self-renewal. Shimono et al. (2009) identified 37 microRNA

that showed differential expression between breast cancer stem cells and non-tumorigenic cancer cells. For example, miR-200c-141, miR-200b-200a-429, and miR-183-96-182 were downregulated in breast cancer stem cells. Guessous et al. (2010) found that microRNA-34a could inhibit glioma stem cell malignancy by reducing glioma stem cell proliferation, preventing cell invasion, inducing G1/S arrest and apoptosis, and glioma stem cell differentiation. However, downregulation of microRNA-34a might induce the occurrence of gliomas. These results suggested that microRNA-34a is involved in the regulation of malignant transformation and differentiation of glioma stem cells. Many types of CSCs have excessive expression in miRNAs. Some miRNAs are upregulated in CSCs, acting as of oncogenes, while other miRNAs are downregulated in CSCs to inhibit tumor cell proliferation, acting as tumor suppressor genes. Abnormal expression of miRNAs can cause CSCs dysfunction, resulting in uninhibited cell differentiation and tumorigenesis.

The separation and identification of cancer stem cells

The discovery of CSCs represented a new breakthrough in the study of tumor biology. CSCs only account for a small part of the total number of tumor cells; therefore, their separation and purification have become the key to tumor biology research. To date, methods to separate CSCs are in their infancy, and identification methods are still in the exploratory stage. The main methods of CSC isolation and identification are side population (SP) sorting, suspension cell culture *in vitro*, fluorescence activated cell sorting (FACS), and magnetic activated cell sorting (MACS) methods.

Side population cell sorting

The SP sorting method exploits the principle that CSCs can emit DNA binding dye to separate and identify CSCs (Szotek et al., 2006). Currently, the use of the SP sorting method has successfully isolated a variety of tumor SP cells (Kondo et al., 2004).

Suspension cell culture *in vitro*

Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) are added into Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1) to make serum-free medium (SFM). CSCs can form floating spheres in SFM, which can be used for CSC isolation and identification (Liu et al., 2010; Ma et al., 2010).

Fluorescence activated cell sorting (FACS) and magnetic activated cell sorting (MACS)

According to the principle that related surface markers, such as CD117, CD133, and CD44 can express different fluorescence intensities, the method is

combined with flow cytometry to classify and isolate CSCs. Different types of CSCs express different specific markers.

Lapido et al. (1994) reported CD34+ CD38- phenotype acute myeloid leukemia (AML) cells for the first time. They found that the isolated CD34+ CD38- phenotype cells could induce human-like leukemia in mice; however, the CD34+ CD38+ phenotype cells did not have this tumor-causing ability. Other stem cell markers include CD133+ and nestin, which have been identified as brain tumor stem cell specific markers (Jordan, 2004). CD44, CD133, CD166, and epithelial cell adhesion molecule (EpCAM) have been identified as surface markers of colon cancer stem cells (Dalerba et al., 2007). Recent studies have shown that ALDH1 is also a specific marker for head and neck cancer stem cells (Ginestie et al., 2007; Zhou et al., 2007). ATP binding cassette subfamily G member 2 (ABCG2), ALDH1, minichromosome maintenance complex component 2 (MCM2), spinocerebellar ataxia 1 (SCA-1), and tumor protein p63 (p63) have been identified as stem cell surface markers of retinoblastoma cells (Fang et al., 2005; Seigel et al., 2005). Levina et al. found that after treatment with chemotherapeutic drugs, lung cancer cells that overexpressed CD133, CD117, and OCT4 survived, which suggested that these proteins could be markers of lung cancer stem cells (Levina et al., 2008). In addition, CD44, CD24, epidermal surface antigen (ESA) and CD133 + can label pancreatic cancer stem cells (Hermann et al., 2007; Li et al., 2007).

There are some limitations of *in vitro* single CSCs enrichment methods. For example, the dye used in the SP sorting method has some toxic effects, which may cause the screened cells to lose their ability to resist staining. Not all CSCs express the same marker, because of the existence of individual differences. Therefore, when using the specific marker screening method, multiple markers are often used in combination to improve the possibility of screening CSCs (Medema, 2013). The suspension cell culture method can easily cause cells to adhere to each other, making it difficult to obtain CSC single cell clones. In addition, the longer culture period can produce abnormally differentiated, unrelated cells (Valent, 2012) or limit the expression of CSCs, which is not conducive to subsequent studies. Therefore, the combination of multiple enrichment methods may improve the enrichment efficiency and accuracy of CSC purification. However, according to the current enrichment methods, which combination of methods has the best enrichment effect and the highest sorting efficiency should be confirmed by further research.

Conclusion and prospects

CSCs are potential therapeutic targets in a broad range of malignancies and provide a new indication and direction for the treatment of malignant tumors. The biggest distinction between tumor stem cells and

differentiated tumor cells is that the former are more radiotherapy and chemotherapy resistant. This means that although the majority of tumor cells can be eliminated by radiotherapy and chemotherapy, the key tumorigenic cancer stem cells survive, allowing them to induce tumor recurrence and metastases. Therefore, a full and clear understanding of the distinct mechanism of tumor stem cells and their regulatory pathways is required to develop treatments for cancer. This article discussed the origin of cancer stem cells, summarized the identification methods of tumor stem cells, and discussed the main regulatory pathways. However, research on CSCs is in its infancy, and many problems remain to be solved. We still have a long way to go before we have a full understanding of CSCs. For example, only a few cell surface markers of specific CSCs have been identified and validated; however, more CSC-specific markers are currently under investigation. The specific molecular mechanisms of CSC radiotherapy and chemotherapy resistance require further study. The core mechanisms underlying the various signal transduction pathways and regulatory pathways of CSCs remain unclear. Further research will resolve many of these issues, and CSCs will ultimately play a more important role in the targeted therapy of malignant tumors.

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Conflicts of interest. The authors have no conflicts of interest to declare.

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