



(REVIEW ARTICLE)



Cancer Stem Cells (CSCs) and its various biomarkers for treatment of human cancers: Review article

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Abstract

Traditional cancer treatments often fail to target specific cells, leading to therapy resistance and recurrence. Stem cell biology offers new potential for cancer treatment, including self-renewal, migration, differentiation, and modulatory effects. This review discusses stem cell use in cancer treatment, clinical applications, risks, and future directions for improving cancer outcomes. The kind of treatment chosen is determined by the kind, stage, and goal of the cancer. The first line of treatment for the direct removal of solid tumors that are localized is surgery. Radiotherapy damages the DNA of cancer cells, which can kill tumors. Chemotherapy helps slow down or stop tumour growth by using extremely toxic drugs. Many stem cell-based approaches are currently being studied in preclinical trials, and they present both exciting opportunities and difficult challenges for the treatment of cancer. Epigenetic mutations in normal stem cells or in precursor/progenitor cells give rise to CSCs, also known as stem-like cells, immature progenitors of tumor cells, or tumor-initiating cells. Tumor tissues contain CSCs, which are crucial for the development, spread, and recurrence of cancer. Thus, targeting CSCs may hold the key to treating different kinds of solid tumors. This review discusses the biological roles of stem cells and their diverse applications in cancer treatment. HSC transplantation is effective for hematologic cancers like leukaemia, multiple myeloma, and lymphomas. Immunomodulatory MSCs have shown high effectiveness in reducing GVHD cases and repairing injured tissues. MSCs and NSCs have also been investigated for tumour-tropic properties.

Keywords: Stem cell therapy; Cancer Stem Cells (CSCs); Cancer Treatment; Biomarkers; Mechanisms

1. Introduction

Cancer stem cells (CSCs) have been viewed as promising therapeutic targets for cancer therapy ever since they were first discovered in leukemia in 1994. These cells can differentiate and self-renew, and they are involved in a variety of tumour malignancies, including those that are resistant to radiation, drugs, recurrence, and heterogeneity. Many pluripotent transcription factors, including OCT4, Sox2, Nanog, KLF4, and MYC, control the biological activities of CSCs. Furthermore, it has been demonstrated that a variety of extracellular factors, including vascular niches, hypoxia, tumor-associated macrophages, cancer-associated fibroblasts, cancer-associated mesenchymal stem cells, extracellular matrix, and exosomes, as well as numerous intracellular signaling pathways, including Wnt, NF- κ B (nuclear factor- κ B), Notch, Hedgehog, JAK-STAT (Janus kinase/signal transducers and activators of transcription), PI3K/AKT/mTOR (phosphoinositide 3-kinase/AKT/mammalian target of rapamycin), TGF (transforming growth factor)/SMAD, and PPAR (peroxisome proliferator-activated receptor), as well as extracellular factors, have been demonstrated to be highly significant regulators of CSCs. Chronologic diseases like cancer pose a serious threat to human life. Numerous approaches have been devised for the management of cancer, such as surgery, radiotherapy, chemotherapy, and targeted therapy. Due to illness of these treatments, the incidence rate of cancer has decreased slightly in men and remained stable in women over the past ten years (2006–2015), and the rate of cancer deaths has decreased as well

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(2007–2016), [1]. However, only some malignant tumors respond well to conventional cancer treatment techniques [2]. Metastasis, recurrence, heterogeneity, resistance to radiation and chemotherapy, and avoidance of immunological surveillance are the primary causes of treatment failure for cancer [3]. The features of cancer stem cells (CSCs) may account for all of these failures [4]. CSCs have the capacity to arrest in the G0 phase and give rise to new tumors, which can result in cancer relapse, metastasis, multidrug resistance, and radiation resistance. [5]. As a result, CSCs may be the most promising targets in the fight against cancer.

2. The concept of cancer stem cells (CSCs)

The biological properties of CSCs Recent years have seen a major advancement in cancer treatment and clinical diagnosis due to the growing body of research on tumour biology. The high death and recurrence rates, however, are still unanswered and are intimately linked to the biological traits of CSCs. Recent advances in CSC characteristics have ushered in a new era of tumour biology research. For this reason, knowing the biological characteristics of CSCs is crucial for both tumour diagnosis and treatment. Because of their potent capacity for self-renewal, CSCs are directly responsible for carcinogenesis [6]. One CSC and one daughter cell or two CSCs can split symmetrically from CSCs [7]. The symmetrical splitting of CSCs causes an excessive increase in cell growth, which in turn causes the formation of tumors [8]. After being transplanted into mice with severe combined immunodeficiency disease (SCID), CSCs isolated from the original tumour tissue went on to form new tumors [9]. Furthermore, there are regulatory signaling pathways that CSCs and normal stem cells share, such as the Wnt/ β -catenin [10]. Sonic Hedgehog (Hh) [11], and Notch pathways play a role in the process of self-renewal. Furthermore, PTEN and the polycomb family of signaling molecules are significant players in the control of CSC proliferation [12]. The secret to comprehending carcinogenesis is to control the process of CSC self-renewal. These investigations will give cancer treatment a specific target [13]. To encourage normal stem cell proliferation and differentiation in a reasonably balanced manner, different signaling pathways typically control the self-renewal and differentiation of these cells. Uncontrollably proliferating stem cells eventually cause tumorigenesis once the regulatory balance is upset [14]. In order to control carcinogenesis, CSCs can also transdifferentiate into different multilineage cells [15]. Bussolati et al. [16], discovered that after injecting human renal CSCs into SCID mice, the majority of the tumors developed into vascular endothelial cells (ECs). Furthermore, CSCs that undergo angiogenesis-promoting differentiation into vascular ECs have been detected in glioblastoma [17] and liver cancer, among other cancers. [18]. The process by which cancer cells spread from their original location through blood, lymphatic, or internal body vessels is known as metastasis [19].

Due to the secretion of signaling molecules in the tumour microenvironment (TME) by stromal cells, including macrophages and granulocytes, these cells induce epithelial–mesenchymal transformation (EMT) to facilitate the invasion of tumour cells [20], which in turn causes differentiated human mammary epithelial cells to form mammary glands [21]. At this point, it is thought that CSCs are the primary "seeds" for tumour development, metastasis, and recurrence [22]. CSCs are extremely diverse and have developed over time [23]. Different surface biomarker expression patterns, including CD44+, CD24–, SP, and ALDH+, are seen in breast CSCs. In SCID mice, tumors can be formed by CD271– or CD271+ melanoma stem cells [24, 25, 26]. Forty Lung cancer, prostate cancer, glioblastoma, and other malignancies have all been linked to the heterogeneity of CSCs [27]. Because CSC heterogeneity is so complex, more potent biomarkers are required to either identify CSCs or discern between their heterogeneity [28].

3. Isolation and identification of CSCs

It is well known that CSCs make up a very small percentage of tumour tissues—typically only 0.01–2% of the entire mass of the tumour. Furthermore, there are similarities between normal stem cells and CSCs in terms of transcription factors and signalling pathways. As a result, it is more difficult to separate and identify CSCs. However, an increasing number of techniques and means have emerged. CSCs have been identified through different biomarkers in human cancers (Table 1).

Table 1 Various bio markers of Cancer stem cells in human cancers

Cancers	Markers	Function
BREAST	CD29+(Shackleton et al.2006), CD49f+(Pec.,et al.2010) CD90+(Lu et al., 2014) CD133+(Liu et al., 2013)	ALDH:An enzyme that plays a role in cell resistance.(Moreb, Schweder, Gray, Zucali, & Zori, 1998) CD44:A glycoprotein involves in cell migration and self-renewal. (Azevedo et al.,2018)

	ALDH+(Ricardo et al., 2011) ESA+/CD44+/CD24,CD44+/CD24 (Fillmore & Kuperwasser, 2008)	CD90:A glycoprotein participates in T cell adhesion and signal transduction. (Kumar, Bhanja, Bhattacharyya, & Jaganathan, 2016) CD133:A transmembrane glycoprotein that maintains lipid composition in cell membranes. (Yin et al., 1997) CD24:A marker that promotes blood flow in the tumor during metastasis.(Baumann et al., 2005) CD49f:A membrane proteins of the integrin family that plays an important role in cell surface adhesion and signaling.(Fan et al., 2019)
PROSTATE	EpCAM+(Deng et al., 2015) CD117+(Kerr et al., 2015) α 2 β 1+ALDH+ CD44+(Patrawala et al.,2006) EZH2+(Ugolkov, Eisengart, Luan, & Yang, 2010) CXCR4+(Darash-Yahana et al., 2004) E-cadherin+(Bae, Parker, Vieweg, & Siemann, 2010) CD133+(Richardson et al.,2004)	α 2 β 1:A receptor involves in cell adhesion and recognition E-cadherin: It plays an important role in tumor migration and invasion. CXCR4:CXC chemokine receptor works with CD4 protein to support HIV entry into cells. EZH2:A member of the Polycomb family plays an vital role in the central nervous system.
LUNG	CD166+(Tachezy et al., 2014) CD90+ (Yan et al., 2013) CD87+(Gutova et al., 2007) ALDH+(Jiang et al., 2009) CD44+ CD133	CD87:A receptor for urokinase plasminogen activator that affects many normal and pathological processes associates with cell surface plasminogen activation and local degradation of extra cellular matrices.
BRAIN	CD49f+ CD90+ CD44+ CD36+(Hale et al., 2014) EGFR+(Mazzoleni et al., 2010) A2B5+ L1CAM+ CD133+	CD36:The main glycoprotein on the surface of platelet has an important function as an adhesion molecule. EGFR: It binds to epidermal growth factor and promote proliferative migration in tumor. A2B5:A ganglioside marker that identifies sub populations of nerve cells in the central nervous system. L1CAM:A adhesion molecule that plays an important role in the development of the nervous system include neuronal migration and differentiation.
STOMACH	ALDH+ CD44+ CD44V8–10+ CD133+(Zhu et al. 2014) CD24+ CD54+ CD90+(Xue et al., 2012) CD49f+ CD71+ 9EpCAM+	CD44V8–10:A variant of CD44 with a specific class of CSCs. CD54:A class of adhesion molecules express in malignant tumour cells.

Cell surface-based indicators that are particular to CSCs can be combined to facilitate their separation.³ The two main methods of separation are magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) [29-

30]. Since Dick JE employed FACS technology to separate CSCs from leukaemia in the first place, FACS has grown to be the most popular method for cell separation. It has good specificity and high purity, and it can sort many biomarkers at once. Since Dick JE employed FACS technology to separate CSCs from leukaemia in the first place, FACS has grown to be the most popular method for cell separation. It has good specificity and high purity, and it can sort many biomarkers at once. A MACS technique is MACS. Although the MACS separation method is laborious, it is rather straightforward. As such, this approach necessitates considerable CSC activity. It is possible to isolate CSCs from a large number of cells using these two efficient approaches. Immunotherapy guided by CSCs Paul Ehrlich first put forth the theory that a healthy immune system inhibits the growth of tumours at the beginning of the 20th century (advancing cancer therapy with current and Emerging ImmunoOncology Approaches). On the basis of our growing knowledge of cellular immune modulation, novel approaches to cancer treatment have been developed. Apart from the antibodies directed against the aforementioned CSC molecules, several innovative anti-CSC immunotherapeutic techniques have been created, including immunologic checkpoint blocking and CAR-T cell treatments. Clinical trials have also been conducted on a number of medications that target the immune checkpoint receptors CTLA-4, PD-1 (nivolumab, pembrolizumab, and cemiplimab) and PD-L1 (avelumab, durvalumab, and atezolizumab).

4. Major transcription factors in CSCs

Stem cells typically share two properties: the capacity for self-renewal and the ability to differentiate into one or more specialised cell types. Transient ectopic overexpression of the transcription factors Oct4, Sox2, Nanog, KLF4, and MYC can reprogramme somatic cells to become induced pluripotent stem cells. Additionally, there are some similarities between CSCs and ES cells. It is reasonable that some transcription factors from embryonic sources can be reactivated or e-expressed in CSCs. Oct4 has been identified as a master regulator that governs pluripotency, self-renewal, and stem cell maintenance. Studies have shown that Oct4 is highly expressed in CSCs. High Oct4 expression is positively correlated with gliomas. and promotes Oct4 self-renewal, chemoresistance, and tumorigenicity of HCC stem cells. High Oct4 expression is also observed in breast CSC-like cells (CD44+/CD24-). Cisplatin, etoposide, adriamycin, and paclitaxel-taxely-irradiation increase Oct4 expression in lung cancer cells.

5. Major signaling pathways in CSCs:

principal routes of signalling in CSCs In cancer or CSCs, a number of signalling pathways that support the survival, proliferation, self-renewal, and differentiation characteristics of normal stem cells are aberrantly activated or inhibited. Numerous microRNAs and endogenous or foreign genes control these intricate processes. In CSCs, these signalling pathways can also cause the expression of downstream genes, including those related to cytokines, growth factors, apoptosis, antiapoptotic, proliferation, and metastasis. In order to control CSC growth, these signalling pathways are made up of intricate networks of signalling mediators rather than a single regulator. Consequently, the regulation of CSC growth by signalling pathways will be discussed in this section. Cancer stem cells possess the Wnt/ β -catenin pathway. The canonical Wnt/ β -catenin pathway controls CSC pluripotency and dictates how they will differentiate. When Wnt signalling is not present, β -catenin binds to the Axin complex, which is made up of APC and GSK3 β . When phosphorylated, it becomes ubiquitinated and is degraded by proteases via the β -Trcp pathway. The β -Trcp pathway is regulated by the complex (TAZ/YAP), the long noncoding RNA TIC1, and the proteins (TRAP1 and TIAM1). The interaction of LRP5/6 and Fzd, in the presence of Wnt signalling, suppresses the Axin complex's activity and phosphorylates β -catenin, causing it to enter the nucleus and bind to TEF/TCF to create a complex that recruits cofactors to start downstream gene expression. Research has verified that human malignancies, including but not limited to breast, lung, bladder, pancreatic, chondrosarcoma, rhabdomyosarcoma, neuroblastoma, medulloblastoma, and gastric cancer, exhibit aberrant activation of the Hh signalling pathway. Hh signalling activity varies, nevertheless, depending on the type of tumour. Germline deletion of the PTCH1 gene is linked to the autosomal dominant disorder Gorlin syndrome (basal cell nevus syndrome). This syndrome is highly prevalent in medulloblastoma, rhabdomyosarcoma, and basal cell cancer.

6. Signaling pathways regulating CSCs

An complicated signalling network regulates the homeostasis of normal stem cells; abnormal activation or inhibition of this network promotes neoplastic transformation. These abnormalities provide CSCs the ability to self-renew and differentiate, which gives malignancies their stemness. CSCs depend on these signalling pathways for both survival and the preservation of their stemness, just like their regular counterparts.

7. Intrinsic signaling pathway in CSCs

7.1. WNT signaling

Being a highly conserved route, the Wnt pathway has long been known to play a crucial role in regulating tissue homeostasis and embryonic development [31,32]. Abnormal activation of Wnt signalling components, such as Axin, β -catenin, Wnt1, and adenomatous polyposis coli (APC), is often detected in a variety of malignancies and is associated with the development and progression of cancer [33,34]. The non-canonical Wnt route is independent of β -catenin, whereas the canonical Wnt pathway depends on it. The canonical Wnt signalling appears to have a significant role in the maintenance of tumour cells' stem cell-like characteristics in the setting of CSC regulation [35]. A bad prognosis is indicated by the active Wnt/ β -catenin pathway, which is enriched in almost half of breast cancer cases. In breast CSCs, the expression of the activated β -catenin protein is also elevated. When β -catenin signalling was inhibited, tumour growth and metastasis in HER2-overexpressing breast cancer cells were drastically reduced.

7.2. NOTCH signaling

A genetically conserved route called notch signalling plays a crucial role in the development of the heart, brain, and several other organs throughout the embryonic stage. Another crucial factor in the development and spread of cancer is the notch signal. [36,37]. There have been reports of five membrane-bound cell surface ligands (JAG 1 and 2, DLL 1, 3, and 4) and four transmembrane receptors (Notch 1–4) in mammalian cells. The Notch intracellular domain (NICD), the active portion of Notch receptors, is released by proteolysis after the ligand-receptor contact [38]. After NICD's nuclear translocation, the NICD-CSL complex was created. This, in turn, attracted coactivators MAML and p300, which in turn activated the downstream genes of Notch signals Hes-1, c-Myc, HER2, NF- κ B, cyclin-D1, and p21 [39].

7.3. HH signaling

The 1980 Nobel Prize winning team identified the HH signalling pathway [40]. Throughout embryogenesis, the HH pathway is essential for the development of several organs [41]. It's interesting to note that, aside from the central nervous system (CNS), skin, hair, and teeth, all postnatal tissues still lack active HH signalling [42]. The three secreted ligand isoforms that make up the HH pathway are Sonic Hedgehog (Shh), Desert Hedgehog, and Indian Hedgehog. The receptors for these isoforms include Smoothed (SMO), Patched, and three Gli transcription factors (Gli1-3) [43].

7.4. TGF β /SMAD signaling

TGF- β is a dual-purpose cancer regulator that functions as a differentiation signal that may prevent tumour initiation in its early stages [44-46]. By activating EMT, TGF- β promotes the CSC-like phenotypes of cancer cells, as opposed to tumour start [47]. When TGF- β binds to TGF- β type I receptor kinase (ALK5), the canonical TGF- β pathway that is dependent on Smad is initiated. [48]. TGF- β -ALK5 phosphorylates Smad2/3, which then combines with Smad4 to form a complex that controls transcription of genes after nucleus translocation [49]. For cancer migration and breast CSC proliferation, there must be an interaction between TGF- β and the bioactive lipid mediator sphingosine-1-phosphate, a regulator of CSC development. [50].

7.5. NF- κ B signaling

The NF- κ B protein family consists of the following five members: c-Rel, p65 (RelA), RelB, NF- κ B1 (p105/p50), and NF- κ B2 (p100/p52) [51]. Both differentiated cells and stem cells contain NF- κ B family proteins in their cytoplasm. When NF- κ B is in an inactive state, its nuclear localization is prevented by its binding to inhibitory I κ B proteins [52]. I κ B proteins are degraded as a result of the I κ B kinase (IKK) complex (IKK α , IKK β , and IKK γ) phosphorylating them in response to different stimuli, like lipopolysaccharide [53]. Upon entering the nucleus, NF- κ B initiates the transcription of target genes that are implicated in several biological processes.

8. CSC-directed immunotherapy

Paul Ehrlich first put forth the theory that a healthy immune system inhibits the growth of tumours at the beginning of the 20th century (advancing cancer therapy with current and Emerging ImmunoOncology Approaches). On the basis of our growing knowledge of cellular immune modulation, novel approaches to cancer treatment have been developed. Apart from the antibodies directed against the aforementioned CSC molecules, several innovative anti-CSC immunotherapeutic techniques have been created, including immunologic checkpoint blocking and CAR-T cell treatments. Clinical trials have also been conducted on a number of medications that target the immune checkpoint receptors CTLA-4, PD-1 (nivolumab, pembrolizumab, and cemiplimab), and PD-L1 (avelumab, durvalumab, and

atezolizumab). The FDA has approved ipilimumab, a CTLA-4 antibody, and preliminary clinical data indicate that it is well tolerated by patients with metastatic melanoma.

8.1. CSCs and cancer therapy resistance

When CSC is injected into an animal with compromised immune function, it can cause a tumour. The majority of CSCs are thought to be resistant to radiation and/or chemotherapy, which highlights the crucial roles CSCs play in metastasis and cancer recurrence. Relapses in cancer may be caused by tumorigenic breast cancer cells that express high levels of CD44 and low or undetectable levels of CD24. These cells may be resistant to chemotherapy. Before and after normal dosage chemotherapy (docetaxel, doxorubicin, cyclophosphamide, and trastuzumab), Li et al. investigated the percentage of cell subpopulations in breast cancer tumour samples (Li et al., 2008). After 12 weeks of treatment, the percentage of CD44+CD24-/low cells rose from a mean of 4.7% at baseline to 13.6%, while the number of epithelial cancer cells remained relatively unchanged. Human ovarian CSCs are resistant to chemotherapeutics like doxorubicin, according to a recent study by Meirelles K et al. (Meirelles et al., 2012). These investigations offer clinical proof of a subset of CSCs resistant to treatment. Radiation resistance of breast CSCs was evaluated using early passage, patient-derived xenografts from two different patients, according to a recent article (Zielske et al., 2011), which revealed a contentious outcome from two patients on CSC resistance to therapeutic treatment. Two weeks after receiving radiation therapy, the CD44+CD24-/low Lineage- and ALDH+ breast CSCs from one patient were rapidly depleted, along with a reduction in tumour sphere frequency and tumorigenic capacity. The CSCs from the other patient, on the other hand, showed resistance to treatment and enrichment following radiation (Zielske et al., 2011). These results might be representative of patient-specific CSC variation. Future therapeutic applications of CSCs will need to take patient differences into consideration.

9. Conclusions and future perspectives

We can draw the conclusion that CSCs are a tiny subset of cancer cells with the ability to differentiate and self-renew, which gives them the ability to cause tumour recurrence, metastasis, multidrug resistance, heterogeneity, and radiation resistance. Crucial regulators of CSCs include a number of pluripotent transcription factors, such as Oct4, Sox2, Nanog, KLF4, and MYC; some intracellular signalling pathways, such as Wnt, NF- κ B, Notch, Hh, JAK-STAT, PI3K/AKT/mTOR, TGF/Smad, and PPAR; and extracellular factors, such as vascular niches, hypoxia, TAM, CAF, cancer-associated MSCs, the ECM, and exosomes. To target CSCs, many therapies have been developed, including CAR-T cells, antibodies, vaccines, and drugs that target these pathways. Crucially, a great deal of clinical research has also been done with CSCs, indicating a bright future for cancer treatment. Nevertheless, there are a number of obstacles that must be overcome in order to successfully eradicate CSCs. First off, it's unclear exactly what traits many CSCs in particular tumour types have. Second, these models do not accurately represent the biological complexity of tumours in the clinic because the majority of studies on CSCs are conducted in mice lacking an adaptive immune system. Third, the existence of CSCs in a particular niche allows them to survive. Nonetheless, the majority of recent research that don't include a microenvironment uses isolated CSCs. Fourth, little research has been done on the interaction between TAMs/CAFs and CSCs, and the environmental variables in CSC niches are poorly known. Fifth, not all of the regulatory elements that support CSCs are suitable to use as therapeutic targets in the treatment of cancer, as CSCs and normal stem cells share some signalling pathways. Sixth, there is disagreement over whether CSCs should be stopped or activated during cancer treatment. Seventh, because they also contribute to the stemness of CSCs, novel signalling and more regulatory levels, including RNA editing, epigenetics, and cellular metabolism, should be taken into account in cancer therapy. Eighth, since certain CSC signalling inhibitors are not highly selective, it is necessary to develop novel inhibitors. Ninth, natural products that target CSCs should also be researched in the future. Lastly, new approaches to targeting the CSC microenvironment are also worth investigating since they show promise.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Statement of ethical approval

Relevant Articles reviewed from several renowned and Authentic resources and independently included in this article for provide theoretical approaches regarding study conducted.

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