*The Pharmaceutical and Chemical Journal*, 2022, 9(3):90-99

*Available online www.tpcj.org*

**Review Article**

**ISSN: 2349-7092**
**CODEN(USA): PCJHBA**

# A Review on Cancer Stem Cell Signaling Pathways

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**Abstract** CSCs have been regarded prospective therapeutic targets for cancer therapy since they were initially discovered in leukaemia in 1994. These cells have the ability to self-renew and differentiate, and they contribute to a variety of tumour malignancies, including recurrence, metastasis, heterogeneity, multidrug resistance, and radiation resistance. Several pluripotent transcription factors, including OCT4, Sox2, Nanog, KLF4, and MYC, regulate the biological activities of CSCs. Furthermore, many intracellular signalling pathways, including Wnt, NF-B (nuclear factor-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/SMAD, and PPAR (peroxisome) prolife. To specifically target CSCs, molecules, vaccines, antibodies, and CAR-T (chimeric antigen receptor T cell) cells have been developed, and some of these factors are already in clinical trials. This review summarises CSC characterization and identification, depicts major factors and pathways that regulate CSC development, and discusses potential CSC targeted therapy.

**Keywords** medulloblastoma, mouse models, stem cells, PI3K pathway

## Introduction

Cancers are chronic diseases that pose a serious threat to human life. Many cancer treatment strategies have been developed, including surgery, radiotherapy, chemotherapy, and targeted therapy. All of these treatments have resulted in a stable incidence rate of cancer in women and a slight decrease in men over the last decade (2006–2015), as has the cancer death rate (2007–2016). Traditional cancer treatment methods, on the other hand, are only effective for a subset of malignant tumours. Metastasis, recurrence, heterogeneity, resistance to chemotherapy and radiotherapy, and avoidance of immunological surveillance are the primary causes of cancer treatment failure. All of these failures could be explained by cancer stem cell characteristics (CSCs). Through their ability to arrest in the G0 phase and give rise to new tumours, CSCs can cause cancer relapse, metastasis, multidrug resistance, and radiation resistance. As a result, CSCs may be considered the most promising cancer treatment targets. In the 1990s, CSCs were discovered in leukaemia and isolated using CD34+ and CD38 surface marker expression. CSCs expressing various surface markers, such as CD133, nestin, and CD44, have since been discovered in many nonsolid and solid tumours, and these cells also constitute the majority of the tumour [1-5].

**The concept of CSC**

CSC biological characteristics Clinical diagnosis and cancer treatment have improved significantly in recent years as tumour biology research has progressed. The high recurrence and mortality rates, however, remain unresolved and are closely related to the biological characteristics of CSCs. CSCs isolated from original tumour tissue and transplanted into mice with severe combined immunodeficiency (SCID) formed new tumours. 1 Understanding tumorigenesis is dependent on the regulation of CSC self-renewal. These studies will identify a specific target for cancer treatment [6-8].

**Isolation and identification of CSCs**

It is well known that the proportion of CSCs in tumour tissues is very low, accounting for only 0.01–2% of the total tumour mass. Furthermore, CSCs and normal stem cells share transcription factors and signalling pathways. As a result, isolating and identifying CSCs is more difficult. However, an increasing number of methods and techniques have emerged [9,10].

**Factors Regulating CSCs**

Normal stem cells, directed group progenitor cells, mature cells, and the fusion of stem cells and other mutant cells can all give rise to CSCs. 68 As a result, transformed CSCs derived from normal cells necessitate multiple gene Targeting cancer stem cell pathways for cancer therapy [11].

**Major transcription factors in CSCs**

Stem cells share at least two characteristics in common: the ability to self-renew 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. Furthermore, CSCs and ES cells share some characteristics. Some embryonic transcription factors may be re-expressed or reactivated in CSCs, which makes sense. As a result, these transcription factors play a critical role in the regulation of CSC growth [12-14].

Oct4, a Pit-Oct-Unc family homeodomain transcription factor, is regarded as one of the most important transcription factors. Oct4 has recently emerged as a master regulator of pluripotency, self-renewal, and stem cell maintenance. According to some studies, Oct4 is highly expressed in CSCs. High Oct4 expression correlates with glioma grade and promotes self-renewal, chemoresistance, and tumoricity of HCC stem cells. Oct4 expression is also high in breast CSC-like cells (CD44+/CD24). Cisplatin, etoposide, adriamycin, and paclitaxel -irradiation increase Oct4 expression in lung cancer cells, and CD133+ cells are more drug resistant than CD133 cells. Oct4 expression is also linked to a poor clinical outcome in hormone receptor-positive breast cancer. Oct4 knockdown also reduces the stemness of germ cell tumours. As a result, these studies have demonstrated that Oct4 is a pluripotent factor in CSCs. Sox2 is a transcription factor in the high-mobility group that plays an important role in the early development and maintenance of undifferentiated ESCs. It is also one of the most important transcription factors in CSCs. Rodriguez-Pinilla et al. discovered that increased Sox2 expression in basal-like breast cancer may aid in the identification of poorly differentiated/stem cell phenotypes. Hagerstrand et al. discovered that high Sox2 levels can cause xenograft glioma [15,16].

**Major signaling pathways in CSCs**

Many signalling pathways that contribute to normal stem cell survival, proliferation, self-renewal, and differentiation are abnormally activated or repressed in tumorigenesis or CSCs. Many endogenous and exogenous genes, as well as microRNAs, regulate these intricate pathways [17-19].

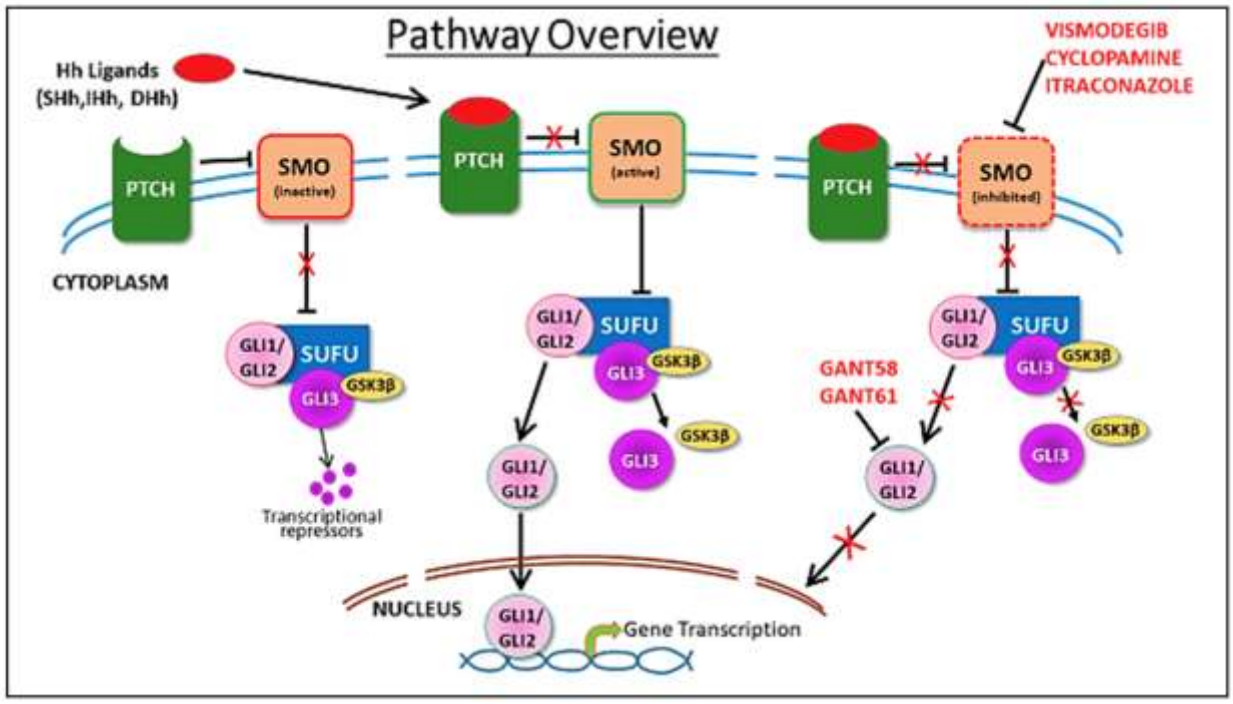
**Wnt signaling pathway in CSCs.**

Wnts are large protein ligands that influence a variety of processes, including cell polarity and cell fate. The Wnt pathway is extremely complex and evolutionary conserved, with 19 Wnt ligands and over 15 receptors. The Wnt

signalling pathway is divided into two parts: canonical Wnt signalling (via the FZD-LRP5/6 receptor complex, which leads to -catenin derepression) and noncanonical Wnt signalling (via FZD receptors and/or ROR1/ROR2/RYK coreceptors, which activate PCP, RTK, or $Ca^{2+}$ signalling cascades) [20,21].

**Hh signaling pathway in CSCs.**

Ligands and receptors are components of the Hh signalling pathway. Extracellular Hh ligands, the transmembrane protein receptor PTCH, the transmembrane protein SMO, intermediate transduction molecules, and the downstream molecule GLI comprise the Hh signalling network. The components of the Hh signalling pathway each play a unique role [22]. The membrane protein SMO regulates positively, while the transmembrane protein PTCH regulates negatively. PTCH is divided into two subtypes, PTCH1 and PTCH2, with 73% homology between them. In vertebrates, GLI, an effector protein, has three subtypes: Gli1, Gli2, and Gli3, and these effector proteins have different functions. Gli1 strongly activates transcription, whereas Gli3 inhibits it. Hh signalling serves different purposes in different types of cancer [23-26].

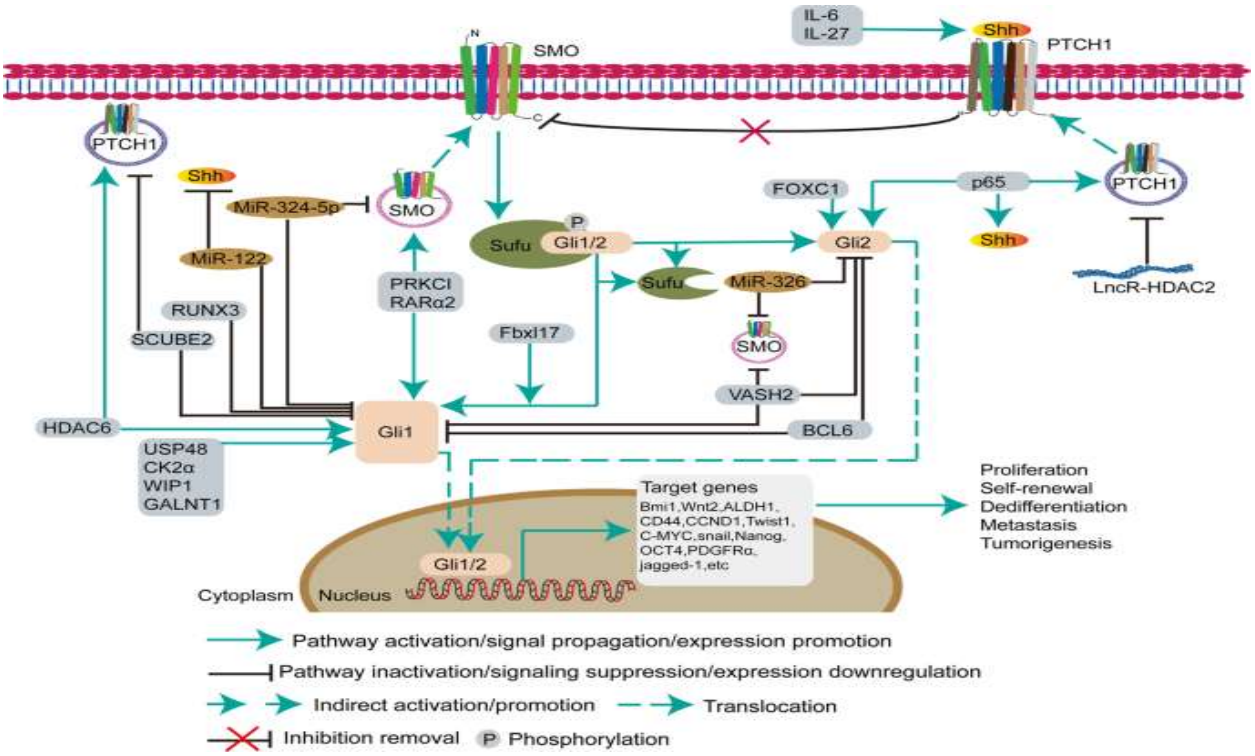



**Hedgehog signaling pathway in cancer stem cells**

Ligands and receptors are components of the Hh signalling pathway. The Hh signalling network is extremely complex, with extracellular Hh ligands, the transmembrane protein receptor PTCH, and the transmembrane protein PTCH. The Hedgehog pathway is essential for CSC stem maintenance, self-renewal, and regeneration. The secreted Hh protein initiates a series of cell responses such as cell survival, proliferation, and differentiation in a concentration- and time-dependent manner [27-29].

CSCs have an NF-B signalling pathway. Nuclear factor-B (NF-B), a rapidly inducible transcription factor, is made up of five proteins (p65, RelB, c-Rel, NF-B1, and NF-B2). The p50-p65 dimer is the main physiological function of NF-B. The primary mode of NF-B regulation takes place at the subcellular level [30].

The NF-B pathway regulates immune and inflammatory responses. Furthermore, the NF-B pathway is involved in cellular survival, proliferation, and differentiation. The NF-B pathway regulates inflammation, self-renewal, and CSC maintenance and metastasis (Fig. 3). CD44+ cells promote ovarian CSC self-renewal, metastasis, and maintenance by upregulating the expression of RelA, RelB, and IKK and mediating nuclear activation of the p50/RelA (p50/p65) dimer. High levels of NIK activate the noncanonical NF-B pathway, which regulates the self-renewal and metastasis of breast CSCs [31-34].




*NF-κB signalling pathway in cancer stem cells NF-B proteins participate in transcription factor dimerization, regulate gene expression, and influence various CSC biological processes such as inflammation, stress responses, CSC growth, and development. The p50-p65 dimer is the main physiological function of NF-B. The active p50-p65 dimer is further activated by post-translational modification (phosphorylation, acetylation, or glycosylation) and transported into the nucleus, where it induces target gene expression in collaboration with other transcription factors.*

**TGF/SMAD signaling pathway in CSCs**

TGF signalling is involved in many cellular processes related to organism and embryo development, including cell proliferation, differentiation, apoptosis, and homeostasis. Despite the fact that the TGF signalling pathway regulates a wide variety of cellular processes, its structure is relatively simple [35]. BMPs, growth and differentiation factors (GDFs), anti-Mullerian hormone (AMH), activin Nodal, and TGF- are all TGF- superfamily ligands [36-38].

CSCs have a PI3K/AKT/mTOR signalling pathway. Phosphatidylinositol 3-kinase (PI3K) is a phosphatidylinositol kinase that is found within cells. It is made up of the regulatory subunit p85 and the catalytic subunit p110, both of which are serine/threonine (Ser/Thr) kinase and phosphatidylinositol kinase. AKT is a serine/threonine kinase with three isoforms: AKT1, AKT2, and AKT3 [39-42].

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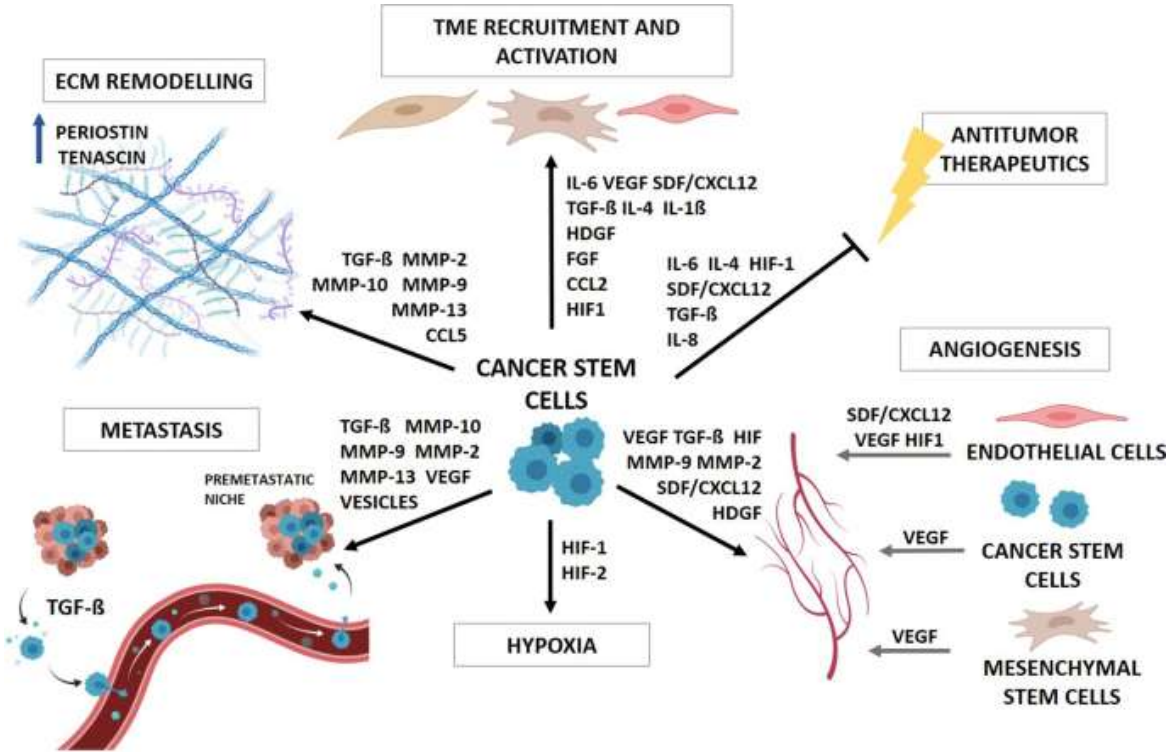
**Interactions between signaling pathways in CSCs**

As previously stated, these complex signal transduction pathways are not linear. Crosstalk between and among pathways to regulate CSCs occurs in some cases. Wnt/-catenin and NF-B signalling cooperate to promote CSC cell survival and proliferation. TNFRSF19, a member of the TNF receptor superfamily, is regulated by -catenin, but its receptor molecules activate NF-B signalling to regulate colorectal cancer development. 400 CD146 knockdown inhibits NFB/p65-initiated GSK3 expression, which promotes nuclear translocation and catenin activation [47,48].

Furthermore, Wnt/-catenin and NF-B signalling are negatively regulated. catenin has been shown in studies to inhibit NF-B activity in liver, breast, and colon cancer cells. Furthermore, crosstalk between pathways promotes cell growth and metastasis by maintaining [49]. Downregulation of Notch1 and IKKα enhances NF-κB activation to promote the CD133+ cell population in melanoma CSCs.409 IL-6/JAK/ STAT3 and TGF-β/Smad signaling induce the proliferation and metastasis of lung CSCs.

Image




*Cancer stem cells' microenvironment CSCs proliferate, self-renew, differentiate, metastasize, and tumorigenesis in the CSC microenvironment. Vascular niches, hypoxia, tumor-associated macrophages, cancer-associated fibroblasts, cancer-associated mesenchymal stem cells, and extracellular matrix are the main components of the CSC microenvironment. In response to hypoxic stress and matrix, these cells produce growth factors and cytokines (such as IL-6 and VEGF) that regulate CSC growth via Wnt, Notch, and other signalling pathways.*

**The microenvironment of CSCs**

CSCs communicate with their surroundings via adhesion molecules and paracrine factors. The microenvironment promotes CSC self-renewal and differentiation, protects CSCs from genotoxicity, and increases chemical and radiological tolerance. The tumour stroma, adjacent tissue cells, microvessels, immune cells, and immune molecules make up the majority of the TME. CSCs not only adapt to changes in the TME, but they also influence it. Concurrently, the microenvironment promotes CSC self-renewal, angiogenesis, immune and stromal cell recruitment, and tumour invasion and metastasis [50].

CSCs and vascular niche microenvironments ECs, basement membranes, and parietal cells make up the normal vasculature. ECs are the building blocks for the inner surface of blood vessels. The concept of the cancer microvascular environment was first proposed after studies revealed that glioblastoma stem cells are found near blood vessels. Calabrese et al. discovered direct contact between ECs and CSCs in brain tumours. CSCs have also been found near ECs in other cancers, including papilloma and colorectal cancer. According to one study, CD133+/CD144 glioma stem cell-like cells differentiate into cancer cells, endothelial progenitor cells, and then mature ECs. CSCs and the hypoxia microenvironment Hypoxia is an important factor in CSC formation and maintenance. The hypoxic microenvironment keeps cancer cells undifferentiated, increases their cloning rate, and

induces the expression of CD133, a CSC-specific biomarker. HIFs are transcription factors that control cellular hypoxia responsiveness and prevent cell apoptosis. HIF is a heterodimer composed of HIF and HIF. HIF-1 regulates CSC proliferation and fate in medulloblastoma and glioblastoma multiforme, as well as activating the NF-B pathway to promote CSC survival and tumorigenesis. HIF-2 maintains CSC survival and phenotype. HIF also controls the expression of target genes such as GLUT1, GLUT3, LDHA, and PDK1. Thus, CSCs can adapt to a new method of cell energy metabolism and avoid apoptosis caused by hypoxia. CSCs and tumor-associated macrophages Macrophages, a group of cells with plasticity and heterogeneity, are an important component of the innate immune response. Infiltrating and inflammatory macrophages develop from bone marrow mononuclear cells. In different microenvironments, these precursor cells infiltrate various tissues via blood vessels and differentiate into different subtypes. Macrophages are classified into two subtypes: M1 and M2. The M1 phenotype has anti-inflammatory and anti-tumor properties and secretes proinflammatory factors such as interleukin-1 (IL-1), IL-12, IL-23, TNF-, chemokine (C-X-C motif) ligand 5 (CXCL5), CXCL9, and CXCL10. M2 macrophages are a tumor-promoting cell type with immunosuppressive and angiogenesis-promoting properties. TAMs are closely related to CSCs, or stem cell transformation. Renal epithelial cells cocultured with macrophages induce EMT, transforming renal cancer cells into CSCs expressing CD117, Nanog, and CD133 [51,52].

**Cancer-associated MSCs and CSCs**

MSCs are capable of self-renewal and multidirectional differentiation. MSCs also migrate specifically to the injured site and tumour tissue and are simple to isolate and expand in vitro. MSCs are thought to be an ideal vector for gene therapy because of their ability to homing to and secrete cytokines in tumours. However, these tumorigenic properties of MSCs need to be investigated further. MSCs not only promote tumour development but also inhibit cancer cell growth. MSCs from bone marrow promote tumour growth by promoting angiogenesis, metastasis, and the survival of CSCs. MSCs in the TME promote the proliferation, carcinogenesis, and metastasis of breast CSCs via ionic purinergic signal transduction. MSCs can differentiate into CAFs, and CAFs regulate CSCs and promote cancer occurrence and metastasis. The potential mechanism involves the spontaneous fusion of cancer cells and MSCs. MSC fusion with breast cancer, ovarian cancer, gastric cancer, and lung cancer cells has been demonstrated *in vitro* and *in vivo* [53].

**Extracellular matrix and CSCs**

In mesenchymal and epithelial vessels, the ECM is an insoluble structural component of the matrix. Collagen, elastin, aminoglycan, proteoglycan, and noncollagen glycoprotein are all components of the ECM. At the moment, evidence suggests that the ECM is an essential component of stem cell niches, regulating the balance of stem cells in three different biological states: static, self-renewal, and differentiation.

Exosomes in TMEs and CSCs Exosomes are nanovesicles (30–100 nm in diameter) secreted by various types of living cells that are widely distributed in peripheral blood, saliva, urine, ascites, pleural effusion, breast milk, and other body fluids. Exosomes contain a large number of functional proteins, RNA, microRNAs, DNA fragments, and other bioactive substances. These bioactive substances mediate material transport and information exchange between cells, thereby influencing cell physiological function. Endocytosis of lipid rafts in MSCs has been linked to increased exosome secretion, according to recent research. Exosome signalling mediates the interaction of CSCs and normal stem cells, regulating oncogenesis and tumour development [54].

**Therapeutic Targeting of CSCs**

Agents targeting CSC-associated surface biomarkers in clinical trials Monoclonal antibodies (mAbs) that target CSC-specific surface biomarkers have become an emerging technology for cancer therapy. Rituximab, a CD20 mAb, is an effective treatment for follicular and mantle-cell lymphoma, but it is not without serious side effects. Following that, a phase II clinical trial for a radioiodine replacement of rituximab was conducted to improve the availability and affordability of radioimmunotherapy for refractory or recurrent non-lymphoma Hodgkin's (NHL), which demonstrated a response rate of 71 percent and a complete remission rate of 54 percent in 35 patients, with

only two cases of grade IV hematologic toxicity observed. Alemtuzumab, a humanised CD52 antibody, has been approved for the treatment of chronic lymphocytic leukaemia (CLL) in patients who have not responded to alkylating agents or purine. Furthermore, the combination of CD20 and CD52 antibodies was safe, nontoxic, feasible, and effective in the treatment of refractory CLL. Another antibody drug, renamed bivatuzumab, is an anti-CD44v6 mAb that was found to be safe when used to treat head and neck SCC. These findings were confirmed in subsequent clinical research and safety/efficacy studies. Unfortunately, one patient with head and neck SCC of the oesophagus died from skin toxicity during a stage I dose escalation study with the CD44v6 antibody. Several CD123 antibodies, including XmAb14045 and MGD006, have been developed with biospecific effects against CD3 and CD123. Talacotuzumab also works against CD16 and CD123. Another CD123 antibody, CSL360, was used to treat relapsed, refractory, or high-risk acute myeloid leukaemia (AML) but showed no anti-leukemic activity in the majority of cases. Another CD123 antibody, 556 SL-401, was used to treat patients with blastic plasmacytoid dendritic cell neoplasm. The findings revealed significant positive responses in seven of nine patients, with five complete responses and two partial responses. An ongoing phase II study of SL-401 in 29 patients with blastic plasmacytoid dendritic cell neoplasms found that it was effective as a single agent, with an 86 percent overall response rate. The most recent antibodies against CSC surface markers are in clinical trials, including XmAb14045 (NCT02730312), flotetuzumab (NCT02152956), and talacotuzumab (NCT02472145). IL-1 receptor accessory protein, CD27/70, CD33, CD38, CD138, CD93, CD99, C-type lectin-like molecule-1, and T cell immunoglobulin mucin-3 are also clinically developed markers that can distinguish LSCs from other cells. Clinical trials have also focused on EpCAM, a common CSC biomarker. Adecatumumab, an EpCAM antibody, was tested in patients with hormone-resistant prostate cancer, and the results demonstrated that the EpCAM-specific antibody has significant clinical potential. Catumaxomab, a multifunctional anti-EpCAM mAb, binds to and recognises EpCAM as well as the T cell antigen CD3 (anti-EpCAM anti-CD3). Catumaxomab intraperitoneal injection has shown high efficacy in killing cancer cells and reducing ascites formation in EpCAM-positive ovarian cancer and malignant ascites. Catumaxomab has been used to treat nonsmall-cell lung cancer and has a high success rate. Other EpCAM antibodies, such as edrecolomab and adecatumumab, have shown limited efficacy in colorectal and breast cancers. Combining EpCAM antibodies with chimeric antigen receptor T cell (CAR-T) technology has been used in phase I trials for a variety of cancers, including NCT02915445, NCT03563326, NCT02729493, and NCT02725125. With a better understanding of CSC surface biomarkers, significant progress has been made in developing antibodies that target CSCs. CSC surface phenotypes, however, can differ between patients or cancers, and different CSC populations with different phenotypes may coexist. CSCs can also diverge or evolve into different types of cancer cells, resulting in distinct phenotypes upon relapse. As a result, clinical trial strategies should be determined based on the phenotypes of the various cancers. Concurrently, combining various surface antibodies with relevant chemotherapy drugs can achieve an optimal therapeutic effect. Early clinical trials for Notch and Hh pathway inhibitors have been successful, but targeting the Wnt pathway has proven difficult. 10 The Notch signalling pathway is important in CSC maintenance and can induce CSC differentiation. Many cancers, including leukaemia, glioblastoma, breast cancer, lung cancer, ovarian cancer, pancreatic cancer, and colon cancer, have abnormal Notch signalling activity [55].

**Conclusions and Perspectives**

We can conclude that CSCs are a small population of cancer cells with self-renewal and differentiation potential, which confers tumour relapse, metastasis, heterogeneity, multidrug resistance, and radiation resistance. Several pluripotent transcription factors, such as Oct4, Sox2, Nanog, KLF4, and MYC, as well as intracellular signalling pathways such as Wnt, NF-B, Notch, Hh, JAK-STAT, PI3K/AKT/mTOR, TGF/Smad, and PPAR, as well as extracellular factors such as vascular niches, hypoxia, TAM, CAF, cancer-associated MSCs, the ECM. CSCs can be targeted with drugs, vaccines, antibodies, and CAR-T cells that target these pathways. Importantly, numerous clinical trials on CSCs have been conducted, indicating a promising future for cancer therapy. However, there are numerous obstacles that must be overcome in order to effectively eliminate CSCs. For starters, the characteristics of many CSCs in specific types of tumours are poorly understood. Second, because most CSC studies are conducted in

immune-deficient mice lacking an adaptive immune system, these models do not accurately represent the biological complexity of tumours in the clinic. Third, CSCs exist in a specific niche that sustains their survival. However, isolated CSCs are used in most current studies that lacks a microenvironment. Fourth, the environmental factors in CSC niches are not well understood, and the relationship between TAMs/CAFs and CSCs has not been well studied. Fifth, since CSCs also share some signaling pathways with normal stem cells, not all the regulatory factors that contribute to CSCs are appropriate for use as therapeutic targets in cancer treatment. Sixth, whether CSCs should be activated or arrested is an open question in cancer therapy. Seventh, novel signaling and more regulatory levels, such as RNA editing, epigenetics and cellular metabolism, should be considered in cancer therapy because they also contribute to the stemness of CSCs. Eighth, some inhibitors that target CSC signaling are not very specific, and so new inhibitors need to be designed. 656 Ninth, natural products that target CSCs should also be studied in the future. finally novel ways of targeting the microenvironment of CSCs are also promising and need to be explored.

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