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Review Article

Cancer stem cells: Review of current state and future directions

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Abstract
Cancer is a group of disorders characterized by the abnormal proliferation of cells. Cancer can infiltrate or spread to different areas of the body. Cancer stem cells (CSCs) are small groups of cancer cells that have the ability to self-renew and develop into tumors by different signaling pathways. CSCs play a significant role in the development of many types of cancer and are a significant factor in tumor metastasis and relapses. They are usually resistant to the existing treatments primarily focusing on most tumor cells. Recent technological advances have made it easier to study the basic role of CSCs in cancer biology and target them for potential therapeutic strategies. In this minireview, we give a brief overview of recent insights into the characteristics, mechanisms, current treatment opportunities, challenges, and future directions of cancer stem cells and their potential to revolutionize cancer treatment.

Key Words: Cancer stem cells, Cancer biology, Therapeutic potential.

Abbreviations
AML: acute myeloid leukemia; ATP: adenosine triphosphate; CD: cluster of differentiation; CSCs: cancer stem cells; CAFs: cancer-associated fibroblasts; CXCR: CX chemokine receptor type 4; DNMT1: DNA methyltransferase 1; ECM: extracellular matrix; EMT: epithelial-mesenchymal transition; FGF: fibroblast growth factor; HCC: hepatocellular carcinoma; MSCs: mesenchymal stem cells; MDSC: myeloid-derived suppressor cells; PDGF: platelet-derived growth factor; TAN: tumor-associated neutrophils; TAM: tumor-associated macrophages; VEGF: vascular endothelial growth factor.

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INTRODUCTION

Cancer is a group of disorders characterized by the abnormal proliferation of cells that have the ability to infiltrate or spread to different areas of the body. Although there have been advancements in cancer therapy, it continues to be a prevalent cause of mortality worldwide. The probability of treating cancers is most significant when they are detected in the early stages and treated with standard methods like chemotherapy, surgery or radiotherapy [1, 2]. However, a significant number of cancers are detected at an advanced stage, by which time they progressed and metastasized to other organs. Despite early diagnosis and treatment of cancers, residual cells persist and can eventually lead to metastasis and tumor recurrence and resulting in a more aggressive form of the disease. There is increasing evidence suggesting that residual cells identified at any stage of cancer development, known as cancer stem cells (CSCs), are responsible for therapy resistance due to their stem cell-like properties [3]. CSCs initially discovered in 1990, are a limited group of cancer cells that possess the ability to self-renew and sustain their stem stem-like properties, as well as exhibit resistance to drugs. The ability to self-renew and specialize into numerous cell types present in the tumor distinguishes them from the majority of other cancer cells [4]. The CSC model provides a conceptual framework for understanding the presence of diverse cell types within a tumor, as it enables the identification of different phenotypes and the maintenance of their population. Tumors lacking stem cells exhibit varying degrees of cellular differentiation, however; they exhibit a high rate of cell proliferation. Extensive research has been conducted on their potential to evade current cancer treatments, since concerns have arisen about their potential to develop into cancer and their suitability for use in therapy. They possess the ability to multiply and become more lethal as a result of their adaptation driven by natural selection to endure in harsh environments [5]. Numerous surface markers, such as CD44 and CD133, and signaling pathways, such as Wnt and notch pathways have significant roles in the regulation of cancer stem cells. Therapeutic treatments, including

tyrosine kinase inhibitors, monoclonal antibodies, chimeric antigen receptors (CAR) T cells, and tumor vaccines, have been developed to specifically target these surface markers and pathways of CSCs [2,4].

The intricate molecular components and pathways comprise a complex and dynamic regulatory network that regulates the diverse development and potential of CSCs in the tumor environment. The balance between the mechanisms of self-renewal and differentiation plays a crucial role in the proliferation and progression of tumors. A detailed understanding of this intricate interplay of molecules is necessary for the progress of targeted therapy, thereby reducing the probability of cancer reoccurrence. Current treatments are costly and mostly ineffective, therefore novel treatments to target CSCs are required. The expanding understanding of fundamental biology has the power to improve existing therapeutic methods in the near future [5]. We summarize the overview of characteristics, mechanisms, and current treatment opportunities of cancer stem cells. We also highlight the challenges and future directions of CSCs implications for revolutionizing cancer treatment.

Discovery of Cancer Stem Cells

The theory about the existence of cancer stem cells (CSC) fundamentally reshapes understanding of tumor biology. It has been proposed that tumors are organized in a hierarchical manner similar to normal tissues. In the tumor hierarchy, CSCs exit at the top followed by progenitor cells and differentiated cancer cells. These cells are supported by the surrounding tumor microenvironment that drives tumor growth, progression and resistance to therapy. CSCs perform dual functions i.e. proliferation and differentiation. The cancer stem cell theory gained strong evidence from the identification of CSCs in several types of cancer, including leukemia, breast cancer, and brain tumors. Notably, only a small fraction of cells within these tumors had the ability to multiply and become cancerous when transplanted into other organisms. Ernest McCulloch and James Till, two Canadian scientists from the University of Toronto, made groundbreaking discoveries in the 1960s that laid the foundation for the concept of cancer stem cells [6, 7]. They demonstrated the existence of stem cells in mouse bone marrow and proposed the idea that cancer might arise from these stem cells. Their work revolutionized the field of hematology and oncology and paved the way for later research on CSCs. In 1997, John and Bonnet made an important discovery in stem cell research. They identified cells with a high potential to multiply in acute myeloid leukemia (AML) and isolated a specific type of stem cell known as CSCs, which are characterized by the CD34+CD38− phenotype [4]. In 2001, this significant discovery recognized the presence of leukemia stem cells and laid the foundation for the concept of cancer stem cells. CSCs have the ability to self-renew and are responsible for driving the formation and growth of tumors, similar to normal stem cell function [8] but with a specific role in the advancement of cancer. The notion of CSCs has broadened to cover diverse solid tumors [9]. In 2003, Al-Hajj initially isolated CD24−CD44+/low CSCs from breast tumors, which demonstrated notable tumorigenicity in mice. Transplanting a small number of CSCs in breast cancer can result in the formation of a tumor, while transplanting a significantly larger number of normal cancer cells does not have the same effect. Similar findings were obtained from biopsies of human carcinomas from colon carcinomas, brain tumors, and head and neck malignancy. Researchers transplanted CSCs into mice with weak immune systems, and these cells grew into new tumors that were identical to the original tumors. CSCs have also been identified in pancreatic carcinoma, lung cancer, and malignant melanoma. Because of their ability to resist apoptosis, both chemotherapy and radiation are ineffective in destroying majority of the CSCs (Figure 1). Chemotherapy and radiation have the ability to decrease the growth of a tumor, but only the resilient cells can survive. This is why remission is frequently followed by a particularly aggressive recurrence. CSCs possess three key survival strategies that enable them to resist cancer treatments including; they can enter a dormant state, employ detoxification mechanisms to remove harmful substances, and activate protective pathways that prevent cell death. The prospective cancer treatments must specifically focus on tumor stem cells to successfully combat cancer [5].

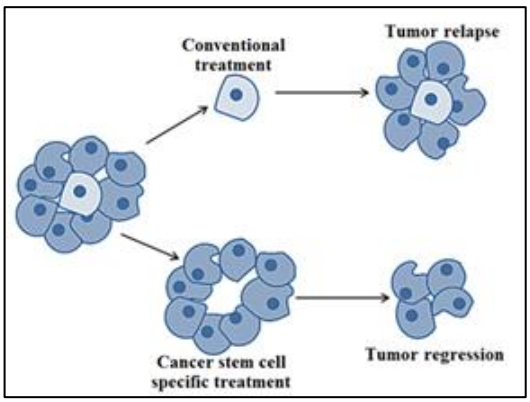

Figure 1: Cancer Therapies. Tumor-relapse after conventional cancer treatment (chemotherapy and radiation therapy) due to the presence of cancer stem cells. Treatments that target cancer-specific stem cells produce tumour regression.

Characteristics of Cancer Stem Cells

Cancer stem cells possess some unique features including self-renewal, differentiation, proliferation, metastasis, angiogenesis, and tumorigenesis. One of their characteristics is the ability to divide and renew them, making them capable of growing indefinitely. This property is often assessed using *in vitro* assays, such as limiting dilution assays or sphere formation assays, where CSCs can grow as non-adherent spheroids in suspension culture, indicating their stem cell-like properties. CSCs have been reported to have increased resistance to conventional treatments such as cytotoxic drugs and irradiation that are known to be effective in proliferating cells. This resistance occurs due to multiple factors that include the upregulation of such proteins as drug efflux pumps, which include the ATP binding cassette transporters, enhanced DNA repair capacity, and the ability to exit the cell cycle and become dormant in response to administered treatments. Additionally, CSCs are multipotent as they can differentiate into various specialized cell types that constitute the tumor, thus contributing to its heterogeneity. This differentiation potential is crucial for tumor adaptation and survival in response to environmental stress. Cell surface proteins including CD44 and CD133 have been used for the selective isolation of CSCs. However, differences in marker expression based on tissue type and tumor stage limit the general applicability of targeting cancer stem cells, making it essential to develop more specific and novel approaches for effective treatment [10].

Molecular Mechanisms of Cancer Stem Cells to Develop into Cancers

The CSC niche consists of several components that keep them in an inactive state. Niche also controls the adaptability and inactivity of the cells by activating multiple essential molecules, processes, and signaling pathways. The mechanisms and pathways in the CSC niche represent the comprehensive network of all component and cells interactions inside the niche.

Signaling Pathways Regulating the CSC Niche

The regulation of cancer stem cells may be complex, which encompasses multiple signaling processes and external microenvironments [11]. Some of the important signaling pathways that have been implicated in determination of CSC properties include Wnt/ β-catenin/TCF, Notch, and Hedgehog signaling pathways. These pathways are involved in self-renewal and tumorigenic properties of CSCs (Figure 2). For example, in the Wnt signaling pathway, a key feature of cancer is the excessive activity of specific proteins, such as Wnt ligands and receptors, which lead to the accumulation of β-catenin. This, in turn, causes β-catenin to move into the nucleus, where it triggers the expression of genes involved in cell growth, self-renewal, and proliferation. Another key pathway implicated in regulation of CSCs and their properties is the Notch signaling pathway, which is essential for the balance between self-renewal and the process of differentiation in CSCs. Overall, this pathway promotes the stemness of these cells. The Hedgehog pathway has been reported to play a role in maintaining self-renewal of CSCs in various cancers. It promotes tumorigenesis and metastasis of these cells. CSC function and activity is influenced by its surrounding stroma. The stroma includes stromal cells, immune cells and the extracellular matrix. CSCs are important in that they can also directly interact with neighboring cells, and this can improve cellular survival as well as promote angiogenesis and metastasis. For example, when macrophages stimulate the CSCs, tumor aggressiveness also increases. This dynamic interplay highlights that intrinsic/endogenous regulation accompanied with extrinsic signals that modulate CSC biology may provide new phenomenal insight to target and affect the function of cancer stem cells [12].

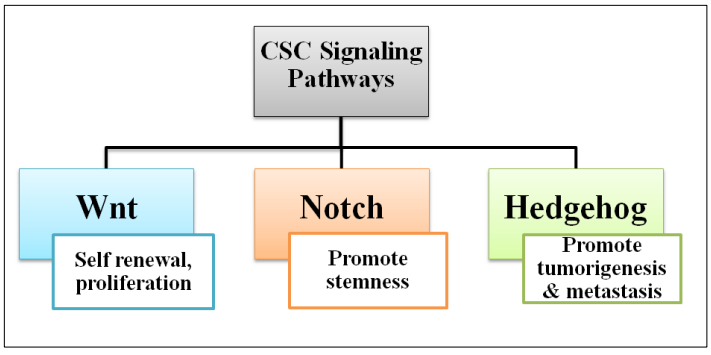

Figure 2: Signaling pathways in Cancer stem cells. Wnt signaling pathways regulate self-renewal and proliferation. Notch and Hedgehog signaling pathways promote stemness and tumorigenesis & metastasis, respectively.

Essential Molecules in the Niche

The maintenance of the balance in the CSC niche depends on the interplay among various components and cells of the microenvironment. Essential molecules that regulate the CSC niche are adhesion molecules, cytokines, and chemokines. Adhesion molecules are proteins on the surface of cells that facilitate cell-to-cell adhesion, binding, and interaction. Stem cell niches have a unique ability to self-renew and connect with other cells, which is crucial for their function. This adhesion is made possible by various molecular pathways and substances present in both stem cells and niche cells. E-cadherin is a well-known cell adhesion molecule that interacts with both intracellular and extracellular parts of a cell thereby modulating cellular adhesion. The interaction of this molecule enhances the binding to proteins associated with cytoskeleton and other cells. Some cadherins can control gene expression and signaling pathways when they bind to specific proteins or receptors on cancer stem cells [13]. For example, N-cadherin in breast cancer stem cells binds to FGFR, leading to activation of PI3K/AKT signaling, promoting stem cell self-renewal and tumor initiation.

Cytokines are small proteins released by cells that act as messengers to coordinate the immune response, inflammation, and cellular communication. They can stimulate or inhibit the activity of various immune cells, such as T cells, B cells, and macrophages. Cytokines are released into the tumor microenvironment by both CSCs and other neoplastic cell types. These cell types include tumor-associated macrophages (TAM), tumor-associated neutrophils (TAN), and myeloid-derived suppressor cells (MDSC) leading to an immunosuppressive environment within tumor stroma. Chemokines are a subgroup of cytokines that specifically direct the movement of immune cells to sites of infection, inflammation, or injury. They act as chemo-attractants, guiding cells to migrate towards the source of the chemokine. SDF-1a is one of the most important chemokines identified together with its receptor CXCR4. CXCR4 plays a vital role in retaining, sustaining and homing of tumor cells on hepatocellular carcinoma (HCC). It is important to note that this receptor is crucial for regulating renewal potential of CSCs. Research has reported that SDF-1 can be produced by mesenchymal stem cells (MSCs), and increased levels of SDF-1 have been associated with metastasis in solid cancers such as breast, liver, or lung cancer. Additional cells inside the niche area that are affected by CSCs are cancer-associated fibroblasts (CAFs). CAFs are a type of cell that plays important role in the development, growth, and progression of cancer CAFs are recognized for their ability to promote cell division, stimulate extracellular matrix (ECM) synthesis, and release critical factors like platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) [14, 15].

Inflammatory components like IL-6, IL-8, TGF-β, NFκ, and TNF-α create a tumor microenvironment that promotes the migration and invasion of cancer stem cells. Inflammatory cytokines upregulate the NFκB pathway in CSCs leading to regulation of gene expressions of epithelial-mesenchymal transition (EMT) factors such as Twist, Snail, and Slug factors or genes. These factors control EMT that is an important event in cancer progression, metastasis, and stemness. This highlights the importance of NFκB in migratory and invasive behaviors of CSCs in diverse human malignancies including pancreatic carcinoma, cutaneous melanoma and ovarian tumors. The inflammatory response in the niche is regulated by a complex network of molecules, some of which also play roles in other pathways and functions [16].

Epigenetic and Genetic Modulations in the CSC Niche

The CSC niche is also influenced by epigenetic modifications, such as DNA methylation and ubiquitination, which interact to regulate CSC behavior and maintenance. DNA methylation is a common epigenetic modification that plays a key role in reprogramming gene expression, with significant implications for CSC biology. For instance, studies have shown that BEX1, regulated by DNA methyltransferase 1 (DNMT1), exhibits varying expression levels in patients with hepatoblastoma, CSC-hepatocellular carcinoma (HCC), and non-CSC HCC. Additionally, activation of the Wnt/β-catenin signaling pathway is essential for maintaining and self-renewing liver CSCs. Furthermore, ubiquitination, a post-translational modification, is vital for CSC maintenance, self-renewal, differentiation, and carcinogenesis. Notably, a recent study demonstrated that targeting MYH9 can inhibit the ubiquitination of GSK3β, leading to the activation of the β-catenin destruction complex. This, in turn, inhibits cancer stemness and EMT in hepatocellular carcinoma, highlighting a potential therapeutic strategy [17].

The genetic diversity of cancer cells has sparked interest in understanding the genetic characteristics and functions of CSCs. Within CSC populations, molecular subgroups can influence their behavior, including their level of dormancy or activity, which in turn affects their ability to survive chemotherapy. The dynamic interaction between CSCs and their microenvironment suggests that changes in the genetic makeup of CSCs or their niches can determine their fate and interactions. This complex relationship allows CSCs to adapt and cycle through different states, enabling them to evade unfavorable changes and thrive in their environment [18].

Implications of CSCs: Opportunities and Challenges

Tailoring cancer therapies to target cancer stem cells can lead to a significant increase in their effectiveness and potentially lead to more durable responses. However, it also comes with substantial risks. The features of CSCs, including their resistance to conventional therapies, and inherent heterogeneity represent challenges in treatment. Present strategies involve using monoclonal antibodies, CAR T-cell therapy, and small-molecule inhibitors to target CSCs selectively. For example, monoclonal antibodies can be produced that selectively target and attach to CSC associated markers and activate the immune system for their elimination. Recently, in cell-based therapy, the treatment that has been made possible through CAR T-cell therapy has been attempted where a patient's T cells have been genetically altered to identify CSC specific antigens, and it has been found effective in the preclinical studies and early phase clinical trails. However, the emergence of resistance mechanisms and the ability of CSCs to respond to therapeutic pressures remain a challenge. Further studies are being conducted to understand changes at the molecular level that possibly make CSCs more resistant or allow them to activate other survival pathways. Furthermore, the isolation of new CSC-specific markers might help in the design of more efficient therapeutic intervention. The challenge lies in developing therapies that can effectively eliminate CSCs without inadvertently creating new populations of resistant cells. Research suggests that CSCs can migrate to other parts of the body, settle, and form new tumors, contributing to cancer metastasis. This highlights the need for a dual treatment approach that targets both the primary tumor and any secondary tumors that have formed, using a combination of invasive (e.g., surgery) and non-invasive (e.g., chemotherapy, radiation) methods [5].

Conclusion and Future Directions

Prospective cancer treatments should focus on targeting cancer stem cells, which are believed to be the root cause of tumor recurrence and resistance to treatment. Due to the developing knowledge of CSC biology, new strategies have been introduced which include the use of combination of conventional therapies together with molecular therapies for elimination of CSCs. For instance, integration of chemotherapy with drugs that work on CSC signaling pathways may increase the therapeutic impact of chemotherapy and minimize the risks of recurrence. The treatment should offer on the specific nature of the tumor. Research is underway to study the molecular mechanisms controlling the CSC behavior and the influence of microenvironment or stem cell niches, which will lead to the development of new therapeutic approaches. Such advanced technologies that include CRISPR gene editing and nanotechnology may also help to further enhance the targeting procedures of CSCs. For example, CRISPR could be used to delete genes that are required for CSC survival, and nanoparticles could be used to deliver chemotherapeutic agents to CSCs selectively or to increase their specificity to decrease side effects. The idea of CSCs in the development and progression of neoplasms will bring a new vision in cancer treatment and help increase the life expectancy of cancer patients in many countries. With more research and the discovery of the intricacies of CSCs, the hope is to translate these findings into clinical applications that not only extend the lives of cancer patients but also improve their overall quality of life.

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