Introduction to Cancer Stem Cells

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Abstract

Cancer is a persistent public health-care issue of modern life that poses a global challenge. It comprises several diseases that basically involve abnormal cell growth and have a potential to invade or metastasize to other distant organ systems, spreading the disease to other part(s) of the body. Development of resistance to conventional therapies and disease recurrence are some common phenomena encountered in almost all types of cancer. Understanding "hallmarks of cancer" and "tumor microenvironment" is therefore important for development of successful therapy for cancer. Numerous drugs have been designed and tested for their anticancer efficacy over decades to find out a complete cure for this lethal disease, but without desirable success so far. The concept and role of "stem cell" therapy in oncology research have drawn considerable interest in recent years. Thus, emphasis has been given on proper identification and characterization of the "cancer stem cells" and "other stem cells" for elucidation of the signaling cascades involved in the process of cancer limitation and progression (and resurgence). In the introductory part of this book, an attempt has been made to provide an overall idea on different aspects of cancer stem cells, optimization of rate and type of cell growth, and their associative cure strategy by adopting a well-defined scientific perspective.

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Keywords

Cancer · Stem cell types · Cancer stem cell · Stem cell therapy · Targeted therapy · Therapy resistance

Abbreviations

AML	Acute myeloid leukemia
CSC	Cancer stem cell (CSC)
DNA	Deoxyribonucleic acid
EMT	Epithelial to mesenchymal transition
EPC	Endothelial progenitor cell
FACS	Fluorescence-activated cell sorter
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
MET	Mesenchymal-epithelial transition
MSC	Mesenchymal stem cell
NSC	Neural stem cell
ROS	Reactive oxygen species
SSC	Somatic stem cell
TSG	Tumor suppressor gene
HSCTs	Hematopoietic stem cell transplants

1.1 Cancer and Cancer Stem Cells

Cancer is a global public health challenge, and according to the latest GLOBOCAN report in 2018, approximately 18.1 million new cases and 9.6 million deaths were recorded [1]. Epidemiological studies showed smoking, alcohol, irregular and unhygienic food habits, lifestyle, genetic polymorphism, susceptible alleles, oncogene regulation, chromatin remodeling, and environmental and genotoxic stress to be the major causes of developing cancer. The knowledge of cancer has now extended toward understanding of "tumor microenvironment," and over the years, genomic, epigenomic, transcriptomic, and proteomic databases of around 33 cancer types have also been established [2]. Overall findings of the pan-cancer atlas reflect the six "hallmarks of cancer" [3] and role of ~140 driver genes which are classified into 12 major cancer signaling pathways [4]. Hence, this new era of translational cancer research is focused on early diagnosis and targeted cancer treatment. For targeted therapy, the aim is to employ single molecule or pathway inhibitors with or without conventional treatment.

The conventional treatments for cancer are surgery, chemotherapy, radiotherapy, and hormone therapy. On the initial stage of chemoradiotherapy treatment, tumor shrinkage commonly takes place, but sooner or later tumor growth is reestablished at the original and/or in new sites [5]. Further, when cancer is diagnosed at its advanced stages, most of the conventional therapies fail, and most patients in due course of

treatment develop chemoradiotherapy resistance with the ultimatum toward death [6]. This alarming situation necessitates the immediate attention for understanding the "hidden mechanism" of disease recurrence for better treatment and management of therapy resistance.

Cancer is a heterogeneous disease phenotypically and genotypically controlled. The heterogeneous nature of this disease is evident even within a single patient. It is evident that the intra- and inter-tumor heterogeneity is due to mutational landscapes in the "driver genes." These driver genes are either tumor suppressor genes (TSGs) or oncogenes. The TSGs are functionally involved in transcription regulation, signal transduction, and angiogenesis. In cancer, the TSGs are inactivated due to genetic and epigenetic alterations. The genetic alterations of TSGs include (1) mutation and (2) deletion [7]. The epigenetic inactivating events are (1) methylation, (2) deregulated imprinting, (3) altered splicing, (4) histone modification, and (5) decreased mRNA stability through miRNA or other processes [3, 4, 7]. Therefore, it can be said that "loss-of-function" mutations in TSG contribute to cancer development. Retinoblastoma is a classic example which occurs due to loss of function of Rb-TSG gene.

An oncogene is capable of transforming normal cells into cancerous one, both for cells growing in cell culture in vitro or in animal models in vivo. Oncogenes are said to be derived from their normal cellular counterparts called proto-oncogenes. A classic example is the Ras gene (a proto-oncogene) that encodes for an intracellular signal transduction. The mutant form called the rasD gene (oncogene) is derived from the original Ras. In this way, the encoded mutant protein thus produced is responsible for uncontrolled cell growth [8]. Cellular transformation of a proto-oncogene into an oncogene occurs due to "gain-of-function" mutation by following any of the mechanisms, namely, (1) point mutation, (2) chromosomal translocation and (3) amplification [8].

According to the "Clonal Evolution Model" of cancer development, the driver gene mutations stimulate cell dedifferentiation and phenotypic regression with loss or gain of function, uncontrolled proliferation, and inability to activate cell death pathways. Whereas the "Alternative Model" of cancer development says, every tumor comprises a rare population of cells termed as cancer stem cells (CSCs) or cancer-initiating cells. The CSC hypothesis also says, within the tumor microenvironment, only a subpopulation of cells with self-renewing and tumorigenic properties are responsible for the generation of cancer cells and their hierarchical organization [9].

The CSCs were first identified in acute myeloid leukemia (AML) by Bonnet and Dick [10]. The population of AML-CSCs (~0.1–1% of the overall tumor population) identified with surface marker CD34 + CD38 was found to develop cancer in mice [10]. The CSCs are identical in nature with normal stem cells in respect of their common self-renewal and differentiation properties [10, 11]. The CSCs were also demonstrated to have the role in developing resistance to conventional cancer therapies and may play a role in developing metastasis [12]. Epigenetic reprogramming mechanism can lead to the metabolic and phenotypic changes to convert non-CSC population into CSC to develop therapy resistance [13]. For tumor invasion, the mechanism of epithelial to mesenchymal transition (EMT) can be a

major factor in which epithelial cells lose their original characteristics and gain mesenchymal properties [14]. The EMT has also been suggested to have the ability to induce intravasation, the process by which cancer cells enter the bloodstream for invading healthy tissue. The reverse program of EMT that is called mesenchymal to epithelial transition (MET) can promote new tumor formation [15, 16]. Therefore, understanding these molecular events associated with CSC is very important for targeted cancer therapy [17].

CSCs are a small proportion of cells within a tumor that is self-sufficient to trigger tumorigenesis. These cells have the ability of self-renewal and can produce different lines of cancer cells [18]. In support of the molecular events associated with CSC as mentioned above, the loss of E-cadherin with a concomitant rise of N-cadherin, expression of transcription factors like Snail and Twist and signal proteins VEGF and TGFβ, and overexpression of Sox2, Oct4, and Nanog are the induction factors to initiate EMT, believed to be a major driving force for metastasis [19]. The stemness pathways like Wnt/β-catenin, JAK-STAT, Notch, etc., are abnormally regulated contributing to resistance to apoptosis, progression, and propagation of cancer cells. In addition to maintaining the ends of chromosomes by expressing the hTERT gene, their microenvironment composed of blood vessels and stromal cells supports the multiplication of tumor cells [20, 21]. Further, the potentiality to produce free radical scavengers to scavenge the reactive oxygen species (ROS) and combat oxidative stress is of prime importance for the sustenance of cells by avoiding DNA damage.

Cell surface markers like CD34+ and intracellular markers like aldehyde dehydrogenase 1 have shown a light to detect their presence and distinguish them from normal stem cells [22, 23]. Methods such as DNA barcoding for tracing CSCs using FACS provide an attempt to separate CSCs from the heterogeneous population of cells. Detecting circulating CSCs to determine the recurrence in patients suffering from cancer, transplanting the isolated CSCs into the mouse model, and colony formation assay are other ways to characterize their nature [10]. Researchers have also found strategies to knock down the gene encoding TERT proteins that lead to cell cycle arrest and modified T cells called chimeric antigen receptors for detecting CSCs which direct another way of evoking our immune system to fight infections [24]. To end the deep-rooted cause of progression of cancers, nowadays, clinical trials are underway to target the stemness pathways for long-term outcomes.

In this short introductory section, we will endeavor only to focus briefly on an overall idea about CSCs and how these are different from the normal stem cells.

"Stem cell," as the name indicates, may be defined as the cell characterized by the unique ability of self-renewal for an indefinite period of time. These cells are endowed with the capability to form single cell-derived clonal cell population. These cells can also differentiate into several other cell types. The property of self-renewal in the stem cell pools plays pivotal roles in tissue regeneration and homeostasis [25, 26]. Stem cells can further be categorized as "embryonic stem cells" (ESCs) or "somatic stem cells" (SSCs). The SSCs, also called adult stem cells, are multipotent in nature and bear the potentiality to differentiate into any other cell type of particular lineage. These might include neural stem cells (NSCs), hematopoietic

stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and many others [27].

Embryonic stem cells (ESCs), just like that of pluripotent cells, can differentiate into many cell types and are thus immensely used as standards for detection of pluripotent cultured cells in vitro with some restriction of usage in scientific studies and clinical trials in human pertaining to ethical considerations [28]. ESCs are now being replaced by *induced pluripotent stem cells* (iPSCs). These iPSCs are reprogrammed adult somatic cells which have enforced expression of pluripotency factors. Embryo destruction is not required for iPSC establishment. iPSCs are like ESCs, except for the fact that they lack immunogenic or ethical limitations, and therefore, they bear the possibility for clinical application more than ESCs [29].

Neural stem cells (NSCs) are a type of stem cells which can self-renew and differentiate into neurons, astrocytes, oligodendrocytes, etc., and express Sox2, nestin, and other classic markers and have been deployed to treat brain, breast, prostate, and lung tumors [30–32].

Mesenchymal stem cells (MSCs), known to be derived from bone marrow, are able to differentiate into mesodermal cells, including bone, cartilage, muscle, stroma, adipose tissue, connective tissue, and tendon. MSCs can be isolated easily, and they are known to propagate in vitro and have huge application in cancer therapy.

Hematopoietic stem cells (HSCs) belong to the most primitive of all the blood lineage cells. They are predominantly found in bone marrow and generally known to produce mature blood cells by proliferation and differentiation of lineage-restricted progenitor cells. HSC transplantation has clinical implication over the last four decades.

Endothelial progenitor cells (EPCs) are primarily concerned with vascular regeneration and thereby have potentiality in cancer therapy by coupling with antitumor drugs or performing transfection or acting with angiogenesis inhibitors [33].

Parthenogenetic stem cells, pluripotent stem cells (PSCs), have now been derived parthenogenetically from activated human oocytes. These cells represent similar characteristics as displayed in the human embryonic stem cells (hESCs) which include the infinite division and in vitro and in vivo modes of differentiation into germ cell lineages [34]. The human parthenogenetic ESCs (pESCs) consisting of homozygous human leukocyte antigen (HLA) are known to strongly increase the degree of matching and significantly increase the histocompatibility among cohorts of cells in human population [35]. The main strategy lies therein is to activate the oocyte artificially without the ample extrusion of second polar body. Further, the events of early recombination in oocyte also results in heterozygous pESC formation.

Now, the question is: how and what properties distinguish the normal stem cells from CSCs? The cellular niche or the surrounding cellular environment helps to maintain the "stemness" property. When a normal stem cell divides to give rise to two daughter cells, a balance is maintained. Among the two daughter cells, one acquires the "self-renewal" property and remains as the stem cell, whereas the other one goes for expansion and differentiation to develop into mature cell. In both cases,

the cells prevent to acquire "tumorigenic" property by sustaining a fine balance of "proliferation inhibition" and "proliferation promotion" [36]. The imbalance may be caused due to mutational "hit" that makes a normal cell to acquire the CSC phenotype. Mutation is a random process, and the frequency to generate a normal stem cell into CSC phenotype varies from cell to cell and organ to organ. It can be said that the greater the number of stem cells, the higher the chances of developing CSC phenotype as well as cancer [37]. Further, as said earlier, the cellular niche or the surrounding cellular environment is also associated with developing the CSC phenotype. Cancer is not just a mass of malignant tumor cells but a complex mix of several components which contribute to its development. This includes the immune cells, cancer-associated fibroblasts, endothelial cells, and blood vessels. These nonmalignant components can comprise up to >50% of the primary or metastatic tumor mass which play a major role as "microenvironment" and in acquiring the CSC phenotype [3]. Some important features of CSCs are their expressivity of the stemness genes, their self-renewal property, and their ability to differentiate and proliferate into other non-stem cancer cells and resist traditional mode of cancer treatment. Non-CSCs in the tumor have been reported to proliferate at a faster rate than that of CSCs but have little tumor-initiating potential [38].

1.2 Identification and Characterization of Cancer Stem Cells

CSCs are cancer cell subpopulation having stem-like properties that can be identified by cell surface markers. The CSCs can be isolated following standard practice from tissues of a patient and cell lines derived from different cancer types. Some of the key features of CSCs for identification, isolation, and characterization can be summarized as follows:

(a) CSC sorting based on biomarkers: CSC subpopulations can be distinctly sorted out from other cancer cells based on their surface markers. Flow cytometric sorting of CSCs is done from the total cancer cell population of a patient's primary tissue as well from cancer cell lines by specific markers, e.g., CD44+, CD133+, Cd117, ALDH1+, Pakt+, Oct4, Sox2, Nanog, ABCG2, ABCC1, Mrp1, Nrf2, BMI 1, etc. The sorted cell populations can be grown in ultralow attachment plates with Matrigel embedded conditions (3D culture condition) for sphere forming assay [39]. In breast cancer of non-responding cases, after neoadjuvant chemotherapy, prevalence of CSCs having CD44+ and CD24−/low has been reported [40]; further, these cells showed CSC renewal and mesenchymal features [41].
(b) Tumor growth study in mice: The flow cytometry-based sorted cell populations can be transplanted in immunodeficient mice (tumor xenograft). The CSCs have the tumorigenic potential and develop tumor when transplanted into immunodeficient mice. The CSCs when they form tumors contain both the tumorigenic and non-tumorigenic cells [38]. In head and neck squamous cell carcinoma (HNSCC), CD44 molecule was first identified as the surface marker of CSC, and

it was also found in only <10% CD44-positive cells with tumorigenic potential but not in the CD44-negative cells [42].

1.3 Cancer Stem Cell Signaling Pathways

Like normal stem cells, CSC follows three major self-renewal pathways, namely, *Hedgehog*, *Wnt*, and *Notch*. Key regulatory genes of these signaling pathways are associated with cancer, and targeting these pathways can be one of the important strategies for cancer therapy [43]. Key regulatory genes of these signaling pathways can be summarized as follows. The *Hedgehog pathway genes* are HHIP, PTCH1, Smo, SuFu, and Gli-1. The *Wnt pathway genes* are DKK1, Wnt, β-catenin, Axin-2, GSK-3β, and APC. The *Notch pathway genes* are Jag1/2, Hey 1, Hes 1, Tace, and presenilin. Increased expression of Gli-1 was observed in HNSCC tumors after developing resistance due to long-term treatment of epidermal growth factor receptor (EGFR) inhibitor [44]. In HNSCC cell line after chemotherapeutic treatment (bortezomib and etoposide), increased frequency of CSC population and overexpression of Wnt signaling proteins DKK1 and AXIN2 were found [45]. Similarly, overexpression of SMO has been recorded in a HNSCC cell line after treatment with cyclopamine [46]. The accumulating data indicates that there is preferential selection of CD44+ CSC populations after treatment with neoadjuvant chemotherapy in HNSCC along with alterations of these self-renewal pathways, particularly Hedgehog and Wnt. Development of chemoresistance in HNSCC might be due to alterations in these CSC pathways. It also seems likely that the prevalence of CD44+ CSCs may be the indicator or biomarker of chemoresistance after neoadjuvant chemotherapy. Increased expression of CD44 might be due to overexpression of Gli/β-catenin, the effector protein of Hedgehog/Wnt pathways [47, 48]. High expression of a Notch signaling ligand DLL4 was reported from HNSCC patients undergoing radio-chemotherapy [49]. Agrawal and his research group have identified mutation in Notch1 mutation in HNSCC patients. Their study further revealed in HNSCC types that Notch1 acted as tumor suppressor rather than oncogene [50].

1.4 Role of Stem Cell and CSC in Developing Disease and Therapy Resistance: Therapeutic Implications and Future Directions

Stem cell therapy is generally based on transplantation of living cells into an organism either to repair a tissue/organ or to restore their optimal functioning which might have been lost completely. Human embryonic stem cells (hESCs) are in use for several cell therapy procedures which accounts for 13% of cases reported so far. However, on the contrary, fetal stem cells (fESC) are used only in 2% of cases. Further, record of usage of umbilical cord stem cells is only 10%, and adult stem cells are in use for treating 75% of cases [51]. Cardiovascular and ischemic

diseases, diabetes, diseases related to liver and hematopoietic organs (more than 25,000 cases of Hematopoietic stem cell transplants (HSCTs)/year and counting), orthopedics, etc., are a few types among myriads of other diseases which are being treated with stem cell transplantation globally [52, 53].

The most common stem cells that are modified by multiple mechanisms for potential use in cancer therapies are NSCs and MSCs which may vary from including the therapeutic enzyme/prodrug system or using a nanoparticle or introducing an oncolytic virus delivery on tumor site. Enzyme/prodrug therapy/suicide gene therapy is one of the promising applications of stem cell against cancer. The NSCs and MSCs can express enzymes which can convert nontoxic prodrugs into cytotoxic products by bioengineering. These modified stem cells, when transplanted into tumor-bearing models, quickly localize to tumor tissues where the exogenous enzyme aided prodrug conversion to cytotoxic molecules ultimately damages the tumor cells [54]. Further, stem cells can overcome the limitations of common cancer therapy and function as in situ drug factories by secreting antitumor agents [55] and through delivery of virus by MSCs toward bio-targets by combining the oncolytic activity with that of the immunoprivileged and tumor-tropic properties of the MSCs [56].

The use of nanoparticles as potent drug delivery systems is now the current trend of treatment of different diseases like diabetes [57–60], cancer [61–65], cardiomyopathy [66, 67], anti-genotoxic [68] and anti-inflammatory [69], based on their bioactive targeted delivery, increased penetration, reduction in drug-dose ratio, sustainable release, faster action, and protection against degradation due to harsh biological environment at administration. However, the efficacy of stem cells as nanoparticle delivery agents has now been a futuristic approach owing to the reduction in unrestricted uptake of different nanoparticles by them, increase in intra-tumor drug distribution, and protecting the drugs from host immunologic reactions [70].

Traditional therapies of cancer cannot eliminate CSCs while they can kill non-stem cancer cells. Chances of relapse of tumors remain usually high when the CSCs which had not been killed during therapeutic processes proliferate and differentiate. Thus, strategies for targeting CSCs may solve several clinical issues of drug resistance and recurrence [71, 72]. Evidences from several studies indicate that the CSCs can develop and maintain different categories of human malignancy which imply great opportunities for assessment of oncologic therapeutic strategies to impart a better life to cancer patients. There exists a minute analysis and comparison between CSC and cells derived from normal tissue. The CSC-targeted therapeutic arsenal often comes across several potential hurdles, like normal stem cell cytotoxicity and acquisition of resistance against the treatment, which need to be addressed to maximize the chances of success [73].

The CSCs have different mechanisms of defense against chemotherapy and radiation. Here, we have highlighted two major mechanisms. CSCs produce antioxidant enzymes to protect against radiation-induced damages. One of the routine treatments of cancer is radiotherapy that produces free radical as a natural byproduct of oxygen metabolism. This oxidative damage causes the damage to DNA to kill

cancer cells. In some studies, increase of resistance to radiotherapy has been accompanied with enhanced DNA repair, less damage to DNA, reduced apoptosis, and increase of angiogenesis [74].

The other mechanism of CSC is through detoxification enzymes which play a role in resistance to chemotherapy. Drug detoxification is done in three stages; in the first stage, detoxification is done through cyto p450, which removes OH^- and free radical $O_2^{\cdot -}$ species. In the second stage, toxins are conjugated using glutathione, glucuronic acid, or sulfate catalyzed by glutathione S-transferase, uridine disulfate, glucuronosyltransferase, and sulfatase. Finally, drug and toxin are also pumped out of the cell through intermembrane channels [74].

Identification of similarities and dissimilarities between normal stem cells, CSCs, non-tumorigenic cells, and normal differentiated cells based on differences in their immunophenotype shall allow the development of CSC-targeted therapeutic strategies which shall definitely impart a relatively low risk toward normal cellular/tissue level cytotoxicity. Evaluating the efficacy of such targeted molecule treatments shall require the advent of modern approaches to determine the CSC frequency and their degree of viability within tumor mass. However, resistance due to clonal selection and tumor microenvironment such as hypoxia might pay hindrance toward the development of the cure and needs utmost care and precautions.

In view of the tremendous importance of CSCs in the management and control of cancer, subsequent chapters of this book have been assigned to deal elaborately and critically with several important aspects, such as types of CSCs, how CSCs can contribute to the development of different types of cancer, isolation and characterization of CSCs, role of other tumor microenvironmental factors in association with CSCs in cancer development, controversies of acceptance for the CSC hypothesis, new strategies or alternative therapies for targeting CSCs for cancer treatment, and some other emerging issues.

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