

Review Article

Molecular Imaging in Tracking Tumor Stem-Like Cells

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Cancer remains a major public health problem in many countries. It was found to contain a subset of cancer stem cells (CSCs) that are capable of proliferation and self-renewal, and differentiation into various types of cancer cells. CSCs often display characteristics of chemotherapy resistance and radiotherapy resistance. Numerous putative biomarkers of CSCs are currently identified including CD133, CD44, CD24, ALDH (aldehyde dehydrogenase), and ABCG2. Interestingly, no single marker is exclusively expressed by CSCs. Thus, the various combinations of different biomarkers will be possible to identify CSCs, and considerable work is being done to recognize new ones. In order to demonstrate the mechanisms of resistance and response to therapy and predict the outcome as well as prognosis, the ways to track and identify CSCs will be extremely important. The technologies of molecular imaging will reveal mechanisms of cancer progression and provide visual targets for novel therapeutics. Limited studies were investigated on the detection of various types of CSCs by molecular imaging. Although the tracking of circulating CSCs is still hampered by technological challenges, personalized diagnosis and therapies of cancers are expected to be established based on increased understanding of molecular imaging of cancer stem-like cells biomarkers.

1. Introduction

The global burden of cancer is increasing, and cancer is a major public health problem in many parts of the world, including China. Based on the American Cancer Society cancer statistics, a total of 1,596,670 new cancer cases and 571,950 deaths from cancer, corresponding to more than 1500 deaths per day, are projected to occur in the United States in 2011 [1]. Among men cancers of the prostate, lung and bronchus, and colorectum will account for approximately 52% of all newly diagnosed cancers. Breast, lung and bronchus, and colorectum will be the three most commonly diagnosed types of cancers among women this year. Cancers of the lung and bronchus, prostate, and colorectum in men, and cancers of the lung and bronchus, breast, and colorectum in women continue to be most common causes of cancer death. These four cancers account for almost half of the total cancer deaths among men and women [1].

Cancer mortality remains high, despite the recent advances made in cancer treatment and diagnosis. One possible cause for therapeutic failure is that many malignant tumors contain a subset of tumor-initiating cells termed cancer stem cells (CSCs) when multipotency and self-renewal have been demonstrated. The CSC model provides an attractive concept that help to explain why cancer cells resistant to chemo- and radiotherapies. There has been acceptance of the idea that the remaining tumor cells after anticancer therapy have a distinct molecular phenotype that confers resistance to the therapies [2, 3]. Identification and purification of the CSC population from clinical samples and cell lines have been proven successfully based on the expression of a particular cell-surface marker, as well as by functional assays. The identification of CSC has undergone rapid development in recent years.

Side population (SP) technology has been widely used to identify the cancer stem-like cells by detection of the

ability to efflux Hoechst 33342 dye through an adenosine-triphosphate-(ATP-) binding cassette (ABC) membrane transporter. In many tumors, SP cells are reported as enriched cells with tumorigenesis and are responsible for chemoresistance and tumor recurrence [4–6]. The current methods for isolation of putative CSCs depend on cell surface biomarkers and enzymatic activity detected by fluorescence-activated cell sorter (FACS). Clarified understanding and eradication of CSCs, in turn, may improve the outcome of some cancer therapy [3, 7]. One of the promising strategies is to track the CSCs and then target them for efficient treatments. Techniques required for real-time imaging and studies of tumor cells in viable fresh tissue or living animals are urgently needed.

Molecular imaging is a new and noninvasive strategy which can provide molecular and physiological information regarding cancer using various molecular-targeted imaging probes specific for cell surface biomarkers that are unique to cancer. It aims to visualize and quantify biological, physiological, and pathological processes on cellular and molecular levels. This has advantages over traditional methods, as they permit real-time tracking *in vivo*. Based on the biological characteristics of cancer stem cells, molecular imaging will provide a novel role for the visualization of cancer cells. Intravital imaging of CSCs could be of great importance for determining prognosis, as well as monitoring therapeutic efficacy and influencing therapeutic protocols. Cancer biomarker-based molecular imaging has become an essential element for cancer diagnosis. In most cases, the accuracy of predicting cancers depends on the specificity and signal sensitivity of molecular probes targeting genes or proteins involved in cancer growth and progression. In this paper, we will briefly discuss and summarize cancer stem biomarkers and the applications of the molecular imaging in tumor stem-like cells or tumor cells.

2. Types of Cancer Stem Cells and Its Biomarkers

CSCs have been defined as a subset of cancer cells with exclusive ability of self-renewal and to cause the heterogeneous lineages of cancer cells that comprise the tumor. Numerous putative markers are currently under investigation, and much work is being done to identify and characterize new marker. The experimental methods to identify CSCs have been developed so far as follows: (a) clonogenic assay; (b) sphere-forming assays; (c) assays to investigate the differentiation potency of isolated cells into the various differentiated cells. In addition to these analyses based on *in vitro* properties of CSCs, the existence of CSCs has been demonstrated by tumor initiation assays, in which the primary human tumor cells were transplanted into NOD/SCID mice [8–10] (Figure 1). Cell surface markers and enzymatic activity detected by FACS have been widely used for the prospective isolation of putative CSCs. Here, the biomarkers to identify CSCs in different types of tumor models are investigated.

2.1. Hematopoietic System: Acute Myelogenous Leukemia (AML). In 1994, Lapidot et al. showed that leukemia can be initiated in mice using a single tumorigenic cell. It implied that a single or a few malignant cells can produce tumors [11]. These leukaemia-initiating cells were found to be CD34⁺ CD38⁻ on the basis of the cell-surface-marker expression. Subsequently, Bonnet et al. also demonstrated that the cell capable of initiating human AML in nonobese diabetic mice with severe combined immunodeficiency disease (NOD/SCID mice), termed the SCID leukemia-initiating cell, was exclusively CD34⁺ CD38⁻ [12]. After the identification of rare CSCs in leukemia, numbers of molecular markers for detecting CSCs in solid tumors have also been identified. The models that have been devised to describe the CSCs are as follows.

2.2. Solid Tumors: Brain/Breast/Lung/Colorectal/Prostate/Pancreas/Liver

2.2.1. Brain. Brain tumors are the leading cause of cancer mortality in children and remain incurable despite advances in surgery and adjuvant therapies [1]. Singh et al. reported that only the CD133⁺ brain tumor fraction contains cells that were capable of tumor-initiation in NOD-SCID mouse brains [13]. They used a xenograft assay that identified human brain tumor initiating cells that initiate tumors *in vivo*. In their studies, injection of 10⁵ CD133⁻ cells did not form a tumor, whereas only 100 CD133⁺ cells produced the patient's original tumor. This identification of an *in vivo* tumor-initiating cell from human brain tumors provided a powerful tool to investigate the tumorigenic process in the central nervous system. Interestingly, Chiao et al. suggested that CD133⁺ glioblastoma stem-like cells (GSCs) and CD133⁻ GSCs shared similar efficacy in tumor formation but showed distinct intratumor vasculature [14]. Compared with CD133⁻ GSC, a highly vascularized anaplastic tumor was found in CD133⁺ GSC-derived tumor xenografts. Both *in vitro* and *in vivo* subsets of CD133⁺ GSC but not CD133⁻ GSC were capable of vascular smooth muscle-like cell differentiation. The detection of endothelium-associated CD31 gene and differentiation of vascular-like cells suggest that GSCs may contribute to form vessel-like structures and provide a blood supply for glioblastoma cells.

Additionally, Tabuse et al. found high expression of the HOXD9 gene transcript in glioma cell lines and human glioma tissues [15]. They suggested that HOXD9 would be a novel marker of GSCs and cell proliferation and/or survival factor in gliomas and glioma cancer stem-like cells, and a potential therapeutic target (HOX genes encode a family of homeodomain-containing transcription factors involved in the determination of cell fate and identity during embryonic development. They also behave as oncogenes in some malignancies).

2.2.2. Breast. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths, based on the GLOBOCAN 2008 estimates [1,

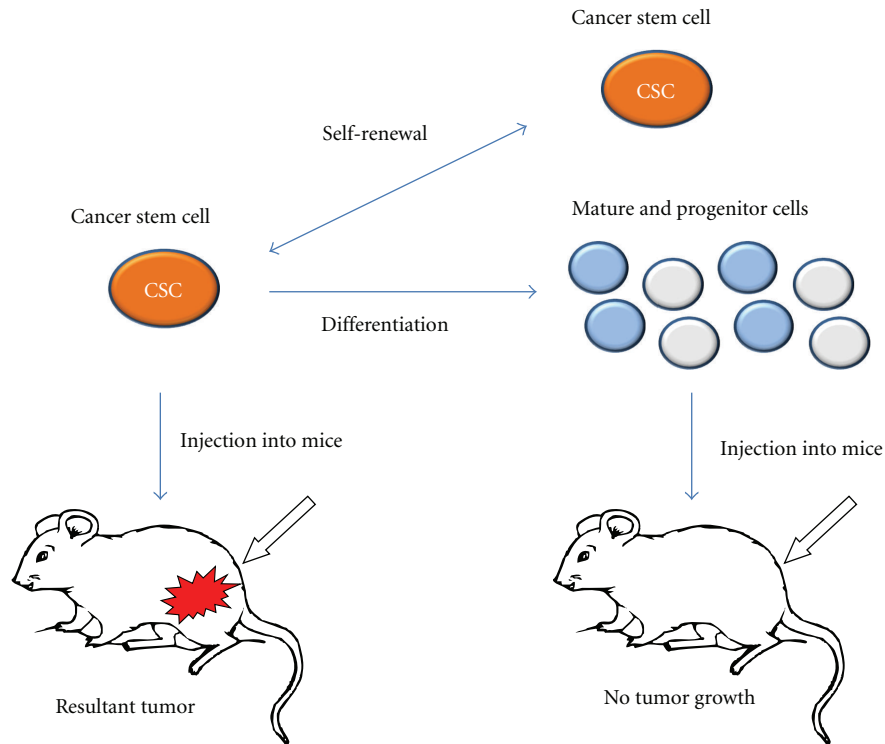


FIGURE 1: Three phenotypes of CSCs: self-renew, multipotentiality, and tumor-initiating capacity. Self-renewal: single-cell culture sphere-forming assay. Differentiation: flow cytometric analysis of differentiation markers and loss of stem cell markers. Tumor-initiating properties were investigated by injection of CSCs in NOD/SCID mice. The most crucial standard for a CSC is its ability to reinitiate a serially transplantable tumor that recapitulates the original heterogeneity and tumor histology. Using this standard, many so-called CSCs reported so far are based on correlative studies rather than being true CSCs.

16]. In the United States, breast cancer is expected to account for 30% (230, 480) of all new cancer cases among women in 2011 [1]. Through analysis of cell surface markers and xenotransplant models, a subpopulation of putative human breast cancer stem cells that is $CD24^-/CD44^+$ and bears high aldehyde dehydrogenase isoform 1 (ALDH1) activity has been isolated in clinical samples of breast cancer tissues [3]. These cells are regarded as having the most prominent tumor-initiating activity. Cells with aldehyde dehydrogenase (ALDH) activity isolated from normal human breast have phenotypic and functional characteristics of mammary stem cells. Analyzing the expression of ALDH1 in hundreds of human breast carcinomas from two independent tumor sets indicated poor clinical outcome [17]. These findings provide support for the “cancer stem cell hypothesis” and further indicated that ALDH1 was a marker of stem/progenitor cells of the normal human breast and breast carcinomas. The ALDH1-positive cell population had a small overlap with the previously described cancer stem cell, $CD44^+/CD24^-/lin^-$ phenotype [18].

The overlap represented approximately 1% or less of the total cancer cell population, which bears both phenotypes appeared to be highly enriched in tumorigenic capability. It was found that as few as 20 cells were able to generate tumors. Since ALDH is also expressed in hematopoietic and neuronal stem cells, this marker may prove useful for

the detection and isolation of cancer stem cells in other malignancies, thus facilitating the application of cancer stem cell biology to clinical practice. In breast CSCs, it was also shown that the activity of TAZ, a transducer of the Hippo pathway, is required to sustain self-renewal and tumor-initiation capacities. TAZ protein levels and activity, which is promoted by the epithelial-mesenchymal transition (EMT), are elevated in prospective CSCs and in poorly differentiated human tumors, which have prognostic value [19]. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells.

2.2.3. *Lung*. Lung cancer is the third most commonly diagnosed type and third most common cause of cancer death both in male and female [1]. Similar to the limited understanding of lung stem cell biology, the identity of the cell of origin in lung tumorigenesis is also largely unknown. Lung cancer contains a rare population of $CD133^+$ cancer stem-like cells able to self-renew and generates an unlimited progeny of nontumorigenic cells. Molecular and functional characterization of such a tumorigenic population may provide valuable information to be exploited in clinic [20]. It was found that human lung CSCs also express ABCG2, c-kit receptors, embryonic markers (SSEA-3, TRA-1-81, Oct-4, and nuclear β -catenin), and low levels of the cytokeratins 8/18 (CK8/18) [21, 22].

2.2.4. Colorectal Cancer. Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females [1]. Colorectal cancer incidence rates are rapidly increasing in several areas historically at low risk, of which possible reasons are a combination of factors including changes in dietary patterns, obesity, and an increased prevalence of smoking.

The existence of human colon cancer tumor-initiating cells was demonstrated by O'Brien et al. [10]. They used the renal capsule transplantation in immunodeficient NOD/SCID mice to identify a human colon cancer-initiating cell (CC-IC), which was CD133⁺. However, the CD133⁻ cells that comprised the majority of the tumor were unable to initiate tumour growth. They calculated by limiting dilution analysis that there was one CC-IC in 5.7×10^4 unfractionated tumor cells, whereas there was one CC-IC in 262 CD133⁺ cells, representing >200-fold enrichment. CC-ICs within the CD133⁺ population were able to maintain themselves as well as differentiate and reestablish tumor heterogeneity upon serial transplantation. The identification of colon cancer stem cells and the existence of tumorigenic and nontumorigenic cells within colon cancers suggested that they must target the cancer stem cells for therapeutic strategies to be effective.

Simultaneously, tumorigenic cells in colon cancer were included in the high-density CD133⁺ population, which accounted for about 2.5% of the tumor cells [9]. The original tumor in NOD/SCID mice can be reproduced by injection of colon cancer CD133⁺ cells. Purified CD133 and carcinoembryonic antigen-positive (CEA⁺) colon cancer cells were unable to transfer the tumor into immunocompromised mice, and the tumorigenic population in colon cancer is restricted to CD133⁺ cells. Importantly, CC-ICs need to remain undifferentiated to maintain tumorigenic potential. During the *in vivo* passages, the tumors were serially transplanted for several generations, and CD133⁺ cells did not lose their tumorigenic potential. Instead, in each of the tumors they observed progressively faster tumour growth without significant phenotypic alterations. Therefore, the small number of undifferentiated tumorigenic CD133⁺ cells should be the target of future therapies.

Using multiple genetic markers, researchers also had evaluated the clinical significance of circulating tumor cells as a prognostic factor for overall survival (OS) and disease-free survival (DFS) in the peripheral blood (PB) of patients with colorectal cancer Dukes' stages B and C who had undergone curative surgery. OS and DFS of patients who were positive for CEA/CK/CD133mRNA were significantly worse than those of patients who were negative for these markers. Detection of CEA/CK/CD133 mRNA in PB is a useful tool for determining which patients are at high risk for recurrence and poor prognosis [23]. Moreover, in human colorectal cancer, CSCs are also defined using CD166, CD44, and CD24 cell-surface markers [24, 25].

The CSC theory suggests that the actual tumorigenic capacity of individual cancer cells may be influenced by homeostatic signals derived from their microenvironment. It was observed that high Wnt activity functionally designates the colon CSC population [26]. Nevertheless, the current

data lend strong support to the influence of the niche on CSC maintenance.

2.2.5. Prostate. Prostate cancer will account for 29% (240,890) and be most commonly diagnosed cancer in men in United States according to the American Cancer Society in 2011 [1]. There continues to be a need for new diagnostic markers and potential new therapeutic targets in prostate cancer. Studies suggested that high ALDH activity and coexpression of CD44 (ALDH^{hi} CD44⁺) cells exhibited a higher proliferative, clonogenic, and metastatic capacity *in vitro* and demonstrated higher tumorigenicity capacity *in vivo* in prostate cancer cell line. However, low ALDH activity and CD44-negative (ALDH^{lo} CD44⁻) cells were able to develop tumors, albeit with longer latency periods. In their study, ALDH activity and CD44 did not appear to identify prostate cancer stem cells; however, they did reveal increased tumorigenic and metastatic potential, indicating their potential importance for further exploration [28].

Rajasekhar et al. identified a minor subset of stem-like human prostate tumour-initiating cells (TICs) that do not express prostate cancer markers, such as androgen receptor or prostate-specific antigen [29]. The cells represent an undifferentiated subtype of basal cells and can be purified from prostate tumors based on coexpression of the human pluripotent stem cell marker TRA-1-60 with CD151 and CD166. Such triple-marker-positive TICs possess stem cell characteristics and multipotency as demonstrated by *in vitro* sphere formation and *in vivo* tumor initiation, respectively.

2.2.6. Pancreas. Pancreatic adenocarcinoma continues to be one of the deadliest cancer-related diseases in the world [30]. Little progress has been made since the past introduction of the chemotherapeutic agent gemcitabine, which remains the first-line chemotherapeutic agent in pancreatic cancer. The prognosis of patients diagnosed with pancreatic cancer remains extremely poor [31]. Emerging evidence suggests that the majority of pancreas tumors display vital functional heterogeneity in their respective cell populations. However, a small subpopulation of tumor cells that have acquired the same somatic mutations as the other tumor cells with epigenetically distinct is responsible for tumor initiation, growth, and metastasis [32, 33].

Researchers identified a highly tumorigenic subpopulation of pancreatic cancer cells expressing the cell surface markers CD44, CD24, and epithelial-specific antigen (ESA) using a xenograft model of immunocompromised mice for primary human pancreatic adenocarcinomas [34]. Pancreatic cancer cells expressing the cell surface markers CD44, CD24, and ESA had at least a 100-fold increased tumorigenic potential compared with nontumorigenic cells. The CD44⁺CD24⁺ESA⁺ pancreatic cancer cells showed the stem cell properties of self-renewal, the ability to produce differentiated progeny, and increased expression of the developmental signaling molecule sonic hedgehog (SHH).

More recent data suggested an abundant expression of ALDH-1 in normal pancreas tissue [35]. It was found that a subset of adult centroacinar cells and terminal ductal epithelial (CA/TD) cells were characterized by high levels

TABLE 1: Comparison of the biomarkers of CSC and normal stem cell.

Cancer type	CSC marker	Normal stem cell	Marker	References
Acute myelogenous leukemia	CD34 ⁺ CD38 ⁻	Mouse embryonic stem cell	Oct-4	[64]
			SSEA-1	[65]
Glioma	Nestin, HOXD9	Human embryonic stem cell	SSEA-3	[65]
			SSEA-4, Oct-4	
Breast	ALDH1, TAZ CD44 ⁺ /CD24 ⁻ /lin ⁻		TRA-1-60	[65]
			TRA-1-81	
Colon	CD166	Neural stem cell	CD133	[66]
Prostate	TRA-1-60 CD151, CD 166		Nestin	[66]
Pancreas	ESA	Hepatic stem cell	EpCAM	[43]
Liver	CD90, EpCAM CD13	Mammary stem cell	CD49f ⁺ EpCAM ^{neg-low}	[67]
Breast, liver, and pancreas	ALDH		ALDH1	[17, 68]
Glioma, lung, colon, pancreas, and liver	CD133			
Pancreas, colon, and liver	CD24, CD44			
Breast, pancreas, and lung	ABCG2			

CD49f: $\alpha 6$ integrin; Lin: Lineage surface antigen; SSEA: stage-specific embryonic antigen.

of ALDH1 enzymatic activity. CA/TD cells carry significant progenitor capacities and may contribute to the maintenance of tissue homeostasis in adult mouse pancreas. Subsequently, Kim et al. sought to determine the relationship of ALDH and CD133 whose marker best enriches for pancreatic TICs and the sufficiency of ALDH^{hi} and ALDH^{lo} cell populations for tumor-initiation when enriched or depleted of cell populations also expressing CD133 [36]. Finally, they demonstrated that cell populations enriched for high ALDH activity alone were more efficient and fulfill the major criteria of a CSC and efficiently recapitulate the phenotype of the original tumor independent of CD133 cell surface expression, using a strict, direct xenograft tumor system and limiting dilutions of sorted human pancreatic cancer cells injected into NOD/SCID mice [36].

It was also suggested that SP cells were enriched in CSCs of pancreas cancer and validated an intriguing regulatory mechanism of the cancer stem cell-related phenotypes, which could represent a novel therapeutic target [4, 37].

2.2.7. Liver. Hepatocellular carcinoma (HCC) accounts for over 90% of primary liver cancer in adults. The first-line treatment for HCC is liver transplantation or surgical resection [37, 38]. However, most HCCs are inoperable because patients present at advanced stages. Even after surgical resection, the long-term prognosis of HCC remains unsatisfactory due to high recurrence rates. Recently, there has been mounting evidence in support of the existence of CSCs in hepatocellular carcinoma. CD24 was found to be a functional liver tumor-initiating cell (T-IC) marker. CD24⁺ HCC cells were found to be critical for the maintenance, self-renewal, differentiation, and metastasis of tumors and to significantly impact patients' clinical outcome [39]. In liver, CSCs can also be identified by several other cell markers, including CD44, CD133, CD90, CD13, EpCAM, OV6, and

ALDH enzymatic activity, or by selecting for SP cells which express an adenosine triphosphate-binding cassette (ABC) membrane transporter, such as MDR1 and ABCG2 [40]. However, generally accepted markers of CSCs in HCC have not been identified.

In summary, recent studies indicate that the expression of the surface markers is not suitable as a universal signature to detect CSCs in cells derived from different tumor types. It is likely that a panel of markers will be required to assess the presence of reliable and true CSCs in tumors. Although the list of CSC markers is not yet finished, a method to isolate and substantially enrich CSCs for cancer research is provided (Table 1).

3. Imaging of Cancer Stem-Like (or Cancer) Cells

Noninvasive molecular imaging technology is an effective and novel approach to visualize living cells in vivo. It also plays a critical role in tracking the location and activity of CSCs [41]. Now that CSCs are being identified and characterized in different tumor types, they are postulated to be responsible for tumor development, metastasis, and relapse after conventional therapies. To date, limited studies were investigated on the detection of various types of CSCs by molecular imaging methods (Table 2).

Examples will be given for the identified biomarkers of the tumor stem-like (or tumor) cells with molecular imaging techniques. Specifically, the feasibility of molecular imaging of prostate stem cell antigen-(PSCA-) positive prostate cancer was evaluated using an intermediate size antibody fragment by micropositron emission tomography (micro-PET) imaging [42], cell tracking with magnetic resonance imaging (MRI) after the hepatic progenitor cells labeled with EpCAM microbeads [43], CD133-specific monoclonal

TABLE 2: Molecular imaging in tracking tumor stem-like cells.

Imaging agent	Molecular imaging	Biomarker	Model
⁶⁴ Cu-ATSM	PET	CD 133	Colon cancer
Antibody magnetic microbead	MRI	ESA	Liver cancer
HA-MNCs	MRI	CD44	Breast cancer
Antibody AC133.1	FMT	CD133	CD133-overexpressing glioblastoma
Optical bifusion reporter genes	BLI	CD44	Breast cancer
GSC-targeting peptide	BLI	Nestin	Glioma
199b Luc-1	BLI	CD133	Medulloblastoma tumor
Adv5-199b	PET/CT		

antibody AC133.1 used for quantitative fluorescence-based optical imaging of mouse xenograft models [44], and so on.

3.1. Positron Emission Tomography (PET). PET imaging is a highly sensitive system that has advantages over traditional methods, as it permits noninvasive, real-time tracking in vivo. To our knowledge, few published studies were found to track the CICs in cancers with PET imaging. We will give an example of a generated stem cell surface marker specific probe to prostate cancer by micro-PET imaging. In the human prostate cancer LAPC-4 xenograft model, a number of candidate molecules were identified with high expression using representational difference analysis (RDA), a PCR-based subtractive hybridization strategy [45, 46]. One promising candidate, PSCA, is a prostate-specific cell surface antigen expressed strongly in both androgen-dependent and -independent LAPC-4 tumors. Elevated levels of PSCA were correlated with increased tumor stage, grade, and androgen independence [47]. PSCA located at chromosome 8, band q24.2, is a prostate-specific gene, encoding a 123-aa protein with an amino-terminal signal sequence, a carboxyl-terminal GPI-anchoring sequence, and multiple N-glycosylation sites [46]. Researchers had isolated and characterized a series of monoclonal antibodies directly against PSCA. The localization of PSCA had been detected using these antibodies.

Leyton et al. revealed a humanized radioiodinated minibody as a PET imaging agent for the detection of PSCA-expressing tumors [42]. Visualization of PSCA-positive prostate cancer is feasible using an intermediate size antibody fragment at 21 hours. The genetically engineered radioiodinated (¹²⁴I or ¹³¹I) 2B3 minibody as a novel antibody fragment for specific targeting and potential PET imaging agent for PSCA-expressing prostate cancers was introduced [48]. Then an affinity-matured minibody that ranked second in affinity for PET imaging of PSCA-expressing tumors was selected as the best tracer [49]. Currently, this candidate is under development for evaluation in a pilot clinical imaging study. Interestingly, two antibodies, anticleudin 4 and anti-PSCA, were radio-labeled with iodine 125 (¹²⁵I) for imaging pancreatic cancer xenografts in mice using gamma scintigraphy and single-photon emission computed tomography-computed tomography (SPECT/CT). It has been demonstrated that they are promising as radiodiagnostic and possibly radiotherapeutic agents for human

pancreatic cancers [50]. Antibodies or their constituent parts can be conjugated with radionuclides to carry them into tumors specifically and effectively. Similar to the specific normal stem cell biomarker detected in prostate cancer, which was used as early diagnosis marker in prostate cancer, tracking the CSCs biomarkers by PET imaging is also promising.

⁶⁴Cu-diacetyl-bis (N4-methylthiosemicarbazone) (⁶⁴Cu-ATSM), a PET imaging agent, accumulated in regions of CD133⁺ high expression, which are then killed by radiation, resulting in a decrease of the percentage of CD133⁺ cells [51]. In vivo (colon 26-bearing mice) studies showed that ⁶⁴Cu-ATSM treatment inhibited tumor growth. The percentage of CD133⁺ cells and metastatic ability in ⁶⁴Cu-ATSM-treated tumors was decreased compared with that in control animals. ⁶⁴Cu-ATSM could be a potential internal radiotherapy agent affecting the tumor's hypoxic regions with a high density of CD133⁺ cells, designated as CSCs. Therefore, tracking CSCs biomarkers and evaluating therapy efficiency are feasible and attractive. Further studies with more kinds of CSCs biomarkers would be needed to clarify the precise applications.

3.2. Magnetic Resonance Imaging (MRI). Magnetic resonance imaging is a noninvasive modality and can provide information on the anatomy, function, and metabolism of tissues in living subjects with no ionizing radiation. This imaging technique is increasingly performed in clinical diagnostics. Epithelial cell adhesion molecule (EpCAM) also known as epithelial-specific antigen (ESA) or CD326, a cell surface antigen, is found on liver CSCs, on hepatic progenitor cells including human hepatic stem cells (hHpSCs) and hepatoblasts (hHBs), and on proliferating epithelial cells in other tissues. McClelland et al. have evaluated the feasibility of cell tracking with MRI after the hepatic progenitor cells labeled with EpCAM microbeads before or after transplantation. Then the transplanted hHpSCs could be monitored and counted repeatedly in the same host by injection of the labeled MRI probe [43]. Additionally, magnetic iron microbeads coupled with HEA-125 monoclonal antibody against the epithelial cell adhesion molecule, abbreviated as EpCAM microbeads, have been developed primarily for the positive selection or depletion of EpCAM-positive cells. The magnetic labeling methodology is applicable to other

surface antigens for which antibody magnetic microbeads are available [43]. Antibodies or their constituent parts can be conjugated with nanoparticles or other substances, to carry them more specifically and effectively into tumors. The biological effects of monoclonal antibodies are supposed to be possible if targeting antigens are present in cancer stem-like cells [52].

CD44 is a cell surface glycoprotein overexpressing in breast CSCs and plays an important role in promoting cancer cell proliferation, invasion, and tumor-associated angiogenesis [53]. First, researchers synthesized pyrenyl HA (Py-HA) conjugates as CD44-targetable surfactants capable of simultaneously encapsulating magnetic nanocrystals and targeting CD44 using HA and 1-pyrenebutyric acid (Py). Finally, Py-HA conjugates and magnetic nanocrystals were used to formulate HA-MNCs. Their study suggested that HA-MNCs can be a potent cancer-specific molecular imaging agent for effective diagnosis of CD44-overexpressing breast cancer via MR imaging [53]. Furthermore, CG-rich duplex containing prostate-specific membrane antigen (PSMA) aptamer-conjugated thermally cross-linked super paramagnetic iron oxide nanoparticles (TCL-SPIONs) was reported as prostate cancer-specific nanotheranostic agents [54]. These agents were capable of prostate tumor detection in vivo by MRI and selective delivery of drugs to the tumor tissue, simultaneously.

3.3. Optical Imaging. CD133/prominin (AC133) has been identified as a CSC marker in a variety of tumors. Tsurumi et al. showed that CD133-specific monoclonal antibody AC133.1 was used for quantitative fluorescence-based optical imaging of mouse xenograft models [44]. Near-infrared fluorescence molecular tomography (FMT), a highly sensitive and fully quantitative technology, was applied as the in vivo imaging modality. Imaging worked well using CD133 overexpressing cell line however, the implanted xenograft HCT116 cells, which express CD133 at an endogenous level, had limitations. The lower CD133 expression level of the HCT116 wild-type tumors led to a generally lower signal to noise ratio compared to CD133-overexpressing tumors. They provided the first evidence that noninvasive antibody-based in vivo imaging of tumor-associated CD133 is feasible and that CD133 antibody-based tumor targeting is efficient.

In breast cancer, it was demonstrated that with noninvasive imaging approaches, as few as 10 CD44⁺ cells of stably labeled breast cancer stem cells (BCSCs) could be tracked in vivo by bioluminescence imaging (BLI) [41]. Imaging revealed that CD44⁺ cells from both primary tumors and lung metastases were highly enriched for tumor-initiating cells. The BCSCs were tagged through expression of optical bifusion reporter genes to facilitate their visualization in the living human-in-mouse breast cancer xenograft model in NOD/SCID mice. Such reporters included firefly luciferase (Luc) for whole-body tracking of cells via BLI and fluorescent proteins. In order to label BCSCs, dual-function BLI-fluorescent reporter constructs constituting the Luc coding sequence (Luc2) fused to that of fluorescent proteins were used. They fused Luc2 to enhanced GFP (*eGFP*) or the red fluorescent protein td-Tomato (*Tom*), which improved

detection in vivo relative to GFP. They established optimized parameters to transduce primary or passaged BCSCs with lentiviral vectors encoding Luc2-eGFP (L2G) or Luc2-Tom (L2T) fusion genes; thereby one cell in vitro and minimum of 10 cells of labeled BCSCs could be noninvasively visualized at 16 hours after implantation, thus greatly enhancing the accuracy and efficiency of tumorigenic assays compared with traditional monitoring assay. This study implied that the optimized strategy for labeling BCSCs with optical reporter genes can be applied to studies with CSCs in other tumors such as colon cancer, brain tumor, head-and-neck cancer, and leukemia, and to evaluate other putative CSC markers (e.g., CD24, CD133, EpCAM, ALDH1, etc.).

In addition, Vlashi et al. demonstrated that reduced 26S proteasome activity is a general feature of CICs for human glioma and breast cancer cells that can easily be exploited to identify, track, and target them in vitro and in vivo [55]. A fluorescent protein (ZsGreen) is a target of the 26S proteasome. The fluorescent positive cells had increased sphere-forming capacity, expressed CSC markers, and lacked differentiation markers in vitro. When injected into nude mice, ZsGreen-positive cells were approximately 100-fold more tumorigenic than the opposite cells. It was required approximately 100 ZsGreen-positive cells to form tumors in vivo. They proposed that ZsGreen-positive cells need to be further purified to obtain a pure CSC population. Tracking of CICs by fluorescence imaging in vivo may facilitate the search for novel therapeutic approaches that improve radiation therapy outcome.

Nestin, one of the most reliable stemness markers, is used to evaluate the identity of normal neural stem cells and glioma stem cells (GSCs). Samuel Beck et al. isolated a GSC-targeting (GSCT) peptide that demonstrated selective binding isotopes of Nestin proteins specifically expressed in GSCs. In human glioblastoma tissue specimens, the fluorescence-conjugated GSCT peptide could visualize putative GSC populations, showing its possible use as a diagnostic agent (Figure 2). Intravenously injected GSCT peptide effectively penetrated into tissues, specifically accumulated in gliomas that arise from implantation, and predominantly targeted Nestin-positive cells in these tumors (Figure 3) [27].

3.4. Multimodal Imaging. Molecular imaging of cancers has been characterized based on the sensitivity and selectivity of a single cancer probe targeting a cancer biomarker of a specific cancer cell line. The goal of multimodality imaging is to combine the best features of separate modalities. Researchers had designed a multimodal nanoparticle-based simultaneously multiple aptamers and RGD targeting (SMART) cancer probe targeting multiple cancer biomarkers to enhance the specificity and signal sensitivity for various cancers. The SMART probe was conjugated to a multimodal nanoparticle that is capable of concurrent fluorescence, MRI, and radionuclide imaging. Unlike a single aptamer or peptide-conjugated multimodal cancer probe targeting a single cancer biomarker, the multimodal SMART cancer probe simultaneously targets multiple cancer biomarkers in various cancer cells, resulting in enhanced targeting efficacy and signals [56].

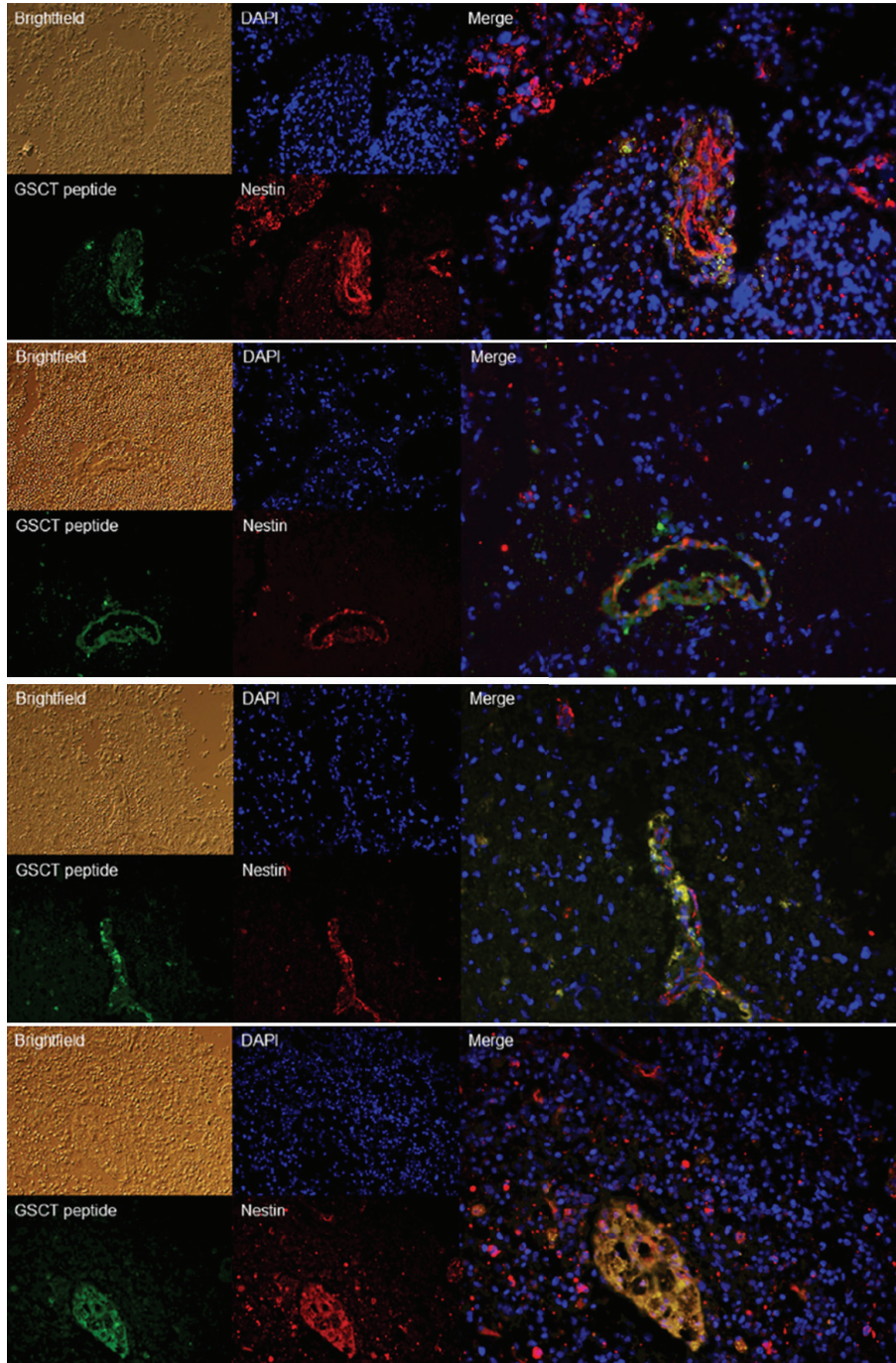


FIGURE 2: GSCT selectively binds Nestin-positive tumor cells in human GBM tissues. Paraffin-embedded human GBM tissue sections were stained with a biotinylated-GSCT (B-GSCT) peptide (Green) and Nestin antibody (Red). This immunofluorescence analysis revealed that most B-GSCT peptide-targeting tumor cells are colocalized with Nestin-positive tumor cells [27]. With reprint permission from Elsevier.

Garzia et al. demonstrated that miR-199b-5p over-expression blocks expression of several CSC genes and impairs the engrafting potential of medulloblastoma tumor (MB) stem-cell-like (CD133⁺) subpopulation of cells in the cerebellum of nude mice. They used PET-CT fusion imaging to investigate the relationship between miRNAs targeting the MB tumors. The expression of miR199b-5p can deplete this

tumor cell compartment, indicating the use of miRNA as an interesting therapeutic approach for the targeting of CSCs in brain tumors [57].

An important recent development is the concept of multimodality fusion reporter systems. Researchers have described fusion modality reporter genes for whole mouse body fluorescent, bioluminescent, and nuclear imaging [58].

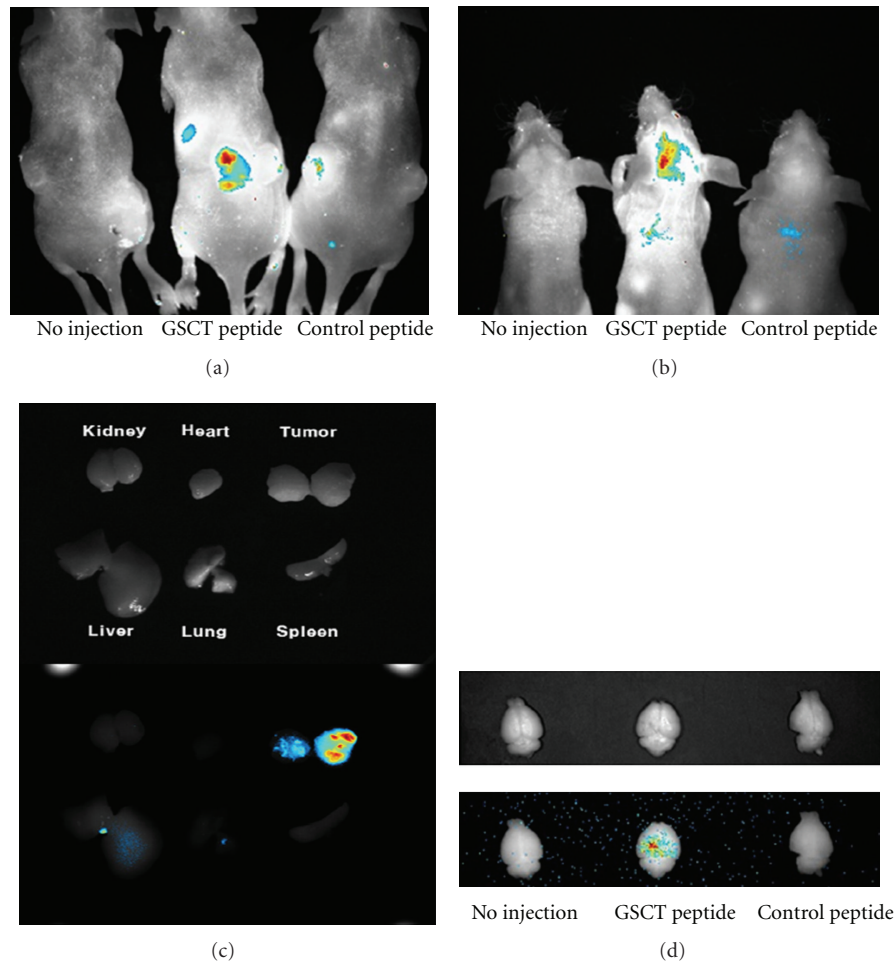


FIGURE 3: Homing of GSCT peptide to GSC-derived tumors in vivo [27]. (a) In vivo biodistribution of GSCT peptide was examined by intravenous injection of Q-dot 800-conjugated GSCT (QGSC) or control peptide in mice bearing subcutaneous tumors derived from the injection of Id4-driven mouse GSCs. Whole-body biofluorescence images were obtained 24 h after injection of Q-GSCT and Q-control peptides. (b) In vivo biodistribution of Q-Dot 800-conjugated GSCT or control peptide in orthotopic brain tumor models. Whole-body biofluorescence images were obtained 4 days after injection of Q-GSCT and Q-control peptides in mice bearing orthotopic brain tumors derived from the injection of Id4-driven mouse GSCs. (c) Fluorescence images of Q-GSCT peptides in tumors and organs extracted from mice bearing subcutaneous tumors are shown in Figure 3(a). (d) Fluorescence images of Q-GSCT peptides in brains extracted from mice bearing orthotopic tumors are shown in Figure 3(b). With reprint permission from Elsevier.

The development of multifunctional reporter platforms has the potential to provide both diagnostic and therapeutic capabilities in cancer treatment.

4. Discussion

Researchers have tried for decades to understand cancer development in the context of therapeutic strategies. The CSC model provides an attractive concept to explain why cancer contains cells resistant to chemo- and radiotherapies [2, 3]. A minority population of CSCs has the ability to self-renew, whereas the majority of cancer cells have the limited or no ability to proliferate in breast and brain tumors [59]. There has been acceptance of the idea that the remaining tumor cells after anticancer therapy have a distinct molecular phenotype that confers resistance to the therapies [2, 3].

CSCs are tumorigenic and possess the metastatic phenotype. Interestingly, many studies show that cells of CSC phenotypes also express stem/progenitor-associated genes which may suggest the possible link between CSCs and normal stem cells. A comparison of the biomarkers of CSC and normal stem cell was shown in Table 1. CSCs share common features with normal stem cells, most obviously the characteristic of self-renewal. Current knowledge indicates that a specialized microenvironment, the stem cell niche, is one of the factors regulating normal stem cell maintenance and self-renewal [59]. In analogy to the normal intestinal stem cell niche, researchers conclude that there is a CSC niche that is probably composed of a combination of stromal cells and more differentiated progeny and delivers crucial signals to the CSCs. Tumors may arise through a series of mutations that disrupt normal development

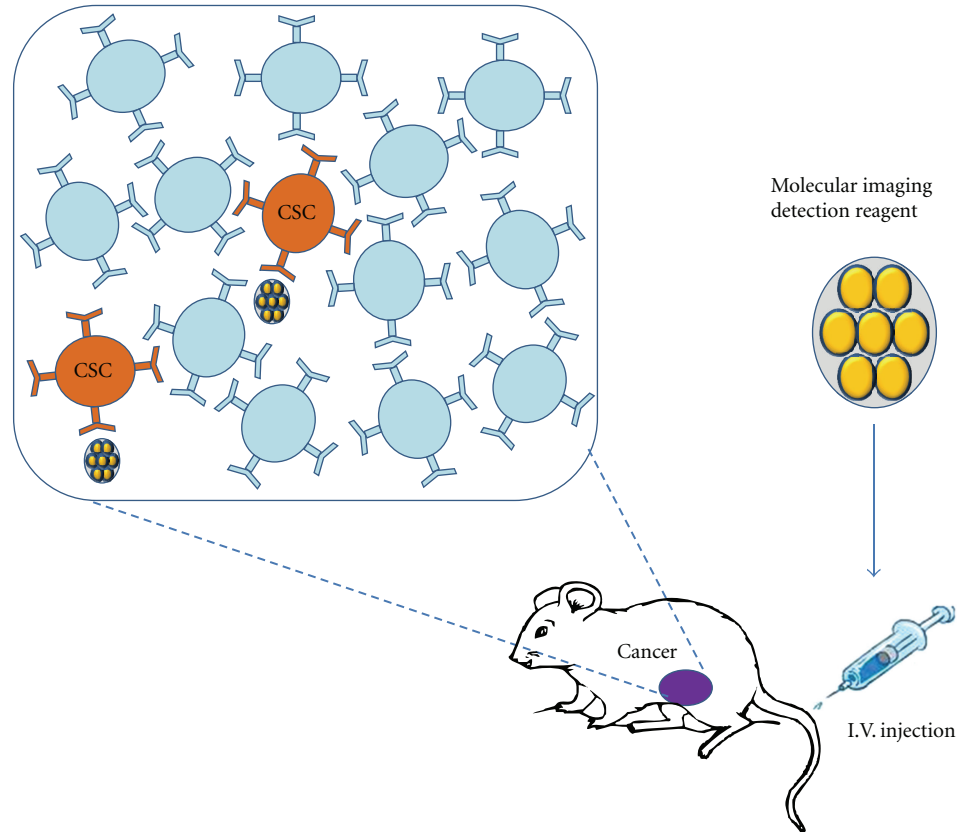


FIGURE 4: Paradigm of the application of molecular imaging in CSCs detection based on cancer stem cell model. Red and blue indicate cancer stem cells (CSCs) and non-CSCs, respectively.

pathways, and a population of continuously self-renewing cells must arise [59]. Genes (e.g., *Bmi1*) that program self-renewal rather than differentiation are likely to be candidate oncogenes [60–62]. Exploring the mechanism regulating stemness features of CSCs in the tumorigenic process will benefit developing treatment aimed at destroying CSCs without adversely affecting normal stem cell characteristics.

CSCs may often constitute only a small population of tumor mass thus, there is a need for imaging modalities and probes that provide high-sensitivity and high-imaging resolution. Molecular imaging is emerging as a promising strategy with which to shed light on the clinical application. This technique has the potential to become a powerful technique for monitoring the history and the fate of CSCs and for evaluating response to therapy. The precise real-time imaging of CSCs in tumors at molecular and cellular levels is challenging. Single-luciferase models require animals to be injected with the luciferin substrate, and the limitation of the signal in xenograft model of human neoplasia requires expression of luciferase is at least 2,500 cells for proper detection [63]. The good option may be concurrently employing more than one fluorophore and highly sensitive detectors. MRI detects the presence of SPIO contrast agents regardless of whether the cells are alive or dead; it is also challenged to count the number of SPIO labeled cells in MRI on account of the contrast agent dilution during cell

division, transfer to other cells, and the exist of other source of iron in tissue. To overcome limitations, the multimodality of imaging techniques (e.g., magnetic and optical) can be developed for applications in medicine.

For noninvasive imaging of CSCs as well as CSC-specific therapies, the targeting of cell surface proteins using antibodies or other receptor ligands is particularly relevant (Figure 4). Noninvasive imaging of CSCs would be critical in the management of malignant cancer disease, for example, for determining prognosis of patients, using as a diagnosis parameter, and monitoring therapeutic efficacy.

5. Conclusions

There is increasing evidence to support the existence of CSC that is identified by several markers, including CD133, CD44, CD24, and ALDH, or by selecting for the SP cells. Interestingly, no single marker is exclusively expressed by CSCs. Thus, in order to identify CSCs, the various combinations of different biomarkers may be possible. Targeting and elimination of the small population of CSCs hold attractive cancer treatment and avoid tumor recurrence.

Tracking of CSCs *in vivo* may facilitate the search for novel therapeutic approaches that improve radiation therapy outcome. The development of therapeutic agents specifically targeting CSCs together with a revised radiotherapy or chemotherapy protocol will alter the traditional poor

prognosis of tumor patients. Novel drugs that selectively target CSCs offer great promise for more completed cancer treatment; however, designing and finding such drugs remain a great challenge.

Still, much work needs to be done in order to completely understand the origin of CSCs, as well as the mechanisms regulating their stemness features in the tumorigenic process and their increased resistance to chemotherapy and radiotherapy. Although the tracking of circulating CSCs is still hampered by technological challenges, we expect personalized diagnosis and therapy of cancers to be established based on increased studies and applications of molecular imaging of biomarkers in cancer stem-like cells.

Abbreviations

CSCs:	Cancer stem cells
CICs:	Cancer initiating cells
T-ICs:	Tumor-initiating cells
SP:	Side population
PSCA:	Prostate stem cell antigen
EpCAM:	Epithelial cell adhesion molecule
ESA:	Epithelial-specific antigen
PET:	Positron emission tomography
BLI:	Bioluminescence imaging
MRI:	Magnetic resonance imaging
TCL-SPIONS:	Thermally cross-linked superparamagnetic iron oxide nanoparticles
FMT:	Near-infrared fluorescence molecular tomography.

Conflict of Interests

The authors declare that they have no conflict of interests.

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