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Review Article

In vivo molecular imaging of cancer stem cells

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Abstract: A rare subpopulation of cancer cells known as cancer stem cells (CSCs) have distinct characteristics resembling stem cells, including cell renewal capability, differentiation into multiple lineages, and endless proliferation potential. Cumulating evidence has revealed that CSCs are responsible for tumorigenicity, invasion, metastasis, and therapeutic resistance. Despite continued investigation of CSCs, *in vivo* behavior of CSCs is not yet fully understood. The *in vivo* imaging modalities of optical, nuclear, and magnetic resonance are currently being employed to investigate the complexity behind the CSCs behavior. Valuable information that were previously obscured by the limitations of *in vitro* techniques now are currently being revealed. These studies give us a more comprehensive insight about what happen to CSCs *in vivo*. This review will briefly discuss the recent findings on CSCs behavior as informed by *in vivo* imaging studies.

Keywords: Cancer stem cell, MRI, PET, fluorescence imaging, *in vivo* imaging, molecular imaging, cell tracking

Introduction

Tumors consist of both tumor cells and those that make up the tumor microenvironment. Tumor cells are composed of heterogeneous subpopulations with distinct genotypes and phenotypes, which may harbor divergent biological behaviors [1]. Recently, there is accumulating evidence on the existence of a tumor subpopulation termed cancer stem cell (CSCs) with distinct characteristics resembling stem cells [2]. This CSCs hypothesis has become one of the most intriguing and challenging concepts in oncology. This hypothesis states that the initiation and growth of cancer is controlled by a small subpopulation that has stem cells-like characteristics. Eradication of the stem-cell compartment of a tumor may be essential to achieve stable, long-lasting remission, and cure of cancer [3].

Many studies have been performed over the past 40 to 50 years to learn about CSCs [4-8]; however, the complex behavior of CSCs *in vivo* remains largely a mystery. Nowadays, *in vivo* imaging technologies provide opportunities to deepen our understanding about CSC characteristics. Advances in experimental and clinical imaging enable researchers and physicians not only to locate the CSCs but also to assess the tumor biological processes involving CSCs. This review will briefly discuss *in vivo* imaging modalities and techniques that have been used to visualize and track CSCs.

Cancer stem cells

CSCs are defined by The American Association for Cancer Research Stem Cell Workshop as cells within a tumor that possess the capacity to self-renew and to inherit heterogeneous lineages of cancer cells that comprise the tumor [9]. Compared to the other tumor subpopulations, CSCs are unique due to three distinctive characteristics (**Table 1**): the capacity of self-renewal (i.e., one or both daughter cells retain the same biologic properties as the parent cell at cell division), the capability to develop into multiple lineages, and the potential to proliferate extensively [2].

The first evidence of CSCs existence was found 50 years ago. A single tumor cell derived from ascites fluid of teratocarcinomas and leukemia had tumorigenic potential and could generate heterogeneous progeny [5, 8, 10]. Later, CSCs

Table 1. Characteristics of CSCs

Characteristics of CSCs	Reference
Capacity of self-renewal	[4, 6, 7, 14]
Capability to develop into multiple lineages	[4, 6, 7, 14]
Potential to proliferate extensively	[4, 6, 7, 14]
Rare subpopulation of cells	[4]
Radioresistance	[22, 23]
Chemoresistance	[19-21]
Promote invasion and metastatic activity	[26-28]

Table 2. CSCs surface biomarker

Tumor entity	Markers	Ref.
Breast cancer	CD44+CD24-/low	[14]
	CD133+	[73]
Glioblastoma	CD133+	[15]
Prostate cancer	CD44+/$\alpha_2\beta_1^{hi}$/CD133+	[6]
Melanoma	CD20+	[74]
Lung cancer	CD133+	[75]
	CD44 high CD90+	[76]
Liver cancer	CD133+	[77]
	CD90+CD44+	[78]
Colon cancer	CD133+	[79]
	EpCAM+CD44+CD166+	[7]
Pancreatic cancer	CD44+CD24+ESA+	[12]
	CD133+CXCR4+	[27]
Head and neck cancer	CD44+	[13]
Acute myeloid leukemia	CD34+CD38+	[42]
Multiple myeloma	CD138+	[41]

were found in hematological tumor. Injection of a small subset of leukemic cells with a primitive hematopoietic progenitor phenotype (CD34+ CD38-) into immunodeficient mice gave them Acute Myeloid Leukemia (AML). The frequency of this subset was found to be rare (± one per million tumor cells) [4]. Since then, many studies were performed to identify and isolate CSCs subpopulations in other tumor types including brain, breast, colon, pancreas, prostate, lung, and head and neck cancer [6, 7, 11-13].

CSCs may come from normal tissue stem cells (TSCs) that undergo mutation. TSCs, which proliferate throughout life, are more susceptible to oncogenic mutations. Moreover, TSC markers and differentiation markers such as CD133 and CD44 have been found in many cancers [14, 15]. Also, stem cells and precursor cells transformed with oncogenic genes can develop cancer *in vivo* [16]. However, several experiments showed that CSCs might also arise from more committed progenitor cells that regained self-renewal properties [16-18].

CSCs are less sensitive to conventional therapies such as chemotherapy [19-21] and radiotherapy [22, 23]. This resistance is due to factors such as the quiescent state of CSCs, enhanced DNA repair, upregulated cell cycle control mechanisms, reactive oxygen species scavenging, and interaction with stromal environment. The detailed mechanisms of radioresistance and chemoresistance are well described in other reviews [24, 25]. CSCs also promote invasion and metastatic activity [26-28].

The initial approach in CSC investigation is isolation and identification. Assays for CSC activity should focus on demonstrating both self-renewal and tumor propagation, because those two properties are the hallmarks of CSC. The gold standard assay that fulfills these criteria is serial transplantation in animal models. Unfortunately, this method is tedious and time consuming [9]. To overcome those limitations, simpler methods have been devised such as cell side population (SP) exclusion [29-32], floating sphere formation [20, 33-36], and aldehyde dehydrogenase (ALDH) activity assay [37-40].

Another important method used for the identification of CSCs is based on knowledge derived from hematopoietic or embryonic stem cell development (**Table 2**). For example, CD138, a marker for terminally differentiated B cells, is negative in multiple myeloma (a plasma cell malignancy) CSCs [41]. Likewise, CSC markers from acute myelogenous leukemia are CD34+CD38- which are the same markers used to identify normal early hematopoietic progenitor cells [42]. Surface markers allow for the definition of a precise population. However, they may not be sufficiently reliable in discriminating cancer stem cells from non-stem cell subpopulations. Another limitation is that markers derived from cell-line studies or mouse tumors have yet to be validated in human tumors [43, 44].

Imaging cancer stem cell

Advances in imaging technology allow us to explore the biology of CSCs. Some of these modalities are promising as clinical applications. Modalities used for *in vivo* imaging of

Table 3. Advantages and limitations of imaging modality for CSC study [46, 48, 80]

Imaging modality	Resolution[1]	Depth	Time for image acquisition	Quantitative[2]	Target	Clinical use	Notes
MRI	10-100 μm	no limit	minutes to hours	yes	Anatomical, physiological, molecular	yes	Non-invasive, non-radioactive, excellent spatial resolution but costly
CT	50 μm	no limit	minutes	yes	Anatomical, physiological,	yes	Usually combined with PET or SPECT, high radiation exposure
PET/SPECT	1-2 mm	no limit	minutes to hours	yes	Physiological, molecular	yes	Versatile imaging modality with many tracers and high sensitivity
Fluorescence reflectance imaging	2-3 mm	< 1 cm	seconds to minutes	no	Physiological, molecular	yes	Suitable for imaging molecular events at surface tumor but have limited depth resolution
FMT	1 mm	< 10 cm	minutes to hours	yes	Physiological, molecular	in development	Quantitative imaging of fluorochrome reporters
Bioluminescence imaging	several mm	cm	minutes	no	Molecular	no	Can detect gene expression rare cells such CSC at high resolution
Intravital microscopy[3]	1 μm	< 400-800 μm	seconds to hours	no	Anatomical, physiological, molecular	in development	Single cell resolution but have limited depths and coverage for CSC study

[1]High resolution for small animal imaging. [2]Quantitative here means inherently quantitative. All approaches allow relative quantification. [3]Laser-scanning confocal or multiphoton microscopy.

CSCs include radionuclide imaging such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), intravital imaging, bioluminescence imaging, and various fluorescence imaging including fluorescence-mediated tomography (FMT) and near infrared (NIR) fluorescence reflectance imaging.

In case of *in vivo* CSCs imaging, there is no "best" imaging modality. The choice of specific imaging modality is depend on the purpose of the study. For investigating therapeutic response or metastatic CSCs, PET and MRI are the most suitable modality. Whereas optical imaging modality is favorable for investigating tumor propagation and plasticity of CSCs with single cell resolution. In **Table 3**, we summarized each of imaging modality's strengths and limitations.

In CSCs imaging, we need to choose the specific imaging modality, the reporter gene or probe, the imaging requirement (single versus repeated acquisition), intended use (animal versus human), and spatial requirements (organ versus cellular resolution) [45]. Because CSCs are rare subpopulation of cells, the imaging modality must be sensitive to the contrast agent at a resolution that can detect cells with frequency as low as one in one thousand [46].

Optical imaging

Optical imaging is unequivocally the most versatile and widely used modality in clinical practice and research. In addition, optical imaging techniques are the easiest to apply to CSCs study at the resolution of a single cell, and relatively cheaper than other imaging modalities [46] .

As CSCs are a rare subpopulation of cells, the main concerns in optical imaging are choosing reporter signal and imaging modalities. Bioluminescence signal can define tumor growth, regression, and metastases but its spatial resolution and sensitivity in identifying rare cells is limited [46]. Luciferase reporter plasmid is highly sensitive for measuring biologic activity in growing tumor, but in luciferase models, animals must be injected with the luciferin substrate. Detection of signal *in vivo* requires expression of luciferase in at least 2500 cells [47].

Fluorescence imaging is currently the best choice for imaging CSCs. Sensitive detectors and the intensity and stability of fluorescence signal allow imaging of fluorescent cells *in vivo* at high resolutions. Multiple fluorophores can be used at the same time, which is useful in imaging the complex biological features of CSCs.

Factors that need to be considered in choosing optical imaging devices for studying CSCs are depth of tissue penetration, imaging time points, and use of multispectral unmixing [46]. Depth of penetration is important in studying and imaging internal organs or subcutaneous xenograft models. Near-infrared fluorescence (NIR) probes have the highest penetration.

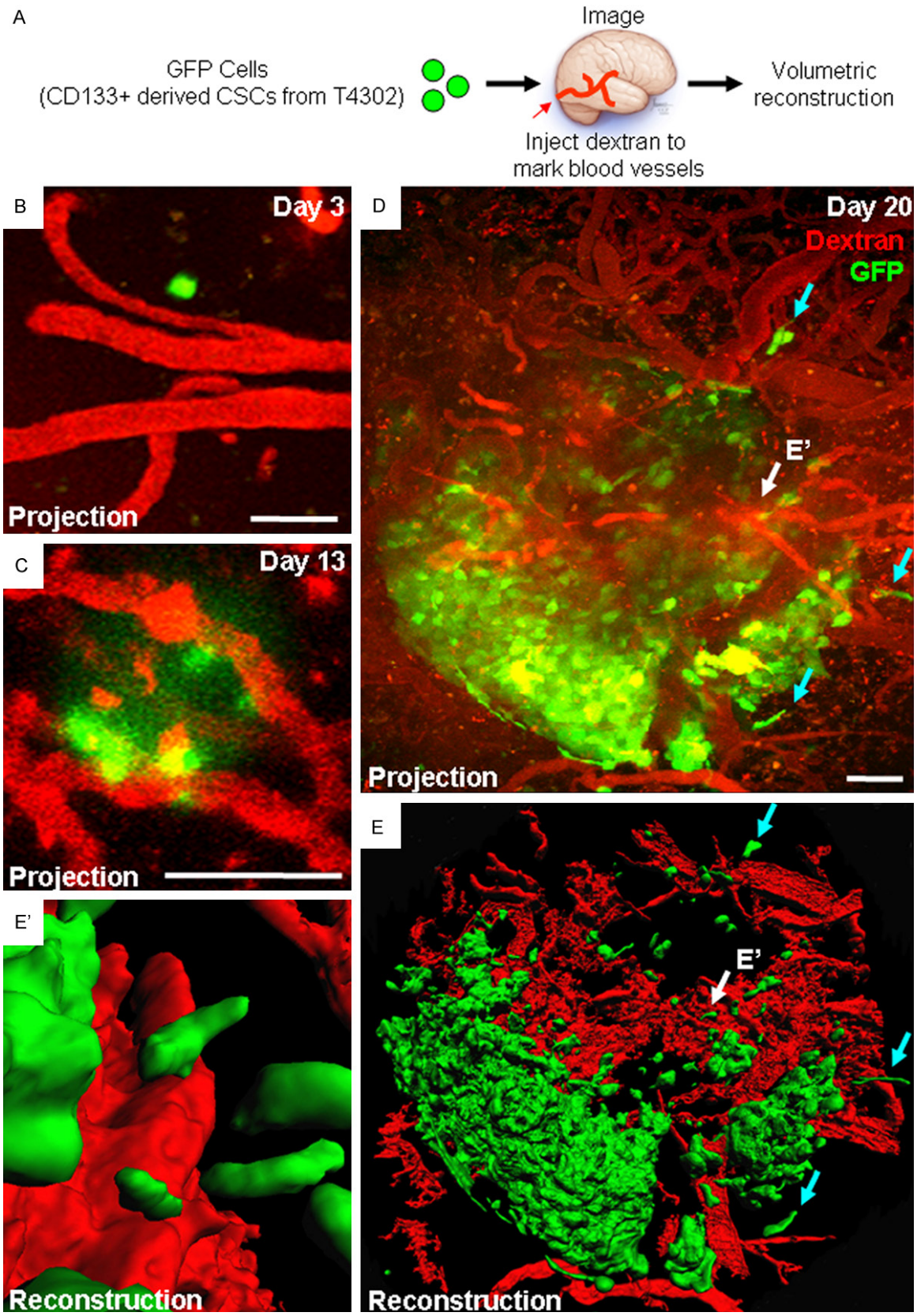

Figure 1. In vivo multiphoton microscopy from experiments depicted in scheme (A) followed up the tumor growth from cancer stem cells. Projection micrographs (B-D) demonstrate tumor formation over time and three-dimensional

reconstructions depicted in micrographs (E, E') revealed CSCs (white arrows) was associated with blood vessels and and grew in proximity with blood vessels (D, E). Over time (from day 13 to 20), a tumor nodule rapidly formed and tumor cells (blue arrows) were observed to infiltrate the peripheral regions (D, E). Fluorescent dextran (shown in red) was injected into the circulation to illuminate blood vessels prior to imaging. Scale bar represents 50 μm [51].

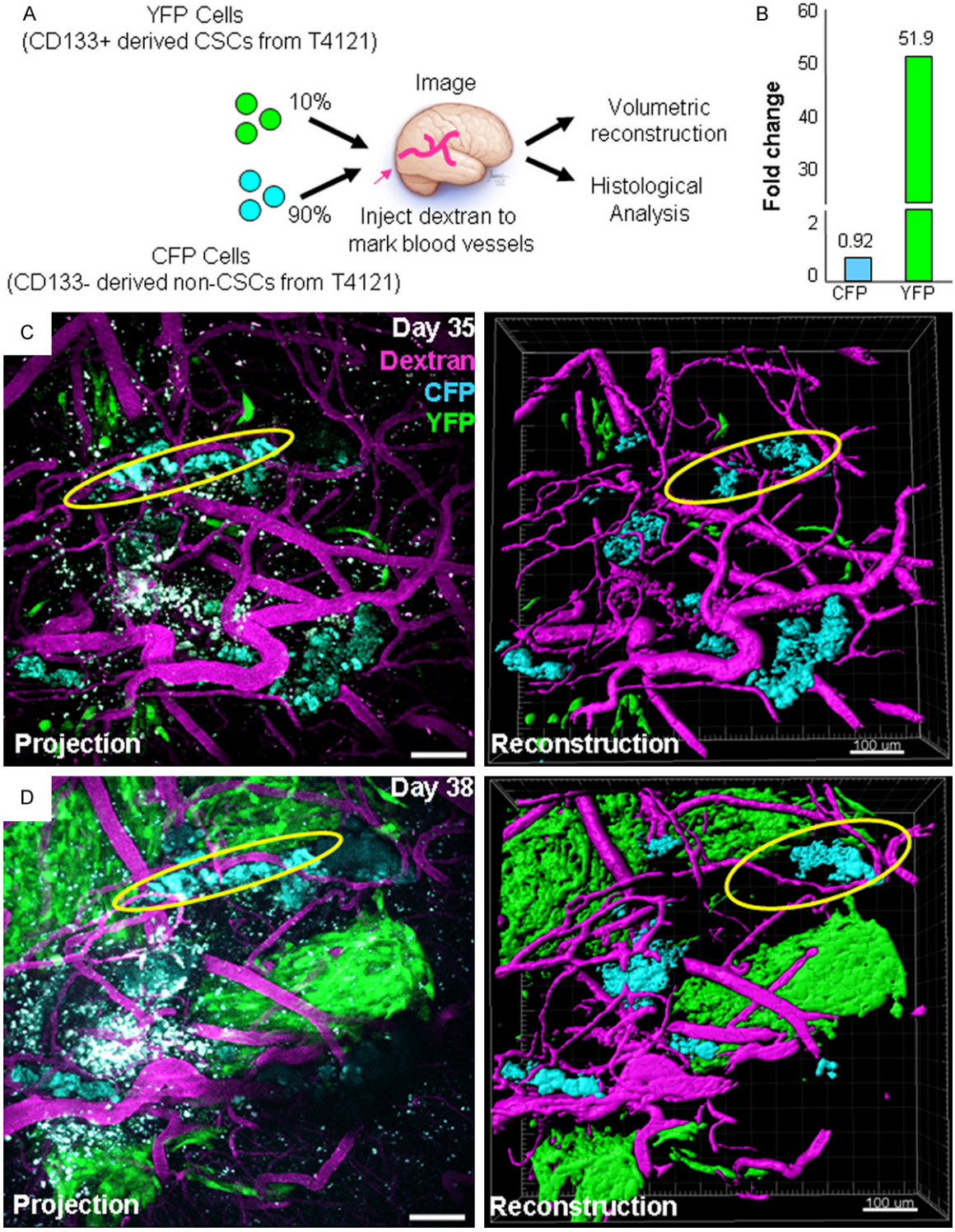

Figure 2. Fractionated CSCs and non-stem tumor cells were labeled with different fluorescent proteins and transplanted into mice at a 10% cancer stem cell (YFP) to 90% non-stem tumor cell (CFP) ratio as shown in experimental

design schematic (A). Graph (B) which is calculated based on three-dimensional reconstructions of projection micrographs (C, D) demonstrated that CSCs outgrew non-CSCs in vivo. Additionally, tumor populations did not intermingle *in vivo* (non-stem tumor population indicated by yellow oval). Fluorescent dextran (shown in purple) was injected into the circulation to illuminate blood vessels prior to imaging. Scale bar represents 100 μm [51].

Surgical manipulation such as skin flaps can also enhance penetration of probes. Multiple imaging time points are necessary in longitudinal studies. Spectral unmixing removes autofluorescence and can distinguish between emission peaks of nominal separation [48].

There have been studies imaging CSCs using optical imaging. Tsurumi *et al* showed *in vivo* detection of CD133, a cancer stem cell surface marker for a variety of tumor entities. They labeled AC133.1, a CD133-specific monoclonal antibody (mAb), with fluorescence dye for quantitative NIR fluorescence molecular tomography (NIR FMT) imaging on mouse subcutaneous xenograft models. They visualized and quantified CD133 in lentivirally-transduced CD133 overexpressing cells lines. Cells that express CD133 at endogenous levels had lower signal to noise ratio [49].

Gaedicke *et al* continued the Tsurumi *et al* study by showing that *in vivo* CD133 imaging using fluorescence labelled-mAb could also be applied to an orthotopic glioblastoma model. The fluorescence signal of ACC133 mAb was significantly higher in the tumor region than in the isotype control antibody. This study revealed that systemically administered antibodies may reach extravascular targets in brain tumors with a disturbed blood brain barrier, and also showed the potential of clinical translation of CD133 imaging [50].

Intravital microscopy techniques can be used study the behavior of cells by visualizing tissues in living animals. Advances in intravital microscopy enable visualization of CSCs at a resolution of up to 1 μm, allowing single cell resolution imaging. Additional developments are deeper penetration, minimal image distortion, signal quantification, and three-dimensional image reconstruction [51, 52].

Using intravital microscopy, Lathia *et al* depicted CSCs growth *in vivo* over a temporal time course using lentivirus-transduced fluorescence-labeled CSCs (CD133+ cells). As the first intravital imaging study using multiphoton microscopy to observe tumor growth from CSCs, this study showed how CSCs flourished from a perivascular niche and outgrew the non-CSCs cells over a temporal time course (**Figures 1** and **2**) [51].

Stem cells-like properties may not be permanent. CSCs may lose stemness properties, and non-CSCs may gain them. This phenomenon is known as stem cell plasticity [53, 54]. Intravital microscopy has been used to observe cancer stem cell plasticity. Zomer *et al* proved this hypothesis by intravital lineage tracing. They lineage traced the growth of genetic mammary tumors that expressed a tamoxifen-activated Cre confetti construct. In this mouse models, Cre activation induced random expression of one of four confetti fluorescent colors (cyan, green, yellow, and red). Cre-expressing cells and their progeny would be genetically marked by one of the confetti colors, which allowed lineage tracing of these cells. They observed that only some of the cell grew and formed single colored patches four days after being activated. This showed that a tumor population developed from a small tumor subpopulation. Several days later, other new patches appeared, while some of the previous patches regressed, suggested that CSC properties were occasionally acquired and may also be lost [55].

A new NIR-sensitive molecular imaging probe based on hydrogel complexes can visualize CD44-expressing cancer stem cells in gastric cancer. The probe, NIR-sensitive supramolecular hydrogels (Cy 5.5-conjugated polyethyleneimine/hyaluronic acidpolyplexes), was fabricated by polyplexing in an aqueous medium. This probe demonstrated good water-stability, biocompatibility, and specificity to CD44 [56].

Another approach to study CSC behavior *in vivo* is by using reporter genes. Liu *et al* applied dual-function bioluminescence imaging-fluorescent reporter constructs (Luc2 fused with eGFP coding sequence) in breast cancer stem cells (BCSCs). Luc2 sequence was employed for whole body tracking by bioluminescence imaging (BLI) while eGFP was used for intravital imaging and *ex vivo* analysis. These optical reporters stably expressed over series of tumor

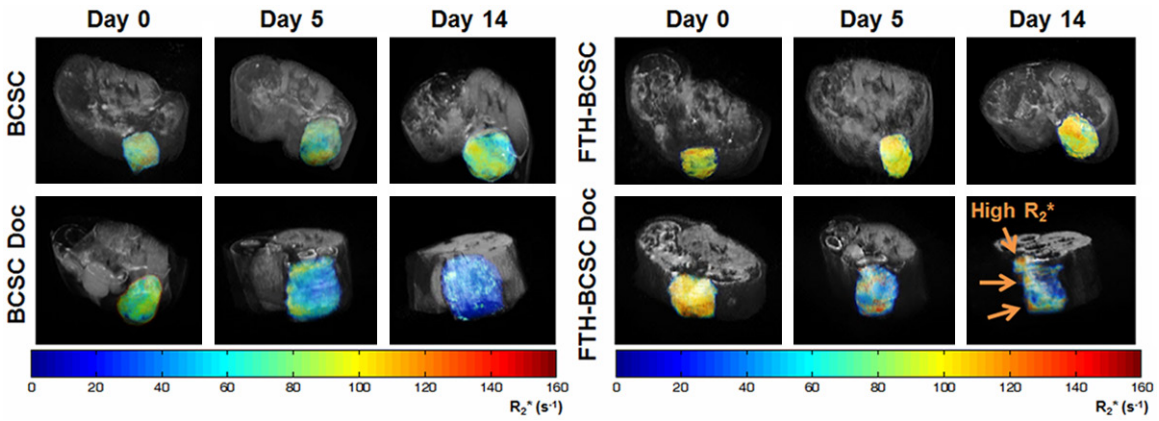

Figure 3. Follow-up volumetric MRI images of BCSC, BCSC treated with docetaxel (BCSC Doc), FTH-BCSC and FTH-BCSC treated with docetaxel (FTH-BCSC Doc). After docetaxel treatment, the mean R2* values of the BCSC Doc and FTH-BCSC Doc tumors were significantly decreased compared with the BCSC and FTH-BCSC tumors at days 5 and days 14. Moreover MRI images, revealed significantly different signal intensities (R2* values) between FTH genes-transduced BCSCs (FTH-BCSC & FTH-BCSC Doc) and wild type BCSCs (BCSC & BCSC Doc) [69].

progeny. Using BLI, as few as ten injected $CD44^+$ BCSCs could be tracked *in vivo*, enabling studies of early tumor growth and spontaneous metastasis [57].

Besides surface protein markers, proteasome activity can also be used for *in vivo* tracking of CSCs. Vlashi *et al* found that 26S proteasome activity was reduced in CSCs. Using this finding, they performed *in vivo* CSCs tracking in human glioma and breast cancer. They engineered cancer cells to stably express fluorescence fusion protein (ZsGreen-ornithine decarboxylase) that accumulates in cells that have reduced 26S proteasome activity. ZsGreen-positive cells were successfully tracked by *in vivo* fluorescence imaging. Compared with the ZsGreen-negative population, secondary sphere-forming capacity and *in vivo* tumorigenicity of ZsGreen-positive cells were significantly higher. Furthermore, ZsGreen-high cells were positive for the stem cell marker nestin, Musashi-1, and Sox2 and negative for differentiation markers such as GFAP and neuron-specific class III β-tubulin *in vitro*. These findings suggest that ZsGreen-high cells are CSCs [58].

Positron emission tomography (PET)

PET quantitatively detects high-energy γ-rays emitted from a subject injected with positron-emitting isotopes or isotope-labeled molecular probes. PET is highly sensitive, non-invasive, permits real time tracking *in vivo*, and independent of the depth from which the signal is emitted. However, until now, there is no PET technology capable of detecting CSC at single cell resolutions.

Jin *et al* investigated the potential of *in vivo* radionuclide imaging of CSCs using Iodine-125-labeled ANC9C5, an anti-human CD133 antibody, in colon carcinoma xenografts. Although a favorable biodistribution profile was not obtained, intratumoral distribution of ^{125}I-labeled ANC9C5 depicted on autoradiography was overlapped with CD133 immunohistochemistry expression in many areas [59]. This study revealed the potential of radioimmunotargeting of CSCs using PET or SPECT.

Gaedicke *et al* performed PET imaging to detect AC133, an epitope of the second extracellular loop of CD133. In this study, two cell lines over-expressing CD133 (engineered and patient-derived) were xenografted in mice and imaged using ^{64}Cu-NOTA-AC133. The imaging yielded accurate and high-resolution images of brain tumor lesions 2-3 mm in size, with significant tumor-to-background contrast. This study disclosed the differences in invasive behavior between orthotopically growing U251 (noninvasive) and NCH421k (invasive) gliomas. More interestingly, PET signal intensity accurately reflected the microscopic pattern of tumor AC133+ expression. This was observed in sharply delineated PET images of U251 tumor that has compact and spherical microscopic appearance. On the other hand, the chaotic and infiltrative growth pattern in NCH421k

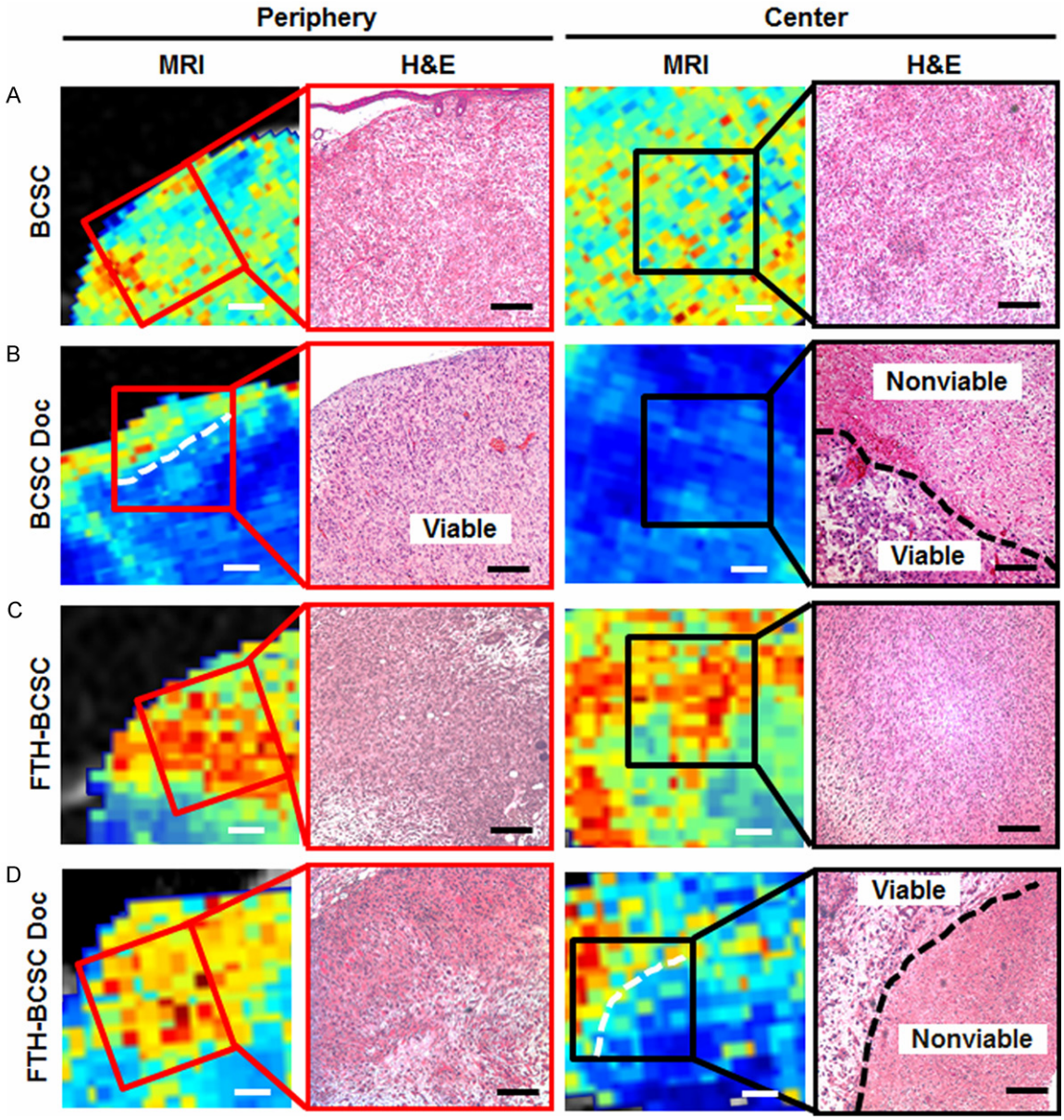

Figure 4. Histological analysis with H&E staining of the area in the red and black boxes of MRI images on FTH genes-transduced BCSCs (FTH-BCSC & FTH-BCSC Doc) and wild type BCSCs (BCSC & BCSC Doc). High R2* value pixel showed the area of viable cells and the FTH gene enhances the contrast in MRI images by further increased the R2* values (A-D). Similar distribution of R2* value pixels in MRI between the periphery and center of both BCSC and FTH-BCSC were confirmed with H&E staining; FTH genes transduction enhanced the R2* values (A & C). After Docetaxel therapy, non viable cells were appeared in the center of the tumors (demarcated with black dotted line) (B & D). Without FTH genes transduction, different R2* value pixel in MRI (demarcated with white dotted line) failed to represent cell viability as shown by H&E staining (B). In FTH-BCSC cells, R2* value pixel correspond well with cell viability in both periphery and center of the tumor (D). Scale bar in H&E and MRI represents 100 μm and 500 μm respectively [69].

tumors was represented by more diffuse PET signal [50].

Magnetic resonance imaging (MRI)

From pre-clinical and clinical point of view, MRI is a non-invasive and robust imaging modality with high spatial resolution. MRI does not rely on radioactive isotopes, which may be an important advantage for longitudinal studies. Moreover, MRI can collect not only morphological but also pathophysiological information in living objects. The ability of MRI with contrast

agents for labeling cells providing dynamic assessment of cell migration into target tissues [60]. MRI has been used to detect single cells in small animals in a wide range of studies extending from stem cells to cancer cell tracking [61].

To pinpoint a cell of interest from background signal, a label capable of producing significant positive or negative contrast on the appropriate pulse sequence is an absolute must. In addition to strong signal-altering properties (T1, T2 relaxation), an "ideal" label should have characteristics such as biocompatibility, lack of genetic modification or perturbation to the target cell, capability of single-cell detection, ability to image target cells over a long period, and ability to quantify cell number at a given locus [62].

Most of the magnetic resonance labels currently used in cell tracking are ultrasmall superparamagnetic iron oxide (USPIO) or superparamagnetic iron oxide (SPIO). Advantages of these contrast agents over conventional paramagnetic gadolinium-based contrast agents are low toxicity, subnanomolar-range detection limits, and higher contrast enhancement [63-65]. SPIO has successfully detected and tracked transplanted human hepatic stem cells *in vivo* and also glioblastoma CSCs *in vitro* [66, 67].

In MRI, the amount of USPIO and SPIO, not the number of cells, determines signal change. Cell proliferation distributes iron into daughter cells, so the signal from individual cells decreases with each generation. Iron released from apoptotic or lytic cells can be internalized by macrophages in nearby tissue nearby, resulting in signal wrongly associated with target cells [68]. Choi *et al* used the MRI reporter gene ferritin to overcome these limitations for detecting human breast cancer stem cells (BCSCs). The overexpression of ferritin enabled cells to uptake more iron, producing low signal intensities in MRI [69]. As MRI reporters are stably expressed, even during cell division, they can be used for studying dynamic processes, for, example, the migration and invasion of cells of interest over an extended period. This can provide temporal and spatial information for anticancer treatment effects on a specific cell population. Quantification of the number of viable cancer cells in deep tissues can be monitored by calculating R_2^* (= $1/T_2^*$) values from T_2^* mapping of MRI images [70, 71].

Choi *et al* utilized dual reporter gene (human ferritin heavy chain [FTH] and enhanced green fluorescence protein [eGFP]) transduced-BCSCs transplanted into NOD/SCID mice to allow noninvasive tracking of BCSC-derived populations and to show viable cell populations of tumors after docetaxel chemotherapy. MRI revealed significantly different signal intensities (R_2^* values) between BCSCs and FTH-BCSCs *in vitro* and *in vivo* (**Figure 3**). Histological analysis revealed that areas showing high R_2^* values in docetaxel-treated FTH-BCSCs tumors by MRI contained more viable cell populations with high percentages of BCSCs (**Figure 4**). This experimental model system can be used to investigate the best treatments of BCSCs-derived tumors [69].

Another labelling technique targeting cancer stem cells has also been developed. Lim *et al* designed hyaluronan-modified magnetic nanoclusters (HA-MNCs) for *in vitro* and *in vivo* detection of CD44-overexpressing breast cancer using a MR imaging. Hyaluronan, an immune-neutral polysaccharide, is a ligand for CD44. Biocompatible magnetic nanoclusters (MNC) of less than 100 nm in size is the optimum vehicle for this ligand since it may hinder reticuloendothelial trap to prolong its circulation and at the same time ensure tumor accumulation via enhanced permeation and retention effect. Hence, HA-MNCs exhibited excellent targeting efficiency to CD44-overexpressing cancer cells. Signal intensity on T2-weighted images was decreased in HA-MNCs-treated mice, demonstrating a remarkably high MR contrast effect. It can be concluded that HA-MNCs has high potential to image cancer stem cells *in vivo* and promising for diverse applications in tumor diagnosis [72].

Conclusion

Even though CSCs have been investigated extensively, their *in vivo* behavior is still unclear. *In vitro* studies may not sufficiently depict the complexity of CSC biology. *In vivo* imaging techniques allow CSCs tracking and obtaining valuable information such as tumor development from CSC populations, stem cell plasticity, and their interaction with the surrounding environ-

ment. These techniques may be used to investigate yet unexplored behavior such as interaction with the immune system, behavior under hypoxic conditions and stem cell-like property inheritance.

CSCs are also responsible for therapeutic resistance and tumor recurrence. Developing imaging modalities to localize CSCs will help ensure CSCs eradication for a complete cancer cure. Discovery of more accurate yet biocompatible tracers, development of better techniques and higher imaging resolution and contrast are in progress. MRI, PET, and fluorescence imaging are the most promising modalities for clinical application of CSCs detection. Integration of all aspects of molecular imaging for CSCs detection will accelerate bench-to-bedside translation.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Abbreviations

CSCs, Cancer stem cells; AML, Acute Myeloid Leukemia; TSC, tissue stem cell; SP, side population; ALDH, Aldehyde Dehydrogenase; PET, Positron Emission Tomography; MRI, Magnetic Resonance Imaging; FMT, Fluorescence-Mediated Tomography; NIR, Near Infrared; mAb, monoclonal antibody; BCSCs, breast cancer stem cells; SPECT, Single Photon Emission Computed Tomography; USPIO, Ultrasmall Superparamagnetic Iron Oxide; SPIO, Superparamagnetic Iron Oxide; HA-MNCs, hyaluronan-modified magnetic nanoclusters.

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References

[1] Fisher R, Pusztai L and Swanton C. Cancer heterogeneity: implications for targeted therapeutics. Br J Cancer 2013; 108: 479-485.

[2] Jordan CT, Guzman ML and Noble M. Cancer stem cells. N Engl J Med 2006; 355: 1253-1261.

[3] Al-Hajj M, Becker MW, Wicha M, Weissman I and Clarke MF. Therapeutic implications of cancer stem cells. Curr Opin Genet Dev 2004; 14: 43-47.

[4] Bonnet D and Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 730-737.

[5] Bruce WR and Van Der Gaag H. A Quantitative Assay for the Number of Murine Lymphoma Cells Capable of Proliferation in Vivo. Nature 1963; 199: 79-80.

[6] Collins AT, Berry PA, Hyde C, Stower MJ and Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 2005; 65: 10946-10951.

[7] Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C and Clarke MF. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A 2007; 104: 10158-10163.

[8] Kleinsmith LJ and Pierce GB Jr. Multipotentiality of Single Embryonal Carcinoma Cells. Cancer Res 1964; 24: 1544-1551.

[9] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL and Wahl GM. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res 2006; 66: 9339-9344.

[10] Makino S. Further evidence favoring the concept of the stem cell in ascites tumors of rats. Ann N Y Acad Sci 1956; 63: 818-830.

[11] Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT and Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 2005; 121: 823-835.

[12] Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF and Simeone DM. Identification of pancreatic cancer stem cells. Cancer Res 2007; 67: 1030-1037.

[13] Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF and Ailles LE. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S A 2007; 104: 973-978.

[14] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ and Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983-3988.

[15] Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD and Dirks PB. Identification of human brain tumour initiating cells. Nature 2004; 432: 396-401.

[16] Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N, Rowan R, Amaral S, Curley

D, Williams IR, Akashi K and Gilliland DG. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. Cancer Cell 2004; 6: 587-596.

[17] Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A, Sawyers CL and Weissman IL. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med 2004; 351: 657-667.

[18] Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, Golub TR and Armstrong SA. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature 2006; 442: 818-822.

[19] Eramo A, Ricci-Vitiani L, Zeuner A, Pallini R, Lotti F, Sette G, Pilozzi E, Larocca LM, Peschle C and De Maria R. Chemotherapy resistance of glioblastoma stem cells. Cell Death Differ 2006; 13: 1238-1241.

[20] Ghods AJ, Irvin D, Liu G, Yuan X, Abdulkadir IR, Tunici P, Konda B, Wachsmann-Hogiu S, Black KL and Yu JS. Spheres isolated from 9L gliosarcoma rat cell line possess chemoresistant and aggressive cancer stem-like cells. Stem Cells 2007; 25: 1645-1653.

[21] Ma S, Lee TK, Zheng BJ, Chan KW and Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene 2008; 27: 1749-1758.

[22] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD and Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006; 444: 756-760.

[23] Phillips TM, McBride WH and Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. J Natl Cancer Inst 2006; 98: 1777-1785.

[24] Baumann M, Krause M and Hill R. Exploring the role of cancer stem cells in radioresistance. Nat Rev Cancer 2008; 8: 545-554.

[25] Moncharmont C, Levy A, Gilormini M, Bertrand G, Chargari C, Alphonse G, Ardail D, Rodriguez-Lafrasse C and Magne N. Targeting a cornerstone of radiation resistance: cancer stem cell. Cancer Lett 2012; 322: 139-147.

[26] Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur MH, Diebel ME, Monville F, Dutcher J, Brown M, Viens P, Xerri L, Bertucci F, Stassi G, Dontu G, Birnbaum D and Wicha MS. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res 2009; 69: 1302-1313.

[27] Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ and Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 2007; 1: 313-323.

[28] Liao J, Qian F, Tchabo N, Mhawech-Fauceglia P, Beck A, Qian Z, Wang X, Huss WJ, Lele SB, Morrison CD and Odunsi K. Ovarian cancer spheroid cells with stem cell-like properties contribute to tumor generation, metastasis and chemotherapy resistance through hypoxia-resistant metabolism. PLoS One 2014; 9: e84941.

[29] Dean M. ABC transporters, drug resistance, and cancer stem cells. J Mammary Gland Biol Neoplasia 2009; 14: 3-9.

[30] Hadnagy A, Gaboury L, Beaulieu R and Balicki D. SP analysis may be used to identify cancer stem cell populations. Exp Cell Res 2006; 312: 3701-3710.

[31] Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA and Brenner MK. A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci U S A 2004; 101: 14228-14233.

[32] Kondo T, Setoguchi T and Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci U S A 2004; 101: 781-786.

[33] He QZ, Luo XZ, Wang K, Zhou Q, Ao H, Yang Y, Li SX, Li Y, Zhu HT and Duan T. Isolation and characterization of cancer stem cells from high-grade serous ovarian carcinomas. Cell Physiol Biochem 2014; 33: 173-184.

[34] Ma B, Lei X, Guan Y, Mou LS, Yuan YF, Yue H, Lu Y, Xu GT and Qian J. Maintenance of retinal cancer stem cell-like properties through long-term serum-free culture from human retinoblastoma. Oncol Rep 2011; 26: 135-143.

[35] Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, Pilotti S, Pierotti MA and Daidone MG. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 2005; 65: 5506-5511.

[36] Shi CJ, Gao J, Wang M, Wang X, Tian R, Zhu F, Shen M and Qin RY. CD133(+) gallbladder carcinoma cells exhibit self-renewal ability and tumorigenicity. World J Gastroenterol 2011; 17: 2965-2971.

[37] Cheung AM, Wan TS, Leung JC, Chan LY, Huang H, Kwong YL, Liang R and Leung AY. Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. Leukemia 2007; 21: 1423-1430.

[38] Clay MR, Tabor M, Owen JH, Carey TE, Bradford CR, Wolf GT, Wicha MS and Prince ME. Single-marker identification of head and neck squamous cell carcinoma cancer stem cells with aldehyde dehydrogenase. Head Neck 2010; 32: 1195-1201.

[39] Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, Fields JZ, Wicha MS and Boman BM. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res 2009; 69: 3382-3389.

[40] Pearce DJ, Taussig D, Simpson C, Allen K, Rohatiner AZ, Lister TA and Bonnet D. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. Stem Cells 2005; 23: 752-760.

[41] Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanhehco Y, Smith BD, Civin CI and Jones RJ. Characterization of clonogenic multiple myeloma cells. Blood 2004; 103: 2332-2336.

[42] Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA and Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994; 367: 645-648.

[43] Clevers H. The cancer stem cell: premises, promises and challenges. Nat Med 2011; 17: 313-319.

[44] Medema JP. Cancer stem cells: the challenges ahead. Nat Cell Biol 2013; 15: 338-344.

[45] Shah K, Jacobs A, Breakefield XO and Weissleder R. Molecular imaging of gene therapy for cancer. Gene Ther 2004; 11: 1175-1187.

[46] Hart LS and El-Deiry WS. Invincible, but not invisible: imaging approaches toward in vivo detection of cancer stem cells. J Clin Oncol 2008; 26: 2901-2910.

[47] Sweeney TJ, Mailander V, Tucker AA, Olomu AB, Zhang W, Cao Y, Negrin RS and Contag CH. Visualizing the kinetics of tumor-cell clearance in living animals. Proc Natl Acad Sci U S A 1999; 96: 12044-12049.

[48] Weissleder R and Pittet MJ. Imaging in the era of molecular oncology. Nature 2008; 452: 580-589.

[49] Tsurumi C, Esser N, Firat E, Gaedicke S, Follo M, Behe M, Elsasser-Beile U, Grosu AL, Graeser R and Niedermann G. Non-invasive in vivo imaging of tumor-associated CD133/prominin. PLoS One 2010; 5: e15605.

[50] Gaedicke S, Braun F, Prasad S, Machein M, Firat E, Hettich M, Gudihal R, Zhu X, Klingner K, Schuler J, Herold-Mende CC, Grosu AL, Behe M, Weber W, Macke H and Niedermann G. Noninvasive positron emission tomography and fluorescence imaging of CD133+ tumor stem cells. Proc Natl Acad Sci U S A 2014; 111: E692-701.

[51] Lathia JD, Gallagher J, Myers JT, Li M, Vasanji A, McLendon RE, Hjelmeland AB, Huang AY and Rich JN. Direct in vivo evidence for tumor propagation by glioblastoma cancer stem cells. PLoS One 2011; 6: e24807.

[52] Ustione A and Piston DW. A simple introduction to multiphoton microscopy. J Microsc 2011; 243: 221-226.

[53] Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyak K, Arendt LM, Kuperwasser C, Bierie B and Weinberg RA. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc Natl Acad Sci U S A 2011; 108: 7950-7955.

[54] Li Y and Laterra J. Cancer stem cells: distinct entities or dynamically regulated phenotypes? Cancer Res 2012; 72: 576-580.

[55] Zomer A, Ellenbroek SI, Ritsma L, Beerling E, Vrisekoop N and Van Rheenen J. Intravital imaging of cancer stem cell plasticity in mammary tumors. Stem Cells 2013; 31: 602-606.

[56] Park J, Ku M, Kim E, Park Y, Hong Y, Haam S, Cheong JH, Park ES, Suh JS, Huh YM and Yang J. CD44-specific supramolecular hydrogels for fluorescence molecular imaging of stem-like gastric cancer cells. Integr Biol (Camb) 2013; 5: 669-672.

[57] Liu H, Patel MR, Prescher JA, Patsialou A, Qian D, Lin J, Wen S, Chang YF, Bachmann MH, Shimono Y, Dalerba P, Adorno M, Lobo N, Bueno J, Dirbas FM, Goswami S, Somlo G, Condeelis J, Contag CH, Gambhir SS and Clarke MF. Cancer stem cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. Proc Natl Acad Sci U S A 2010; 107: 18115-18120.

[58] Vlashi E, Kim K, Lagadec C, Donna LD, McDonald JT, Eghbali M, Sayre JW, Stefani E, McBride W and Pajonk F. In vivo imaging, tracking, and targeting of cancer stem cells. J Natl Cancer Inst 2009; 101: 350-359.

[59] Jin ZH, Sogawa C, Furukawa T, Saito Y, Aung W, Fujibayashi Y and Saga T. Basic studies on radioimmunotargeting of CD133-positive HCT116 cancer stem cells. Mol Imaging 2012; 11: 445-450.

[60] Liu W and Frank JA. Detection and quantification of magnetically labeled cells by cellular MRI. Eur J Radiol 2009; 70: 258-264.

[61] Heyn C, Ronald JA, Mackenzie LT, MacDonald IC, Chambers AF, Rutt BK and Foster PJ. In vivo magnetic resonance imaging of single cells in mouse brain with optical validation. Magn Reson Med 2006; 55: 23-29.

[62] Frangioni JV and Hajjar RJ. In vivo tracking of stem cells for clinical trials in cardiovascular disease. Circulation 2004; 110: 3378-3383.
[63] Arbab AS, Bashaw LA, Miller BR, Jordan EK, Lewis BK, Kalish H and Frank JA. Characterization of biophysical and metabolic properties of cells labeled with superparamagnetic iron oxide nanoparticles and transfection agent for cellular MR imaging. Radiology 2003; 229: 838-846.
[64] Leuschner C, Kumar CS, Hansel W, Soboyejo W, Zhou J and Hormes J. LHRH-conjugated magnetic iron oxide nanoparticles for detection of breast cancer metastases. Breast Cancer Res Treat 2006; 99: 163-176.
[65] Weissleder R, Elizondo G, Wittenberg J, Rabito CA, Bengele HH and Josephson L. Ultrasmall superparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging. Radiology 1990; 175: 489-493.
[66] McClelland R, Wauthier E, Tallheden T, Reid LM and Hsu E. In situ labeling and magnetic resonance imaging of transplanted human hepatic stem cells. Mol Imaging Biol 2011; 13: 911-922.
[67] Wang X, Wei F, Liu A, Wang L, Wang JC, Ren L, Liu W, Tu Q, Li L and Wang J. Cancer stem cell labeling using poly(L-lysine)-modified iron oxide nanoparticles. Biomaterials 2012; 33: 3719-3732.
[68] Rogers WJ, Meyer CH and Kramer CM. Technology insight: in vivo cell tracking by use of MRI. Nat Clin Pract Cardiovasc Med 2006; 3: 554-562.
[69] Choi Y, Kim HS, Cho KW, Lee KM, Yi YJ, Eun SJ, Kim HJ, Woo J, Choi SH, Whangbo TK, Choi C, Noh DY and Moon WK. Noninvasive identification of viable cell populations in docetaxel-treated breast tumors using ferritin-based magnetic resonance imaging. PLoS One 2013; 8: e52931.
[70] Choi SH, Cho HR, Kim HS, Kim YH, Kang KW, Kim H and Moon WK. Imaging and quantification of metastatic melanoma cells in lymph nodes with a ferritin MR reporter in living mice. NMR Biomed 2012; 25: 737-745.
[71] Townson JL, Ramadan SS, Simedrea C, Rutt BK, MacDonald IC, Foster PJ and Chambers AF. Three-dimensional imaging and quantification of both solitary cells and metastases in whole mouse liver by magnetic resonance imaging. Cancer Res 2009; 69: 8326-8331.
[72] Lim EK, Kim HO, Jang E, Park J, Lee K, Suh JS, Huh YM and Haam S. Hyaluronan-modified magnetic nanoclusters for detection of CD44-overexpressing breast cancer by MR imaging. Biomaterials 2011; 32: 7941-7950.
[73] Wright MH, Calcagno AM, Salcido CD, Carlson MD, Ambudkar SV and Varticovski L. Brca1 breast tumors contain distinct CD44(+)/CD24(-) and CD133(+) cells with cancer stem cell characteristics. Breast Cancer Res 2008; 10: R10.
[74] Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE and Herlyn M. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res 2005; 65: 9328-9337.
[75] Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C and De Maria R. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ 2008; 15: 504-514.
[76] Wang P, Gao Q, Suo Z, Munthe E, Solberg S, Ma L, Wang M, Westerdaal NA, Kvalheim G and Gaudernack G. Identification and characterization of cells with cancer stem cell properties in human primary lung cancer cell lines. PLoS One 2013; 8: e57020.
[77] Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, Zheng BJ and Guan XY. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroenterology 2007; 132: 2542-2556.
[78] Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, Lam CT, Poon RT and Fan ST. Significance of CD90+ cancer stem cells in human liver cancer. Cancer Cell 2008; 13: 153-166.
[79] Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C and De Maria R. Identification and expansion of human colon-cancer-initiating cells. Nature 2007; 445: 111-115.
[80] Hermann PC, Bhaskar S, Cioffi M and Heeschen C. Cancer stem cells in solid tumors. Semin Cancer Biol 2010; 20: 77-84.