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REVIEW ARTICLE

Invincible, but Not Invisible: Imaging Approaches Toward In Vivo Detection of Cancer Stem Cells

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ABSTRACT

With evidence emerging in support of a cancer stem-cell model of carcinogenesis, it is of paramount importance to identify and image these elusive cells in their natural environment. The cancer stem-cell hypothesis has the potential to explain unresolved questions of tumorigenesis, tumor heterogeneity, chemotherapeutic and radiation resistance, and even the metastatic phenotype. Intravital imaging of cancer stem cells could be of great value for determining prognosis, as well as monitoring therapeutic efficacy and influencing therapeutic protocols. Cancer stem cells represent a rare population of cells, as low as 0.1% of cells within a human tumor, and the phenotype of isolated cancer stem cells is easily altered when placed under in vitro conditions. This represents a challenge in studying cancer stem cells without manipulation or extraction from their natural environment. Advanced imaging techniques allow for the in vivo observation of physiological events at cellular resolution. Cancer stem-cell studies must take advantage of such technology to promote a better understanding of the cancer stem-cell model in relation to tumor growth and metastasis, as well as to potentially improve on the principles by which cancers are treated. This review examines the opportunities for in vivo imaging of putative cancer stem cells with regard to currently accepted cancer stem-cell characteristics and advanced imaging technologies.

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INTRODUCTION

Clonal evolution, the classic and widely accepted model of carcinogenesis, attributes tumor growth to a single cell's acquiring a combination of genetic mutations resulting in unlimited proliferation.[1-3] This model suggests that maintenance and progression of tumors occurs by continual selection of the strongest and most resistant cells within the tumor. Therefore, every cell within a tumor has the potential to acquire the proper mutations to become invasive and/or metastatic. The cancer stem-cell (CSC) model of carcinogenesis was initially described in the context of liquid malignancies in the 1930s and has recently garnered attention with regard to solid tumors.[1,4] In contrast to that of clonal evolution, the CSC theory maintains that cancers develop from, and are maintained by a CSC arising from a resident normal stem/progenitor cell within the tissue bearing the malignancy. The theory holds that the CSC arises from either a stem cell with genetic or epigenetic mutations resulting in the cancer phenotype or from a transformed progenitor cell that also acquires the stem-cell ability of self-renewal. CSCs possess the ability to produce progeny of both stem-cell and differentiating fates, resulting in tumors of a heterogeneous phenotype.[4,5] In addition, recent evidence suggests that stem cells are the culprits of metastasis,[6] supported by the observation that metastatic tumors tend to reproduce a similar heterogeneity as the primary tumor.[7] Furthermore, CSCs from solid tumors have been identified as chemo- and radioresistant, pointing to their potential role in recurrence.[8-12] Therefore, the CSC model suggests that the true culprit for tumor maintenance and metastasis could possibly lie in the ability of CSCs to survive microenvironmental challenges, resist genotoxic chemotherapy and radiation therapy, repopulate the tumor through unlimited rounds of proliferation, and maintain CSC numbers through asymmetric division.

CSCs are a rare population of cells, making up as little as 0.1% of cells in primary specimens and cell lines tested.[9] A low target number is of therapeutic advantage as long as chemotherapy and radiation are effective in killing CSCs. Unfortunately, an ever-growing body of publications suggests that the opposite is true. CSCs are resistant to genotoxic chemotherapeutics (via increased drug pump activity and/or their slow doubling time) and radiation therapy (possibly by increased DNA repair activity).[8,10-14] The CSC model raises the concern that treatments targeting rapidly proliferating and/or non–stem-like cells are

destined to fail.[15] Therefore, the ability to identify and quantify CSCs has therapeutic and possibly even prognostic value. Currently, the gold standard in confirming the identification of a CSC population requires serial passage of the cells in vivo. These assays are difficult to perform, but have led to the identification of a CSC population in brain, breast, skin, prostate, lung, and colon cancers (Table 1). The ability to use identified markers of CSCs for in vivo imaging is of utmost value for expanding our knowledge of CSC function and translating such knowledge to clinical use.

Identification and purification of the CSC population from clinical samples and cell lines has proven successful when based on the expression of a particular combination of cell-surface markers,[16-22] as well as by functional assays including the exclusion of the Hoechst-33342 DNA-binding dye (the "side population"),[23-26] or the presence of increased aldehyde dehydrogenase activity.[27] There exists a small body of knowledge regarding the differential gene expression patterns of CSCs; however, the identification of CSCs on the basis of the expression pattern of particular genes has yet to be described, with the exception of a recent publication by Liu et al[28] describing a relationship between the loss of *BRCA1* expression and an increased stem/progenitor phenotype in human breast cancer patients. Nevertheless, such an identification method is certainly capable of being exploited for the in vivo imaging of CSCs. In addition, information regarding CSC biology can be obtained by imaging CSCs in conjunction with microenvironmental probes, such as markers of angiogenesis or protease activity.

With regard to the choice of imaging equipment, cellular resolution is a primary concern. Optical fluorescence imaging provides the highest resolution to date; however, magnetic resonance imaging (MRI) and positron emission tomography (PET) are making advancements in spatial resolution that warrant their inclusion in this review.[29-31] Considering that the CSC population will be as low as one cell among 1,000, the imaging device must be sensitive to the contrast agent at a resolution on the order of 100 nm. This necessity all but excludes use of the VisEn FMT (VisEn Medical, Woburn, MA), the Explore Optix (GE/ART, Montreal, Quebec, Canada), the Odyssey (LI-COR Biosciences, Lincoln, NE), the Maestro (CRi, Woburn, MA), and the popular IVIS (Xenogen/Caliper, Hopkinton, MA), while still including basic imaging devices, such as a dissecting microscope outfitted with a Nuance imaging module or other high-resolution imagers (CRi, Woburn, MA; Table 2, Fig 1A). The ability to image CSCs in vivo will provide novel information regarding the biology of these cells in the presence of a stromal compartment, microenvironmental cues, and most importantly, in three dimensions. As clinical trials are altered to include measurements of stem-like cells, the imaging of CSCs in vivo will provide a powerful tool to translate CSC biology into clinical application by driving the development and testing of CSC-specific therapies, prognostic indices, and measures of therapeutic efficacy.

Table 1. Stem-Cell Markers Identified Among Various Tumor Types

Type of Cancer	Stem-Cell Marker	Expression Level	References
Breast	CD44	Positive	16
	CD24	Negative/low	16
	lin	Negative	16
	ALDH	High activity	27, 28
Brain	CD133	Positive	94, 95
Colon	CD133	Positive	20, 21
Prostate	CD44	Positive	6, 17
	$\alpha 2\beta 1$	High	17
	CD133	Positive	17, 22
Acute myelogenous leukemia	CD34	Positive	93
	CD38	Negative	93
	CD96	Positive	18
Pancreas	CD44	Positive	19
	CD24	Positive	19
	ESA	Positive	19

IMAGING CSCs IN HUMAN TISSUES

To best appreciate the approaches for imaging CSC populations in vivo, we will first highlight data using ex vivo human specimens from which such approaches are extrapolated. As already mentioned, the ability to simultaneously image cellular antigens and functional markers of CSCs is of great value. This has been explored in vitro within a range of cell lines and tissues by a number of laboratories,[28,32,33] as well as with animal and human specimens ex vivo.[28,34-36] In addition, similar studies have also gathered information with regard to tissue organization and the distinct localization of subpopulations of cells, such as stem cells.[37-40] Two-photon fluorescence has contributed useful information as to cellular organization in both two-dimensional cultures[41] and three-dimensional reconstruction of xenografted tumors.[42] The use of multiple contrast agents necessitates the ability to differentiate spectral profiles and remove background signal. Therefore, the spectral unmixing of multispectral images has proven powerful in the detection of rare subsets of cells within cell lines, spheroid cultures, and tumor sections. Perhaps the most elegant example of this is the discrimination of human mesenchymal stem cells from osteoblasts by seven-color fluorescence and spectral unmixing performed by Schieker et al.[43] Because CSCs are identified by increasing numbers of markers, the need for imaging seven markers at once is not difficult to foresee. In addition, Byers et al[44] have demonstrated elegant multispectral imaging by performing gene expression profiling with oligonucleotide probes (labeled with quantum dots) in conjunction with immunohistochemistry, as a means of semiquantitative, high-throughput analysis of cellular lineage and gene expression patterns in human tissues. Interestingly, Chung et al[45] have employed optical imaging to demonstrate unique cancer profiles in terms of spectral reflectance and autofluorescence measurements without the use of exogenous contrast agents. These types of in vitro analysis provide important information regarding the discrimination of CSCs from neighboring cancer cells within a cell line or tumor. In addition, it is easy to envision the use of such analyses as a means of determining the prognosis of cancer patients via CSC numbers in bone marrow, blood, CSF, or tumor biopsies, a strategy already being explored.[35,46,47]

IN VIVO IMAGING OF CSCs

Established Imaging Models

The imaging of cancer cells at single-cell resolution is not a novel undertaking. Rather, it is the in vivo imaging of CSCs specifically that remains to be accomplished. On the basis of convincing reports thus

Table 2. Comparison of the Specifications of High-Resolution Optical Imaging Devices

Criterion	OV100*	IV100*	Nuance/Nikon AZ100†	Multiphoton Laser Scanning Microscopy
Hardware				
Numerical aperture	0.03-0.43	0.14-0.7	0.05-0.5	Approximately 1.2
Camera pixels	4080×3072 (DP70 CCD*)	64×64-4096×4096	1434×1050 (Sony ICX285)‡§	512×480§
Magnification	0.14×-16×	6×-27×	5×-400×	60×
Surgical manipulation				
Subcutaneous	Skin flap	Very small incision	Skin flap or excised	Excised
Internal organ	Skin flap	Small skin flap/incision	Skin flap or excised	Excised
Advantages	4 individual parcentered and parfocal lenses; spectral unmixing; NIR	3 simultaneous detection channels for fluorescence; 4 lasers; NIR	Spectral unmixing; NIR	Lack of out-of-focus absorption; low excitation light scatter; NIR

Abbreviation: NIR, near-infrared.
*Olympus, Center Valley, PA.
†Nikon, Tokyo, Japan.
‡Sony, Tokyo, Japan.
§Varies according to camera chosen.

far, CSCs should be a high priority of oncologic imaging studies. Multiple publications in the last decade have successfully imaged cancer cells at single-cell resolution under ex vivo conditions, as well as longitudinally for studies of tumor growth and response to therapy.[48-52] In 1997, Chishima et al[53] imaged micrometastases ex vivo with an MRC-600 confocal imaging device mounted on a Nikon microscope. They orthotopically implanted GFP-expressing CHO-K1 ovarian cells and identified GFP-positive micrometastases in the excised lungs and liver of the mice. Nonproliferative lung micrometastases of GFP-expressing breast tumor cells have also been imaged ex vivo following mammary fat pad injection.[54] In 2000, Chen et al[50] published a longitudinal study detecting fluorescent neurons at single-cell resolution with two-photon laser scanning microscopy. Initial cellular tracking studies evolved into fluorescence reporter assays by establishing cell lines with specific promoter-driven fluorescent proteins, such as that of Fukumura et al,[55] whereby vascular endothelial growth factor transcriptional activity was measured in response to microenvironmental factors in orthotopic brain tumors in vivo. In addition to gene expression imaging profiles, MRI and PET imaging techniques provide a functional component that can be more readily applied in clinical translational studies. Both MRI and PET have recently advanced to become more accessible for high-resolution needs,[56] and therefore, are discussed further in this review.

In addition to specifically tracking cells of interest via fluorescence or other agents of contrast, a number of physiological probes have been developed to image tumor microenvironmental events, such as angiogenesis, protease activity, and apoptosis. Injection of the blood pooling agent Angiosense (VisEn Medical) results in tumor-specific accumulation over time, likely a result of the leaky nature of tumor vasculature. VisEn also provides tumor-associated protease probes, MMPSense reporting matrix metalloproteinase activity (MMPs 2, 3, 9, and 13), ProSense, which is activated by Cathepsins B, L, and S, and plasmin within the extracellular milieu of solid tumors, and the osteogenic probe OsteoSense. Many studies have taken advantage of these physiological indicators.[57-59] The tracking of apoptosis has also been successful with Annexin V probes that bind cells with extracellular phosphatidylserine exposure, and are therefore valuable in monitoring therapeutic response, albeit with limitations.[60,61] Tracking rare cells, such as CSCs, in conjunction with microenvironment-specific probes will be useful in providing additional information as to the behavior of the cells of interest under specific environmental conditions. Imaging a rare population of cells on the basis of gene expression, biochemical activity, or cell surface protein levels is possible with current knowledge of CSC characteristics. Additional microenvironment-specific CSC survival and growth patterns will be revealed when imaged in vivo, and the influence of other components of the tumor microenvironment such as hypoxia or pH changes can be determined.

High-Resolution Optical Imaging

For the purpose of imaging at the resolution of a single cell, optical imaging techniques are most readily applicable to the CSC model. When optically imaging a rare population of cells, the choice of both reporter signal and imaging device are equally important. With regard to the choice of optical signal, a fluorescent signal provides the greatest advantage at visualizing small numbers of cells. Despite the success with which bioluminescent signals define tumor growth, regression, and metastasis, the spatial resolution and sensitivity limitations of bioluminescence render it incapable of identifying and localizing rare cells at high resolution or with tomographic capability. Luciferase reporter plasmids have been extremely valuable at providing measurements of biologic activity in growing tumors with high sensitivity; however, luciferase models require animals to be injected with, and every cell in question be exposed to, the luciferin substrate. Furthermore, the limitation of the signal in vivo requires expression of luciferase in at least 2,500 cells for proper detection.[62] Therefore, for the purposes of imaging a very rare population of cells, such as CSCs, readout of fluorescence is the ideal choice. The detection of fluorescence is made practical by the high intensity and stability of signal, the highly sensitive detectors available, and the option to concurrently employ more than one fluorophore. There is a large variety of potential fluorophores from which to choose, including fluorescent proteins such as GFP, RFP, YFP, and DsRed, the DsRed variant fruit fluorescent proteins such as mCherry,[63] cyanine fluorochrome conjugates, quantum dots and probes,[44] and chimeric transmembrane fluorescent proteins,[64] and others. As already described, the ability to

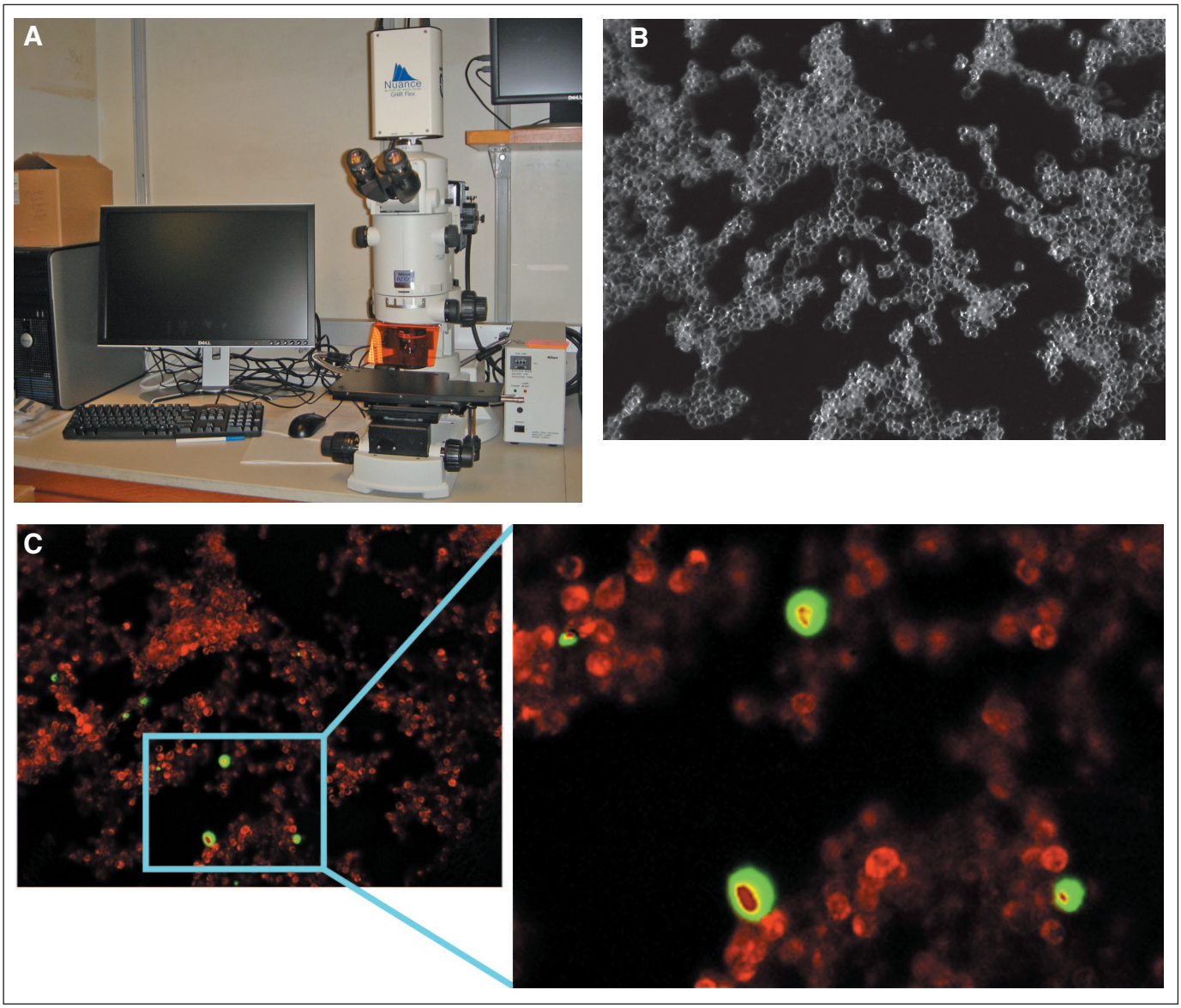

Fig 1. High-resolution fluorescence imaging of CD133$^+$ putative cancer stem cells in a live population of SW620 human colorectal carcinoma cells. (A) Photograph of the Nuance module (CRi, Woburn, MA) mounted on a Nikon AZ100 dissecting microscope (Nikon, Tokyo, Japan). (B) Phase contrast image of SW620 cells, and (C) spectrally unmixed image of CD133$^+$ cells (green) and live cells counterstained with MitoTracker Red (Molecular Probes, Eugene, OR).

image fluorescent cells in vivo at high resolution has been demonstrated by many labs with models requiring relatively minor adaptations for the specific visualization of CSCs.

In terms of imaging technology, there are a number of optical imaging devices capable of high-resolution optical imaging, including the OV100 and IV100 imagers (Olympus, Tokyo, Japan), multiphoton laser scanning microscopes, Cellvizio (Mauna Kea Technologies/Leica, Paris, France), and the relatively simple yet effective set-up of a dissecting microscope (such as a Nikon AZ100; Nikon, Tokyo, Japan) equipped with the Nuance multispectral imaging module (CRi). Each of these devices provides high-resolution imaging; the advantages of one over another merely stem from the particular objectives of the experiment at hand and cost of the equipment. Factors concerning the choice of imaging device include, but are not limited to, depth of tissue penetration, imaging time points, and option for multispectral unmixing. Depth of penetration is a significant factor in subcutaneous xenograft models and when imaging internal organs, whereby creation of a skin flap is necessary to avoid skin-associated light scatter. The IV100 is most advanced in this regard as the largest of the objectives is 3.5 mm in diameter allowing it to be easily inserted into small incisions (Table 2). The number of imaging time points desired is a significant factor because longitudinal studies with the OV100 and IV100 require incisions or transparent window models, but these are relatively easy to perform and have been demonstrated in many mouse models.[65-69] In the absence of surgical manipulation, the highest penetration (with the least tissue damage) is achieved with near-infrared (NIR) fluorescence probes. Each of the imaging devices discussed here is capable of detecting signal in the NIR range. Depth of penetration and surgical manipulation become especially significant when longitudinal studies are desired. Multiple time points are not

possible with multiphoton laser scanning microscopes, which are mainly useful for high-resolution end point analysis of excised tumors, but nonetheless provide valuable information. Lastly, the advantages of spectral unmixing are insurmountable. The unmixing feature allows for the removal of autofluorescence, as well as distinguishing between emission peaks of nominal separation. Autofluorescence is less of a complication with the IV100 or OV110 (because of the lasers' being narrowly focused on the specimen), but unmixing capabilities are a necessity for devices like the Nuance, where light scatter is more of a problem. Of course, considerations such as these are dependent on the model and end points chosen.

We have compared some of the instruments discussed based on important features of the hardware and prospective experimental design considerations (Table 2). Resolution is primarily dependent on the numerical aperture (NA) of the objective. NA is a value attributed to the ability of the objective to collect light and resolve detail at a fixed distance, with a higher NA value associated with better resolution. However, the final level of resolution achieved is also dependent on the magnification, the pixels of the image sensor, and the wavelength of the light. Each of the imaging devices listed is capable of cellular resolution, for example, using the OV100 MicroZoom objective with an NA of 0.43 (and with their DP70 CCD camera) will result in resolution of approximately 0.7 μm. Also noted in Table 2 are the experimental limitations of the devices with regard to the manipulation of the specimen. It is possible for NIR fluorophores to penetrate the skin for imaging subcutaneous tumors, but to achieve the best images possible and for the imaging of internal organs each of the devices requires exposure of the tumor or organ in question. Various advantages specific to the imaging devices have also been highlighted in Table 2.

The Cellvizio instrumentation has introduced the capability to perform in vivo confocal microscopy during clinical GI endoscopy for visualization of tubular adenomas or Barrett's esophagus. A similar capability has been developed for imaging lung alveoli. It is not too difficult to imagine use of such capabilities or others for endoscopic or laparoscopic applications to visualize human CSCs in vivo.

High-Resolution MRI

Surprisingly, MRI is not to be left out of this discussion. Historically, MRI has been employed as a means of gaining anatomic information; however, recent advances in the evolution of contrast agents have provided the opportunity to image cells and gene regulation at cellular resolution with MRI.[70] MRI image resolution is excellent, on the order of 100 μm, but only recently has MRI technology been manipulated with the intent of detecting subtle molecular changes. By overexpressing an altered form of the transferrin receptor, Moore et al[71] have increased the iron load within a target population of cells, thereby increasing contrast and detection. Similarly, Louie et al[72] have engineered a beta-galactosidase reporter system in which cells with increased contrast are representative of transgene expression. These studies have succeeded in fusing the excellent in vivo detection capability of MRI with the cellular resolution and functional advantages of reporter activity. More recently, single-cell resolution imaging via MRI has successfully been employed to detect invading glioma cells and metastasizing breast carcinoma cells labeled with micrometer-sized particles of iron-oxide (MPIOs).[29,30] Heyn et al[30] demonstrated high-resolution MRI of MPIO-labeled MDA-MB231BR/EGFP cells in the brains of mice at just 5 hours after intracardiac injection (Fig 2). They confirmed that the MRI signal voids were indeed the cells of interest by titrating the cell number and correlating the voids with fluorescent signal detected by high-resolution confocal microscopy. The noninvasive nature of MRI makes it one of the most attractive imaging techniques for longitudinal imaging. The possibility of noninvasive longitudinal imaging combined with the single-cell resolution models discussed make MRI a primary modality in the in vivo imaging of CSCs.

PET

PET quantitatively detects high-energy γ-rays emitted from a subject injected with positron-emitting isotopes or isotope-labeled molecular probes.[73,74] PET is a sensitive and noninvasive imaging technique because the detection of positron emission is independent of the depth from which the signal is emitted. However, only modified PET instruments, such as those with pinhole and micropinhole apertures or micro-PET small-animal scanners are able to achieve resolution approaching (200 μm and/or 1 mm^3) what is necessary for CSC imaging.[75-77] PET technology is not yet at the level of resolution necessary for detecting single CSCs within a tumor; however, the level of spatial resolution has improved significantly, progress is being made in the development of radiolabeled antibodies, substrates, and reporter probes,[78-80] and PET advancements are readily translated to the clinic. A common isotope-associated gene reporter system used with PET imaging is the detection of herpes simplex virus type 1 thymidine kinase (*HSV1-tk*) gene expression with an ^{18}F-fluoropenciclovir probe, which when phosphorylated by HSV1-TK is retained within cells.[80,81] In addition, Doubrovin et al[82] have examined the regulation of endogenous genes with dual reporters for *p53* and *p21*, resulting in the ability to simultaneously analyze the transcriptional activity of p53 and correlate those changes to effects on *p21*. PET strategies (via mutant *HSV1-tk*) have been combined with bioluminescence (via firefly luciferase expression) in an effort to image infused human mesenchymal stem cells with dual imaging modalities during bone formation and adipogenesis (Fig 3).[79] This is a potentially useful technique with the potential for adaptation to a CSC model to monitor the engraftment, propagation, and subsequent differentiation of CSCs at a primary or metastatic site. These studies highlight powerful capabilities of PET imaging that, when combined with the advantage of noninvasive detection, makes PET an attractive technique for imaging CSCs, with the requirement of further improvements in sensitivity and resolution. It is important to note there are two important goals: (1) human CSC tracking in vivo, which may be achieved by injecting ex vivo imaging agent-labeled cells and monitoring their fate over time in vivo; and (2) high-resolution imaging of endogenous human CSCs using exogenously administered, highly specific and sensitive probes. The second goal is the one that is extremely relevant to diagnostic and prognostic applications as well as the monitoring of the effects of therapy in patients.

NOVEL OPPORTUNITIES FOR IMAGING CSCs IN VIVO

Models that have proven successful for single-cell imaging include xenograft models of cells stably transfected with reporter constructs, transgenic mice, in vivo immunodetection of cell-surface proteins, and the application of exogenous probes or contrast agents. In applying what is currently known about CSCs, each of these models could

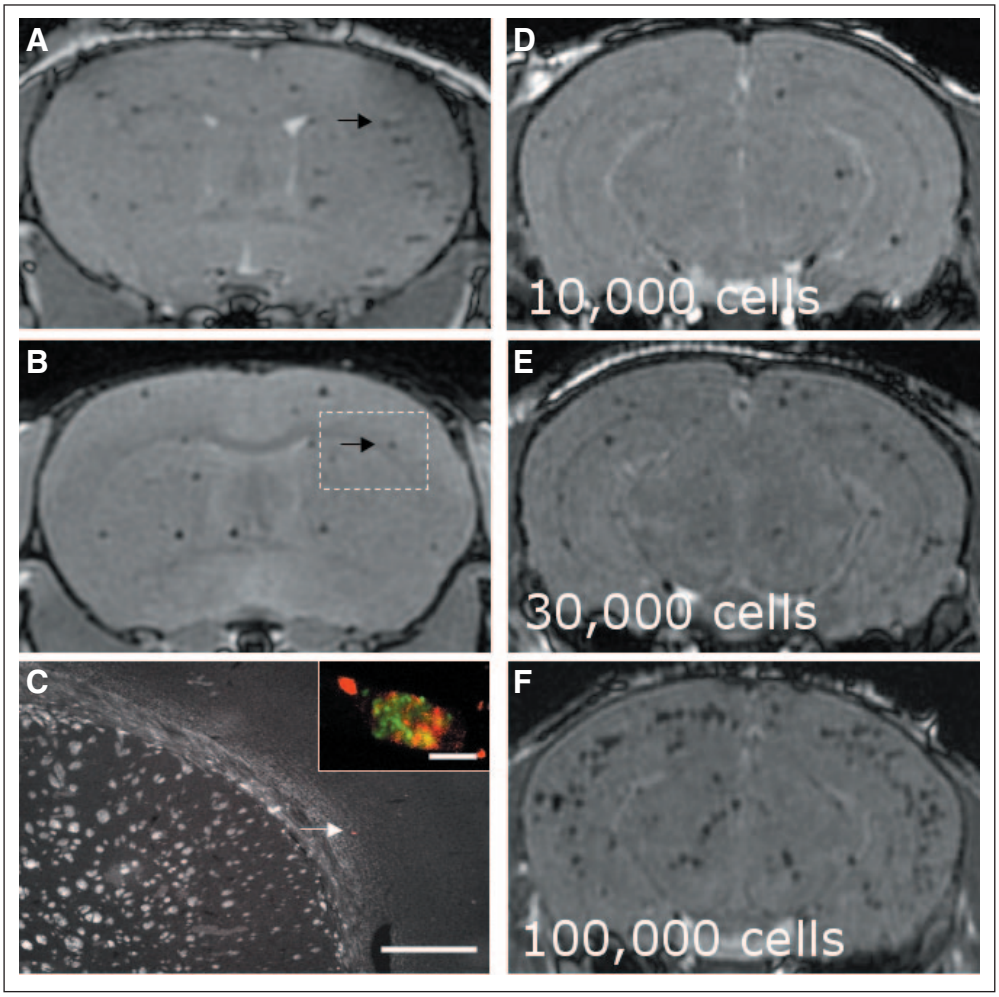

Fig 2. Magnetic resonance detection of a single MPIO-labeled MDA-MB-231BR/EGFP cells at day 0, and the effect of cell dose on the number of detectable cells are shown. (A) In vivo (100 × 100 × 200 μm^3) and (B) high-resolution ex vivo (100 μm^3) magnetic resonance imaging (MRI) of mouse brain demonstrates the presence of discrete signal voids (black arrow) throughout the brain of a mouse injected with 30000 Dragon Green MPIO and DiI-labeled GFP+ cells. (C) A discrete signal void detected on MRI [black arrow in (A) and (B)] was correlated optically to a DiI-positive region (white arrow). High-resolution confocal microscopy revealed this region to be a solitary DiI-labeled cell (red) with green fluorescence attributable to either GFP expression or MPIO-labeled beads (inset of C). (D-F) In vivo MRI of mouse brains demonstrates the presence of increasing numbers of signal voids with increasing cell dose. Scale bar: (C) 500 μm (inset, 10 μm). Figure and legend reprinted with permission John Wiley and Sons Inc.[30]

be adjusted to specifically image CSCs. The CSC model exposes an important opportunity for the use of such techniques in characterizing the in vivo biology of CSCs and validating in vitro results. In vivo imaging of CSCs has yet to be explored beyond the realm of developmental biology, as much of what is known about the molecular signature of CSCs has been extrapolated from studies of normal stem-cell biology.

For example, the polycomb repressive complex 1 (PRC1) protein Bmi-1 is involved in gene repression and is required for the maintenance of self-renewal in stem cells.[83-85] Hosen et al[86] have generated a

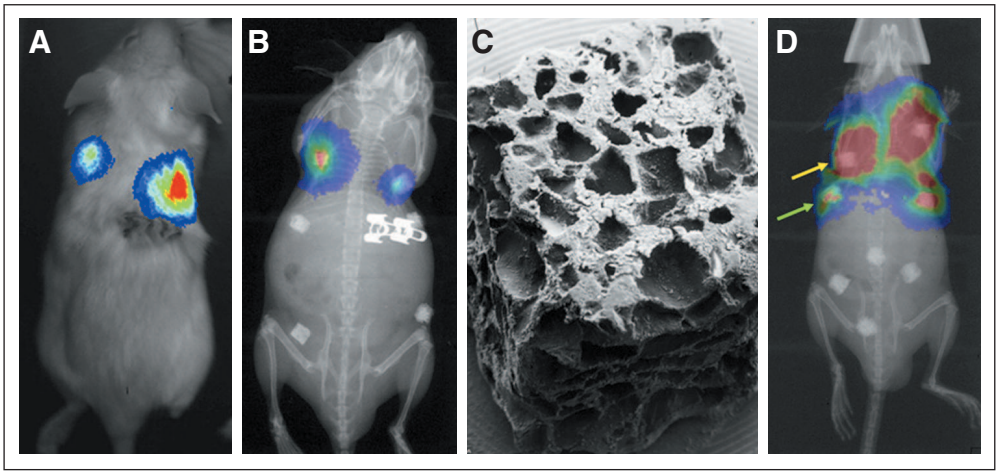

Fig 3. Bioluminescence (BLI) of cube implants. BLI overlaid on (A) photograph and (B) radiograph. Both mice had six cubes: top two loaded with reporter-transduced human mesenchymal stem cells (hMSCs), middle two loaded with wild-type hMSCs, and bottom two loaded with empty-vector–transduced hMSCs located at caudal midline, and empty ceramic cubes located caudally and laterally. (C) Scanning electron micrograph of representative ceramic cube for cell loading and implantation. (D) BLI overlaid on radiograph in animal implanted with reporter-transduced hMSCs (top row, yellow arrow), mixture of empty-vector–transduced and reporter-transduced hMSCs (second row, green arrow), empty-vector–transduced hMSCs located at midline, and empty ceramic cube located laterally. Figure and legend reprinted with permission the Society of Nuclear Medicine.[79]

knock-in mouse under which GFP expression is regulated by the *Bmi-1* transcriptional promoter to better understand the role of Bmi-1 signaling in the self-renewal mechanism. They found that *Bmi-1* expression was highest within the most stem-like hematopoietic cells and was decreased on differentiation. This is an excellent example of a model system for studying stem cell–associated genes and should be expanded on to include an analysis of *Bmi-1* regulation during tumor growth. Normal stem-cell imaging studies have also been performed in the context of *Nestin*[87] and *Sox1*[88] transgenic mice. Similar experiments can also be performed in mice bearing reporters of the *Oct 3/4*, *Notch*, *Klf4*, *Sox2*, *BRCA1*, *NF-κB*, and c-*Myc* genes, members of a short, but growing list of stem-cell and differentiation genes with potential roles in CSC function.[28,89-92] In addition, such gene reporters can be generated under tissue-specific promoters in models of spontaneous tumor formation for a more detailed analysis of carcinogenesis with regard to tissue-specific stem-cell gene activity. These studies may lend invaluable information as to the true nature and/or existence of CSCs in growing tumors.

In addition to stem-cell gene regulation, the identification of cancer-specific stem-cell markers is of great value for in vivo imaging purposes. Similar to studies with the Annexin V probes, immunodetection of cell-surface markers with fluorophore-conjugated (or other isotope- or magnetic contrast–conjugated) antibodies is a great strategy for visualizing CSCs in the context of a growing tumor. CSC-specific cellular antigens have been identified for a number of tumor types (Table 1), particularly AML ($CD96^+$ and/or $CD34^+/CD38^-$),[18,93] breast ($CD44^+/CD24^{low/-}$/lin-negative),[16] brain ($CD133^+$),[94,95] colorectal ($CD133^+$),[20,21] prostate ($CD44^+/\alpha2\beta1$-high/$CD133^+$),[17,22] and pancreas ($CD44^+/CD24^+/ESA^+$).[19] Difficulty would be encountered when imaging cells with low- or negative-expressing antigens, but a combination of high expression of antigens could easily be detected with a variety of fluorophores and spectral unmixing capabilities. It is therefore possible to image prostate cancer cells expressing CD44, $\alpha2\beta1$, or CD133, and by definition, prostate CSCs expressing all three. In fact, we have recently imaged putative human CSCs within living SW620 human colon cancer cells with the Nuance module via preincubation with fluorescent-conjugated anti-CD133 antibody (Figs 1B-1C). The spectral unmixing feature of the Nuance allows for imaging of more than one cell-surface molecule given that different fluorophores are employed. In the example shown, Mito Tracker Red was used to document cell viability and the presence of viable $CD133^+$ as well as $CD133^-$ cells in the tumor cell population. Expanding on the identification of cell-surface proteins, LI-COR Biosciences has developed a probe to identify cells with epidermal growth factor receptor activity by labeling epidermal growth factor ligand with an NIR fluorophore. This represents a model system toward which the CSC field should be moving. The ability to identify new CSC-specific extracellular proteins will lead to the development of novel probes providing the opportunity to visualize CSCs in vivo, and possibly even therapeutically target[96] functional proteins necessary for CSC survival and resistance.

With regard to CSC-specific gene expression, there exists the option of imaging the functional characteristics of aldehyde dehydrogenase (ALDH) activity and dye efflux or retention in vivo. Both increased ALDH activity, efflux of Hoechst 33342, and retention of labeling dyes such as CFSE are functions associated with CSC populations; sorting cells on the basis of these characteristics enriches for tumorigenic CSCs.[25,27,97,98] Although ALDH activity has only been measured under in vitro conditions, it is reasonable to envision the in vivo detection of ALDH concurrent with the identification of cellular antigens or biologic events such as angiogenesis. It is less likely that imaging Hoechst 33342 efflux in vivo would be practical; however, dye-retaining cells could potentially be visualized during tumor growth in vivo as long as the loss of dye does not precede measurable tumor burden. This would provide the opportunity to image the in vivo activity of quiescent cells with regard to the microenvironment. The alteration of organelle structure as a cell moves throughout capillaries[99] and the progression of a cell through the cell cycle in real time[100] have also been achieved and can be applied to our further understanding of CSC biology. These experiments relied on the tracking of specific proteins via fluorescence, a strategy that could be readily adapted to visualize the interplay between cells at differing levels of differentiation, and therefore expressing different cellular antigens in a dynamic manner. These suggestions, along with those regarding CSC gene expression, bring to light the possibility of imaging events more functional than the expression of cell-surface markers. However, it is likely that a combination of the imaging tools discussed herein will prove most useful in garnering novel information of in vivo CSC biology.

CLINICAL IMPLICATIONS FOR CSC IMAGING

With the ability to perform high-resolution imaging of CSCs comes new knowledge of CSC behavior in a relatively undisturbed environment compared with current in vitro models. Whether CSCs exist is still the subject of intense debate, hence use of the term "putative CSC." In vivo imaging of cancer cells thought to be stem-like will put to rest some of the unanswered questions pertaining to the CSC model, and potentially validate already accepted theories. Are CSCs the culprit of the well established chemo- and radioresistance of tumors? Monitoring CSC levels within a tumor responding to treatment would help to answer such a question. Do CSCs possess the metastatic ability within a tumor? With fluorescent- or MPIO-labeled CSCs it becomes possible to track the invasion and metastasis of very small numbers of cells.[30,53,54,72] Similar questions are on their way to being answered in the clinic; however, the heterogeneous nature of patient populations makes it less likely that a consensus will be met in the next few years, as is possible with in vivo preclinical modeling.

Progress is being made in the development of prognostic measures of CSCs. Putative CSCs have been identified in the bone marrow of early breast cancer patients on the basis of the $CD44^+/CD24^{-/low}$ phenotype.[35] In terms of clinical imaging advancements, Wang et al[101] have successfully performed confocal optical imaging on colonic mucosa in human patients undergoing screening colonoscopy. They found that the movement pattern of fluorescein (applied topically within the colon) varies between normal and hyperplastic crypts, providing valuable real-time histologic analysis of cancer in vivo. Such studies could be modified to analyze CSC markers via optical imaging of fluorescently labeled antibodies during colonoscopy, and perhaps advancements in resolution MRI and PET imaging could also be employed. Such imaging techniques would produce a measure of the CSC component within primary tumors, lymph nodes, and sites of metastasis, thereby providing prognostic information as is already being explored ex vivo,[35,46,47] and could potentially influence the therapeutic protocols for individual patients, as well as monitor therapeutic response during treatment. The human studies discussed require further characterization for the purpose of thoroughly assessing

the factors driving cancer progression, metastasis, and CSC existence and behavior. However, these are excellent early steps in the identification and analysis of tumor cells at the single-cell level in living patients, and have the potential to drive the way in which CSCs are identified and quantified, prognoses are defined, and therapeutic strategies are developed.

SUMMARY

The recent high-profile reports describing the generation of pluripotent stem cells from fibroblasts raise the possibility that as few as four genes are required in maintaining the stem-cell phenotype.[91,92] This observation lends support to the possibility that cancer arises from just a few mutations in resident tissue stem, progenitor, or even differentiated cells. It remains to be undeniably verified that CSCs are the sole culprit of carcinogenesis; however, in the study of CSC biology, high-resolution imaging must be readily accessible because CSC biology requires analysis within the least manipulated and most controlled environments possible. Never before has in vivo modeling been such an integral necessity to clarifying the role of a subpopulation of cells with regard to tumor growth, progression, and resistance to therapy. The field of oncology must exhaust all capabilities in the identification of CSCs in mouse models and human patients on the chance that the now putative CSCs will soon be accepted as bona fide and responsible for a disease that has rejected most therapeutic tactics and claims a life every minute. CSCs may not ultimately be invincible.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Campbell L, Polyak K: Breast tumor heterogeneity: Cancer stem cells or clonal evolution? Cell Cycle 6:2332-2338, 2007

2. Merlo LMF, Pepper JW, Reid BJ, et al: Cancer as an evolutionary and ecological process. Nat Rev Cancer 6:924-935, 2006

3. Nowell P: The clonal evolution of tumor cell populations. Science 194:23-28, 1976

4. Reya T, Morrison SJ, Clarke MF, et al: Stem cells, cancer, and cancer stem cells. Nature 414: 105-111, 2001

5. Bissell MJ, LaBarge MA: Context, tissue plasticity, and cancer: Are tumor stem cells also regulated by the microenvironment? Cancer Cell 7:17-23, 2005

6. Patrawala L, Calhoun T, Schneider-Broussard R, et al: Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene 25:1696-1708, 2006

7. Brabletz T, Jung A, Spaderna S, et al: Migrating cancer stem cells: An integrated concept of malignant tumour progression. Nat Rev Cancer 5:744-749, 2005

8. Bao S, Wu Q, McLendon R, et al: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444:756-760, 2006

9. Huang EH, Heidt DG, Li C-W, et al: Cancer stem cells: A new paradigm for understanding tumor progression and therapeutic resistance. Surgery 141:415-419, 2007

10. Sussman R, Ricci M, Hart L, et al: Chemotherapy-resistant side-population of colon cancer cells has a higher sensitivity to TRAIL than the non-SP, a higher expression of c-Myc and TRAIL-receptor DR4. Cancer Biol Ther 6:1490-1495, 2007

11. Tavaluc R, Hart L, Dicker D, et al: Effects of low confluency, serum starvation and hypoxia on the side population of cancer cell lines. Cell Cycle 6:2554-2562, 2007

12. Rich JN: Cancer stem cells in radiation resistance. Cancer Res 67:8980-8984, 2007

13. Eramo A, Ricci-Vitiani L, Zeuner A, et al: Chemotherapy resistance of glioblastoma stem cells. Cell Death Differ 13:1238-1241, 2006

14. Tazzari PL, Cappellini A, Ricci F, et al: Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase//Akt signal transduction network in human acute myelogenous leukemia blasts. Leukemia 21:427-438, 2007

15. Dalerba P, Cho R, Clarke M: Cancer stem cells: Models and concepts. Annu Rev Med 58:267-284, 2007

16. Al-Hajj M, Wicha M, Benito-Hernandez A, et al: Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100:3983-3988, 2003

17. Collins AT, Berry PA, Hyde C, et al: Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 65:10946-10951, 2005

18. Hosen N, Park C, Tatsumi N, et al: CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci U S A 104: 11008-11013, 2007

19. Li C, Heidt DG, Dalerba P, et al: Identification of pancreatic cancer stem cells. Cancer Res 67: 1030-1037, 2007

20. O'Brien CA, Pollett A, Gallinger S, et al: A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445:106-110, 2007

21. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al: Identification and expansion of human colon-cancer-initiating cells. Nature 445:111-115, 2007

22. Richardson GD, Robson CN, Lang SH, et al: CD133, a novel marker for human prostatic epithelial stem cells. J Cell Sci 117:3539-3545, 2004

23. Goodell M, McKinney-Freeman S, Camargo F: Isolation and characterization of side population cells. Methods Mol Biol 290:343-352, 2005

24. Hadnagy A, Gaboury L, Beaulieu R, et al: SP analysis may be used to identify cancer stem cell populations. Exp Cell Res 312:3701-3710, 2006

25. Ho MM, Ng AV, Lam S, et al: Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res 67:4827-4833, 2007

26. Patrawala L, Calhoun T, Schneider-Broussard R, et al: Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. Cancer Res 65:6207-6219, 2005

27. Christ O, Lucke K, Imren S, et al: Improved purification of hematopoietic stem cells based on their elevated aldehyde dehydrogenase activity. Haematologica 92:1165-1172, 2007

28. Liu S, Ginestier C, Charafe-Jauffret E, et al: BRCA1 regulates human mammary stem/progenitor cell fate. Proc Natl Acad Sci U S A 105:1680-1685, 2008

29. Bernas L, Foster P, Rutt B: Magnetic resonance imaging of in vitro glioma cell invasion. J Neurosurg 106:306-313, 2007

30. Heyn C, Ronald J, Ramadan S, et al: In vivo MRI of cancer cell fate at the single-cell level in a mouse model of breast cancer metastasis to the brain. Magn Reson Med 56:1001-1010, 2006

31. Quon A, Gambhir SS: FDG-PET and beyond: Molecular breast cancer imaging. J Clin Oncol 23: 1664-1673, 2005

32. Povsic TJ, Zavodni KL, Kelly FL, et al: Circulating progenitor cells can be reliably identified on the basis of aldehyde dehydrogenase activity. J Am Coll Cardiol 50:2243-2248, 2007

33. Storms RW, Green PD, Safford KM, et al: Distinct hematopoietic progenitor compartments are delineated by the expression of aldehyde dehydrogenase and CD34. Blood 106:95-102, 2005

34. Yi L, Zhou Z-h, Ping Y-f, et al: Isolation and characterization of stem cell-like precursor cells from primary human anaplastic oligoastrocytoma. Mod Pathol 20:1061-1068, 2007

35. Balic M, Lin H, Young L, et al: Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. Clin Cancer Res 12: 5615-5621, 2006

36. Congdon KL, Voermans C, Ferguson EC, et al: Activation of Wnt signaling in hematopoietic regeneration. Stem Cells [Epub ahead of print February 28, 2008]

37. Lardon J, Corbeil D, Huttner W, et al: Stem cell marker prominin-1/AC133 is expressed in duct cells of the adult human pancreas. Pancreas 36: e1-6, 2008

38. Schrot R, Ma J, Greco C, et al: Organotypic distribution of stem cell markers in formalin-fixed

brain harboring glioblastoma multiforme. J Neurooncol 85:149-157, 2007

39. Hayashi R, Yamato M, Saito T, et al: Enrichment of corneal epithelial stem/progenitor cells using cell surface markers, integrin [alpha]6 and CD71. Biochem Biophys Res Commun 367:256-263, 2008

40. Zhuo S, Chen J, Jiang X, et al: Visualizing extracellular matrix and sensing fibroblasts metabolism in human dermis by nonlinear spectral imaging. Skin Res Technol 13:406-411, 2007

41. Rice W, Kaplan D, Georgakoudi I: Quantitative biomarkers of stem cell differentiation based on intrinsic two-photon excited fluorescence. J Biomed Opt 12:060504, 2007

42. Calabrese C, Poppleton H, Kocak M, et al: A perivascular niche for brain tumor stem cells. Cancer Cell 11:69-82, 2007

43. Schieker M, Pautke C, Haasters F, et al: Human mesenchymal stem cells at the single-cell level: Simultaneous seven-colour immunofluorescence. J Anat 210:592-599, 2007

44. Byers R, Di Vizio D, O'connell F, et al: Semiautomated multiplexed quantum dot-based in situ hybridization and spectral deconvolution. J Mol Diagn 9:20-29, 2007

45. Chung A, Karlan S, Lindsley E, et al: In vivo cytometry: A spectrum of possibilities. Cytometry A 69A:142-146, 2006

46. Huttner HB, Janich P, Kohrmann M, et al: The stem cell marker prominin-1/CD133 on membrane particles in human cerebrospinal fluid offers novel approaches for studying CNS disease. Stem Cells 26:698-705, 2008

47. Glinsky G, Berezovska O, Glinskii A: Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. J Clin Invest 115: 1503-1521, 2005

48. Bennett J, Duan D, Engelhardt J, et al: Real-time, noninvasive in vivo assessment of adeno-associated virus- mediated retinal transduction. Invest Ophthalmol Vis Sci 38:2857-2863, 1997

49. Contag C, Jenkins D, Contag P, et al: Use of reporter genes for optical measurements of neoplastic disease in vivo. Neoplasia 2:41-52, 2000

50. Chen BE, Lendvai B, Nimchinsky EA, et al: Imaging high-resolution structure of GFP-expressing neurons in neocortex in vivo. Learn Mem 7:433-441, 2000

51. Wang S, El-Deiry WS: Inducible silencing of KILLER/DR5 in vivo promotes bioluminescent colon tumor xenograft growth and confers resistance to chemotherapeutic agent 5-fluorouracil. Cancer Res 64:6666-6672, 2004

52. Kim S-H, Nakagawa H, Navaraj A, et al: Tumorigenic conversion of primary human esophageal epithelial cells using oncogene combinations in the absence of exogenous Ras. Cancer Res 66: 10415-10424, 2006

53. Chishima T, Miyagi Y, Wang X, et al: Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression. Cancer Res 57:2042-2047, 1997

54. Goodison S, Kawai K, Hihara J, et al: Prolonged dormancy and site-specific growth potential of cancer cells spontaneously disseminated from nonmetastatic breast tumors as revealed by labeling with green fluorescent protein. Clin Cancer Res 9:3808-3814, 2003

55. Fukumura D, Xu L, Chen Y, et al: Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. Cancer Res 61:6020-6024, 2001

56. Massoud TF, Gambhir SS: Integrating noninvasive molecular imaging into molecular medicine: An evolving paradigm. Trends Mol Med 13:183-191, 2007

57. Binstadt BA, Patel PR, Alencar H, et al: Particularities of the vasculature can promote the organ specificity of autoimmune attack. Nat Immunol 7:284-292, 2006

58. Montet X, Figueiredo J-L, Alencar H, et al: Tomographic fluorescence imaging of tumor vascular volume in mice. Radiology 242:751-758, 2007

59. Nahrendorf M, Sosnovik DE, Waterman P, et al: Dual channel optical tomographic imaging of leukocyte recruitment and protease activity in the healing myocardial infarct. Circ Res 100:1218-1225, 2007

60. Dicker D, Kim S, Jin Z, et al: Heterogeneity in non-invasive detection of apoptosis among human tumor cell lines using annexin-V tagged with EGFP or Qdot-705. Cancer Biol Ther 4:1014-1017, 2005

61. Ntziachristos V, Schellenberger E, Ripoll J, et al: Visualization of antitumor treatment by means of fluorescence molecular tomography with an annexin V-Cy5.5 conjugate. Proc Natl Acad Sci U S A 101: 12294-12299, 2004

62. Sweeney T, Mailänder V, Tucker A, et al: Visualizing the kinetics of tumor-cell clearance in living animals. Proc Natl Acad Sci U S A 96:12044-12049, 1999

63. Shaner NC, Campbell RE, Steinbach PA, et al: Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp red fluorescent protein. Nat Biotechnol 22:1567-1572, 2004

64. Winnard PJ Jr, Kluth JB, Kato Y, et al: Development of novel chimeric transmembrane proteins for multimodality imaging of cancer cells. Cancer Biol Ther [Epub ahead of print September 1, 2007]

65. Askenasy N, Stein J, Farkas DL: Imaging approaches to hematopoietic stem and progenitor cell function and engraftment. Immunol Invest 36: 713-738, 2007

66. Ciancio SJ, Coburn M, Hornsby PJ: Cutaneous window for in vivo observations of organs and angiogenesis. J Surg Res 92:228-232, 2000

67. Dewhirst M, Shan S, Cao Y, et al: Intravital fluorescence facilitates measurement of multiple physiologic functions and gene expression in tumors of live animals. Dis Markers 18:293-311, 2002

68. Jayaraman S, Song Y, Vetrivel L, et al: Noninvasive in vivo fluorescence measurement of airway-surface liquid depth, salt concentration, and pH. J Clin Invest 107:317-324, 2001

69. Sorg B, Moeller B, Donovan O, et al: Hyperspectral imaging of hemoglobin saturation in tumor microvasculature and tumor hypoxia development. J Biomed Opt 10:44004, 2005

70. Contag CH: In vivo pathology: Seeing with molecular specificity and cellular resolution in the living body. Annu Rev Pathol 2:277-305, 2007

71. Moore A, Josephson L, Bhorade RM, et al: Human transferrin receptor gene as a marker gene for MR imaging. Radiology 221:244-250, 2001

72. Louie AY, Huber MM, Ahrens ET, et al: In vivo visualization of gene expression using magnetic resonance imaging. Nat Biotechnol 18:321-325, 2000

73. Adonai N, Nguyen K, Walsh J, et al: Ex vivo cell labeling with 64Cu-pyruvaldehyde-bis(N4-methylthiosemicarbazone) for imaging cell trafficking in mice with positron-emission tomography. Proc Natl Acad Sci U S A 99:3030-3035, 2002

74. Massoud TF, Gambhir SS: Molecular imaging in living subjects: Seeing fundamental biological processes in a new light. Genes Dev 17:545-580, 2003

75. Beekman F, McElroy D, Berger F, et al: Towards in vivo nuclear microscopy: Iodine-125 imaging in mice using micro-pinholes. Eur J Nucl Med Mol Imaging 29:933-938, 2002

76. Chatziioannou AF, Cherry SR, Shao Y, et al: Performance evaluation of microPET: A high-resolution lutetium oxyorthosilicate PET scanner for animal imaging. J Nucl Med 40:1164-1175, 1999

77. Chatziioannou A, Tai Y, Doshi N, et al: Detector development for microPET II: A 1 microl resolution PET scanner for small animal imaging. Phys Med Biol 46:2899-2910, 2001

78. Namavari M, Barrio J, Toyokuni T, et al: Synthesis of 8-[(18)F]fluoroguanine derivatives: In vivo probes for imaging gene expression with positron emission tomography. Nucl Med Biol 27: 157-162, 2000

79. Love Z, Wang F, Dennis J, et al: Imaging of mesenchymal stem cell transplant by bioluminescence and PET. J Nucl Med 48:2011-2020, 2007

80. Gambhir S, Bauer E, Black M, et al: A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. Proc Natl Acad Sci U S A 97:2785-2790, 2000

81. Chin F, Namavari M, Levi J, et al: Semiautomated radiosynthesis and biological evaluation of [(18)F]FEAU: A novel PET imaging agent for HSV1-tk/sr39tk reporter gene expression. Mol Imaging Biol 10:82-91, 2008

82. Doubrovin M, Ponomarev V, Beresten T, et al: Imaging transcriptional regulation of p53-dependent genes with positron emission tomography in vivo. Proc Natl Acad Sci U S A 98:9300-9305, 2001

83. Molofsky AV, Pardal R, Iwashita T, et al: Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. Nature 425:962-967, 2003

84. Park I-K, Qian D, Kiel M, et al: Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature 423:302-305, 2003

85. van der Lugt NM, Domen J, Linders K, et al: Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. Genes Dev 8:757-769, 1994

86. Hosen N, Yamane T, Muijtjens M, et al: Bmi-1-green fluorescent protein-knock-in mice reveal the dynamic regulation of Bmi-1 expression in normal and leukemic hematopoietic cells. Stem Cells 25:1635-1644, 2007

87. Amoh Y, Yang M, Li L, et al: Nestin-linked green fluorescent protein transgenic nude mouse for imaging human tumor angiogenesis. Cancer Res 65:5352-5357, 2005

88. Barraud P, Thompson L, Kirik D, et al: Isolation and characterization of neural precursor cells from the Sox1-GFP reporter mouse. Eur J Neurosci 22:1555-1569, 2005

89. Beltrami AP, Cesselli D, Bergamin N, et al: Multipotent cells can be generated in vitro from several adult human organs (heart, liver, and bone marrow). Blood 110:3438-3446, 2007

90. Guzman ML, Neering SJ, Upchurch D, et al: Nuclear factor-{kappa}B is constitutively activated in

primitive human acute myelogenous leukemia cells. Blood 98:2301-2307, 2001

91. Takahashi K, Tanabe K, Ohnuki M, et al: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861-872, 2007

92. Wernig M, Meissner A, Foreman R, et al: In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 448:318-324, 2007

93. Bonnet D, Dick J: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730-737, 1997

94. Singh SK, Clarke ID, Terasaki M, et al: Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821-5828, 2003

95. Singh SK, Hawkins C, Clarke ID, et al: Identification of human brain tumour initiating cells. Nature 432:396-401, 2004

96. Shah K, Weissleder: Molecular optical imaging: Applications leading to the development of present day therapeutics. NeuroRx 2:215-225, 2005

97. Cheung AMS, Wan TSK, Leung JCK, et al: Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD//SCID engrafting potential. Leukemia 21:1423-1430, 2007

98. Hirschmann-Jax C, Foster A, Wulf G, et al: A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci U S A 101:14228-14233, 2004

99. Yamauchi K, Yang M, Jiang P, et al: Real-time in vivo dual-color imaging of intracapillary cancer cell and nucleus deformation and migration. Cancer Res 65:4246-4252, 2005

100. Sakaue-Sawano A, Kurokawa H, Morimura T, et al: Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. Cell 132:487-498, 2008

101. Wang T, Friedland S, Sahbaie P, et al: Functional imaging of colonic mucosa with a fibered confocal microscope for real-time in vivo pathology. Clin Gastroenterol Hepatol 5:1300-1305, 2007

