Medical Chemistry to Spy Cancer Stem Cells from Outside the Body

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Abstract: Accumulating evidence indicates that cancer is maintained by cancer stem cells (CSC). The goal of molecular imaging is to detect pathologic biomarkers, which can lead to early recognition of cancer, better therapeutic management, and improved monitoring for recurrence. The main focus of this review is to describe the different classes of tracers, contrast agents and dyers, and their putative application to improve cancer stem cells detection and follow-up. Although the *in vivo* cancer diagnosis has not significantly changed for the past three decades, however, in the future it might be possible to trace all cancer cells, including the cancer stem cells.

Key Words: Biomarkers, molecular image, cancer, mouse models, cancer stem cells, translational oncology, experimental therapeutics.

INTRODUCTION

Molecular imaging is an emerging technology at the life science/physical science interface which is set to revolutionize our understanding and treatment of disease [1, 2]. The tools of molecular imaging are the imaging modalities and their corresponding contrast agents. The diverse nature of molecular imaging requires knowledge from life, chemistry and physical sciences for its successful development and implementation (Fig. **1**). The physical basis of these imaging modalities, the chemistry behind contrast agents and the imaging parameters of sensitivity, temporal resolution and spatial resolution are described. Then, the specificity of contrast agents for targeting and sensing molecular events, and some applications of molecular imaging in biology and medicine are given.

Progress toward a molecular characterization of cancer would have important clinical benefits, including (i) detecting cancer earlier based on molecular characterization, (ii) predicting the risk of precancerous lesion progression, (iii) detecting margins in the operating room in real time, (iv) selecting molecular therapy rationally and (v) monitoring response to therapy in real time at a molecular level. Current therapies to treat advanced cancers are not effective, these types of neoplasias evade many of the current treatments because tumoral cells in these advanced states reach numerous genetic alterations which makes very difficult to find a way that effectively revert all the processes that have already occur [3]. People who develop cancer have a very advanced illness when they are diagnosed. The survival index for people diagnosed with advanced cancer has changed little in the last 20 years [4].

New technologies of not invasive diagnosis based on image can now be used to screen continuously the development of the tumor "*in vivo*", the effects of the therapeutic products in the different cellular populations, or even, on certain biological molecules [5-8] and to establish biomarkers in blood that allow an early, rapid and effective diagnosis of the tumor [4, 9-12]. Non-invasive image systems *in vivo* can be used to detect although primary tumors as metastasis, as well as to monitor the physiological associated-events, such as blood volume or tumor perfusion [13-16]. These skills also can be used to trace new molecular markers. The advances in the biomedical sciences have been accelerated by the introduction of the new non-invasive image technologies *in vivo* in the last years. These skills are a valuable hardware in the development of the basic and preclinical sciences that use animal models for research [12, 17].

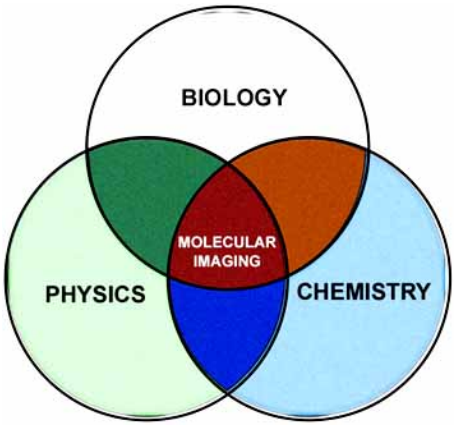

Fig. (1). Molecular Imaging refers to the characterization and measurement of biological processes at the molecular level. Molecular Imaging is inherently a multimodality approach from several disciplines as biology, physics or chemistry.

The concept of early detection - finding the initial phases of the tumor development, before it spread and become incurable - has supposed a more and more attractive field of investigation in the last years. Up to this moment, nevertheless, there have been few ones successes in the process of early detection, if only they have not proved to be sufficiently effective and practical for habitual use [4].

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It is necessary to do ourselves some important questions: why do we need an early detection in tumors? And: what offers this technology to the control of the illness development? What are the requisites in order that the early detection turns out to be effective and practical?, and: why cannot the majority of the tests that are in use at present satisfy these requisites?.

CANCER STEM CELLS

There is an increasing evidence supporting the cancer stem cell hypothesis [18-21]. The stem cells are defined as cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation. Many cancers, like normal organs, seem to be maintained by a hierarchical organization that includes slowly dividing stem cells, rapidly dividing transit amplifying cells (precursor cells) and differentiated cells [22]. It was first extensively documented for leukaemia and multiple myeloma that only a small subset of cancer cells is capable of extensive proliferation [23-25]. For example, when mouse myeloma cells were obtained from mouse ascites, separated from normal haematopoietic cells and put in clonal *in vitro* colony-forming assays, only 1 in 10,000 to 1 in 100 cancer cells were able to form colonies [23]. A substantial characteristic of stem cells is their ability for self-renewal without loss of proliferation capacity with each cell division. The stem cell paradigm postulates that within every tissue there is a small fraction of cells that are the progenitors of every other cell in that tissue (Fig. **2**) [26, 27]. Stem cells have three distinct properties: (i) self-renewal – every time a stem cell divides at least one of the daughter cells is another stem cell; (ii) pluripotency – stem cells give rise to all the other cells within that cell lineage; (iii) longevity – the stem cell may even be immortal [23, 28]. Whether cancer stem cells exist has profound implications for both the understanding and the treatment of cancer.

All these observations have covered the way to a new model of cancer: the 'stem cell model' and, as a result, the term 'cancer stem cell' has been introduced. This model is consistent with some clinical observations. Although standard chemotherapy kills most cells in a tumor, cancer stem cells remain viable [29-32]. Despite the small number of such cells, they might be the cause of tumor recurrence (30).

Further characterization of cancer stem cells is needed in order to find ways to destroy them, which might contribute significantly to the therapeutic management of malignant tumors [33-35]. Here, imaging-based technology to follow-up and positioned CSC inside the body were essential to this therapeutic management (Fig. **3**).

IMAGING TECHNIQUES AND CONTRAST AGENTS

Molecular imaging techniques span the electromagnetic spectrum from ultrasonic to gamma ray and X-ray frequen-

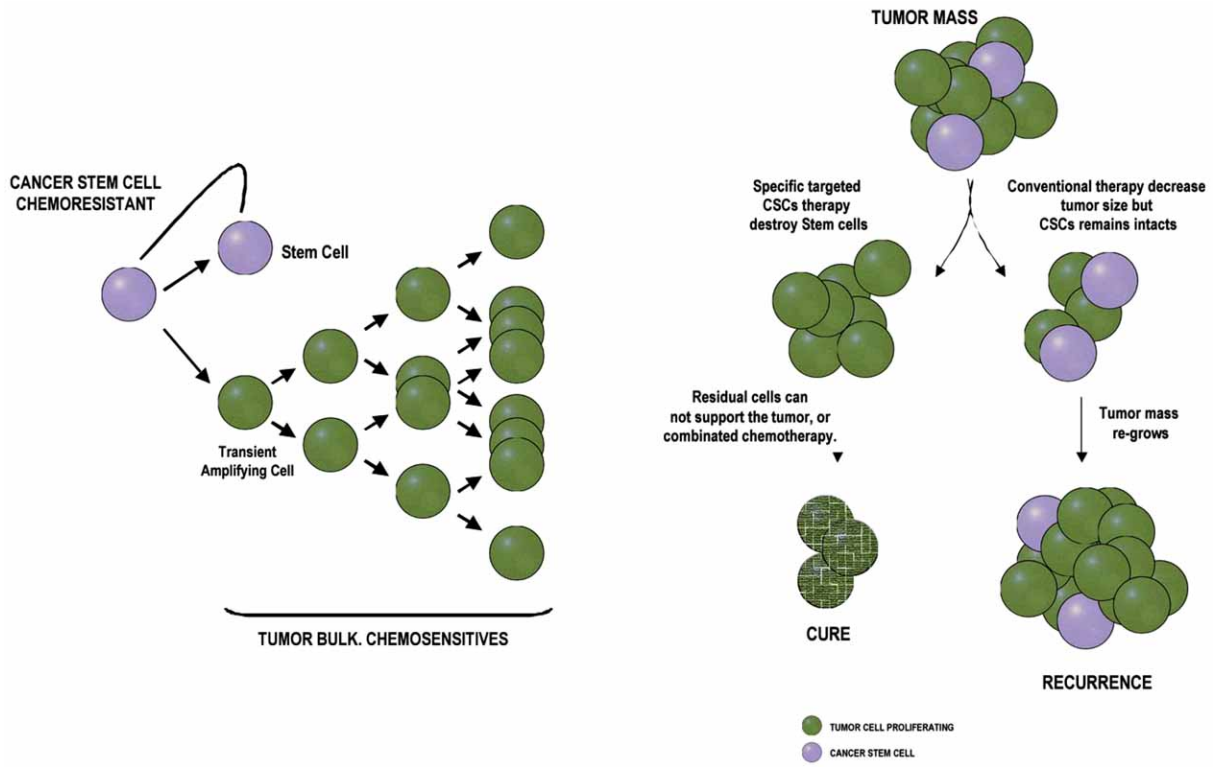


Fig. (2). Cancer Stem Cell (CSC) biology in tumor formation and maintenance. Just few cells inside the tumor contain the ability to maintain it. These Cancer Stem Cells when divided, gives an identical immortal daughter cell (violet), resistant to conventional chemotherapy, and Transient Amplifying cells (green) responsible for tumor bulk cell proliferation, and sensitive under classical chemotherapy (left). Outcome of CSCs targeted therapy *versus* conventional one. From a tumor mass, conventional therapy just decrease tumor bulk (green) but leaves cancer stem cells (violet) intact, this treatment produce tumor recurrence; in contrast CSCs-targeted therapy destroy stem cells and cause cancer cure (right).

Fig. (3). Molecular Imaging technology based on CSCs research allows single Cancer Stem Cell identification inside tumor bulk. Conventional technology could not discriminate between Tumor Cell Proliferating and Cancer Stem Cells.

cies with boards differences between sensitivity and spatial resolution (Fig. **4**). To gain an understanding of these techniques, and the issues that affect their performance, we will describe their physical basis, instrumentation and chemistry behind use of contrast mechanisms and agents. Contrast agents described here could be found in **MICAD** - Molecular Imaging and Contrast Agent Database, a key component of the "Molecular Libraries and Imaging" program of the NIH Roadmap; developed by the National Center for Biotechnology Information (NCBI), at the National Institutes of Health (NIH): http://www.micad.nih.gov. (MICAD) is an online source of information on *in vivo* molecular imaging agents based on recommendations from the scientific community.

MAGNETIC RESONANCE IMAGING

MRI is based on the detection of molecules that contain nuclei that possess the property of nuclear spin [36]. The application of an oscillating radio frequency (RF) magnetic field causes some of the spinning nuclei from a lower energy state to move to a higher one. Spins that have moved to the higher energy state will eventually return to the lower energy state, with the emission of a RF signal, this is the detected signal in MRI. These two energy-loss mechanisms have time constants T1 and T2, respectively, which are sample/tissue and static-field strength dependent [37, 38]. MRI has a number of contrast agent mechanisms for molecular imaging that modify either T1 or T2 relaxation time constants, proton density, or nuclear polarization, for improved sensitivity and specificity.

T1 contrast agents increase the rate of energy exchange between the nuclear spin system and the thermal reservoir, and hence reduce T1 and increase the number of spins that can absorb energy from the next RF pulse [39, 40]. T1 contrast agents are usually based on paramagnetic ions or stable free radical molecules [41], such as Gadolinium III complexes [42, 43] with a short circulating half-life (minutes).

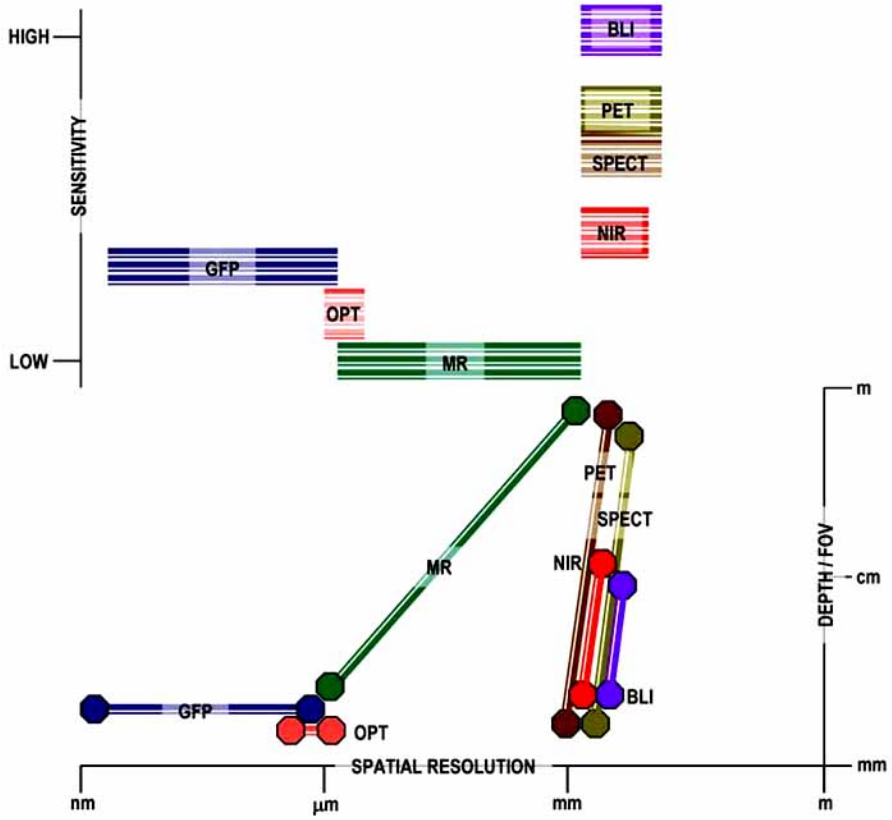


Fig. (4). Ratio between spatial resolution, sensitivity and depth of penetration/field of view (FOV) for different molecular imaging modalities. GFP (Green Fluorescent Protein); OPT (Optical Projection Tomography); MR (Magnetic Resonance); BLI (BioLumIniscence); PET (Postiron Emission Tomography) and SPECT (Single Photon-Emission Computed Tomography).

T1 CONTRAST AGENTS (Fig. 5):

Gadobenate. (Fig. **5a**) [44]

(MultiHance®, B-19036, Gd-BOPTA/Dimeg, gadobenate dimeglumine).Gd-BOPTA

Gadobutrol. (Fig. **5b**) [45]

([1,4,7-Tris(carboxymethyl)-10-(1-(hydroxymethyl)-2,3-dihydroxypropyl)-1,4,7,10-tetraazacyclododecanato]; Gadovist®; 10-[(1SR,2RS)-2,3-dihydroxy-1-hydroxymethylpropyl]-1,4,7,10-tetraazacyclodecane-1,4,7-triacetic acid, gadolinium). Gd-DO3A-butrol.

Gadoversetamide. (Fig. **5c**) [46]

(OptiMARK®).Gd-DTPA-BMEA

Gadoteridol. (Fig. **5d**) [47]

(ProHance®, SQ 32692, MOLI001032).Gd-HP-DO3A

Gd-DTPA-Cystine diethyl ester copolymers. (Fig. **5e**) [48]

T2 contrast agents increase the rate of energy loss within the nuclear spin system by introducing magnetic field perturbations, which are manifested by a reduction in T2 and decreases in the local signal intensity. T2 contrast agents are based on ferromagnetic or superparamagnetic centres. These may take the form of monocrystalline, polycrystalline, cross-linked iron oxide magnetic cores (5–30 nm) embedded in a polymer coating with a total particle diameter of 17–50 nm, with larger molecules having a higher flexibility [49, 50].

Superparamagnetic iron oxide (SPIO) particles are potent and versatile contrast media for magnetic resonance imaging (MRI), and their efficacy has been shown to increase as their diameter decreases [51, 52]. SPIO particles were originally developed as liver MRI contrast agents to improve tumor detection at T2-weighted imaging. Nevertheless, SPIO particles administered as a bolus (e.g., ferucarbotran [53, 54]) only produce moderate signal enhancement, especially in the early stage of a dynamic T1-weighted MRI. Ultrasmall superparamagnetic iron oxide particles (USPIO), such as feruMoxtran-10 [55, 56], can only be administered as an infusion. Current SPIO and USPIO particles present very limited benefits compared with the low-molecular weight gadolinium-based contrast media [57, 58], which acquire their blood pool effect by binding to plasma proteins after intravenous injection.

T2 CONTRAST AGENTS (FIG. 5):

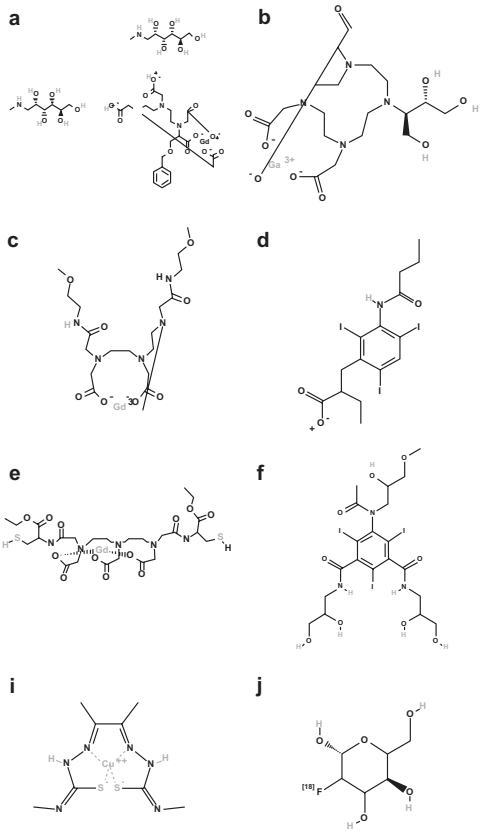
Cross-linked iron oxide-transactivator transcription CLIO-Tat. [59]

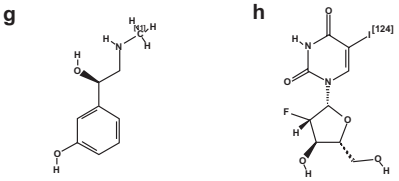
(FITC-CLIO-Tat, CLIO-Tat(FITC), Tat-CLIO).

MRI CONTRAST AGENTS:
T1 :
Gadobenate (a).
Gadobutrol (b).
Gadoversetamide (c).
Gadoteridol (d).
Gd-DTPA-Cystine diethyl ester copolymers (e).
T2 :
Cross-linked iron oxide–transactivator transcription CLIO-Tat .
Bombesin peptide conjugated–cross-linked iron oxide-Cy5.5.
Cross-linked iron oxide-Cy5.5.
Cross-linked iron oxide–C-AHA-AREPPTRTFAYWGK(FITC).
Citrate-coated very small superparamagnetic iron oxide particles.
Annexin V-cross-linked iron oxide-Cy5.5.AnxCLIO-Cy5.5.

CT CONTRAST AGENTS:
N,N´-Bis(2,3-dihydroxypropyl)-5-[N-(2-hydroxyethyl-3-methoxypropyl)-acetamidol]-2,4,6-triiodoisophthalamide (f).

PET TRACERS:
R-[11C]Phenylephrine. [11C]PHEN (g).
2'-Fluoro-2'-deoxy-5'-[124I]iodo-1â-d-arabinofuranosyluracil. [124I]FIAU (h).
Copper(II) diacetyl-di(N4-methylthiosemicarbazone). Cu-ATSM (i).
[18F]Fluoro-2-deoxy-2-d-glucose. [18F]FDG, FDG (j).





(Fig. 5. Contd….)

SPECT TRACERS:
111Indium-diethylenetriaminepentaacetic acid-d-phenylalanine-octreotide (k).
99mTc-Ethylenedicysteine-folate. (99mTc-EC-folate) (l).
5-[123I]Iodo-3-[2(S)-2-azetidinylmethoxy]pyridine (m).

OPTICAL TRACERS:
Cyclo(RGDyK)-Cy5.5. RGD-Cy5.5 (n).

FLUORESCENT QDs TRACERS:
Arginine-glycine-aspartic acid peptide-labeled quantum dot 705. QD705-RGD.
NIR2-Folate (o).

ULTRASOUNDS CONTRASTS AGENTS
Microbubbles-echistatin. MBE.
Perflexane-Lipid Microspheres. AFO150.
Air-Filled, Cross-Linked, Human Serum Albumin Microcapsules.
Perflutren Lipid Microspheres. DMP 115.

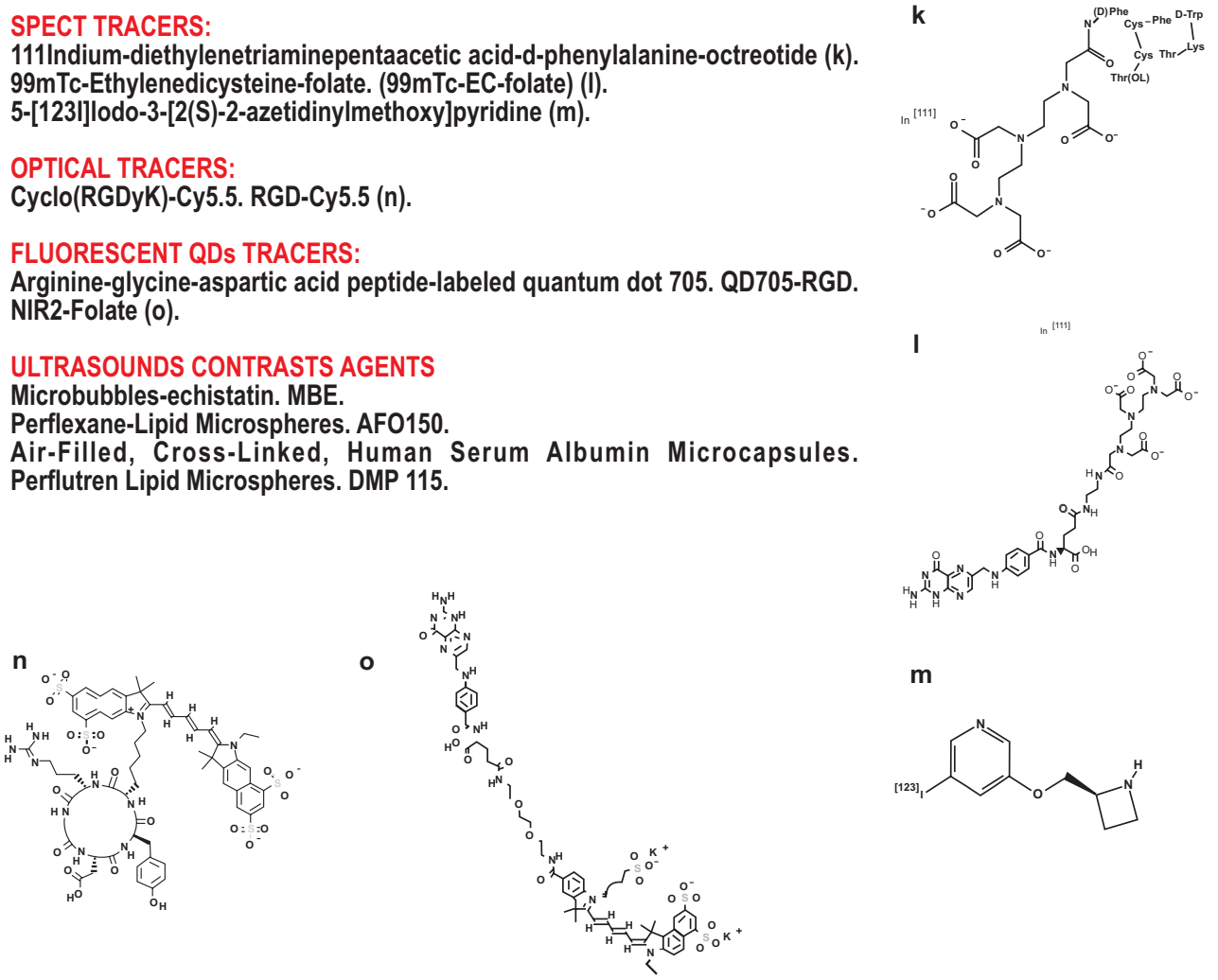


Fig. (5). Supramolecular chemistry and molecular structure of imaging tracers. Contrast agents clustered by imaging technique; common name are display, for synonyms or chemistry name refers to text. Additional information about chemical structure could be found in http://pubchem.ncbi.nlm.nih.gov/.

Bombesin peptide conjugated–cross-linked iron oxide-Cy5.5. [60]

BN-CLIO-Cy5.5. ((FITC)BCDDDGQRLGNQWAVGHLM-CLIO(Cy5.5), BN-CLIO(Cy5.5))

Cross-linked iron oxide-Cy5.5. [61]

CLIO-Cy5.5.

Cross-linked iron oxide–C-AHA-AREPPTRTFAYWGK (FITC). [62]

CLIO-EPPT.

Citrate-coated very small superparamagnetic iron oxide particles. [63]

VSOP-C184.

Annexin V-cross-linked iron oxide-Cy5.5.AnxCLIO-Cy5.5. [64]

Others MRI contrast agents are: **Proton density contrast agents**. This can be achieved using magnetization transfer (MT; [65]), chemical exchange saturation transfer (CEST; [48, 66, 67]), or paramagnetic chemical exchange saturation transfer (PARACEST; [67]). MT is based on the exchange of magnetization between tissue-bound water and the larger bulk water pool, which results in a decrease in proton density. CEST is based on the slow exchange of magnetization between low molecular weight diamagnetic compounds, which introduces proton density contrast that can be turned on and off. PARACEST is based on the unusually slow water exchange of paramagnetic lanthanide complexes that increases the utility of CEST and has the potential to be used as biological y responsive agents capable of sensing molecular exchange phenomena in tissue [67]; and **Nuclear polarization contrast agents.** Those greatly increase (hyperpolarize) the weak nuclear polarization of atomic nuclei from

parts per million (ppm) towards unity. Hyperpolarization can be achieved using a variety of methods, but recent advances in dynamic nuclear polarization (DNP) have shown tremendous potential for molecular imaging [68, 69].

CT IMAGING

Computed tomography (CT), originally known as computed axial tomography (CAT or CT scan) and body section roentgenography, is a medical imaging method employing tomography where digital geometry processing is used to generate a three-dimensional image of an individue from a large series of two-dimensional X-ray source that rotates around the object where sensors are positioned on the opposite side of the circle. Many data scans are progressively taken as the object is gradually passed through the gantry. They are combined together by the mathematical procedure known as tomographic reconstruction.

X-Ray imaging (planar and tomographic) techniques depend on tissue density differences that provide the image contrast produced by X-ray attenuation between the area of interest and surrounding tissues [6, 70, 71]. Contrast enhancement (opacification) with use of contrast agents increases the degree of contrast and improves the differentiation of pathologic processes from normal tissues. Because iodine, an element of high atomic density, causes high attenuation of X-rays within the diagnostic energy spectrum, water-soluble and reasonably safe iodinated contrast agents in intravenous injectable forms have been developed for clinical applications [6, 72].

CT CONTRAST AGENT:

N,N´-Bis(2,3-dihydroxypropyl)-5-[N-(2-hydroxyethyl-3-methoxypropyl)-acetamidol]-2,4,6-triiodoisophthalamide. (Fig. 5f)

NUCLEAR IMAGING

Nuclear imaging is based on the administration and detection of decaying radioisotopes *in vivo* [73]. The radioisotopes are combined with biologically active compounds to form radiopharmaceuticals, which target specific biochemical events. The decay of a radioisotope emits a positron or a gamma ray, which produces either two or a single high-energy photon. The detection of these photons is performed using Positron emission Tomography (PET) and single photon-emission computed tomography (SPECT), respectively [8, 74, 75].

Positron-Emission Tomography (PET)

PET is based on the decay of a radioisotope, which emits a positron and annihilates with an electron to produce two high-energy (511 keV) photons that propagate in nearly opposite directions [76-79]. For PET imaging, a positron emission event is recorded every time two photons are detected within a short timing window (10 ns) by opposing crystals in the cylindrical ring, i.e. the photons are coincident.

PET is inherently a contrast-agent (tracer) based imaging method which uses a number of positron emitting isotopes including 11C, 13N, 15O, 18F, 64Cu, 68Ga, 76Br and 94mTc [78, 80], which emit two photons upon annihilation. The introduction of LSO crystals with optic fiber readout has enabled one-to-one coupling to multiplexed PMTs, and thus has significantly reduced crystal size and improved spatial resolution to less than 2 mm, and therefore has enabled small animal micro- PET applications [70].

There are more than 120 PET tracers in **MICAD** . Here we show a brief overview of some of then divided by source of signal: 11C; 60,61,62,64,67Cu; 18F and 124I.

PET TRACERS

R-[11C]Phenylephrine. [11C]PHEN. (Fig. **5g**) [81]

2'-Fluoro-2'-deoxy-5'-[124I]iodo-1β-d-arabinofuranosyluracil. [124I]FIAU. (Fig. **5h**) [48, 82]

Copper(II) diacetyl-di(N4-methylthiosemicarbazone). Cu-ATSM. (Fig. **5i**) [83]

[18F]Fluoro-2-deoxy-2-d-glucose. [18F]FDG, **FDG.** (Fig. **5j**) [84]

Use of PET imaging techniques for detection and localization of cancer in the body is based on the unique capability of PET to evaluate metabolic activity in human neoplasms. The glucose analog [18F]FDG has proven useful as an oncologic PET probe for many forms of cancer on the basis of accelerated rates of glycolysis in malignancies [85-87]. However, FDG PET has limited sensitivity for detection of certain cancer types, such as androgen-dependent prostate cancer, motivating efforts to develop new oncologic PET tracers.

Single Photon Emission Tomography (SPECT)

SPECT is similar to PET, but the radioisotopes used for SPECT emit only a single high-energy (gamma) photon, and hence SPECT does not require the detection of coincidence, and, therefore, a different detector archi- tecture is required. SPECT detects gamma photons using a gamma camera which is step-rotated around the subject and forms an image using a back-projection algorithm [88-90]. SPECT is also inherently a contrast agent (tracer) based imaging method whic h uses a number of gamma emitting isotopes including 133Xe, 99mTc and 123I. These are heavy radioisotopes, they produce a single photon upon decay, and are more readily available and have longer decay times (tone-half hours to days) than those used in PET [91]. SPECT tracers can illuminate other areas rather than their target area, and may require coregistration with another imaging modality such as computer tomography for confirmation of target site [92]. The sensitivity of SPECT is lower than PET because it uses a mechanical collimator which absorbs many photons, whereas in PET, collimation is done electronically. The sensitivity of SPECT is one to two orders of magnitude less than PET (approximately 10κ10 M; [91]).

There are 29 SPECT tracers in **MICAD**: http://www.micad.nih.gov. Here we show a brief overview of some of then divided by source of signal: 111In; 99mTc and123I.

SPECT TRACERS:

111Indium-diethylenetriaminepentaacetic acid-d-phenylalanine-octreotide. (Fig. **5k**) [93]

(111In-DTPA-octreotide; 111In-DTPA-OC; 111In-DTPA-d-Phe-octreotide;111In-pentetreotide. OctreoScan®).

99mTc-Ethylenedicysteine-folate. (99mTc-EC-folate). (Fig. **5l**) [94]

5-[123I]Iodo-3-[2(S)-2-azetidinylmethoxy]pyridine. (Fig. **5m**) [95]

(5-[123I]IA,5-[123I]Iodo-A-85380).

SPECT, or Single Photon Emission Computed Tomography, is the most commonly used form of tomographic imaging. SPECT cameras are usually used with radiopharmaceuticals that have longer half-lives than those used with PET. Combination scanners improve the diagnostic accuracy of diagnosis for many diseases, enhance physicians' understanding of diseases, and also reduce the number of imaging appointments patients require.

OPTICAL IMAGING

Optical imaging is based on detecting the transmission of light (photons) through biological tissue. The propagation of light through biological tissue experiences both absorption and scattering simultaneously [96-98]. Absorption and scattering are wavelength and penetration-depth dependent. Absorption is large in the ultraviolet (UV), near visible and infrared (IR), but low in red and near-infrared (NIR; 650–1000 nm). For animal and human *in vivo* and *in vitro* molecular imaging, the optical imaging technologies of diffuse optical tomography (DOT), OPT, NIR fluorescence imaging, fluorescence protein imaging and bioluminescence imaging (BLI) are emerging as powerful tools for measuring dynamic metabolic processes and probing protease, protein and enzymatic activity (Fig. **4**).

Optical Fluorescence

Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets [99]. However, the intrinsic fluorescence of biomolecules poses a problem when visible light (350-700 nm) absorbing fluorophores are used. Near-infrared (NIR) fluorescence (700-1000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection [100]. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a non-invasive alternative to radionuclide imaging.

OPTICAL TRACERS (Fig. 5):

Cyclo(RGDyK)-Cy5.5. RGD-Cy5.5. (Fig. **5n**)

Fluorescent Semiconductor Quantum Dots (QDs).

Fluorescent semiconductor quantum dots (QDs) are nanocrystals made of CdSe/CdTe-ZnS with radii of 1-10 nm [101-104]. They can be tuned to emit in a range of wavelengths by changing their sizes and composition, thus providing broad excitation profiles and high absorption coefficients. They have narrow and symmetric emission spectra with long, excited-state lifetimes, 20-50 ns, as compared with 1-10 ns of fluorescent dyes. They process good quantum yields of 40-90% and high extinction coefficients. They are more photo-stable than conventional organic dyes. They can be coated and capped with hydrophilic materials for additional conjugations with biomolecules, such as peptides, antibodies, nucleic acids, and small organic compounds, which were tested *in vitro* and *in vivo* [105-108]. Although many cells have been labeled with QDs *in vitro* with little cytotoxicity, there are only limited studies of long-term toxicity of QDs in small animals [109-112]. However, little is known about the toxicity and the mechanisms of clearance and metabolism of QDs in humans.

QDs as biological probes have lived up to the hopes of their initial promoters [105, 112]. They will not replace the well-established fluorophores or fluorescent protein-fusion technologies, but will complement them for applications needing better photostability, NIR emission, or single-molecule sensitivity over long time scales. Undoubtedly, biologists will catch on to these exciting developments and will find as yet unforeseen applications for this new toolkit, thus enhancing and complementing their existing arsenal of bioimaging tools.

FLUORESCENT QDs TRACERS (Fig. 5):

Arginine-glycine-aspartic acid peptide-labeled quantum dot 705. QD705-RGD. [113]

NIR2-Folate. (Fig. **5o**)

ULTRASOUNDS

Ultrasound is the most widely used imaging modality [114-116] and expanding its role in noninvasive molecular imaging with ligand-carrying microbubbles [115]. Microbubbles are comprised of spherical cavities filled by a gas encapsulated in a shell. The shells are made of phospholipids, surfactant, denatured human serum albumin or synthetic polymer. Ligands and antibodies can be incorporated into the shell surface of microbubbles. Microbubbles are usually 2 to 8 microns in size. They provide a strongly reflective interface and resonate to ultrasound waves. They are used as ultrasound contrast agents in imaging of inflammation, angiogenesis, intravascular thrombus, and tumors [117-119]. They are also potentially used for drug and gene delivery [120]. Contrast agents or echopharmaceuticals are designed to change the attenuation (absorption, reflection, and refraction) or impedance (resistance to sound propagation) of sound for enhancing the differentiation of the signal (echo) of a target organ from that of the surrounding tissue [117-120]. Gas-liquid emulsions (microbubbles or gaseous particles) are highly echogenic *in vivo* because of the nonlinear rarefaction and compression effects that lead to volume pulsations of microbubbles [116, 118]. Human serum albumin, synthetic polymers and phospholipids have been used to construct the membrane of these bubbles. Microbubble preparations of various formulations have been developed, and their clinical usefulness depends very much on the size and stability of these bubbles *in vivo*.

Six sources of signal were described in MICAD (http://www.micad.nih.gov) for ultrasound method of detection. Here we focus four of the more common ones in biomedicine.

ULTRASOUNDS CONTRASTS AGENTS (Fig. 5):

Microbubbles-echistatin. MBE. [121]

Perflexane-Lipid Microspheres. AFO150. [122]

Air-Filled, **Cross-Linked**, **Human Serum Albumin Microcapsules.** [123]

(Air-filled HSA microcapsules. Quantison™).

Perflutren Lipid Microspheres. DMP 115. [124]

(DMP 115, YM454. Definity®; octafluoropropane-lipid microspheres; liposome-encapsulated perfluoropane microspheres)

IMAGING TECHNOLOGY AND CANCER STEM CELLS. GENE EXPRESSION IMAGING.

There is a critical need for improved methods to noninvasively detect and monitor treatment of cancer. Molecular Imaging technology holds unique promise in this regard because of its ability to exploit genetic and biochemical abnormalities present in cancer cells through the use of specific molecular imaging probes. Molecular imaging seeks to understand the components, processes, dynamics and therapies of disease from a molecular perspective, by using and developing imaging technologies and contrast agents. The key to success in this diverse endeavour is the multi-disciplinary interaction between the life and physical sciences (Fig. **1**). For example, the disciplines of physics and engineering have provided the hardware; mathematics and computing the software and analysis tools; chemistry, materials science and biology the contrast agents; and biology and medicine have posed the biological and medical questions to be answered.

Effective use of the tools of molecular imaging requires knowledge of the basis of detection of the imaging modality, contrast agent mechanism and the biological environment. Molecular imaging provides an interface between the life and physical sciences at the level of the contrast agent. Recent developments in contrast agent technology have increased their specificity and therapeutic potential. This will revolutionize the manner in which disease is managed and will rely on a multi-modal and multi-disciplinary approach drawn from specialists from the life and physical sciences, who understand each other's language and share the common goal of combating disease.

The main problem in the cell detection based on molecular imaging is the sensitivity level that provides these techniques [125]. When the objective is to detect a huge tumor mass (or not so massive), the implemented technology usually is sufficient to detect it. The problem begin when the mass is restricted to about thousands of cells [126, 127].

Cancer Stem Cells (CSCs) represents a very small percentage of the total tumor cells in proliferation, If we can be able to differentiate and discriminate between these cellular types we perform an essential tool for the studies on this type of cells (Fig. **3**). The techniques we had reviewed here cover the more important aspects of the molecular imaging in live individuals, from small animals to normal clinic. To these technology is necessary to add all the approaches based on cell microscopy, which, focus on CSCs provide very helpful information based on their sensitivity and magnification. Nevertheless, our first goal, at the moment, is to be able to "track" these cells in whole individuals, for a correct diagnosis and therapy follow-up through complete remission.

In addition to dramatic advances in new high-resolution imaging instrumentation that now make studies in small animals possible, related progress in the development of highly specific probes as sources for imaging contrast, and in molecular and cell biology techniques that can be adapted for *in vivo* imaging studies [30, 128-130], have helped encourage the rapid expansion of imaging methods to the study of disease biology.

Classically, monitoring gene expression usually requires tissue sampling to, for instance, measure messenger RNA levels, but such methods are invasive and unattractive as routine procedures for clinical investigators [30, 34, 35]. In contrast, gene expression imaging, at least ideally, can provide a seamless transition from studies in animals to later studies in humans [30, 128-130]. Gene expression imaging is one form of molecular imaging used to visualize, characterize, and quantify, spatially and temporally, normal as well as pathologic processes at cellular and subcellular levels within intact living organisms. These characteristics made this technology as one of the most promised for CSCs detection and follow-up [30].

Gene expression can be imaged using a PET-*reporter gene*, which codes for an enzyme or receptor that can be imaged using a PET tracer, and which can be introduced into the cell using a viral vector [131, 132]. For example, the herpes simplex 1 thymidine kinase (HSV1-*TK*) gene is imaged using labeled thymidine and acycloguanosine analogs, which are trapped in cells expressing the gene. If another gene, for example a therapeutic gene, is linked to the reporter gene, such that it is controlled by the same promoter, its expression can be inferred [131-135]. This technique has potential for monitoring gene therapy, whether for cancer or other disorders.

To date, almost all studies of reporter gene expression imaging at cell levels, have used adenoviral vectors carrying mutant variants of the murine herpes simplex virus type 1 thymidine kinase gene (mHSV1-tk) as the PET Reporter Gene (PRG) and the nucleoside 9-(4-[18F]fluoro-3-hydroxymethylbutyl)guanine ([18F]FHBG) as the PET Reporter Probe (PRP) [82, 131-137]. This strategy can be applied to Cancer Stem Cells inside the body; whit this system transgenic CSCs carrying a fusion gene of mHSV1-tk with/or not other reporter gene [enhanced green fluorescent protein (egfp)], driven by the constitutive cytomegalovirus promoter, is administered intratracheally *via* a replication-deficient adenovirus. At various times after administration of the virus, expression of the reporter tk gene can be assessed by intravenous administration of [18F]FHBG, a substrate for the thymidine kinase protein. Only tissues expressing the viral tk gene will trap this radiopharmaceutical and generate an imaging signal, which can be detected with an appropriate PET scanner.

On the other hand, SPECT technology allows antibodies labeling against specific membrane molecules that could

detect and follow-up those particular cells. To this SPECT approach (mainly based on Iodine) is necessary to add genomics and proteomics research to differentiate CSCs from the normal cells and the normal Stem Cells. Finally, the new technologies in optic contrast agents, mainly Quantum Dots, will allow, in a near future, to follow CSCs at the cellular level [125, 138].

CONCLUDING REMARKS

There is a critical need for improved methods to non-invasively detect and monitor treatment of cancer [30,136-138]. Molecular Imaging technology holds unique promise in this regard because of its ability to exploit genetic and biochemical abnormalities present in cancer cells through the use of specific molecular imaging probes. Molecular imaging seeks to understand the components, processes, dynamics and therapies of disease from a molecular perspective, by using and developing imaging technologies and contrast agents. Gene expression imaging is the last step inside new technology development for molecular imaging, now we can move from outside the body to inside, from organism to cell levels using the same approach. Therefore, in the future it might be possible to trace all cancer cells, including the cancer stem cells.

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