Molecular Imaging: Basic Approaches

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Introduction

Molecular imaging is an emerging research tool and clinical discipline that aims to discover and apply novel molecules (probes) and methods to image normal and pathological biological processes on a cellular and molecular level in vivo (Weissleder and Mahmood 2001; Wang et al. 2006; Rudin 2005; Pomper 2001; Hoffman and Gambhir 2007; Herschman 2003). One might think of molecular imaging as performing histology and pathology in vivo, without harming the subject. The molecular imaging probes that target specific cells, molecules, or biological events are equivalent to the stains and antibodies used in histology and pathology. The imaging technologies and methods, much like the microscope, provide the means to visualize these probes and report on the in vivo process.

Molecular imaging research is broadly multidisciplinary, integrating many areas of basic and clinical sciences (biology, physics, imaging sciences, chemistry, bioinformatics, engineering, to name a few). It has applications across nearly all clinical disciplines, including radiology, neurology, oncology, cardiology, and rheumatology. Molecular imaging has started to change the clinical practice, especially in the use of ^{18}F-2-fluoro-deoxy-D-glucose (FDG) as a probe for positron emission tomography (PET), in oncological and neurodegeneration imaging. In addition, of particular interest and relevance to drug discovery is the development of rodent imaging systems, which when combined with novel animal models of diseases and molecular imaging probes, would allow efficient assessment of the effectiveness of novel therapeutic drugs.

The current goals of molecular imaging are: (a) to synthesize highly sensitive and specific probes, targeting cells and molecules of interest to biological and pathological processes, (b) to develop new imaging technologies with high probe

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detection sensitivity, high image resolution, and fast acquisition time, (c) to detect disease in the early stage of development, and (d) to facilitate drug development and therapeutic monitoring.

In this chapter, we will introduce the imaging modalities commonly used in molecular imaging, describe relevant applications, and illustrate strategies for designing molecular imaging probes. The readers are encouraged to consult the references included in this chapter as a foundation for a more detailed understanding of the topics discussed.

Imaging Modalities and Imaging Agents

The imaging modalities used in molecular imaging include nuclear imaging (positron emission tomography (PET) and single photon emission computed tomography (SPECT)), magnetic resonance (MR) imaging, optical imaging (fluorescence and bioluminescence), and ultrasound (US) imaging. In Table 1, advantages and disadvantages based on sensitivity, spatial and temporal resolution, depth of signal penetration and cost are summarized. The selection of the imaging system will depend on the problem to be addressed.

Nuclear Imaging (Phelps 2004; Beekman and Vastenhouw 2004)

The main advantages of nuclear imaging are the high intrinsic sensitivity in the nano- to pico-molar concentration scale (only a very small dose of the molecular imaging probe will be needed) and the high penetration depth. Nuclear imaging modalities detect radioactive emission from decaying radionuclides injected into the subject. Nuclear imaging includes PET and SPECT. PET detects the simultaneous arrival of two photons emitted in opposite directions, resulting from the destructive collision between a positron and an electron within the tissue (dual photon detection). SPECT detects single gamma ray photons emitted from the tissue after gamma isotope emission (e.g., ^{99m}Tc, ^{111}In, ^{201}Tl, ^{123}I, and ^{131}I).

The main disadvantage of nuclear imaging is the low spatial resolution of the images. However, this limitation has been partially addressed with the development of integrated systems such as PET-CT (clinical and research) and SPECT-CT (research) scanners. The integration of computed tomography to the nuclear imaging modalities allows one to correlate the active regions with anatomical positions. Despite the low doses of the radiopharmaceuticals needed, radiation risk to both the subject and the staff performing the synthesis, handling, and injection of radiopharmaceuticals is a real concern. Another concern, especially in the FDG PET imaging, is the need for a nearby cyclotron to synthesize the radionuclides because of the short half-lives of some of the agents.

PET radioisotopes: the most common PET isotopes are ^{18}F, ^{11}C, ^{13}N, ^{15}O which can be incorporated within the tracer without a chelation chemistry pathway. ^{11}C is

Table 1 Imaging modalities commonly used in molecular imaging and their characteristics

Modality	Electromagnetic spectrum	Spatial resolution	Temporal resolution	Penetration depth	Imaging agent	Cost
PET	γ-Rays	1–2 mm	Low	High	^{11}C, ^{18}F, ^{124}I, ^{68}Ga, ^{64}Cu	High
SPECT	γ-Rays	<1 mm	Low	High	^{99}mTc, ^{123}I, ^{125}I, ^{111}In	Medium
MRI	Radio-frequency	100 μm	High	High	Paramagnetic and superparamagnetic agents	High
Fluorescence	UV–vis and near infrared	Low	High	Low	Fluorescent protein and fluorescent dyes	Low
Bioluminiscence	Visible	Low	High	Low	Luciferases	Low
Ultrasound	N/A	0.5 mm	Very high	Medium	Microbubbles and liposomes	Low

the most versatile isotope because it can label a large number of functional groups and then be incorporated into organic molecules. The main advantage of using these radioisotopes is that their incorporation will not modify the molecular properties of the final compound, thereby making them good candidates for studying the pharmacological biodistribution of new drugs as well as for targeting neurotransmitter and neuroreceptor systems. The limitation of ^{11}C is its short half-life (20 min). Fluorine-18 overcomes this problem because its half-life is 109 min, allowing more time for image acquisition. But in contrast to ^{11}C, fluorine incorporation may produce slight chemical changes that alter the biodistribution or binding properties of the initial molecule, hence fluorinated probes must be carefully evaluated before they can be used to substitute for the unlabeled compounds. The most common PET probe used is FDG (Sokoloff et al. 1977). The accumulation of the FDG in cells is directly related to high glucose uptake. Other nuclei being employed in clinical research include ^{124}I and ^{64}Cu.

SPECT radioisotopes: radioisotopes used for SPECT present longer half-lives than PET isotopes, from hours to several days. In order to target biological probes, radioisotopes such as ^{99m}Tc can be chelated with DTPA or DOTA, which themselves can be conjugated to different moieties for targeting to specific molecules and tissues. On the other hand, iodine-123 isotope, due to its similarities with endogenous iodine, can be used to monitor gene expression (Tjuvajev et al. 1996).

MR Imaging (Merbach et al. 2001)

More than 60 million clinical imaging procedures are now performed worldwide each year with magnetic resonance imaging (MRI), and emerging preclinical and clinical fusion technologies are becoming available. The main advantage of MRI is that no ionizing radiation or radiopharmaceutical agent is used. Instead, MRI detects the magnetic signals from nuclei (mainly protons). Unlike other tomographic modalities, MRI allows any arbitrary plane of imaging. High-resolution images with excellent soft tissue contrast between different tissues can be used to assess the anatomy with excellent spatial resolution. Despite these attractive parameters, the main obstacle in using MRI as a molecular imaging modality is its relatively low sensitivity to molecular probes compared to nuclear methods such as PET and SPECT.

The use of imaging agents has become an integral part of MR imaging for many applications because the use of exogenous imaging agents allows for better image contrast to be obtained between pathological and healthy tissues. About 35% of the MRI examinations make use of imaging agents, but this percentage is likely to increase further following the development of more effective and specific molecular imaging probes than are currently available. Unlike contrast agents used in X-ray computed tomography and in nuclear medicine, MR imaging agents are not directly visualized in the image. Only their effects are observed: increased contrast is brought about by the effect the imaging agent causes on (usually shortening of) the proton relaxation times, which consequently alters the intensity of the MR signal.

MR imaging agents can be divided into two main groups: positive or negative agents, depending on whether the contrast mechanism predominately affects the T_1- or T_2-weighted signal of the protons.

Positive imaging agents increase the T_1-weighted signal on the images by shortening the T_1 (as well as T_2, although the effect of T_2 shortening is usually not apparent on imaging unless very high local concentration is achieved, such as in the kidneys), and are predominately paramagnetic complexes. The choice of Gd(III) and Mn(II) as metals from MR applications stem from their optimal electronic properties (Lauffer 1987). Of the six clinically approved imaging agents used worldwide for intravenous administration, four of them are based on Gd(III). The first imaging agent approved for clinical use was the anionic Gd(DTPA)$^{2-}$ (Magnevist®, Schering AG, Germany) that, in more than 10 years of clinical experience, has been administered to more than 20 million patients. Other Gd(III) based imaging agents similar to Magnevist® are: Gd(DOTA)$^-$ (Dotarem®, GE Health, USA) and Gd(HPDO3A) (Prohance®, Bracco Imaging, Italy). These first generation imaging agents have been very useful, and have contributed to the rise of MRI utilization in clinical and preclinical imaging. They distribute mainly into the intravascular and interstitial space, and therefore are non-specific (Weinmann et al. 2003). As such these agents cannot cross intact blood–brain barrier, and have become useful in identifying lesions that cause disruption of the blood–brain barrier (e.g., tumors).

Negative imaging agents decrease the signal on T_2-weighted imaging by causing a large shortening of the proton T_2, and are exemplified by superparamagnetic iron oxide nanoparticles (SPIO) (Weissleder et al. 1990; Jung and Jacobs 1995). Iron oxide nanoparticles without explicit molecular specificity are well known in the literature to be used as reporters for several physiological processes and diseases. These include non-targeted cellular uptake, enhanced retention in tumors, macrophage phagocytosis, as well as accumulation in the liver, spleen, and lymph nodes. T_2- and T_2*-weighted sequences allow the detection of nanoparticle accumulation through T_2 shortening effect to provide dark contrast in the areas with the iron oxide uptake. Biodistribution of these particles and their efficient delivery depends mostly on their hydrodynamic radius and surface characteristics, as these parameters control the circulation time of the nanoparticles, accessibility to tissues, opsonization, and rate of cell-type uptake.

Ultrasound Imaging (Foster et al. 2000)

Ultrasonography in clinical imaging is highly useful because of its relatively low cost, the small size of the equipment, as well as its safety. Ultrasound (US) is based on the emission and detection of sound waves that are absorbed, transmitted, reflected, or refracted as the waves travel through tissues.

While US has low detection sensitivity compared to nuclear imaging, US imaging can provide real time imaging (high temporal resolution). The use of US imaging agents to increase the sensitivity is becoming more common. US agents include encapsulated

microbubbles, liposomes, and perfluorocarbon emulsions. Microbubbles contrast relies on differences in the acoustic impedance (differences in the backscattered wave) due to the higher compressibility of gases, making them more reflective than normal tissues (Lanza and Wickline 2001). To obtain higher scattering, bigger bubbles are necessary. The main drawback of these probes is the unusually large size (6–8 μm), which prevents these probes from crossing the intravascular space, confining their applications to vascular anomalies. Microbubbles with smaller size (~3 μm) that can cross into the intravascular spaces are more amenable for non-vascular in vivo applications (Rudin 2005). To increase the lifetime in circulation, some microbubbles incorporate perfluorocarbon (PFC) (Schutt et al. 2003). PFC is an inert liquid that forms relatively good solvents for gases because of the very low intermolecular forces. In contrast to microbubbles filled with air that dissolve quickly in the blood, PFC microbubbles increase the lifetime of the air bubble from seconds to minutes. Another type of US imaging agents is liposome formulations, mainly constituted by phospholipids, glycerol, and cholesterol. Because these particles are of similar density as most tissues, they do not cause backscatter as much as the microbubbles. Another option is to fill the liposomes with PFC; the resultant nano-particles have increased reflectivity (Hall et al. 1977). Functional groups that target cells or molecules can be added to these bubbles to include molecular imaging capabilities (Villanueva et al. 2007; Rychak et al. 2007; Behm and Lindner 2006).

Optical Imaging

While the abovementioned modalities have been translated to human applications, the optical imaging modalities are currently predominately used in the research settings. The optical imaging modalities are fluorescence molecular (mediated) tomography (FMT) and bioluminescence (Ntziachristos 2006; Negrin and Contag 2006).

Physical principles of fluorescence rely on the initial absorption of photons in the optical region of the electromagnetic spectrum by a specific molecule. A fluorophore or fluorescent protein can be excited by an external energy source, resulting in a transition from the electronic ground state to an excited state. The transition from the excited state to a lower energy state results in the emission of light photons which will be detected with highly sensitive charge-coupled device (CCD) camera using appropriate filters (in fluorescence spectroscopy, the emission is at longer wavelength compared to that of the excitation).

Bioluminescence means production of light by a living organism. This technique is based on the observation that organisms such as fireflies and some deep sea fishes can produce light as a result of a chemical reaction, usually through enzymatic cleavage, during which chemical energy is converted to light energy.

One of the drawbacks of the optical imaging is that both hemoglobin and water are the major absorbers in the visible and infrared light regions, resulting in a strong signal attenuation (loss of imaging resolution). FMT overcomes this by utilizing the near infrared (NIR) region of the spectrum (650–900 nm), which has the lowest absorption coefficient in the UV–vis region. Fluorescence imaging has received particular

attention in recent years because of the increasing number of NIR fluorochromes. Bioluminescence presents low background noises since the enzyme (luciferase) as well the substrates are usually not endogenous to the animal being imaged, resulting in an increase of the sensitivity. However, bioluminescence requires genetic manipulation in order to insert the genes necessary for the enzyme expression.

Another disadvantage of the optical techniques is the very low spatial resolution and low penetration depth. Optical tomographic techniques employ techniques to overcome some of these limitations. Currently, FMT can achieve a penetration depth as great as 1–2 cm.

Fluorescence probes: the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*, is widely used in biological applications (Contag and Bachmann 2002). GFP has an excitation maximum at 395 nm and emits green light at 509 nm, but due to its lower wavelength, autofluorescence of the endogenous tissues is a concern. To solve the problem of autofluorescence, agents based on the cyanine scaffold to increase the wavelength of absorption and emissions are being explored (Lin et al. 2002). Several modifications to the π-system, such as elongation of the conjugated bonds, have a direct influence on the spectroscopic properties of the compounds, and also affects the solubility properties (Bouffard et al. 2008).

Quantum dots consisting of semiconductor nanostructures that were originally developed for optoelectronic applications with high quantum yield can be used as fluorescent probes (Yoffe 2001). Quantum dots, for instance, consists of a CdSe core with a ZnS shell. They have to be coated by an organic matrix in order to become biocompatible (Bruchez et al. 1998; Ballou et al. 2004). The main disadvantage of quantum dots for in vivo applications is the toxicity from released cadmium, though efforts are underway to minimize this toxicity.

Bioluminescent probes: the best-studied bioluminescent enzymes are luciferases where light is produced by the oxidation of a luciferin (pigment) sometimes involving ATP. The reaction is energy efficient. Luciferase can be produced in the laboratory through genetic engineering to label molecules and cells.

Design and Examples of Molecular Imaging Probes

There are several important considerations in the development of new molecular imaging probes: (a) high specificity for the target and reasonable pharmacodynamics; (b) the ability of these probes to overcome biological delivery barriers (vascular, interstitial, cell membrane); and (c) suitable amplification strategies to enhance the signal (chemical or biological).

Design of Target-Specific Probes

Target identification and validation with high-affinity probes can be performed using two different strategies of interaction between the probe and the target: direct and indirect (or activatable, also called "smart" probes) (see Fig. 1).
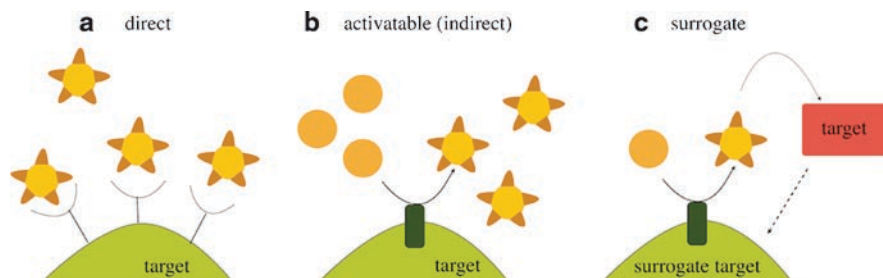
Fig. 1 Mechanisms of target–probe interaction: (**a**) direct mechanism, (**b**) activatable (indirect, smart) mechanism, and (**c**) surrogate mechanism. The figure illustrates a surrogate probe with an activatable mechanism, but a direct probe can also be used

Probes based on the "direct" strategy (Fig. 1a) can be attached to the molecule of interest or are taken up by the cell of interest. The image intensity will be relative to (but also limited by) the amount of the target present. Because of the lack of signal amplification, this kind of strategy is predominately used in nuclear imaging. Antibodies can be conjugated to the probe to increase the affinity and simultaneously, the biocompatibility. However, the use of antibodies can still result in a high background noise due to non-specific interactions. Moreover, due to their size (200–400 kDa) only endovascular and extracellular receptors can be targeted.

"Indirect" or "activatable" probes (Fig. 1b) possess the ability to alter their structure in response to changes in the local environment, often resulting in signal amplification. The most common method to accomplish this is by enzymatic cleavage. This is most often used in fluorescent probes. For example, several activation-sensitive peptide–fluorochrome conjugates have been recently introduced, described with specificity for cathepsins, matrix metalloproteinases, and other enzymes (Law et al. 2007; Ho et al. 2007).

Moats and co-workers were the first to introduce this cleavage strategy for MRI probes (Moats et al. 1997). They designed a gadolinium-based complex consisting of a galactopyranose moiety positioned in the ninth coordination position of the gadolinium complex. In the presence of β-galactosidase, this blocking sugar group is removed by enzymatic cleavage and the T_1 relaxivity of the imaging agent increased 20% because of the improved access of the water molecules to the metal after enzymatic cleavage.

Another strategy to design activatable probes is to design a molecule that can be chemically modified by the target enzyme into a different molecule. This class of enzymatic activation is best illustrated by peroxidase sensitive MRI probes. The endogenous peroxidase (such as myeloperoxidase, which is a key enzyme secreted in active inflammation) amplifies the T_1-weighted signal by oxidizing and radicalizing the parent probe, causing it to polymerize. The activated probe is significantly larger in size and has slower rotational dynamics compared to the parent compound, resulting in higher signal (200% higher) and prolonged pharmacokinetics in vivo (Chen et al. 2004, 2006; Bogdanov et al. 2002).

A third probe type, called the surrogate probe, is sometimes discussed. This is a probe that targets a downstream entity remote from the molecule/event of interest (Fig. 1c). This probe can be either a direct or an indirect probe. The myeloperoxidase probe discussed above is an indirect probe for myeloperoxidase but is also a surrogate probe for active inflammation.

Probe Delivery

Another important issue is the ability of the probes to reach the intended target with high enough concentration and persist in that location long enough to be detectable in vivo. Probe delivery is one of the most challenging issues, in particular, when targeting intracellular receptors and enzymes. One method to overcome this problem is by designing peptide sequences that facilitate the intracellular incorporation of the target.

One example is the use of the HIV-tat peptide because it allows uptake across cell membranes. HIV-tat peptides have been conjugated to CLIO (cross-linked iron oxide nanoparticles) to visualize and track stem and progenitor cells by MR imaging (Josephson et al. 1999; Lewin et al. 2000). Cell specific uptake can also be achieved via receptor mediated endocytosis. Asialoglycoprotein (ASG) receptors are present on normal hepatocytes but are missing in primary malignant or metastatic tumors. Weissleder et al. described an ultrasmall superparamagnetic iron oxide (USPIO) probe conjugated to asialoglycoprotein (ASG) to differentiate hepatic tumors from normal tissues (Reimer et al. 1990). As ASG receptors on normal cells have a high affinity for the terminal galactose groups, AG-USPIO would accumulate in normal tissues but not in tumor cells, thus improving the tumor-liver contrast.

Suitable Amplification Strategies (Chemical or Biological)

In addition to the amplification strategies described in "Design of Target-Specific Probes," another approach to facilitate the differentiation of target and background fluorochromes is to use "molecular switches or beacons". The probes are optically silent in their native (quenched) state and become highly fluorescent after enzyme-mediated release of the fluorochrome (Tung et al. 2000; Ntziachristos et al. 2002). In addition, this highly specific approach has the advantage that one target (e.g., an enzyme) can convert many individual beacons, resulting in several levels of amplifications (10–1,000-fold) over simple tagging.

Another strategy is magnetic relaxation switching (MRS). The MRS technology is based on the conversion of the assembly of iron oxide nanoparticles into highly stable nanoassemblies (clusters), with a concomitant decrease in the spin–spin relaxation time (T_2) of adjacent water protons. Recently, Weissleder et al. have developed MRS biosensors to detect molecular interactions (DNA–DNA,

protein–protein, protein–small molecule, and enzyme reactions) and analyte levels (Perez et al. 2002; Sun et al. 2006). In the low analyte state, the nanoparticles are microaggregated, resulting in a decrease in T_2 (low T_2 state). While in the high analyte state, the nanoswitch is dispersed, causing the nanoswitch to move to a high T_2 state. The degree of T_2 change is proportional to the amount of analyte (e.g., glucose) present.

Applications in Neurological Diseases

Unlike other organs, the brain presents a special challenge with the blood–brain barrier. Only small and lipophilic agents could go through it. However, in pathological states, the blood–brain barrier may be compromised allowing agent access. Molecular imaging in the central nervous system (CNS) can be performed with nuclear imaging, MR imaging, or optical imaging modalities. An interesting application for each modality to illustrate the power of molecular imaging is highlighted below.

One of the disadvantages of using FDG PET in the CNS is the intrinsic high glucose metabolism of gray matter. New molecular imaging agents, designed to target the amino acid transport or incorporated into DNA, have been described (Chen et al. 2005; Jacobs et al. 2005). Unlike FDG, the main advantage of using these agents for PET imaging is that in the normal brain the uptake is low. An example of these agents is the compound L-[methyl-^{11}C]methionine or [^{11}C]MET (Kim et al. 2005), which is responsive to the degree of protein synthesis, providing significantly higher sensitivity for detecting brain tumors compared to FDG PET (Fig. 2a).

Optical molecular neuroimaging is useful to monitor cell trafficking events. Recently, Shah et al. designed a bioluminescence method to validate and track the use of neural progenitor cells (NPCs) in therapy for brain tumors. NPCs were transfected with luciferase gene and implanted intracranially and intraventricularly into nude mice. After 2–3 weeks, there was clear NPCs migration toward the tumor (Fig. 2b) (Tang et al. 2003), which can be labeled with selective chemotherapeutic agents to perform focused therapy (Corsten et al. 2007; Kock et al. 2007; Shah et al. 2005a, b).

It is well known that enhancement in the CNS from conventional, non-specific MRI contrast agents such as Gd-DTPA reflects breakdown in the blood–brain barrier rather than active inflammation. In multiple sclerosis imaging, this poses a particular problem because lesions at all stages demonstrate some breakdown in the blood–brain barrier, hence breakdown in the blood–brain barrier and active inflammation may not always correspond (Cotton et al. 2003; Bruck et al. 1997). Chen and co-workers have recently reported an MR imaging agent (Chen et al. 2006) that is highly specific and sensitive to the enzyme myeloperoxidase, a key enzyme secreted in inflammation and in multiple sclerosis plaques (Nagra et al. 1997). They demonstrated that this myeloperoxidase imaging sensor can detect and confirm more smaller, and earlier active inflammatory lesions in living mice by in vivo MR imaging, and that MPO expression corresponded with areas of inflammatory cell infiltration and demyelination
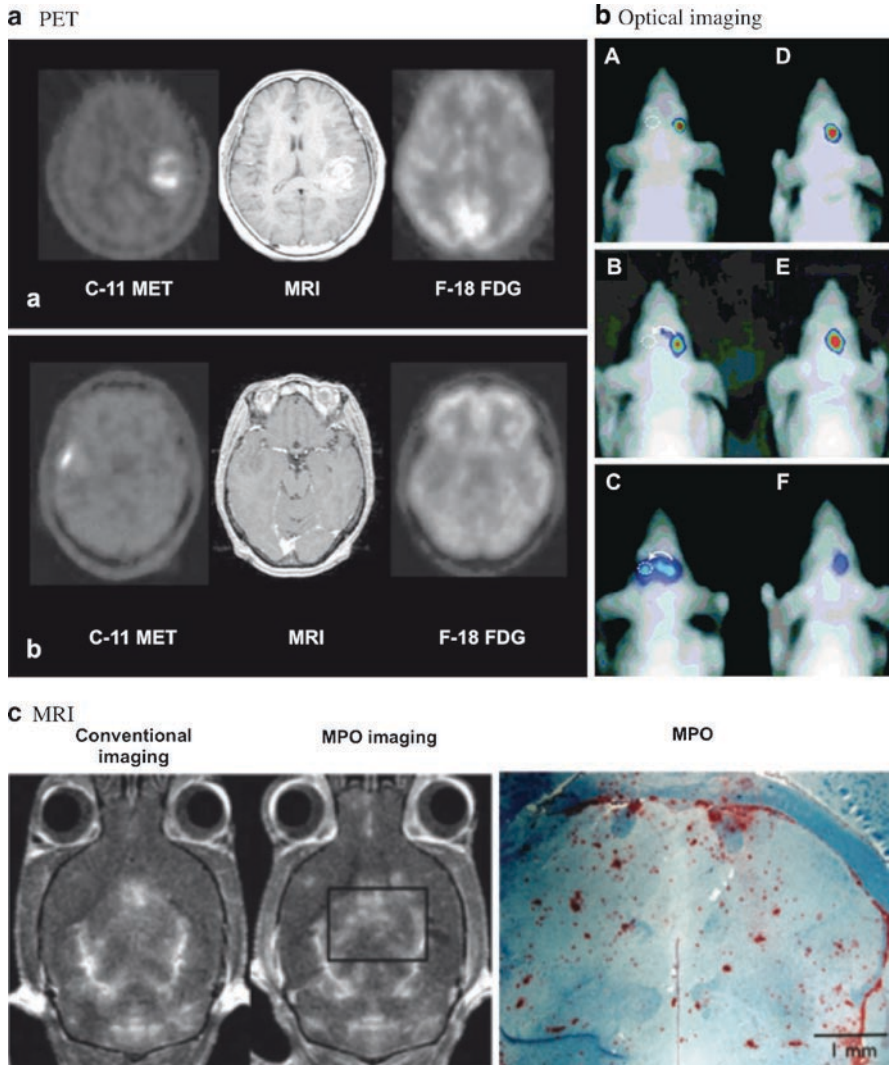
Fig. 2 Examples of molecular neuroimaging: (**a**) [^{11}C]MET PET, transverse enhanced MR, and FDG PET images of two glioma patients (top and bottom). [^{11}C]MET PET has a higher sensitivity for the detection of brain tumors. (Reprinted with permission from Kim et al. (2005).) (**b**) Optical images showing neural progenitor cells migrating towards the tumor obtained at (A) 0 day, (B) 1-week follow-up, and (C) 2-week follow-up. (D), (E) and (F) represent the time series for an animal without glioma and no migration of the NPCs was observed. (Reprinted with permission from Shah et al. (2005b).) (**c**) In a mouse experimental autoimmune encephalomyelitis model for multiple sclerosis, myeloperoxidase MR imaging revealed more and smaller lesions at an earlier time than imaging with the conventional Gd-DTPA. (Reprinted with permission from Chen et al. (2008).)

(Fig. 2c) (Chen et al. 2008). They also found that higher MPO activity, as detected by MPO imaging, biochemical assays, and histopathological analyses, correlated with increased clinical disease severity. This approach could be used in longitudinal studies to identify active demyelinating plaques as well as to more accurately track disease course following treatment in clinical trials.

Drug Development

Molecular imaging has and will continue to play an important role in drug development. An important example is in characterizing the biodistribution of a new drug to assess unfavorable pharmacokinetic properties (e.g., poor absorption or rapid excretion) (Rudin and Weissleder 2003). Classical biodistribution studies in animals have been performed using autoradiography. The drug is labeled with a long-lived radioisotope (^{3}H or ^{14}C) and then the biodistribution is quantified postmortem in histological sections. However, despite the excellent sensitivity and spatial resolution, no drug metabolism information is obtained. Nuclear imaging provides the best way to evaluate these parameters as the incorporation of ^{11}C and ^{18}F to the drug does not affect the properties of the molecule.

Molecular imaging can be a very efficient means to monitor the therapeutic efficacy by imaging the functional repercussions of drug–target interactions. To accomplish this, the probe should be highly specific to the chosen target, and depending on the imaging modality used, the resultant response may need to be amplified. Even in the event that no specific probe can be designed for a particular target, a surrogate strategy may be used, in which a probe may be designed that is specific to downstream molecules or cells that are produced or affected by the target of interest (Fig. 1c).

Molecular imaging can also be helpful to discover new drugs by providing imaging end points instead of invasive and often fatal animal manipulations (e.g., invasive surgical dissection and non-survival histology). Longitudinal and serial studies can be performed where the effect of a drug is followed non-invasively over time in the same animals. As a result, statistical significance of a study can be achieved with fewer animals, and the effect of a drug can be more easily identified.

Conclusions

In this chapter, we have covered the basic concepts of molecular imaging and showed both the advantages and disadvantages of different imaging approaches. It is important to keep in mind that many more molecular probes and applications are in development and in clinical trials. While most are not yet available for clinical use, many techniques and probes can be readily used in preclinical trials to study the efficacy of a drug.

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