

# Molecular Imaging: A Primer for Interventionalists and Imagers

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The characterization of human diseases by their underlying molecular and genomic aberrations has been the hallmark of molecular medicine. From this, molecular imaging has emerged as a potentially revolutionary discipline that aims to visually characterize normal and pathologic processes at the cellular and molecular levels within the milieu of living organisms. Molecular imaging holds promise to provide earlier and more precise disease diagnosis, improved disease characterization, and timely assessment of therapeutic response. This primer is intended to provide a broad overview of molecular imaging with specific focus on future clinical applications relevant to interventional radiology.

J Vasc Interv Radiol 2006; 17:1405–1423

**Abbreviations:** CEA = carcinoembryonic antigen, FDG = fluorodeoxyglucose, HSV1-tk = herpes simplex virus type 1 thymidine kinase, MMP = matrix metalloproteinase, NIR = near-infrared, PET = positron emission tomography, SPECT = single photon emission computed tomography, VEGF = vascular endothelial growth factor

IN this era of molecular medicine, our approach to patient care is evolving as disease is increasingly being defined by underlying molecular and genomic aberrations rather than by clinical signs and symptoms alone. Molecular imaging is an emerging diagnostic discipline that aims to visually characterize normal and pathologic processes at the cellular and molecular levels in living organisms (1–4). Broadly multidisciplinary, molecular imaging incorporates methods and concepts from

molecular and cell biology, imaging sciences, chemistry, high-throughput biology (eg, genomics, proteomics), nanotechnology, pharmacology, and bioinformatics (1,5–7). It is through molecular imaging that radiology is expected to play a critical role in advancing molecular medicine and potentially revolutionize patient care and biomedical research (8,9).

The potential benefits and applications of molecular imaging stem from two fundamental paradigm shifts, one clinical and the other preclinical. Conventional clinical imaging, as practiced by the modern radiologist, generally relies on macroscopic anatomic and/or physiologic variations for disease diagnosis and assessment. Such morphologic changes are often nonspecific and late phenotypic manifestations of underlying molecular derangements (1,2). By contrast, molecular imaging exploits the use of directed imaging probes to sense the specific molecular alterations underlying diseases rather than downstream end effects at the tissue or organ level. This shift in focus from the nonspecific morphologic to the more specific molecular allows for earlier and more precise disease diagnosis, improved disease characterization, and more meaningful monitoring of disease

progression. Moreover, imaging at the molecular level also confers patient specificity; thus, molecular imaging stands to greatly facilitate the practice of personalized medicine in predicting therapeutic response and guiding treatment selection (10). Although the clinical potential of molecular imaging has yet to be realized, several human trials of molecular imaging are under way (11–13).

The potential impact of molecular imaging in biomedical research is equally promising. Although past decades have witnessed explosive growth in our understanding of the fundamental basis of physiology and disease, it has become clear that current paradigms of biomedical investigation have inherent limitations. Traditional *in vitro* research employs a reductionist approach whereby events under investigation are extracted and studied in artificial environments (eg, cell culture studies). This is problematic because biologic processes rarely occur in isolation and are instead mediated through a complex and dynamic interplay of gene expression, signaling pathways, environmental factors, and inherent feedback mechanisms. Therefore, molecular imaging, in interrogating molecular phenomena

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None of the authors have identified a conflict of interest.

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DOI: 10.1097/01.RVI.0000235746.86332.DF

**Table 1**  
**Attributes of Molecular Imaging Modalities (1,2)**

Modality	Sensitivity	Spatial Resolution	Temporal Resolution	Penetration Depth	Cost
SPECT	Medium	Low	Low	High	Medium
PET	High	Low	Low	High	High
MR imaging	Low	High	High	High	High
US	Medium	Medium	High	Medium	Low
Optical imaging	High	Low	High	Low	Low

in living individuals, preserves the context of whole biologic systems and enables the transition from a reductionist to an integrative and holistic approach to research (14). In studying disease in physiologically intact environments, molecular imaging assays are therefore more predictive and relevant. This technology also broadens the capabilities of *in vivo* research. Because molecular imaging interrogates events remotely and noninvasively, analyses can be performed in real time with minimal disturbance to the model system, allowing for continuous observation of dynamic processes. For example, whereas traditional *in vivo* temporal profile studies required animals to be serially killed at fixed time points to obtain tissue for *in vitro* analyses, molecular imaging enables real-time monitoring of molecular phenomena through repetitive imaging of a single animal. These distinct advantages, combined with the commercial development of dedicated small-animal imaging instrumentation, have driven the application of molecular imaging tools in the preclinical arena. Imaging of experimental small animal models, usually mice, has been rapidly adopted into the basic science research of dynamic biologic processes such as hypoxia (15), inflammation (16), apoptosis (17), angiogenesis (18), tumorigenesis (19), and gene expression (20,21). More translational applications include stem cell trafficking (22) and monitoring of the distribution and efficacy of novel therapeutic moieties (23). The potential to streamline and accelerate drug discovery and development is perhaps one of the most promising applications of molecular imaging and has garnered considerable interest from academia and industry (23–25).

Interdisciplinary collaborations form the essential foundation for the contin-

ued advancement of molecular imaging. Naturally, active engagement by physicians will be critical for successful clinical translation of this novel technology (26). The goals of this communication are to provide a basic introduction to molecular imaging and to stimulate discussion among interventional radiologists about whether and how it could be incorporated into future practice. This review begins with an overview of the imaging modalities used, followed by a discussion of basic molecular imaging approaches. To conclude, specific applications in cardiovascular diseases and oncology are detailed. Because the roots of molecular imaging are in molecular biology, readers are referred elsewhere for a review of molecular biology terminology and concepts (27–31). Several other in-depth reviews of molecular imaging are recommended for further reading (2,4,32–35).

## IMAGING MODALITIES

Molecular imaging encompasses a broad set of technologies that couple imaging modalities and contrast agents with molecular specificity. Analogous to stains used in histopathology, these agents, called molecular probes or tracers, consist of a signaling component that emits a detectable signal and a targeting component that confers localization. This latter component can be a peptide, receptor ligand, enzyme substrate, oligonucleotide, or antibody. The imaging instrumentation must then be able to remotely detect this signal with sufficient spatial resolution and sensitivity. Modalities used in molecular imaging include positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance (MR) imaging, optical imaging, and ultrasound (US). These modalities

differ in terms of spatial resolution, temporal resolution, sensitivity in probe detection, depth of signal penetration, availability of biocompatible molecular imaging agents, and, of course, cost. Each has its unique advantages and disadvantages, and the choice of imaging system ultimately depends on the question to be addressed. **Table 1** summarizes the characteristics of each modality.

## Nuclear Imaging

Many argue that nuclear medicine specialists have been practicing molecular imaging since  $^{131}\text{I}$  was first used for thyroid imaging in the 1940s. Indeed, PET and SPECT are routinely used clinically and are the most prevalent molecular imaging modalities to date. Hundreds of nuclear imaging probes have been developed. They include radiolabeled enzyme substrates, receptor ligands, antibodies, drugs, and oligonucleotides (36). Examples of U.S. Food and Drug Administration–approved nuclear probes for SPECT and PET include the  $^{111}\text{In}$ -labeled octreotide analogue Octreoscan (Mallinckrodt Medical; Hazelwood, MO) (37) and the  $^{18}\text{F}$ -labeled glucose analogue  $^{18}\text{F}$ -fluorodeoxyglucose (FDG), respectively.

Nuclear imaging modalities remotely sense molecular events by detecting radioactive emissions from targeted radionuclides. PET specifically detects pairs of coincident  $\gamma$ -rays that result from positron/electron collisions after positron emission. Common positron-emitting isotopes include  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$ , and  $^{124}\text{I}$  (2,36). SPECT, by contrast, detects  $\gamma$ -rays directly from  $\gamma$ -emitting isotopes such as  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{123}\text{I}$ , and  $^{131}\text{I}$  (2).

Nuclear medicine modalities have been at the forefront of molecular imaging because of their high intrinsic sensitivity, their unlimited depth pen-

etration, and the relative ease of radiolabeling molecular probes (38). PET is superior to SPECT for imaging molecular processes because it is 10–100 times more sensitive and because positron-emitting isotopes can be readily substituted for naturally occurring atoms (eg, substituting  $^{18}\text{F}$  for hydrogen or  $^{11}\text{C}$  for elemental carbon) (2,6). In fact, the sensitivity of PET is in the nanomolar to femtomolar range, and therefore radiolabeled probes are needed in only tracer quantities (2). This enables better visualization of targets of low concentration and minimizes perturbation of the system under investigation. Similarly, the availability of positron-emitting radionuclides of common elements allows for radiolabeling by direct isotopic substitution, which minimizes alterations in the biologic properties of the parent molecule (39). This is particularly advantageous in the use of imaging to evaluate novel drugs (25). Last, PET offers the additional benefit of providing quantitative measures of tracer uptake.

Although nuclear imaging modalities provide exceptional sensitivity, they lack the high anatomic detail and spatial resolution of MR imaging and US. The limited spatial resolution of current clinical PET scanners ranges from 3 mm to 6 mm but is approaching 1 mm for small-animal PET (microPET) scanners (38). However, this constraint is being addressed by the clinical introduction of integrated PET/CT systems that simultaneously achieve high probe detection sensitivity and spatial resolution, allowing for direct anatomic correlation and more precise target localization (40). Combined PET/CT systems are expected to replace most stand-alone PET systems in the next decade (38). In addition, prototype PET/MR imaging scanners are on the developmental horizon (41). Another drawback of PET is the need for an on-site cyclotron to synthesize a broad range of radioactive tracers because the half-lives of positron-emitting radionuclides are short and the decay process cannot be controlled. Additional limitations include patient exposure to radiation and the high cost of associated equipment.

### MR Imaging

MR imaging is based on the detection of a signal generated from proton

spin relaxation after the application of a radiofrequency pulse. Paramagnetic agents (eg, gadolinium) and superparamagnetic agents (eg, iron oxide) alter relaxation times and are used as the signaling component of MR molecular probes. Paramagnetic agents shorten T1 relaxation times to produce signal enhancement on T1-weighted images, whereas superparamagnetic agents shorten T2 relaxation times to produce negative contrast on T2-weighted images (42,43). The signaling component of gadolinium ( $\text{Gd}^{3+}$ )-based imaging probes consists generally of larger nanoparticles containing multiple  $\text{Gd}^{3+}$  ions in the form of liposomes, dendrimers, or perfluorocarbons (42,44). Similarly, T2 imaging probes contain monocrySTALLINE or polycrystalline superparamagnetic iron oxide nanoparticles coated with dextran or other polysaccharides (42,43).

In direct contrast to nuclear imaging, MR imaging offers the principal advantages of exceptional spatial resolution (10–100  $\mu\text{m}$ ) and the ability to concurrently provide anatomic and molecular information. Further benefits include the absence of ionizing radiation and good depth penetration. The main disadvantage of MR imaging is its relatively low sensitivity in the detection of imaging probes, necessitating high probe concentrations (millimolar to micromolar range) and/or highly efficient signal amplification techniques (1). Either may lead to potential toxicities or complex amplification steps that may inherently limit molecular imaging with MR imaging. However, the development of higher field strengths and super-fast pulse sequences may potentially open new avenues in the detection of future MR imaging probes.

### Ultrasound

Image generation by US is rendered by detection of differential reflection of sound waves. US contrast agents used in molecular imaging include encapsulated microbubbles, liposomes, and perfluorocarbons emulsions (45,46). Signal enhancement is generated because these agents oscillate strongly in response to acoustic pulses, making them more reflective than normal tissue (47). However, as a result of the size of these agents (0.1–8  $\mu\text{m}$  in diameter), they are restricted to the in-

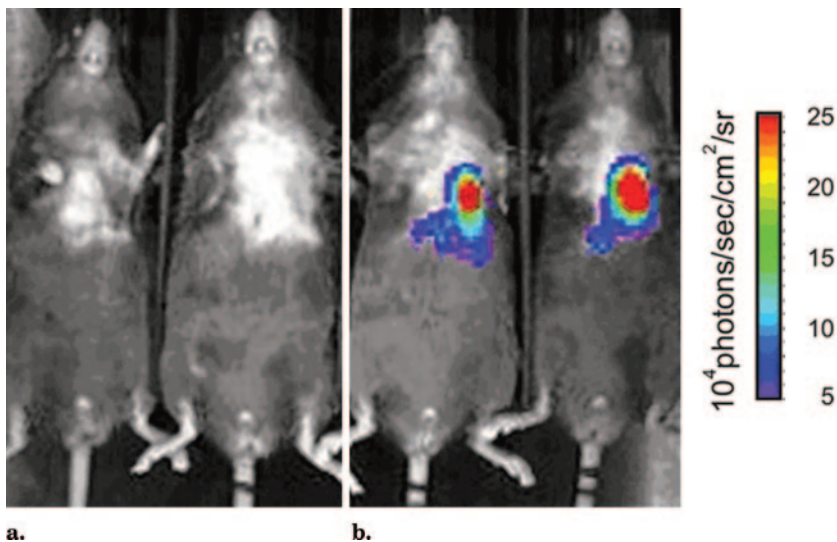
travascular space; therefore, molecular imaging applications have been limited to vascular processes such as angiogenesis (48,49), inflammation (16), and thrombosis (50). Notably, efforts are under way to design hybrid probes that can also carry therapeutic agents, providing simultaneous targeted delivery of contrast and drug (51).

Although it is less sensitive than nuclear imaging and of lower spatial resolution than MR imaging, US offers a good balance between detection sensitivity and spatial resolution (50–500  $\mu\text{m}$ ). Higher spatial resolution is achieved with higher frequencies, but at the expense of depth penetration (47). Like MR imaging, US can also extract anatomic and molecular information simultaneously. US furthermore offers higher temporal resolution, allowing for greater operator control and the ability to capture dynamic processes. Additional advantages include portability, low cost, absence of ionizing radiation, and the potential for hybrid imaging/drug delivery probes.

### Optical Imaging

Although most radiologists are least familiar with the modality of optical imaging, techniques such as fluorescence microscopy have been a mainstay of in vitro molecular and cellular biology research for decades. Optical imaging detects light photons with the use of sensitive CCD cameras that use an absorptive process to convert light photons to an electric signal (52). In in vivo optical imaging, light photons emitted from imaging probes must travel through intervening tissue to reach the detector. In its path, signal attenuation occurs as a result of scattering and absorption, mainly by hemoglobin (for visible light) and water (for near-infrared [NIR] light) (2,53). Therefore, the depth at which light photons can penetrate through opaque tissue has become the primary barrier in the translation of optical imaging from in vitro to in vivo application.

The two principal optical approaches to molecular imaging are fluorescence and bioluminescence imaging (53,54). In fluorescence reflectance imaging, a fluorophore or fluorescent protein is first illuminated and excited by an external light source of one wavelength and then emits visible



**Figure 1.** Bioluminescence imaging of mouse myocardium at 8 weeks after firefly luciferase gene transfer and 8 minutes after luciferin infusion. **(a)** Control mice received injections of saline solution. As expected, no luciferase activity was detected in these mice. **(b)** In experimental mice, the firefly luciferase gene was introduced into myocardial cells by direct injection of recombinant viral vectors into the left anterior wall. Luciferase activity is demonstrated. Color scale indicates signal intensity. Images courtesy of K.M.R. Prasad and S.S. Berr of the University of Virginia Health System.

light at a lower wavelength. Green fluorescent protein from the jellyfish *Aequorea victoria* is perhaps the most well characterized of fluorescent proteins, but has an emission wavelength (510 nm) that overlaps with the autofluorescence of many tissues (55). Efforts to optimize signal-to-noise ratio have led to the development of fluorochromes that emit light in the NIR range (650–900 nm), in which there is minimal tissue absorption and autofluorescence and therefore improved signal penetration (53,56). Several NIR fluorochromes are now available (57), with one, indocyanine green, approved by the Food and Drug Administration for human use (58).

Bioluminescence imaging exploits the use of naturally occurring enzymes that convert chemical energy to light. The genes for these enzymes have been cloned from many light-emitting organisms including firefly (*Photinus pyralis*), coral (*Renilla reniformis*), and jellyfish (*A. aequorea*) (59). In these reactions, the enzyme, generically called luciferase, oxidizes its substrate, generically called luciferin, to produce an electronically excited molecule that emits light (54). Oxygen, adenosine triphosphate, and other cofactors are often required in such processes. The light emitted from these

bioluminescence reactions are light blue to yellow-green in color (490–620 nm) (59). However, as with fluorescence, novel red-shifted luciferases and luciferins (550–700 nm) have been engineered to improve depth penetration (59). In practice, the tissue being studied must first be genetically modified to express the luciferase enzyme with use of established gene-transfer techniques (60). The subject then receives systemic administration of the luciferin substrate before data acquisition. An example of in vivo bioluminescence imaging is shown in **Figure 1**.

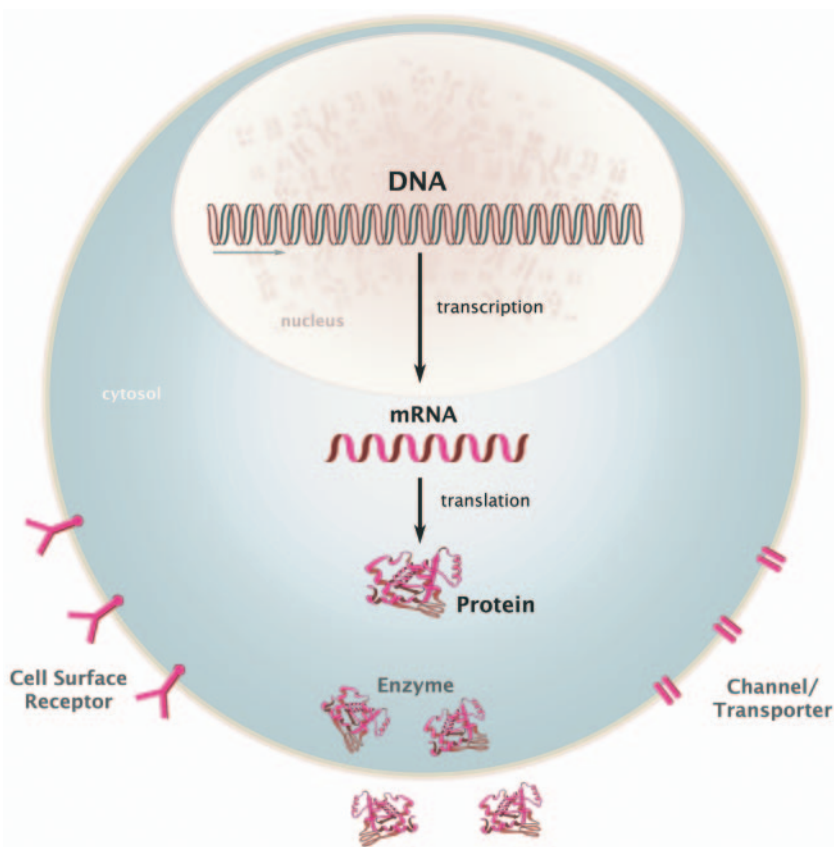
In comparing fluorescence and bioluminescence imaging, bioluminescence has the significant advantage of not requiring external illumination to fluoresce; therefore, background noise is minimal and detection sensitivity is increased. As few as 1,000 luciferase-expressing tumor cells have been detected in mouse models (61). The main limitation of bioluminescence is the need for genetic manipulation. However, the recent development of novel recombinant luciferase fusion reporter proteins composed of luciferase and a targeting moiety promises to expand the application of bioluminescence imaging. In a proof of concept study, recombinant luciferase–vascular endothelial growth factor (VEGF) proteins

were produced in bacteria, purified, and then infused to target VEGF receptors and image angiogenesis in a mouse tumor model (62).

Because both optical imaging modalities are limited by the depth at which probes can be visualized (<2 cm) (2), applications are currently restricted to in vivo imaging of small animals and superficial structures. Clinical uses being explored include dermatologic (63), intraoperative (64), endoscopic (65), and intravascular imaging (53). To overcome this depth limitation, research in optical probes and detector technology are under way. Fluorescence-mediated tomography is a particularly promising new technology that is expected to improve spatial resolution and achieve depth penetrations as great as 10 cm (66). Despite these limitations, small-animal optical imaging has demonstrated tremendous growth in the laboratory setting. Optical imaging is fast, easy to perform, and relatively inexpensive while providing high sensitivity and spatial resolution at the limited depths required for small-animal imaging. Moreover, the plethora of optical reporters and dyes available renders the technology highly versatile and enables the possibility for imaging multiple processes simultaneously (34).

## MOLECULAR IMAGING STRATEGIES

The four imaging systems and their respective contrast agents having been discussed, our focus turns to how molecular imaging is specifically practiced. The imaging strategies most widely used can be classified as direct and indirect. Direct molecular imaging is characterized by the direct and specific interaction of the molecular probe with a target, resulting in probe localization, accumulation, and/or activation. As shown in **Figure 2**, the target can be a cell surface receptor, antigen, enzyme, transporter, channel, or nucleotide. Targets can be located on the cell surface or cell periphery, or in organelles or the nucleus of the cell. Probes designed for direct imaging are highly specific and can be used to interrogate only the intended molecular target. Hence, for each novel target, a new probe must be developed, characterized, and validated, all of which are time-consuming and costly endeavors.



**Figure 2.** Targets for direct molecular imaging. Targets can be at the DNA, RNA, or protein level. Proteins can be intracellular, on the cell surface, or in the cell periphery and function as receptors, antigens, enzymes, channels, or transporters. The number of targets per cell increases as gene expression progresses to the final product.

The second strategy embodies a more generalized and flexible approach in which established imaging systems can be linked to various molecular processes of interest and indirectly report on their activity. As illustrated in **Figure 3**, reporter gene imaging, the most common practice of indirect imaging, entails expression of a reporter gene encoding for a targetable receptor, enzyme, or transporter on the occurrence of a specific molecular event (21,32,67). Complementary reporter probes are then infused systemically and—instead of interacting with endogenous targets as in direct imaging—are bound, trapped, or activated by the exogenous reporter gene product to generate a detectable signal. In some instances, the reporter gene product itself provides the imaging signal (eg, fluorescent proteins), obviating reporter probes. Reporter gene products therefore act as surrogate markers for the level of expression of the

protein being studied. Although reporter gene systems can be engineered to interrogate a variety of biologic events, they are constrained by the need for genetic manipulation of the tissue being studied. In all cases, the reporter gene construct must be first introduced into the cells of living subjects.

### Direct Imaging

The direct strategy is used across all four imaging modalities and can be further categorized by the specific target type (**Fig 2**). Careful target selection is critical for any direct imaging study to be informative. Naturally, the target should play an important role in the initiation or progression of the disease pathway being studied and should also be available in sufficiently high quantities to ensure generation of a detectable signal. Imaging the end products of gene expression is therefore preferred because transcription

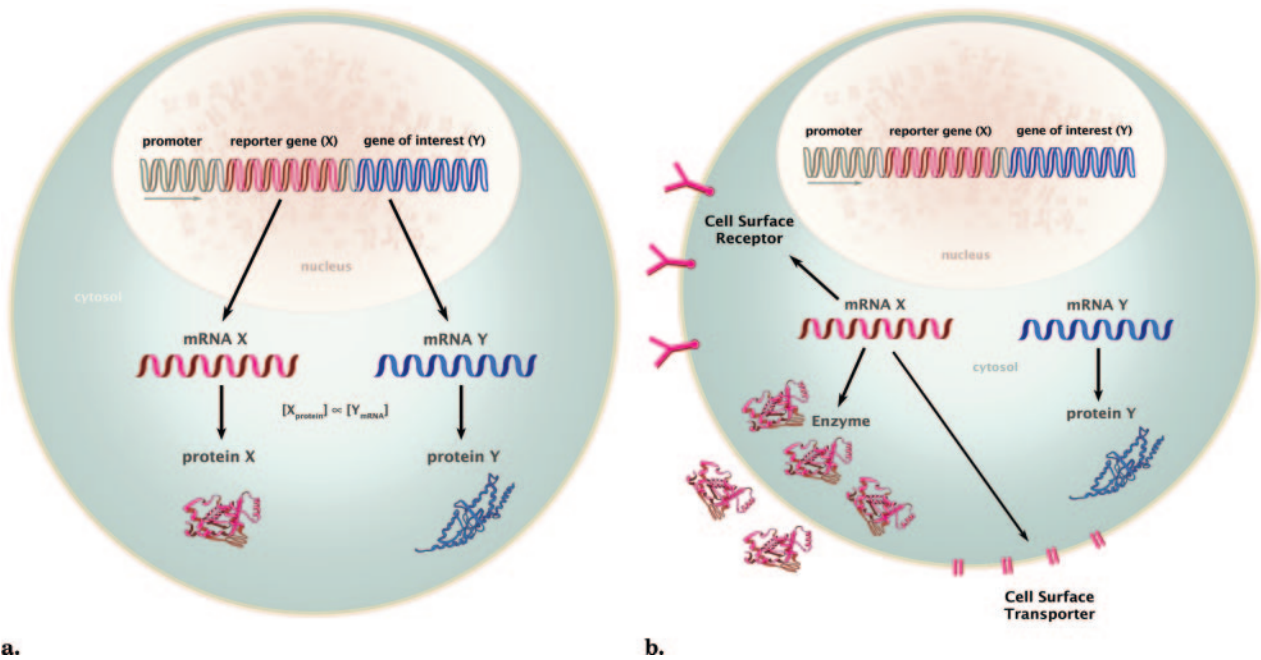
and translation are intrinsic amplification mechanisms (68).

Imaging cell surface receptors and antigens is a common form of direct imaging used in all imaging modalities (**Table 2**). In one example shown in **Figure 4**, colon carcinoma xenografts in mice were selectively imaged by PET with use of  $^{64}\text{Cu}$ -labeled antibody fragments specific for carcino-embryonic antigen (CEA) (69). Direct imaging of enzymes entails enzyme-mediated trapping of a labeled substrate or activation of a silent probe. PET with FDG, a widely known example of the former, specifically images the activity of hexokinase as an indicator of glucose use and metabolic activity. FDG is transported into cells by glucose transporters and then phosphorylated by hexokinase to FDG-6-phosphate, a modification that prevents its diffusion out of cells (72). Relative to receptor imaging, direct enzyme imaging provides inherent signal amplification because enzymes can act on multiple probes, whereas receptors generally interact with only a single probe. By contrast, direct imaging of messenger RNA has been particularly challenging, given the relatively low number of target messenger RNA available per cell (73).

### Activatable Probes

The aforementioned approaches to direct imaging often suffer from high background noise. Because these probes emit signal constitutively, it is not possible to differentiate probes that have reached their target from those that have not. This necessitates a time delay between tracer injection and imaging to allow washout of excess unbound probes from the tissue and systemic circulation. Despite this, significant background noise can remain as a result of nonspecific binding. To circumvent this problem, activatable probes (also called “smart probes” or “molecular beacons”) have been developed for optical and MR imaging. Such agents are designed to emit signal only after activation by the intended target, usually an enzyme. It is estimated that this strategy, through background suppression, amplifies signal generation as much as several hundred fold (74,75).

As illustrated in **Figure 5**, optical activatable probes are composed of an



**Figure 3.** (a) Concept of reporter gene imaging. Reporter DNA constructs are engineered to encode for a reporter gene (X) and a gene of interest (Y) that are linked such that the two genes are expressed together. In this illustration, genes X and Y are driven by the same promoter. The construct is then delivered into the cell nucleus via a viral or nonviral vector. Because the expression of genes X and Y are linked, the level of reporter gene expression and activity are proportional to the degree of expression of gene Y. Imaging of the reporter gene product therefore provides a surrogate marker for expression of gene Y. (b) The reporter gene encodes for a detectable protein product with use of established reporter gene/reporter probe combinations detailed in Table 3. Similarly to direct imaging, reporter probes interact with the specific cell surface receptor, enzyme, or transporter encoded by the reporter gene. Reporter receptors selectively bind reporter probes, whereas transporters selectively internalize them. Reporter enzymes either activate reporter probes or entrap them by phosphorylation.

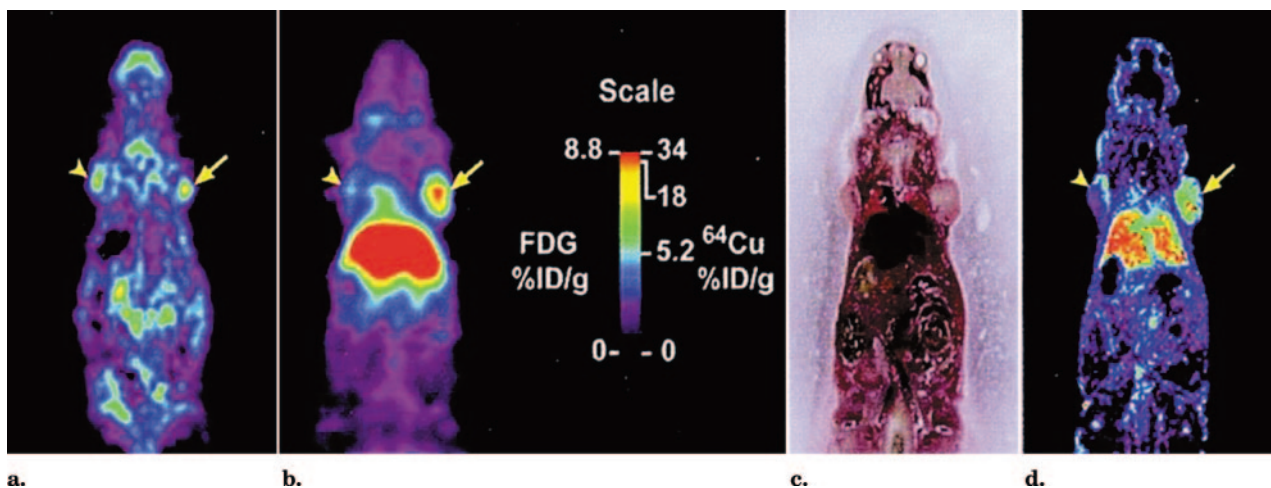
Modality	Target	Application	Molecular Probe		Reference
			Imaging Component	Targeting Component	
SPECT	Somatostatin receptor subtype-2	Neuroendocrine tumor	<sup>111</sup> In	Octreotide analogue*	37
PET	CEA	Colon carcinoma	<sup>64</sup> Cu	Anti-CEA antibody fragment	69
MR imaging	HER-2/ <i>neu</i> receptor	Breast and other cancers	Gadolinium	Anti-HER2/ <i>neu</i> antibody	70
US	$\alpha_v\beta_3$ integrin	Angiogenesis	Microbubble	RGD-containing peptid†	49
Optical imaging	Epidermal growth factor receptor	Multiple cancers	Cyanine 5.5	Epidermal growth factor	71

\* Octreoscan, a Food and Drug Administration–approved agent for imaging neuroendocrine tumors in humans.  
 † Peptides containing the amino acid sequence arginine-glycine-aspartate (RGD).

NIR fluorescent molecule and a quenching molecule attached in close proximity by a linker that can be cleaved only by the target enzyme (74). If exposed to excitation light in its native state, the probe is spectrally si-

lenced by the quencher. When the probe encounters its target, enzyme-mediated cleavage of the linker releases the inhibiting quencher, effectively “turning on” the probe. Activatable NIR probes have been used

to visualize the activity of several enzymes, including cathepsin B and D (74), HIV protease, matrix metalloproteinase (MMP)–2 (Fig 6) (76), protein kinase A (77), caspases (78), and thrombin (79).



**Figure 4.** Selective in vivo microPET imaging of colon cancer xenografts with  $^{64}\text{Cu}$ -anti-CEA antibody fragments. **(a)** MicroPET scan of a mouse administered 200  $\mu\text{Ci}$  of FDG and scanned 1 hour after injection. The left shoulder was implanted with a CEA-negative C6 glioma xenograft (arrowhead), and the right shoulder was implanted with a CEA-positive LS174T colon carcinoma xenograft (arrow). Both xenografts are imaged here. **(b)** MicroPET scan of the same mouse injected with 26  $\mu\text{Ci}$  of  $^{64}\text{Cu}$ -anti-CEA antibody fragments and imaged at 5 hours with the highest retention shown in the LS174T tumor (arrow) and liver and lower retention in the control tumor (arrowhead). **(c)** The mouse was killed for whole-body autoradiography, and an anatomic photograph was taken at the time of sectioning. **(d)** Digital autoradiograph of adjacent section. Color scale indicates signal intensity for  $^{64}\text{Cu}$ -anti-CEA antibody fragment and FDG PET imaging. Reproduced with permission from Wu et al (69).

An MR imaging activatable probe, named EgadMe, has also been designed and consists of a caged  $\text{Gd}^{3+}$  ion that is undetectable in its native state because the cage prevents water molecules from accessing the paramagnetic core, a requirement for T1 signal enhancement (80). However, one wall of the cage is a galactopyranose ring that can be cleaved by the enzyme  $\beta$ -galactosidase. Probe activation is thereby mediated by  $\beta$ -galactosidase through opening of the cage, allowing water molecules to enter and  $\text{Gd}^{3+}$  to exert its T1-lengthening effect on water molecule protons.

### Indirect Imaging

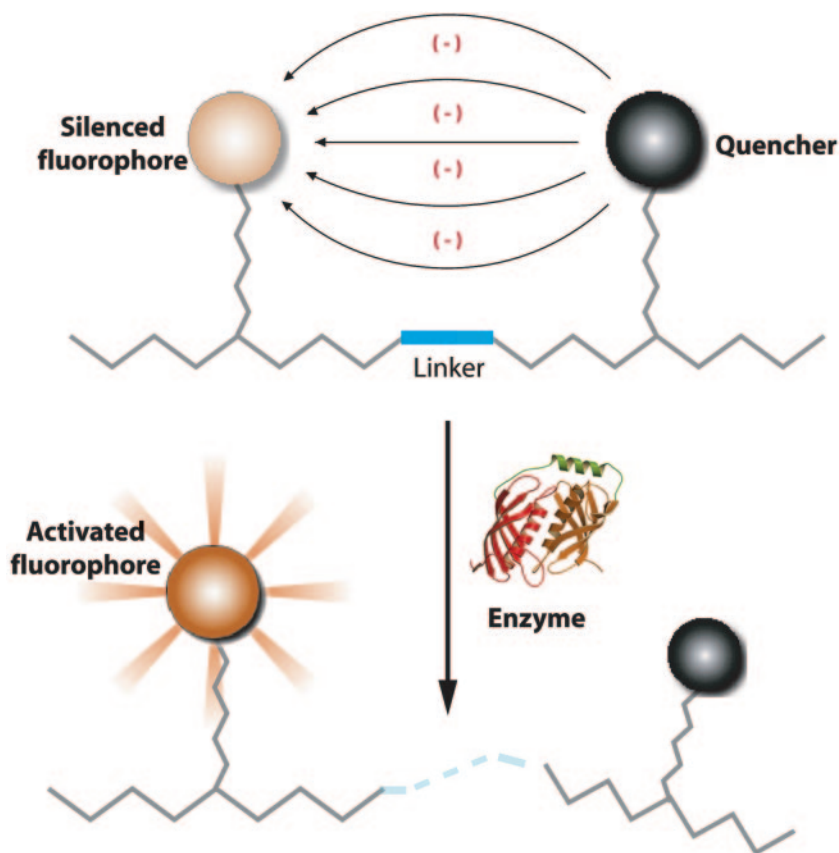
Reporter gene imaging methods have been developed for nuclear (21,81,82), optical (61,83), and MR imaging (80,84). Classification of reporter systems can also be based on whether the reporter gene product is an enzyme, receptor, or transporter (Table 3, Fig 3b). The most common enzyme-based system uses herpes simplex virus type 1 thymidine kinase (HSV1-tk) or its mutant derivative (HSV1-sr39tk) as the reporter gene product, which enables imaging through phosphorylation and subsequent intracellular

entrapment and accumulation of radiolabeled acycloguanosine (eg, ganciclovir, penciclovir) or pyrimidine nucleoside derivatives (82,89). The thymidine kinase of herpes simplex virus type 1 is used because it can phosphorylate acycloguanosine derivatives, whereas the mammalian version, which is more specific in its activity, cannot. In the exploitation of this difference in substrate specificity, background noise is minimized. HSV1-tk has the added advantage of also being a therapeutic gene in its ability to convert ganciclovir, a prodrug, into a cytotoxic compound that inhibits DNA synthesis. This strategy, called suicide gene therapy, has been actively pursued in oncology (90).

Reporter gene imaging has been used to study a variety of molecular and cellular processes such as cell trafficking, gene delivery methods, endogenous gene expression, signal transduction pathways, and gene expression regulation (32). For example, the p53 gene, the most commonly mutated gene known in human cancer, encodes for a key transcription factor that halts cell cycle progression in response to DNA damage. Doubrovina et al (88) studied p53 regulation of gene expression by placing a dual reporter

gene encoding for HSV1-tk and green fluorescent protein under the control of a p53-specific enhancer, and the resulting construct was subsequently transduced into tumor cells in rats. DNA damage-induced upregulation of p53 transcriptional activity was then imaged by PET through expression of HSV1-tk and accumulation of its reporter probe and confirmed by fluorescence microscopy of green fluorescent protein expression.

Although clinical application of this technology is limited by the need to introduce reporter gene constructs into target tissue, reporter gene imaging promises to play a vital role in the development of human gene therapy (60,91). Gene therapy has been significantly hampered by the inability to effectively evaluate the delivery and expression of therapeutic genes. Various genetic engineering strategies have been developed to link the expression of therapeutic genes with that of reporter genes (92,93), allowing for in vivo reporter gene imaging to be used to monitor the location, magnitude, and duration of therapeutic gene expression (91,93). Indeed, reporter gene imaging has been used extensively in animal models of gene ther-



**Figure 5.** Concept of activatable optical probes. In its native state, the fluorophore is structurally linked in close proximity with a quenching molecule that prevents the fluorophore from emitting fluorescent signal on excitation. The linker, usually a short peptide, is designed to be specifically cleaved by an enzyme of interest. When the probe encounters this enzyme, enzyme-mediated cleavage of the linker releases the quencher, allowing the fluorophore to emit signal on excitation. The probe is therefore activated on encounter with the specific activating enzyme.

apy, and initial studies in humans have also been completed (13).

## CLINICAL APPLICATIONS

In its capacity to noninvasively interrogate cellular, molecular, and genetic processes fundamental to diseases, molecular imaging has the potential to affect all disciplines of clinical medicine. A survey of the molecular imaging literature reveals that its application to human ailments is being explored in a range of disparate fields from neurology and psychiatry to infectious diseases and drug resistance. Fortuitously, cardiovascular disease and cancer, the leading causes of mortality and areas most relevant to interventional radiology, have been at the forefront of translational molecular imaging research.

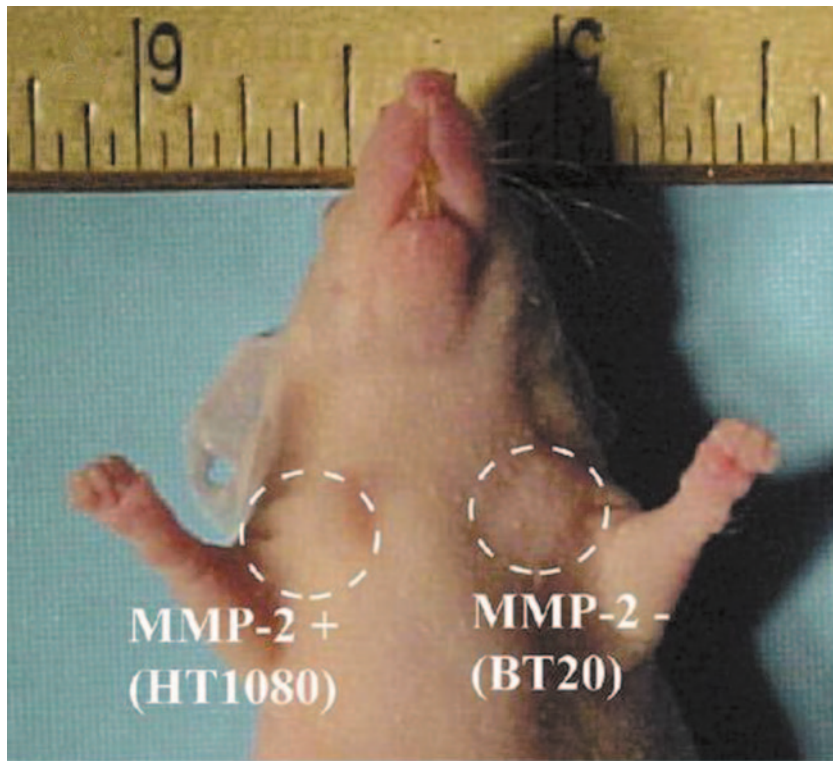
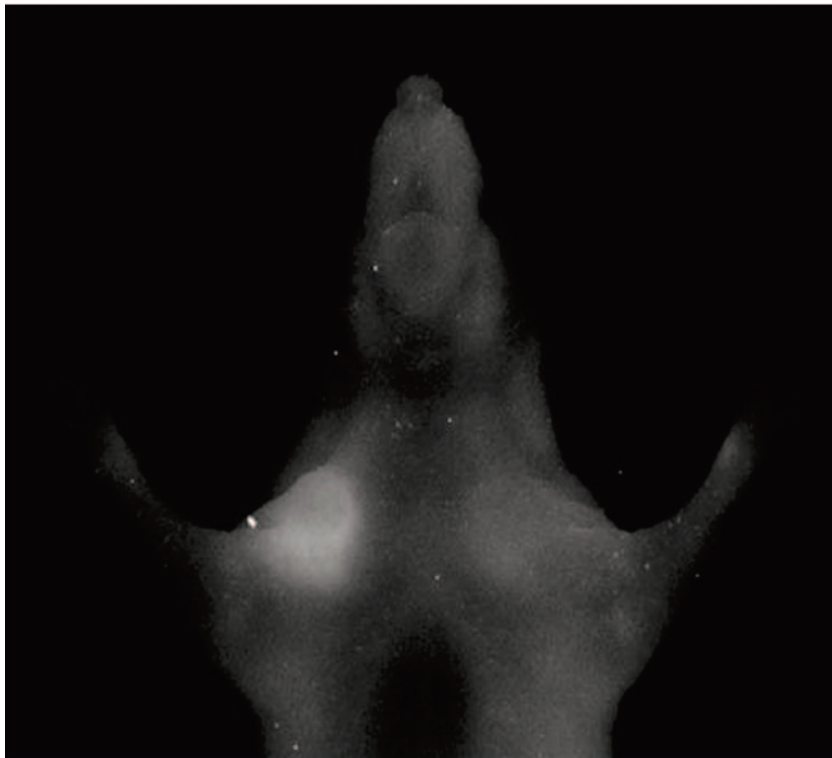
## Molecular Imaging of Cardiovascular Disease

*Atherosclerosis and restenosis.*—Atherosclerosis, the leading cause of morbidity and mortality in developed countries, is in essence a chronic inflammatory process (94,95). Macrophages are the principal inflammatory cell mediator of atheroma formation, progression, and eventual disruption (96). Because unstable plaques are morphologically characterized by a preponderance of macrophages (97,98), there has been considerable interest in *in vivo* imaging of macrophage density in plaques as a means to assess vascular inflammation and risk of rupture. Exploiting the innate ability of macrophages to phagocytose foreign materials, two dis-

tinct human studies (99,100) infused patients with iron oxide nanoparticles before carotid endarterectomy for direct macrophage imaging (99,100). En ensuing MR imaging demonstrated preferential signal attenuation in rupture-prone plaques with histopathologic confirmation of high macrophage density and phagocytosis of iron oxide nanoparticles.

Other studies of atherosclerosis have focused on the underlying mechanisms of macrophage-mediated plaque rupture (101,102). Macrophages secrete proteolytic enzymes, such as cathepsins and MMPs, that weaken the structure of atherosclerotic plaques. As illustrated in **Figure 7**, fluorescence-mediated tomography with an activatable probe specific for cathepsin B in a mouse model of atherosclerosis recorded fluorescence signal that was spatially correlated to regions high in cathepsin B activity, macrophage infiltration, and lipid content (103). Investigative application of activatable protease-specific probes could be extended to other vascular diseases, given that cathepsins and MMPs have been implicated in the pathogenesis of aneurysms and restenosis (104–106).

The application of molecular imaging in restenosis remains in its early stages. Postangioplasty and in-stent restenosis are maladaptive responses to injury characterized by smooth muscle cell proliferation and migration, neointimal hyperplasia, and vascular remodeling (107). Many groups have therefore used molecular imaging tools to study the pathologic activities of vascular smooth muscle cells (108,109). An MR imaging study designed probes directed toward tissue factor (109), a molecule overexpressed by arterial smooth muscle cells after vascular injury (110). Directed probes were synthesized by conjugating perfluorocarbon emulsion  $Gd^{3+}$  nanoparticles with tissue factor antibody fragments (109). Remarkably, these nanoprobe were also loaded with antiproliferative drugs, either paclitaxel or doxorubicin, to illustrate their potential as an imageable and targetable drug delivery platform. After administration of these therapeutic nanoparticles, MR imaging not only confirmed smooth muscle cell-specific probe delivery but also provided a nonin-

**a.****b.**

**Figure 6.** Selective in vivo NIR optical imaging of tumor xenografts expressing MMP-2 with use of MMP-2-sensitive activatable probes. **(a)** Photograph of mouse bearing tumor xenografts. The left shoulder was implanted with an MMP-2-positive HT1080 fibrosarcoma xenograft, and the right shoulder was implanted with an MMP-2-negative BT20 mammary adenocarcinoma xenograft. **(b)** Raw NIR imaging of the same mouse 2 hours after intravenous injection of the MMP-2-sensitive probe. The MMP-2-positive fibrosarcoma generated significantly higher fluorescent signal intensity versus control. Signal attenuation was observed on pretreatment with MMP-2 inhibitors (not illustrated). Reproduced with permission from Bremer et al (76).

**Table 3**  
**Reporter Gene Systems**

Reporter Gene Product	Reporter Gene Product Category	Reporter Probe	Imaging Modality	Reference
HSV1-tk, HSV1-sr39tk	Enzyme	<sup>18</sup> F-labeled uracil or acycloguanosine analogues	PET	81,82
Luciferase	Enzyme	Luciferin	Bioluminescence	61
$\beta$ -galactosidase	Enzyme	EGadMe	MR imaging	80
Dopamine-2-receptor	Cell surface receptor	<sup>18</sup> F-fluoroethylspiperone	PET	85
Somatostatin receptor subtype-2	Cell surface receptor	<sup>111</sup> In-labeled DTPA-octreotide	SPECT	86
Transferrin receptor	Cell surface receptor	Transferrin-MION	MR imaging	84
Sodium iodide symporter	Cell membrane transporter	<sup>131</sup> I	SPECT	87
Green fluorescent protein	Fluorescent protein	None	Fluorescence imaging	88

Note.—DTPA = diethylenetriaminepentaacetic acid; MION = monocrySTALLINE iron oxide nanoparticle.

vasive measure of local drug delivery. Antiproliferative activity was significantly enhanced with the targeted probes relative to nonspecific controls.

**Thrombosis.**—Thrombosis is the pathologic hallmark of acute coronary syndromes, stroke, deep vein thrombosis, and pulmonary embolism. Several mediators of thrombosis have been visualized in attempt to further elucidate their role and function and for in vivo thrombus detection and characterization. Because many proteins of the coagulation cascade are proteases, they are particularly amenable to imaging with activatable probes (111). Indeed, activatable NIR fluorescence probes selective for thrombin and factor XIII have been synthesized and tested in experimental models of thrombosis (112,113). However, for the purpose of detecting thrombus clinically, fibrin is the more appropriate target because it is present in all thrombi, regardless of type and age. In a series of animal studies of different thrombotic pathologic processes (114–116), MR imaging detection of acute, subacute, and chronic thrombi was significantly improved with the use of a fibrin-targeted contrast agent, EP-2104R (Epix Pharmaceuticals; Cambridge, MA), compared with conventional methods. EP-2104R is currently in clinical trials. Other fibrin-targeted molecular probes have been tested for nuclear imaging (117), US (118), and MR imaging (119). Last, several nuclear imaging tracers have been devel-

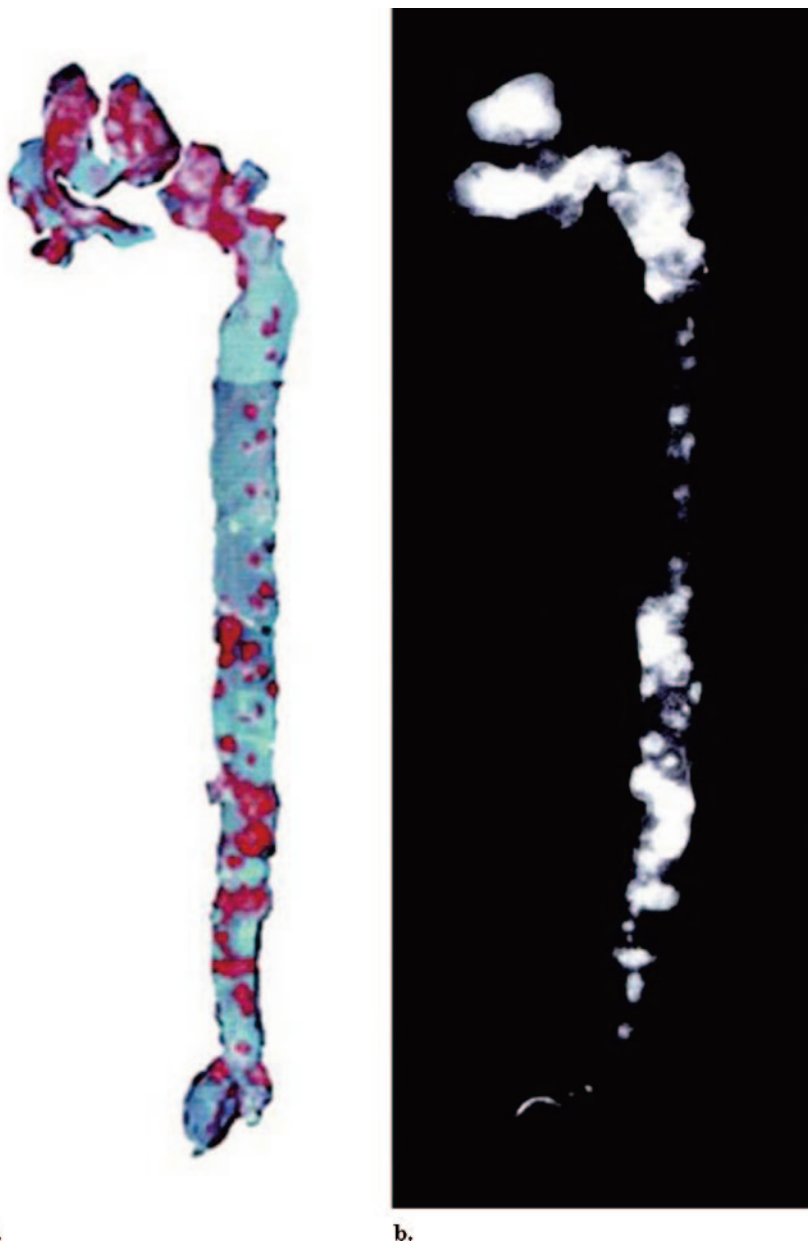
oped to label platelets by specifically targeting glycoprotein IIb-IIIa receptors (117). One such agent has been approved by the Food and Drug Administration and was found to detect acute deep vein thrombosis with sensitivity and specificity rates of 92% and 86%, respectively (120). Platelet-specific US and MR imaging probes have also been reported (121,122).

**Angiogenesis.**—Angiogenesis, the formation of blood vessels, is a highly dynamic process that occurs in a variety of physiologic and pathologic states (123). Pathologic angiogenesis plays a critical role in tumor progression, atherogenesis, proliferative retinopathies, and various other inflammatory and ischemic diseases. Several imaging strategies have centered on assessing new vessel formation. Because  $\alpha_v\beta_3$  integrins are selectively expressed in angiogenic but not quiescent vessels, they represent a promising biomarker for distinguishing neovasculature from existing blood vessels. Molecular probes specific for  $\alpha_v\beta_3$  integrins have been developed for nuclear imaging (124,125), MR imaging (126,127), US (49), and optical imaging (128).

Whether angiogenesis occurs depends on a fine balance between angiogenic promoters and inhibitors. Because VEGF is a potent inducer of angiogenesis, there has been considerable interest in imaging VEGF to study its regulation and biologic activities. For example, hypoxia has been shown to induce expression of VEGF and its receptors to mediate

hypoxia-induced compensatory angiogenesis (129). In a rabbit hind limb ischemia model, uptake of <sup>111</sup>In-labeled VEGF<sub>121</sub> was significantly enhanced in ischemic tissue versus control tissue, suggesting that VEGF receptor imaging could be used to precisely identify ischemic tissue (130). VEGF has also been used for therapeutic angiogenesis via exogenous application and gene therapy (131). In an example of reporter gene monitoring of gene therapy, Wu et al (132) linked the HSV1-sr39tk reporter gene with the VEGF<sub>121</sub> therapeutic gene and then introduced this fusion gene into ischemic myocardium in a rat model of myocardial infarction. After administration of the reporter probe, noninvasive imaging by microPET was found to correlate closely with VEGF<sub>121</sub> and HSV1-sr39tk expression as assessed by immunohistochemistry and enzyme assays. As expected, VEGF<sub>121</sub> gene therapy also induced a significant increase in angiogenesis.

**Cardiac stem cell therapy.**—Corrective or regenerative cell therapy is the use of cells as therapeutic agents. Headlined by the promising therapeutic potential of stem cells, cell therapy is being investigated for the restoration of tissue and function in a broad range of selective or territorial cell-loss pathologic processes such as type I diabetes and myocardial infarction, respectively (133). To date, more than 15 clinical trials have evaluated the use of stem cells to regenerate myocardial tissue in ischemic heart disease patients, with the ma-



**Figure 7.** NIR optical imaging of atherosclerosis in mice aortas with use of cathepsin B-sensitive activatable probes. **(a)** Sudan IV staining of longitudinally opened aorta, in which red areas represent lipid-rich areas stained with Sudan IV. **(b)** Corresponding NIR image shows prominent cathepsin B signal from atherosclerotic lesions that matches Sudan IV staining. Native atherosclerotic lesions had NIR autofluorescence similar to that in normal aorta. Reproduced with permission from Chen et al (103).

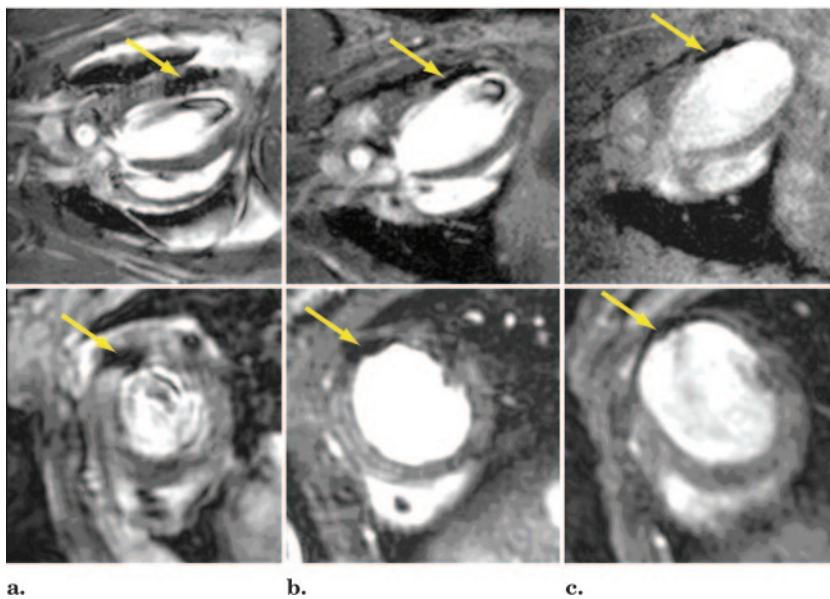
majority demonstrating improved myocardial function (134). Central to stem cell therapy is the remarkable ability of such cells to migrate from the site of transplantation to relevant foci of disease and differentiate into the appropriate cell type in response to chemical cues in the microenviron-

ment. Reliable noninvasive means to assess the delivery, distribution, and fate of transplanted stem cells are therefore imperative and are being established with use of molecular imaging techniques.

There are two distinct approaches to imaging cell-based therapies: di-

rect labeling and genetic integration of reporter genes; both modifications are rendered before transplantation (135). In the first approach, therapeutic cells are loaded intracellularly with a biocompatible imaging agent. Although all imaging modalities can theoretically be used for direct labeling, MR imaging with iron oxide nanoparticles is by far the most widely used method (Fig 8). The high spatial resolution of MR imaging is ideally suited for tracking stem cells *in vivo*, and iron oxide nanoparticles are less toxic, more stable, and more sensitively detected than  $Gd^{3+}$ . In fact, *in vivo* MR imaging of a single iron oxide-loaded cell with a 7-T scanner has been reported (136). Applied to a swine model of myocardial infarction, labeled stem cells were successfully imaged with a standard 1.5-T clinical scanner for as long as 21 days after percutaneous injection under fluoroscopy (137). Cell viability, proliferation, and differentiation capacity were not adversely affected by labeling *in vitro*. Despite these preliminary results, there are significant limitations to this approach. Detection becomes more difficult over time as contrast agents are progressively diluted with each cell division. In addition, imaging agents that persist after cell death can be engulfed by local macrophages and compromise imaging specificity.

In the second approach, reporter genes are engineered for constitutive expression and subsequently integrated into the genome of therapeutic cells. Longer-term imaging is therefore theoretically possible because the reporter genes are preserved and passed down to progeny. All previously described reporter gene systems can be potentially applied for cell tracking. In an elegant animal study (22), embryonic stem cells were transduced with a fusion reporter gene encoding for firefly luciferase and a derivative of HSV1-sr39tk and then injected into the myocardium of rats. Stem cell survival, proliferation, and migration were noninvasively assessed by bioluminescence and PET imaging after infusion of luciferin and 9-(4- $^{18}F$ -fluoro-3-[hydroxymethyl]butyl)guanine, respectively, over a period of 4 weeks. Bioluminescence and PET signals correlated closely, and both actually increased over time. Anatomic context,



**Figure 8.** Longitudinal assessment of cardiac stem cell transplantation in a mouse model of myocardial infarction by MR imaging with use of a 4.7-T scanner. Myocardial infarction was surgically induced by coronary occlusion in experimental mice. Stem cells loaded with iron oxide nanoparticles were directly injected into the infarcted region and tracked by MR imaging at day 1 (a), day 7 (b), and day 28 (c) after injection. Short-axis and corresponding long-axis images are shown in the first and second columns, respectively. Note that iron-loaded stem cells, seen as negative contrast, were localized to the infarcted anterolateral wall at each time point (arrows). Images courtesy of A.J. Katz and B.A. French of the University of Virginia Health System.

to determine the intracardiac location of the transplanted cells, was provided by FDG PET imaging of the hypermetabolic myocardial tissue. The main disadvantage of this approach, again, is the need to genetically manipulate cells—in this case, cells that hold the potential to transform and proliferate unchecked. Remarkably, this same study (22) showed preliminary data that HSV1-tk and its derivatives, which double as suicide genes, can be exploited as a safety mechanism against tumor formation by administration of the prodrug ganciclovir.

Molecular imaging of cell therapy has been demonstrated in experimental models of several other disorders, including ischemic stroke (138), Parkinson disease (139), type I diabetes (140), and muscular dystrophy (141). In human and large animal studies, transplantation is performed by direct injection or intravascular techniques under the guidance of conventional imaging. Interventional ra-

diologists are therefore projected to play a large role in stem cell delivery (142,143).

### Molecular Imaging of Cancer

*Cancer diagnosis and staging.*—Early diagnosis and accurate staging remain the mainstays for successful cancer therapy (144). Despite recent advances in imaging technologies, the reliance of diagnostic imaging on morphologic imaging leaves a considerable void in the accurate detection of early-stage and small-volume tumors. Molecular imaging, in sensing molecular alterations in tumor expression patterns, holds much promise to sensitively detect small cancers, to better distinguish malignant from benign lesions, and to reduce the delay between initial malignant transformation and diagnosis.

A common approach involves direct imaging of receptors or antigens that are exclusively or highly expressed by cancer cells (145). Numer-

ous examples exist, including the previously described Octreoscan and  $^{64}\text{Cu}$ -labeled anti-CEA antibody fragments (Fig 4) (37,69), as well as efforts to image dopamine and folate receptors overexpressed by pituitary adenomas and ovarian carcinomas, respectively (146,147). An extension of this is the use of NIR activatable probes to image enzymes upregulated in cancer cells such as cathepsins and MMPs (75). By imaging tumor-associated proteases, Weissleder et al (75) were able to detect tumors smaller than 0.3 mm in diameter in animal models. Central to these strategies is the identification and validation of reliable and specific molecular markers of cancer (148). High-throughput screening technologies such as microarray analysis can comprehensively analyze differences in expression patterns between normal and malignant cells and have proved particularly important in the discovery of novel tumor markers.

Instead of imaging specific tumor-associated markers, FDG PET detects the unusually high metabolic activity common to most tumors and has rapidly emerged as a widely used oncologic imaging tool. FDG PET has been shown to be more accurate than CT alone in the detection and staging of several cancers, such as non-small-cell lung cancer, breast cancer, lymphoma, and melanoma (149). However, because of its spatial resolution limitations, PET alone is being replaced by combined PET/CT for its improved anatomic detail and more precise lesion localization (38). In a prospective study comparing CT, PET, and PET/CT for staging non-small-cell lung cancer, PET/CT provided additional information in 41% of patients and demonstrated a significantly improved staging accuracy of 88% versus 40%–58% for PET or CT alone (150).

Because macrophage imaging has been used to assess atherosclerosis, MR imaging of *in vivo* macrophage monocrySTALLINE uptake is being investigated for diagnosis of lymph node metastases. Because nodal tissues invaded by cancer cells are unable to physically accommodate macrophages, the absence of T2 hypointensity of lymph nodes on MR imaging would provide an imaging marker for nodal invasion. In a human study of pros-

tate cancer (12), MR imaging after infusion of lymphotropic monocrySTALLINE particles was able to detect nodal metastases smaller than 2 mm in diameter and yielded sensitivity and specificity rates greater than 95%.

**Tumor characterization.**—Current methods of biologically characterizing tumors require invasive tissue acquisition for analysis. Receptor- and enzyme-based molecular imaging methods may also provide noninvasive means of gaining real-time insights into tumor biology. For example, high expression levels of cathepsins, MMPs, and epidermal growth factor receptors have been linked to highly aggressive and invasive tumors and poor prognosis (151–153). In a mouse study comparing aggressive and well-differentiated breast cancer tissue (154), differential levels of cathepsin activity were depicted by NIR fluorescence imaging of a cathepsin B–sensitive activatable probe. Relative to the well-differentiated cancer tissue, NIR fluorescence signal was 1.5 times greater in the aggressive breast cancer tissue. This correlated well with increased cathepsin B expression and rapid tumor growth. Receptor imaging may also prove useful for tailoring the use of targeted molecular therapeutics such as trastuzumab (Herceptin; Genentech; South San Francisco, CA), tamoxifen (AstraZeneca; Wilmington, DE), and gefitinib (Iressa; AstraZeneca). Methods have been devised to image HER-2/*neu* expression by MR imaging (70), estrogen and progesterone receptor status by nuclear imaging (155), and epidermal growth factor receptor expression by optical imaging (71).

**Therapy assessment.**—Decreased tumor size remains the traditional criterion to assess oncologic therapeutic response (156). As with disease detection, morphologic changes after therapy are late and nonspecific, precluding timely adjustments in therapeutic regimen. In addition, many novel oncologic therapies aim to inhibit cell proliferation and angiogenesis and may therefore arrest tumor growth but not necessarily reduce tumor size (33). Surrogate imaging endpoints for monitoring therapeutic efficacy are therefore needed. Be-

cause molecular imaging allows for noninvasive and repetitive probing of cellular and molecular processes, treatment response can be monitored by performing serial imaging and noting changes in the surrogate endpoint.

These endpoints can be specific to the drug's therapeutic mechanism or a nonspecific change in tumor cell activity and viability. For example, antiangiogenesis drugs and MMP inhibitors can be monitored by imaging decreases in angiogenesis markers and MMP enzyme activity, respectively (157,158). In a more generalized approach, posttreatment decreases in tumor cell metabolism as measured by FDG PET have been shown to correlate well with a reduction of viable tumor cells (159). As presented in **Figure 9**, serial FDG PET was used to assess the efficacy of imatinib (Gleevec; Novartis, East Hanover, NJ) in patients with gastrointestinal stromal cancer (160). Imaging of apoptosis has been another proposed means to evaluate oncologic treatment efficacy. Apoptotic cells express the membrane phospholipid phosphatidylserine on the outer surface of cell membranes early in the apoptotic process (161). Annexin V binds to phosphatidylserine selectively and with high affinity and has therefore been a commonly used ligand for apoptosis imaging (17). In a mouse model of radiation therapy, 15 Gy of radiation was given to one of two mouse hind legs inoculated with murine mammary tumor cells. Ensuing fluorescence imaging with cyanine 5.5–labeled annexin V demonstrated greater signal from the irradiated leg than from the contralateral control, indicating an increase in radiation-induced apoptosis (unpublished data, H. Sun and D. Pan, 2006).

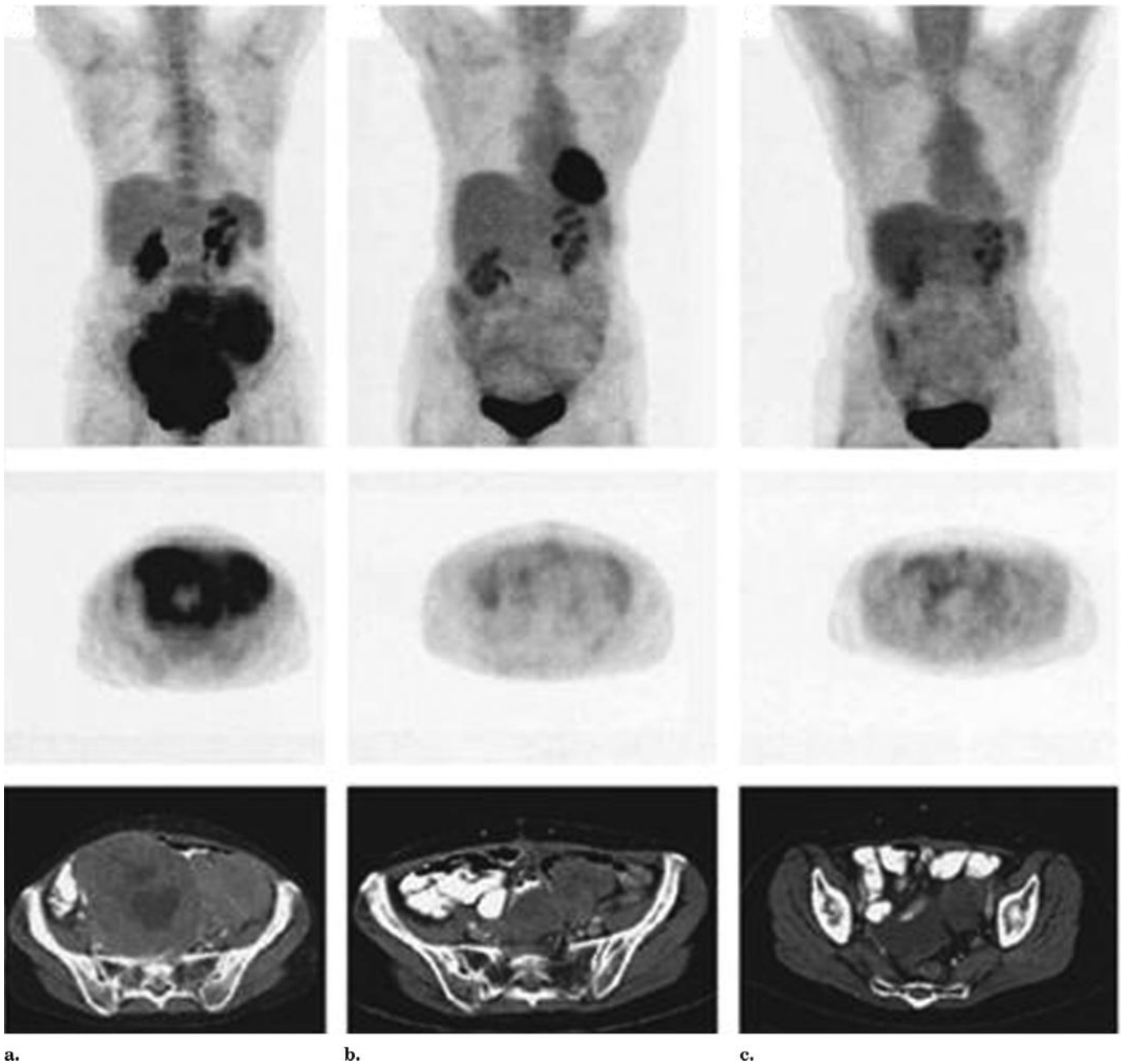
## CONCLUSIONS

The advent of molecular imaging has brought about fundamental paradigm shifts in our approach to research and patient care. In the laboratory setting, molecular imaging provides innovative tools for dissection of the cellular and molecular features of various biologic processes within the milieu of living organisms.

In clinical practice, molecular imaging holds promise to ultimately allow earlier and more precise disease diagnosis, disease characterization, and assessment of therapeutic response. The practice of molecular medicine will consist of prevention, diagnosis, and treatment methods that directly target the molecular, cellular, or physiologic defects responsible for disease. Molecular imaging sits at the interface of this transition and offers a clear opportunity for interventional radiology to have a strong presence in this age of molecular medicine (162).

The convergence of molecular imaging and interventional radiology benefits both fields. As with diagnostic radiology, interventional radiology has traditionally relied on morphologic imaging. In its promise to image molecular and cellular processes with high spatial and temporal resolution, molecular imaging tools can provide significant improvements in target visualization and characterization, therapy planning, procedural guidance, and treatment monitoring. In addition, the adaptation of MR, US, and optical molecular imaging methods for application in the interventional suite will allow interventional radiology to advance from its reliance on ionizing radiation–based imaging. With the continuing development of interventional MR (163), intravascular US (164), and intravascular optical systems (53), the use of these imaging modalities is not far from reality. Conversely, the translation of molecular imaging technologies to clinical application may be accelerated through the use of interventional techniques. Molecular imaging probes are rendered useless if they cannot reach their target as a result of inherent biologic barriers, degradation, or nonspecific binding (1). Although there has been much research to overcome these limitations, local delivery may be necessary for certain classes of molecular probes.

Historically, those who invest in the research and development of new technologies are able to assume ownership of the innovation and implement it in practice. As molecular imaging matures, its first clinical applications are likely to be in cardiovascular and oncologic imaging. Fortunately, cardiovascular disease and cancer are the likely principal clinical



**Figure 9.** Longitudinal assessment of imatinib treatment in a patient with a pelvic gastrointestinal stromal tumor by FDG PET. Sequential PET scans were obtained in the same patient before treatment (**a**), 1 month after imatinib treatment (**b**), and after 16 months of continuous treatment (**c**). Images at each time point include a two-dimensional PET scan of the body (top row), an axial PET scan through the pelvic tumor (middle row), and a corresponding axial CT scan (bottom row). FDG uptake in the cardiac blood pool, the myocardium, the liver, the bowel, the bilateral renal collecting system, and the bladder were within physiologic limits in this patient. The patient had similar blood glucose concentrations at each of these time points. Reproduced with permission from Demetri et al (160).

domains of interventional radiology in the intermediate future (162). With mutual benefits in the integration

of molecular imaging technologies with interventional techniques and the arrival of molecular medicine,

we believe it is time for interventional radiologists to assume key roles in the development of molecular imaging.

## APPENDIX I: GLOSSARY OF TERMS

**Angiogenesis:** formation of new blood vessels.

**Antisense:** sequence of DNA or RNA that is complementary to and binds target DNA or RNA.

**Apoptosis:** programmed cell death.

**Autofluorescence:** the inherent fluorescence of tissues.

**Cell surface receptor:** protein and/or polysaccharide structure on the surface of a cell that selectively binds certain molecular messengers.

**Clone:** production of multiple copies of a single gene.

**Dendrimer:** branching polymer used to transfer genetic material into cells.

**Enhancer:** nucleotide sequence that enhances transcription of a particular gene.

**Gene construct:** an engineered sequence of several genes spliced together.

**Gene expression:** process by which proteins are made from the instructions encoded in DNA.

**In vitro:** in an artificial environment outside the living organism.

**In vivo:** within a living organism.

**Ligand:** molecule that binds to a receptor.

**Liposome:** closed lipid vesicle that may be used to encapsulate materials for delivery into cells.

**mRNA:** messenger RNA; serves as the template for protein synthesis.

**Oligonucleotide:** small chain of DNA or RNA subunits, consisting of a base, a phosphate, and a sugar molecule.

**Phagocytose:** internalization of particles by cells.

**Phosphorylation:** the addition of phosphate to an organic compound through the action of a phosphorylase or kinase.

**Progenitor cell:** multipotential intermediate stem cells that are direct precursors for tissue-specific mature cells.

**Promoter:** binding site in a DNA chain at which RNA polymerase binds to initiate transcription of messenger RNA of one or more nearby genes.

**Protease:** enzymes that catalyze the breakdown of proteins.

**Reporter gene:** gene that encodes for a protein that is readily detectable, with or without the use of reporter

probes, and serves as a marker for expression of the gene construct.

**Stem cell:** primitive precursor cells that possess the capability to proliferate, self-renew, and differentiate into a variety of cell types.

**Substrate:** molecule acted upon by an enzyme.

**Transcription:** synthesis of messenger RNA by RNA polymerases using information encoded in DNA.

**Transduction:** introduction and integration of exogenous DNA into a recipient cell's genome by use of a viral system.

**Transfection:** introduction and integration of exogenous DNA into a recipient cell's genome by use of a nonviral system.

**Translation:** synthesis of protein using the information encoded in messenger RNA.

**Tumorigenesis:** development of tumor cells.

**Tumor markers:** specific biomolecules, such as enzymes or antigens, that are associated with the presence of cancer.

**Vector:** viral or nonviral vehicle used to deliver genes into a target cell.

## APPENDIX II: WEB SITES

The Academy of Molecular Imaging: <http://www.ami-imaging.org>

The Society for Molecular Imaging: <http://www.molecularimaging.org>

Molecular Imaging Central: <http://www.mi-central.org>

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