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Cardiovascular Molecular Imaging

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The recent introduction of novel gene therapies for treatment of cardiac and noncardiac diseases has caused a remarkable need for noninvasive imaging approaches to evaluate and track the progress of these therapies. In the past we have relied on the evaluation of the physiological consequences of therapeutic interventions. With advances in targeted molecular imaging we now have the ability to evaluate early molecular effects of these therapies. The development of dedicated high resolution small animal imaging systems and the establishment of transgenic animal models has enhanced our understanding of cardiovascular disease and has expedited the development of new gene therapies. Noninvasive targeted molecular imaging will allow us to directly track biochemical processes and signaling events that precede the pathophysiological changes. The examples of targeted molecular imaging outlined in this seminar provide some insight into the bright and growing future of cardiovascular molecular imaging. The success of this new field rests on the development of targeted biological markers of molecular and physiological processes, development of new instruments with improved sensitivity and resolution, and the establishment of multidisciplinary teams of experimental and clinical investigators with a wide range of expertise. Molecular imaging already plays a critical role in the experimental laboratory. We expect that, in the near future, targeted molecular imaging will be routinely used in clinical cardiovascular nuclear medicine laboratories in conjunction with existing imaging modalities for both diagnostic and prognostic purposes, as well as for evaluation of new genetic based therapeutic strategies.
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Health care has become so expensive that, to provide it to a maximum number of people, a shift is need in emphasis from treatment to the prevention of disease. This shift presents new challenges to basic research as well as to clinical practice and stimulates technological adaptations of existing diagnostic procedures. In the past, prognostication and evaluation of therapy were assessed by studying physiological consequences expressed by changes in blood flow, function, and metabolism. Current strategies involve the use of targeted markers of biological processes. The development of biologically targeted markers has become possible with recent advances in molecular biology, including genomics and proteomics. The effective application of these technological advances requires the establishment of multidisciplinary teams with a wide range of expertise. Nuclear medicine is expected to play a key role in this interdisciplinary approach to understanding origins, pathogenesis, and progress of diseases and in evaluating therapeutic interventions. Nuclear medicine has shown its enormous potential in observing in vivo complex system functions at the molecular, cellular, organ, as well as the whole-body level.

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Molecular Imaging

The concept and practice of molecular imaging, defined as the in vivo characterization and measurement of biological processes at the cellular and molecular level within living organisms, has been present for decades and originated with targeted nuclear imaging.[1] Targeted imaging can be defined in terms of a probe-target interaction, whereas the probe localization and magnitude are directly related to the interaction with the target epitope or peptide. Nuclear medicine is particularly suited for target imaging that initially involved monoclonal antibody targeting of a specific cell membrane epitope, imaging the activity of a particular enzyme or transporter-specific probe.

Historical Perspective

Imaging cell-specific surface antigens or epitopes with radiolabeled monoclonal antibodies represent some of the earliest

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molecular imaging applications still used in both experimental and clinical nuclear medicine research. Initial studies involved tumor imaging with direct radiolabeled native antibodies (ie, IgG).[2] The introduction of genetic engineering provided a powerful tool to design recombinant fragments that retained high affinity for target antigens and expressed both a high tumor targeting and concomitant rapid clearance from normal tissues. More recently, the imaging of various cell surface receptors using radiolabeled regulatory peptides represents a novel strategy in basic research. The regulatory peptides are small, readily diffusible molecules that express a broad range of receptor-mediated actions. These high affinity receptors are over expressed in many pathological states and provide molecular targets for the diagnosis and assessment of the therapy.

The most widely used paradigm in clinical studies is the assay of enzymatic activity imaging glucose use with 2′-fluoro-2′-deoxyglucose (FDG). This imaging approach is based on an enzyme-specific radiolabeled probe and an enzyme-metabolic trapping mechanism that provides an amplification of the signal in target tissue. Such enzymatic signal amplification (increase in image intensity) offers substantial enhancement for targeted molecular imaging. Currently, ^{18}F-labeled FDG is the most widely used positron emission tomographic (PET) imaging tracer. PET ^{18}F-FDG has become the prerequisite imaging study before the treatment in a wide variety of diseases and often is used to assess treatment response.

Another classic targeted molecular imaging approach involves the imaging of cardiac neuroreceptors in the heart. Most of this work has focused on imaging of the sympathetic nervous system. Important alterations in both pre- and postsynaptic cardiac sympathetic function occur in several cardiovascular diseases, including ischemic heart disease. Presynaptic function can be measured using ^{11}C-meta-hydroxyephedrine a PET radiotracer, or ^{123}I-meta-iodobenzylguanidine, a SPECT radiotracer. Postsynaptic function can be assessed with ^{11}C-CGP12177, a radiolabeled beta-blocker for PET imaging.[3,4]

Newer Applications

Recent progress in the understanding of the molecular-genetic mechanisms as well as technological development of new imaging strategies has led to the application of new biologically based approaches. Methods actively are being developed for controlled gene delivery to various somatic tissues using novel gene constructs. Moreover, gene expression can be controlled and imaged using cell-specific, drug-controlled expression systems.

Recently, new imaging strategies have been proposed and successfully employed in clinical as well as basic research studies. Radiolabeled thymidine analogs, such as 5-iodo-2′-deoxy-uridine (^{123}IdU, ^{124}IdU, and ^{131}IdU) and 5-bromo-2′-deoxy-uridine (^{76}BrdU), have been used to image cell proliferation and DNA synthesis.

In contrast to the direct, targeted imaging paradigm, indirect molecular imaging is more complex and involves multiple components. "Reporter imaging" is an example of an indirect imaging strategy. This paradigm includes a marker/reporter gene and a marker/reporter probe. The reporter gene product can be an enzyme that converts a reporter probe to a metabolite that is selectively trapped within transduced cells. The main advantage of this approach is the enzymatic amplification of the probe-signal that facilitates imaging the magnitude and location of reporter gene expression.

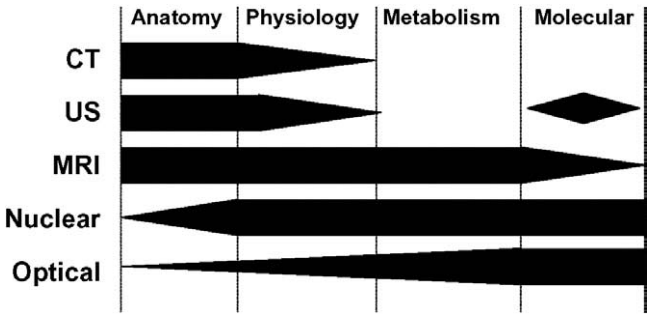

Figure 1 Comparison of the relative strengths of different current imaging modalities for evaluation of anatomy, physiology, metabolism, and molecular processes.

Another important novel imaging paradigm is the imaging of molecular markers and biological pathways that give insight into the pathogenesis and progress of diseases and assessment of therapeutic intervention. These include novel imaging strategies for heart failure, thrombosis, apoptosis, atherosclerosis, and angiogenesis.

Imaging Technology

Significant progress in the technological advancement of imaging instrumentation has been observed during recent decades. However, because of practical limitations of different imaging modalities, the broad use of molecular imaging is restricted to a few techniques (Fig. 1). Nuclear and optical modalities provide remarkable ability to study molecular processes and biological pathways; however, because of their relatively poor spatial resolution, their use in imaging the anatomy is somehow limited. Magnetic resonance imaging (MRI) overcomes problems with spatial resolution but because of the lack of molecular MRI-compatible and widely available probes, the ability of MRI to study molecular processes is still inadequate.

Table 1 shows selected operational parameters for different imaging modalities. It is evident that nuclear techniques, both single photon emission computed tomography (SPECT) and PET possess a unique set of advantages that make them particularly suitable for molecular imaging.

The greatest problems arise when accurate quantitative information about the radiopharmaceutical distribution is required. The accuracy of SPECT imaging is fundamentally limited by the attenuation of the low energy photons by body tissues. This introduces an error in relating the density of detected photons to the concentration of the radiopharmaceutical in an organ. Moreover, the presence of scattered radiation limits spatial resolution. PET imaging can over-

Table 1 Selected Operational Parameters for Different Imaging Modalities

Modality	Spatial resolution	Depth	Temporal resolution	Sensitivity (mol/L)	Molecular probe
PET	1-2 mm	No limit	10 s-min	10^{-11}-10^{-12}	ng
SPECT	0.5-1.5 mm	No limit	min	10^{-10}-10^{-11}	ng
Optical					
Bioluminescence	3-5 mm	1-2 mm	sec-min	10^{-15}-10^{-17}	μg-mg
Fluorescence	2-3 mm	<1 mm	sec-min	10^{-9}-10^{-12}	μg-mg
MRI	25-100 μm	No limit	min-hrs	10^{-3}-10^{-5}	μg-mg
CT	50-200 μm	No limit	min	–	N/A
Ultrasound	50-500 μm	mm-cm	sec-min	–	μg-mg

come some of the attenuation problems however compared with SPECT it is an expensive technique.

The introduction of hybrid systems for imaging of small animals (microSPECT/CT and microPET/CT) greatly enhanced the performance and accuracy of nuclear imaging. The CT component could be used for anatomical localization as well as for attenuation correction. However, the question addressed here is what is the optimal nuclear technology for small animal imaging?

MicroSPECT imaging offers several advantages over microPET imaging, and these include availability of targeted tracers, improved physics of SPECT radiotracers, and general availability and affordability of SPECT technology. In contrast to SPECT technology, inherent resolution of PET radiotracers is fundamentally limited by physical behavior of positron decay (1-3 mm). Moreover, both the movement of the positron before annihilation and deviation from an exact 180° angular separation have a profound effect on PET resolution. The major advantage of targeted SPECT imaging approaches is production and transport of SPECT tracers that are readily available and simultaneous multiple-isotope imaging capability. On the other hand, microPET technology offers several unique advantages over microSPECT imaging. A major advantage of microPET imaging is provided by use of ^{11}C isotopes, which permits labeling a given molecule without perturbing the biological function. Additionally, microPET imaging provides better sensitivity and an established approach for attenuation correction that gives a greater potential for image quantification.

Specific Applications of Cardiovascular Molecular Imaging

Imaging of Angiogenesis

Angiogenesis represents the formation of new capillaries by cellular outgrowth from existing microvessels.[5] It occurs as part of the natural healing process after ischemic injury. The process of angiogenesis includes local proliferation and migration of vascular smooth muscle and endothelial cells as well as potential participation of blood-derived macrophages and circulating stem cells. The principal stimuli for angiogenesis include tissue ischemia and hypoxia, inflammation, and shear stress. A large number of local and circulating angiogenic factors are involved in this process, including vascular endothelial growth factor (VEGF), angiopoietins, basic fibroblast growth factor, and transforming growth factor-β. The angiogenic response is also modulated by the composition of the extracellular matrix (ECM) and intercellular adhesions, including integrins.[6,7] Integrins are a family of heterodimeric cell surface receptors capable of mediating an array of cellular processes, including cell adhesion, migration, proliferation, differentiation, and survival.[8] During angiogenesis endothelial cells must adhere to one another and to the ECM to construct and extend new microvessels. The specific $\alpha v\beta 3$ integrin has been identified as a critical modulator of angiogenesis.[6] Thus, the angiogenic process is a complex multistep phenomenon that involves many stimuli, growth factors and interactions between multiple cell types.[9]

Potential targets for imaging of angiogenesis relate to the imaging of the favorable conditions or molecular events associated with the initiation of the angiogenic process. Approaches for the targeted imaging of angiogenesis have been extensively discussed in a recently published review.[10] Only the most established approaches for targeted imaging of angiogenesis will be highlighted in the current article. This includes evaluation of the altered expression of VEGF receptors, and $\alpha v\beta 3$ integrins.

Imaging of VEGF and Receptors

VEGF is a fundamental mediator of angiogenesis and plays a critical role in vascular development during embryogenesis.[11] The mitogenic activity of VEGF is primarily for endothelial cells, although VEGF also interacts with bone marrow-derived cells, monocytes, and tumor cell lines. The expression of VEGF is induced by hypoxia, indicating that it is a key natural mediator of angiogenesis in response to ischemia. VEGF mediates many cellular functions including, release of other growth factors, cell proliferation, migration, survival, and angiogenesis.[12]

VEGF receptors are reasonable targets for imaging of mediators of ischemia-induced angiogenesis, using $VEGF_{121}$ as the targeting ligand. Indium-111 (^{111}In)-labeled $VEGF_{121}$ recently was evaluated in a rabbit model of unilateral hindlimb ischemia.[13] Planar imaging demonstrated significantly higher ^{111}In-$VEGF_{121}$ activity in the ischemic hindlimb compared with the contralateral nonischemic hindlimb. These differences were confirmed by postmortem gamma well counting of skeletal muscle at multiple time points post injection in subsets of the rabbits. Gamma well counting demonstrated a

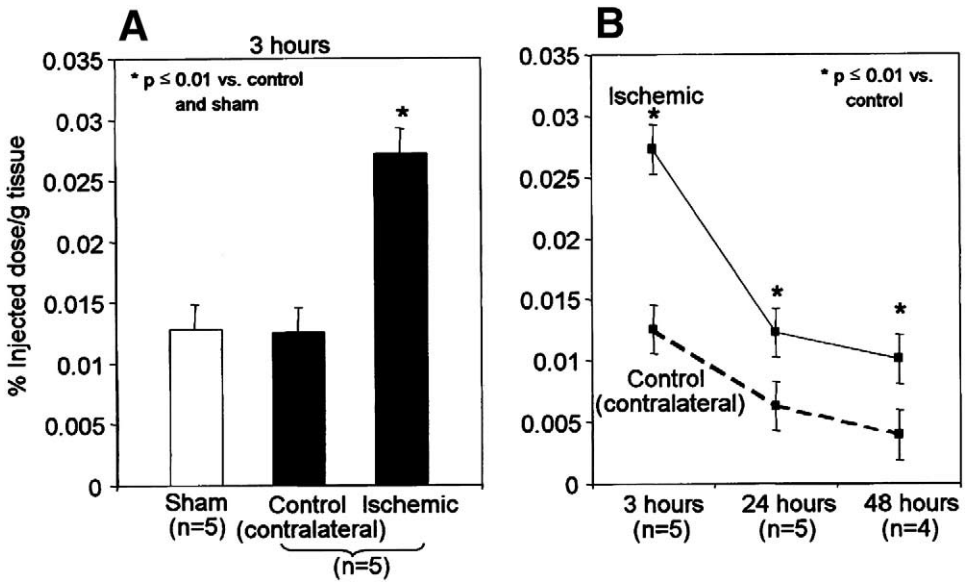

Figure 2 ^{111}In-VEGF retention in model of rabbit hindlimb ischemia. ^{111}In-VEGF activity was measured by gamma well counting in deep muscles of hindlimb) at 3 hours (A) and up to 48 hours (B) after radiotracer injection. Control indicates contralateral nonischemic hindlimb in rabbits with unilateral hindlimb ischemia. Solid bars, group with unilateral ischemia; open bars, group with sham operated hindlimb. (Reprinted with permission.[13])

2-fold greater ^{111}In-VEGF$_{121}$ retention in ischemic muscle compared with contralateral nonischemic muscle and muscle from sham-operated rabbits. The findings are summarized in Figure 2. Immunohistochemistry confirmed increased expression of KDR and Flt-1 receptors within the ischemic hindlimbs, although the skeletal muscle was not evaluated for capillary density to confirm angiogenesis.

This preclinical study suggests that it is possible to identify ischemic tissue by radiolabeling angiogenic receptors using a naturally occurring ligand as the imaging probe. The use of radiolabeled VEGF$_{121}$ as an imaging agent takes advantage of the specificity of VEGF$_{121}$ for hypoxia-inducible endothelial cell VEGF receptors.

The availability of a recombinant human form of VEGF$_{121}$ avoids the potential problem of immunogenicity associated with the use of antibodies as targeting ligands.[14] However, this approach may be in part limited by the total VEGF$_{121}$ receptor density, and the retention of ^{111}In-VEGF$_{121}$ in other critical organs (ie, kidney, liver).

VEGF receptor imaging could provide complementary information to routinely available clinical assessments of flow by imparting physiologic information on hypoxic stress within viable tissue.[15] The application of dual isotope ^{111}In-VEGF$_{121}$ and ^{99m}Tc-sestamibi imaging could be useful for identifying hibernating myocardium or peri-infarct tissue at risk for necrosis. VEGF receptor imaging also could be potentially useful in the evaluation of therapeutic angiogenic strategies but again would probably require complementary perfusion imaging. In peripheral vascular disease, identification of VEGF receptors could help guide the selection of sites for local injection of angiogenic treatments. In patients with clinical myocardial ischemia receiving angiogenic treatments, the serial scintigraphic identification of changes in receptor expression could provide evidence of a therapeutic effect, which could be incremental to, or more sensitive than, scintigraphic measures of flow alone. Further studies in more clinically relevant models will be required to validate the concept of angiogenic receptor labeling as a clinically useful imaging approach.

Imaging of $\alpha v\beta 3$ Integrin

The $\alpha v\beta 3$ integrin is expressed in angiogenic vessels. It is known to modulate angiogenesis and therefore represents another potential novel target for imaging angiogenesis.[16,17] Investigators first proposed the noninvasive detection of tumor angiogenesis in vivo using magnetic resonance imaging and a paramagnetic contrast agent targeted to endothelial $\alpha v\beta 3$ via the LM609 monoclonal antibody.[17] However, targeted in vivo imaging using similar monoclonal antibodies has been limited in the past by slow clearance of the tracer from the blood. Haubner and coworkers subsequently reported the synthesis and characterization of a series of radiolabeled $\alpha v\beta 3$ antagonists, reporting kinetics in both in vitro and in vivo preparations.[16,18,19] Their work has focused on the use of cyclic Arg-Gly-Asp (RGD) peptides, which are known to bind to the $\alpha v\beta 3$ integrin. These radiolabeled RGD peptides exhibited high affinity for the $\alpha v\beta 3$ integrin, and specific binding in several tumor cell lines expressing $\alpha v\beta 3$. This preliminary work supports the potential for radiolabeled targeting of $\alpha v\beta 3$ for imaging of angiogenesis. Although these compounds demonstrated rapid clearance from blood, they are cleared predominantly through the hepatobiliary system, which may complicate imaging of myocardial angiogenesis.

Harris and coworkers recently reported the high affinity

and selectivity of an ^{111}In-labeled quinolone (^{111}In-RP748) for the $\alpha v\beta 3$ integrin using assays of integrin-mediated adhesion.[20] These investigators also demonstrated rapid blood clearance and a favorable biodistribution of ^{111}In-RP748, as well as the feasibility for tumor imaging. Sadeghi and coworkers evaluated a cy3-labeled homolog (TA145) of ^{111}In-RP748 using cultured endothelial cell preparations incubated in the presence and absence of established integrin activators.[21] TA145 localized to $\alpha v\beta 3$ at focal cell-cell contact points. Under these in vitro experimental conditions, TA145 appears to exhibit preferential binding to the activated form of $\alpha v\beta 3$ integrin. This suggests that ^{111}In-RP748 may also exhibit selective binding to activated $\alpha v\beta 3$ integrin.

Meoli and coworkers were the first to report the potential of ^{111}In-RP748 for in vivo imaging of myocardial angiogenesis.[22] ^{111}In-RP748 demonstrated favorable kinetics for imaging of ischemia induced angiogenesis in the heart. Established canine models of myocardial infarction, which are known to produce nontransmural infarction and peri-infarct ischemia resulting in myocardial angiogenesis, initially were used in the application of in vivo SPECT imaging of myocardial angiogenesis. In these studies, focal uptake of ^{111}In-RP748 was observed in the infarct region associated with activation of the $\alpha v\beta 3$ integrin. However, reconstruction and interpretation of the ^{111}In-RP748 "hot spot" images of the $\alpha v\beta 3$ integrin required careful coregistration of the targeted images with perfusion images. In vivo and ex vivo dual isotope SPECT ^{111}In-RP748 and ^{99m}Tc-sestamibi images are shown for two different dogs at 3 weeks following myocardial infarction are shown in Figure 3. The value of the $\alpha v\beta 3$ targeted imaging approach for assessment of myocardial angiogenesis was recently confirmed by another group of investigators that injected an ^{123}I-labeled RGD peptide in pigs with chronic ischemia treated with direct intramyocardial injection of phVEGF$_{165}$.[23]

An additional series of rat studies demonstrated that the regional myocardial retention of ^{111}In-RP748 in the reperfused infarcted region correlated with the uptake of a radiolabeled nitroimidazole (BRU-5921), which has been shown to be retained in hypoxic myocardium.[24] This supports the role of ^{111}In-RP748 as a targeted marker of angiogenesis, which is stimulated in regions of myocardial hypoxia.

Additional studies by the group at Yale University have demonstrated the value of a ^{99m}Tc-labeled peptide (NC100692) for targeted imaging of the $\alpha v\beta 3$ integrin in rodent models of hindlimb ischemia using high resolution pinhole planar imaging.[25] Mice underwent a surgical right femoral artery occlusion and were injected with NC100692 at multiple times after occlusion. In vivo pinhole planar imaging was performed to evaluate temporal changes in angiogenic process within ischemic hindlimb. Nuclear imaging results have been confirmed by in vitro gamma well counting.

These preliminary experimental studies suggest that radiolabeled $\alpha v\beta 3$ targeted agents may be valuable noninvasive markers of angiogenesis after ischemic injury. Additional experimental studies will be required to define the duration of $\alpha v\beta 3$ integrin expression/activation after ischemic injury or after stimulated angiogenesis. The changes in expression/activation of $\alpha v\beta 3$ integrin also will need to be related to changes in more functional parameters like myocardial perfusion, regional mechanical function, permeability, and regional hypoxia. The potential for targeted imaging of other integrins, like $\alpha v\beta 5$, must also be considered.

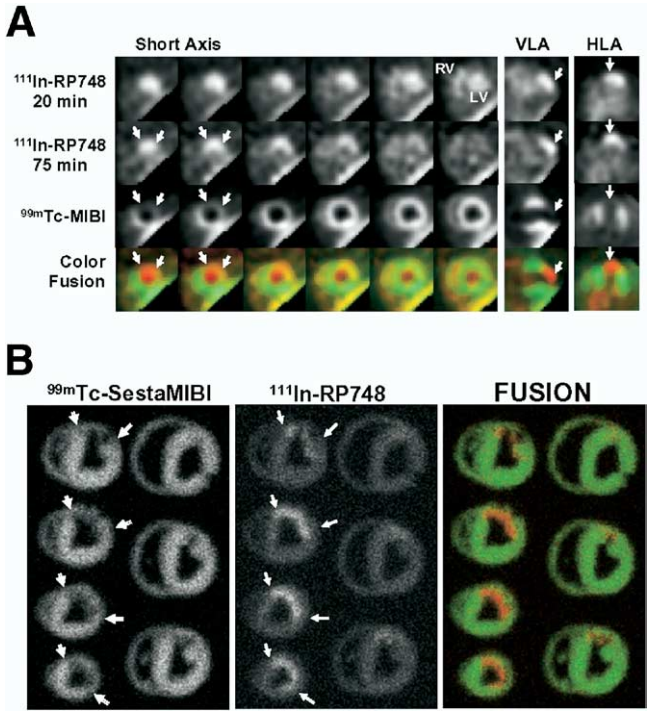

Figure 3 In vivo and ex vivo ^{111}In-RP748 and ^{99m}Tc-sestamibi (^{99m}Tc-MIBI) images from dogs with chronic infarction. Serial in vivo ^{111}In-RP748 SPECT short axis, vertical long axis (VLA), and horizontal long axis (HLA) images in a dog 3 weeks after LAD infarction at 20 min and 75 min after injection in standard format (A). ^{111}In-RP748 SPECT images were registered with ^{99m}Tc-MIBI perfusion images (third row). The 75-min ^{111}In-RP748 SPECT images were colored red and fused with MIBI images (green) to better demonstrate localization of ^{111}In-RP748 activity within the heart (color fusion, bottom row). Right ventricular (RV) and left ventricular (LV) blood pool activity is seen at 20 min. Filled arrows indicate region of increased ^{111}In-RP748 uptake in anterior wall. This corresponds to the anteroapical ^{99m}Tc-sestamibi perfusion defect (open arrow). Ex vivo ^{99m}Tc-sestamibi (left) and ^{111}In-RP748 (center) images of myocardial slices from a dog 3 weeks after LAD occlusion, with color fusion image on right (B). Short axis slices are oriented with anterior wall on top, RV on left. Open arrows indicate anterior location of nontransmural perfusion defect region, and filled arrows indicate corresponds area of increased ^{111}In-RP748 uptake. (Reprinted with permission.[22])

Imaging of Atherosclerosis and Vascular Injury

Integrins, particularly $\alpha v\beta 3$, also have emerged as a promising target for imaging injury-induced vascular remodeling and proliferation. Antagonists of $\alpha v\beta 3$ have been shown to limit neointimal hyperplasia and lumen stenosis in experimental models of vascular injury. Sadeghi and coworkers

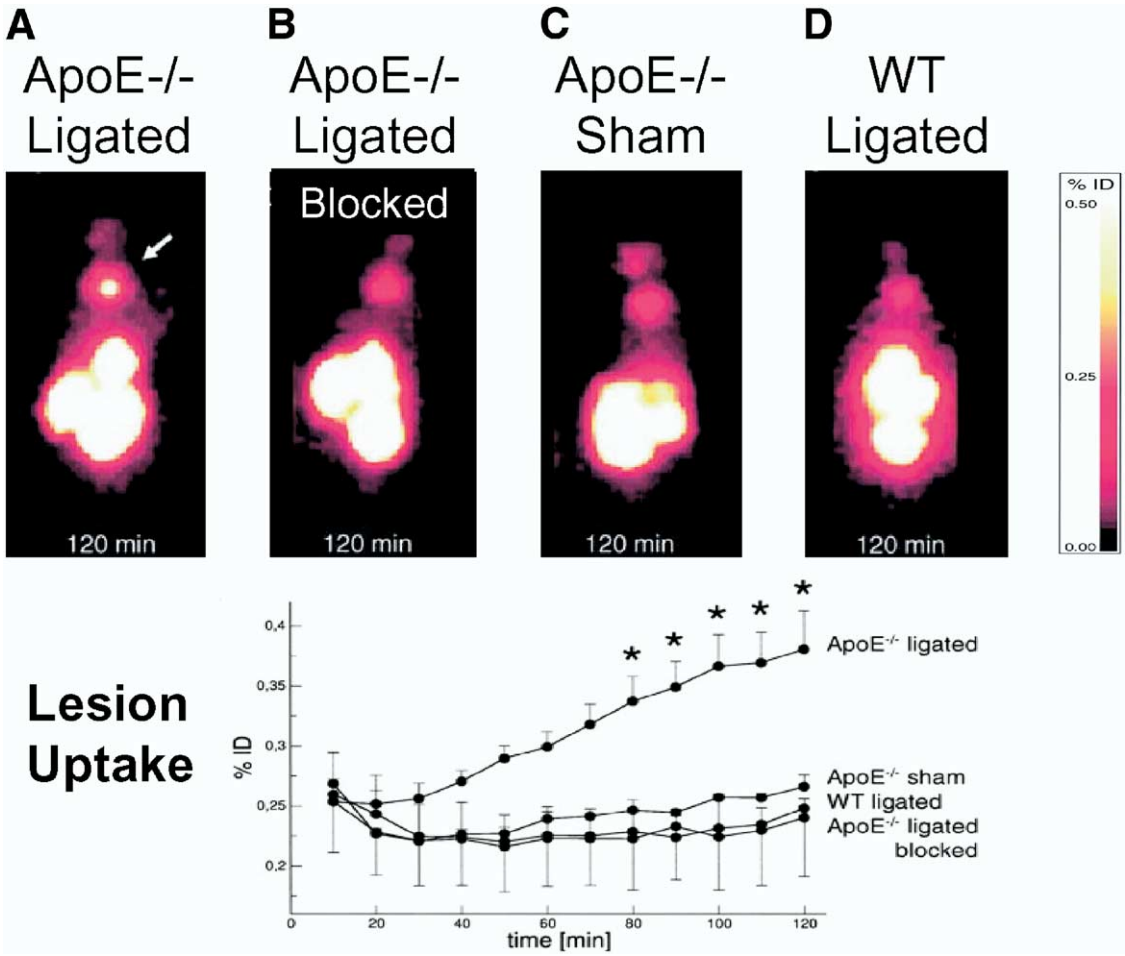

Figure 4 In vivo scintigraphic uptake of [^{123}I]HO-CGS 27023A a MMP targeted radiotracer. Representative planar images taken 120 min after injection in apolipoprotein E-deficient mice (A-C) and wild-type mice (D) 4 weeks after carotid ligation. A, Unblocked; B, after predosing with 6 mmol/L CGS 27023A; C, sham-operated; D, wild-type. Graph illustrates quantitative uptake of the radioligand in the carotid lesion over time is expressed as % ID. $^*P < 0.05$ between unblocked and predosed lesional uptake. The signal in the abdominal cavity is unspecific and probably reflects metabolism of the original compound because there is no inhibition after predosing in all experiments. (Reprinted with permission.[28])

have demonstrated that the novel ^{111}In-labeled $\alpha v\beta 3$ integrin-specific molecule, RP748 and its homologues bind preferentially to activated $\alpha v\beta 3$ on endothelial cells (ECs) in vitro and exhibit favorable binding characteristics for in vivo imaging.[26] Indeed, there was an approximately 15-fold increase in RP748 affinity for $\alpha v\beta 3$ integrin on ECs in suspension in the presence of Mn^{2+} (activated ECs), compared with nonactivated cells. Moreover, investigators presented evidences that RP748 uptake can track the proliferative process associated with carotid artery injury by targeting activated $\alpha v\beta 3$ integrin expression in vivo in apolipoprotein E-negative (apoE$^{-/-}$) mice. Using immunohistochemistry they demonstrated that very low levels of αv and $\beta 3$ staining could be detected in uninjured carotid arteries. In conjunction with vascular wall expansion, the injured arteries expressed significantly higher levels of both αv and $\beta 3$ in the media and neointima. The temporal changes in the expression of αv and $\beta 3$ were observed with maximum at 1 to 3 weeks after injury. These findings may potentially lead to the development of noninvasive imaging strategies for vascular cell proliferation-associated states, whether focal (ie, postangioplasty restenosis) or diffuse (ie, pulmonary hypertension).

Schäfers and coworkers investigated the feasibility of scintigraphic imaging of matrix metalloproteinases (MMPs) in vivo using a radiolabeled broad-spectrum MMP inhibitor (CGS27023A) in an established animal model of arterial remodeling and lesion development where MMPs are induced and activated.[27,28] The MMPs constitute a large family of proteolytic enzymes responsible for degradation of myocardial extracellular matrix (ECM) that is associated with vascular remodeling. These investigators evaluated an ^{123}I-labeled MMP inhibitor (CGS27023A) in cholesterol-fed apolipoprotein E-deficient mice after ligation of the carotid artery. The mice developed an arterial lesion, which was macrophage- and MMP-rich, with MMP-9 as the most prominently expressed enzyme. The in vivo imaging of the carotid lesion showed an approximately 1.5-fold increase in tracer accumulation attributable to specific uptake (Fig. 4). To confirm

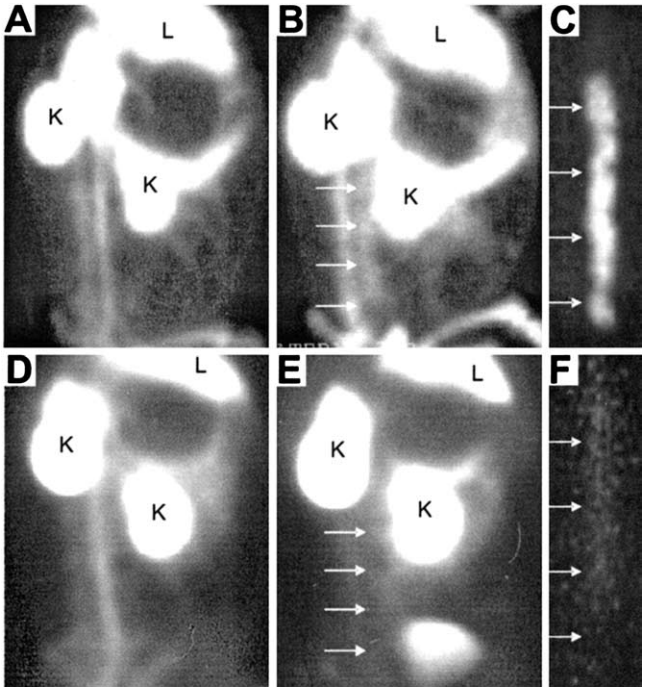

Figure 5 Feasibility of noninvasive imaging of apoptosis by radiolabeled annexin V. Left lateral oblique gamma images of experimental atherosclerotic (A-C) and control (D-F) rabbits injected with ^{99m}Tc-annexin V; L and K mark liver and kidney activities, respectively. Images at the time of injection (A and D) and at 2 h after injection (B and E) are shown. Although blood pool activity is seen at the time of injection (A) in the atherosclerotic animal, tracer uptake is clearly visible in the abdominal aorta (with lesions) at 2 h (B). C, Ex vivo image of B shows intense ^{99m}Tc-annexin V uptake in the arch and abdominal region. Annexin-positive areas were confirmed to contain macrophage and apoptosis-rich regions in the atherosclerotic plaque by histology. D through F show the corresponding images in the control animal. Note that the aorta is indistinguishable from background at 2 hours after injection (E). The blood pool at the time of injection in control animal (D) is comparable to atherosclerotic animal. F, Ex vivo aortic image of the control animal demonstrates the absence of ^{99m}Tc-labeled Annexin V uptake. (Reprinted with permission.[30])

imaging data, carotid arteries were excised and counted in a gamma counter. Radiotracer retention in the left carotid lesion was approximately threefold that of the control right carotid. The specificity of lesion uptake was established using an unlabeled (cold) MMP inhibitor.

Other investigators have focused on the imaging of vascular smooth muscle cell proliferation[29] and apoptosis of these cells or macrophages within the vessel wall (Fig. 5).[30] One useful marker of smooth muscle cell proliferation is the negative charge-modified Z2D3 antibody, which has been used for immunoscintigraphic targeted imaging of atherosclerosis.[29]

Targeted radiotracer imaging of the atherosclerosis or vascular remodeling presents a unique problem, in that the target lesion has a very low mass and may be located deep in the body. Existing PET and SPECT instrumentation may be insufficiently sensitive to detect these small deep lesions. Accordingly, several groups of investigators are developing intravascular scintillation catheters that can be use to detect local uptake of radiotracers targeted to components of the atherosclerotic or unstable vascular plaque. Most of these intravascular detectors use plastic scintillators linked to fiberoptics, which transmit the signal down a flexible catheter system.

Imaging of Postinfarction Remodeling

The MMPs also are responsible for degradation of the myocardial ECM that is associated with post-MI myocardial LV remodeling that often leads to heart failure. A clear cause/effect relationship between MMPs and the LV remodeling process has been demonstrated using animal models of heart failure, both wild type and transgenic as well as through the use of MMP inhibitors. The importance of detecting and quantifying of MMP activity in vivo during the evolution of post-MI remodeling is the driving force to develop a noninvasive method that will help to translate basic observations to clinical applicability.

The measurement of MMP and tissue inhibitors of MMPs (TIMP) levels in tissue and plasma can provide important insight into a critical determinant of MMP activity. However, because of the multiple posttranslational steps that regulate MMP activity, several approaches have been developed to directly quantify MMP activity in situ and more recently in vivo. One approach is in situ zymography. An additional approach for measuring total MMP activity in crude homogenates or in situ is through the use of fluorogenic labeled peptide substrates, however these peptide constructs are unstable in plasma, bound by nonspecific proteins, and have a limited half-life that limits their clinical in vivo applicability. In light of the fact that defining MMP activity in vivo holds great clinical significance in both cancer as well as in cardio-

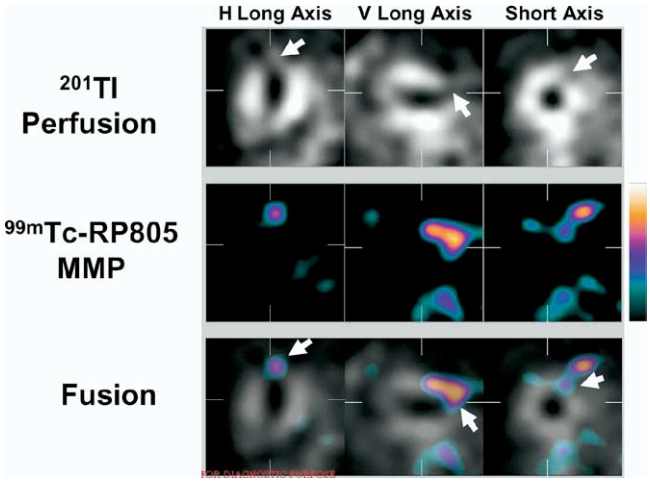

Figure 6 In vivo ^{99m}Tc-RP805 and ^{201}Tl images from mouse with chronic infarction. Serial in vivo ^{201}Tl SPECT (first row) and ^{99m}Tc-RP805 SPECT (second row) short axis, vertical long axis, and horizontal long axis images in a mouse 1 week after LAD infarction. ^{99m}Tc-RP805 SPECT images were coregistered with ^{201}Tl perfusion images (third row). Arrows in the first row indicate ^{201}Tl perfusion defect that corresponds to the area of increased ^{99m}Tc-RP805 uptake marked by arrows in the third row.

vascular disease, recent effort has been placed in developing nonpeptide markers for MMP activity. These constructs have been developed around the structural configuration of pharmacological MMP inhibitors, but do not possess innate MMP inhibitory activity. For example, a compound developed initially by Parke-Davis (PD166703) has been used as a backbone for a number of radiolabeled and spin-labeled compounds that permit visualization of MMP activity using either PET or magnetic resonance imaging. More recently, Bristol-Myers-Squibb has developed MMP targeted SPECT radiotracers which displayed selective binding kinetics to the active MMP catalytic domain. These radiotracers use a nonselective MMP inhibitor as the template that has been conjugated with ^{99m}Tc or ^{111}In. Binding of these radiotracers to the exposed catalytic domain of active MMPs provides a potential method detect and image MMP activation in vivo using a conventional gamma camera for both planar and SPECT imaging.

We have demonstrated the feasibility of a noninvasive MMP imaging using an ^{111}In-labeled nonspecific MMP inhibitor (^{111}In-RP782) to evaluate temporal changes in MMP activation in murine model of myocardial infarction (MI).[31] ^{111}In-RP782 or a negative control ^{111}In-labeled enantiomeric compound (^{111}In-RP788) was injected into mice at 1 week after surgically induced MI or in noninfarcted control mice. Microautoradiography demonstrated increased ^{111}In-RP782 retention within MI region and no local myocardial retention of ^{111}In-RP788. Gamma well counting revealed a significant increase in ^{111}In-RP782 activity in MI regions of post-MI group compared with control group. ^{111}In-RP782 retention correlated well with MMP activity defined by in situ zymography. Similar studies have been performed using ^{99m}Tc-labeled RP805 (Fig. 6) confirming feasibility of that approach.

Imaging of Apoptosis

Apoptosis, or programmed cell death, occurs in association with many cardiovascular diseases. This preprogrammed cell death often occurs in combination with cell death by necrosis. Apoptosis, first described by Kerr and coworkers, is characterized by morphological changes, including cell shrinkage and formation of membrane-bound apoptotic bodies.[32] In contrast, necrosis is characterized by an early loss of membrane permeability, cell swelling and random fragmentation of DNA. Cells undergoing apoptosis express phosphatidyl serine (PS) on their cell membrane, which is a constitutive plasma membrane anionic phospholipid that is not expressed in normal cells; thus, it presents a favorable target for imaging of apoptotic processes. Annexin-V is a medium-size (MW = 36 kDa) physiological human protein with a high (K_a = 7 nM), Ca^{2+}-dependent affinity toward the PS on the outer leaflet of the cell membrane. Annexin-V could be readily labeled with either fluorescent or radionuclide agent and used in apoptosis imaging.

Vermes and coworkers used fluorescein-labeled Annexin-V to identify apoptotic cells in vitro.[33] Recently, a novel real-time imaging model has been reported that visualizes apoptotic membrane changes of single cardiomyocytes in the injured heart of a living mouse by using fluorescent labeled Annexin-V.[34] Others have been used Annexin-V labeled with radionuclide agent to image apoptotic cell death in vivo.[35] In the clinical practice, ^{99m}Tc-labeled Annexin-V is now commercially available for imaging cardiac apoptosis in vivo (Thesus Imaging, Inc.). In patients with myocardial infarction undergoing reperfusion therapy, the gamma camera delayed images clearly showed an intense accumulation of the tracer at the site of infarct, indicating presence of externalized PS on the cardiomyocyte membrane. Compared with ^{99m}Tc-sestamibi, Annexin-V uptake can be seen in a smaller defect.

Recently, ^{99m}Tc-labeled Annexin-V has been used to track the heart transplant rejection process. In fact, apoptosis has been noninvasively identified in an animal model of heart transplant rejection[36] and allograft rejection in rat liver transplantation. Others have investigated the clinical role of imaging with Annexin-V for the detection of apoptosis in cardiac allograft recipients.[37] These studies have demonstrated the usefulness of radionuclide imaging with Annexin-V in human subjects who have undergone heart transplantation. Endomyocardial biopsies were performed within the first 4 days after imaging to confirm imaging data by histology. Patients with focal ^{99m}Tc-Annexin-V uptake had histologically verified transplant rejection (grade $\geq$2). The advantage of the proposed noninvasive approach is that if such correlation between biopsies and imaging results were to be confirmed in larger studies, Annexin-V imaging could help obviate the need for highly invasive endomyocardial biopsies.

Apoptosis imaging would also be useful in various other disease states, both cardiac and noncardiac, that are characterized by increased or decreased apoptosis. Moreover, the monitoring the efficacy of interventions directed toward induction of apoptosis is possible.

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