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Targeted Molecular Imaging of Angiogenesis in PET and SPECT: A Review

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Over the past few decades, there have been significant advancements in the imaging techniques of positron emission tomography (PET†) and single photon emission tomography (SPECT). These changes have allowed for the targeted imaging of cellular processes and the development of hybrid imaging systems (e.g., SPECT/CT and PET/CT), which provide both functional and structural images of biological systems. One area that has garnered particular attention is angiogenesis as it relates to ischemic heart disease and limb ischemia. Though the aforementioned techniques have benefits and consequences, they enable scientists and clinicians to identify regions that are vulnerable to or have been exposed to ischemic injury via non-invasive means. This literature review highlights the advancements in molecular imaging techniques and specific probes as they pertain to the process of angiogenesis in cardiovascular disease.

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†Abbreviations: PET, positron emission tomography; SPECT, single photon emission tomography; CT, computed tomography; MRI, magnetic resonance imaging; VEGF, vascular endothelial growth factor; SMC, smooth muscle cells; HIF-1, hypoxia-inducible factor 1; FGF-2, fibroblast growth factor-2; TGF-β, transforming growth factor beta; PDGF, platelet-derived growth factor; VEGFR, vascular endothelial growth factor; MI, myocardial infarction; ^{18}F-FDG MR, fluorodeoxyglucose; eNOS, endothelial nitric oxide synthase; IGF-1, insulin-like growth factor; RGD, arginine-glycine-aspartate.

Keywords: molecular imaging, angiogenesis, PET, SPECT, CT, myocardial ischemia, hind limb ischemia

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INTRODUCTION

Along with other imaging modalities such as ultrasound and magnetic resonance imaging (MRI), positron emission tomography (PET), and single photon emission tomography (SPECT), imaging techniques have continued to evolve over the last 30 years, advancing the assessment of cardiovascular diseases. These imaging modalities each have advantages and disadvantages. Ultrasound has traditionally been the most widely available and relatively inexpensive means for imaging of the cardiovascular system without exposure to radiation; however, this modality has limited penetration depth and does not have molecular probes available that permit interrogation beyond the intravascular compartment. MRI is another imaging method that does not expose patients to radiation, although it is much more expensive. This modality has good spatial resolution and tissue penetration, but much lower sensitivity for targeted imaging than nuclear imaging techniques such as PET and SPECT. Additionally, MRI has a susceptibility to motion artifacts and currently does not have the availability of as many molecular probes as PET and SPECT. PET and SPECT imaging systems possess superior sensitivity, and there is availability of a wide range of imaging probes for *in vivo* analysis of cellular processes. However, a disadvantage associated with both of these modalities is their lower resolution and exposure of patients to ionizing radiation [1,2].

PET and SPECT have been the traditional nuclear modalities used for *in vivo* imaging of molecular and cellular processes [1-4]. More recent developments in nuclear imaging in the last decade have seen the emergence of hybrid imaging systems that also incorporate the use of X-ray computed tomography (CT) with both PET and SPECT, providing the co-localization of both functional and anatomical information for a wide range of clinical applications. These hybrid PET/CT and SPECT/CT imaging systems offer unique insight into critical cellular processes contributing to the development of a variety of cardiovascular disease states, as multiple radiolabeled probes are available that specifically target a variety of molecular and biological processes of interest [5-8]. Additionally, the creation of hybrid imaging systems has facilitated the combination of high sensitivity radiotracer-based imaging with high resolution CT imaging, allowing for co-localization of function images with anatomical images. These hybrid systems permit attenuation correction, minimizing attenuation artifacts from soft tissue, and correction of partial volume effects, ultimately resulting in enhanced quantification of radiotracers. These imaging approaches ultimately will result in better individualized health care as physicians more accurately quantify molecular signals within specific anatomical structures of interest. Additionally, the increased availability of microPET and microSPECT imaging systems should allow for enhanced translational research from small animal preclinical models of cardiovascular disease into the clinical environment [9-13].

Angiogenesis, or the growth of new capillaries from existing microvessels, is a specific process that can occur following ischemic injury and thus has gained attention as a critical target within the cardiovascular imaging community in recent years [1,2,7,8,14]. The purpose of this review is to focus on specific molecular imaging targets directed at the process of angiogenesis in cardiovascular disease. A PubMed search of "imaging, angiogenesis" returned nearly 3,000 results, with articles being selected based on relevance to the topic at hand (i.e., PET and SPECT imaging).

MOLECULAR IMAGING

Molecular imaging, in general, is defined as the *in vivo*-targeted imaging of biological processes. This imaging approach incorporates molecular probes that localize to specific molecular events associated with physiological or pathological processes [2,3]. The magnitude of these events occurring within biological systems is assessed based upon the magnitude of probe uptake within the region of interest. The high sensitivity of nuclear imaging systems contributes to the

identification of probe uptake, while high resolution information from X-ray CT contributes important information related to the anatomical localization of probe. Successful molecular imaging relies on multiple factors, such as the availability of the probe that is specific and sensitive to the molecular process being examined, as well as the appropriate instrumentation to allow for desirable visualization and quantification of probe uptake [1]. The application of molecular imaging already has proven to be valuable for those within the oncology community by assisting with early detection and intervention strategies [15]. Further development of the molecular imaging approach should continue to improve disease management for additional disease states by not only early identification and detection in vulnerable patient populations, but also by facilitating the direction of pharmacological, cell-based, and genetic therapeutic regimens in the future. One field of study within the molecular imaging community that continues to evolve is targeted imaging of angiogenesis, a process that plays a critical role both in tumor biology and the repair following ischemic injury.

ANGIOGENESIS

Angiogenesis is generally defined as the development of new capillaries from pre-existing microvessels. This complex, multistep process involves a variety of cells, along with both stimulatory and inhibitory factors [16]. Commonly, differentiation of angioblasts into endothelial cells leads to endothelial cell sprouting [17]. Growth factors serve to activate pre-existing endothelial cell receptors, leading to the release of proteases and regulating the degradation of the basement membrane, ultimately allowing for the release of endothelial cells from parent vessels. Endothelial cell sprouting occurs as endothelial cell proliferation and migration into the extracellular matrix takes place with the help of adhesion molecules and integrins. These sprouting endothelial cells develop into new vessels with the addition of pericytes or vascular smooth muscle cells (VSMCs), which serve to stabilize the developing vessel and regulate blood flow. In addition to sprouting angiogenesis, splitting angiogenesis, or intussusception, also exists. In this form of angiogenesis, single vessels are split into two as the capillary wall extends into the vessel lumen. Reorganization of existing cells ultimately results in the formation of an additional vessel lumen, which splits from the parent vessel [16-19].

Several conditions known to stimulate the angiogenic process include ischemia, hypoxia, inflammation, shear stress, and traumatic injury [17]. Previous research has largely focused on imaging of angiogenesis within tumors [20-22], as well as skeletal [9,23-25] and cardiac muscle [26-29] exposed to surgically induced ischemia. The addition of recently developed vessels within these tissues has important clinical implications, as they can ultimately lead to increased perfusion to injured or ischemic tissues [30].

A variety of factors have been found to contribute to the process of angiogenesis following hypoxia-induced conditions, including hypoxia-inducible factor 1 (HIF-1), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), transforming growth factor beta (TGF-β), and angiopoietins (interacting with their Tie receptors) [31-34]. Through the actions of these angiogenic-induced factors, a number of other important mediators are then signaled, including endothelial cells, SMCs, blood-derived macrophages, and circulating stem cells [35]. Although various angiogenesis-stimulating factors exist, VEGF is considered the most potent and predominant factor [18,19]. VEGF ligands, of which there are four known isoforms, mediate their angiogenic effects by binding to specific VEGF receptors (VEGFR-1, VEGFR-2, and VEGFR-3), leading to receptor dimerization and subsequent intracellular signal transduction via tyrosine kinases [20]. In addition to the previously mentioned angiogenic factors, integrins also have been implicated in a number of processes related to angiogenesis, including cell adhesion, migration, proliferation, differentiation, and survival [36].

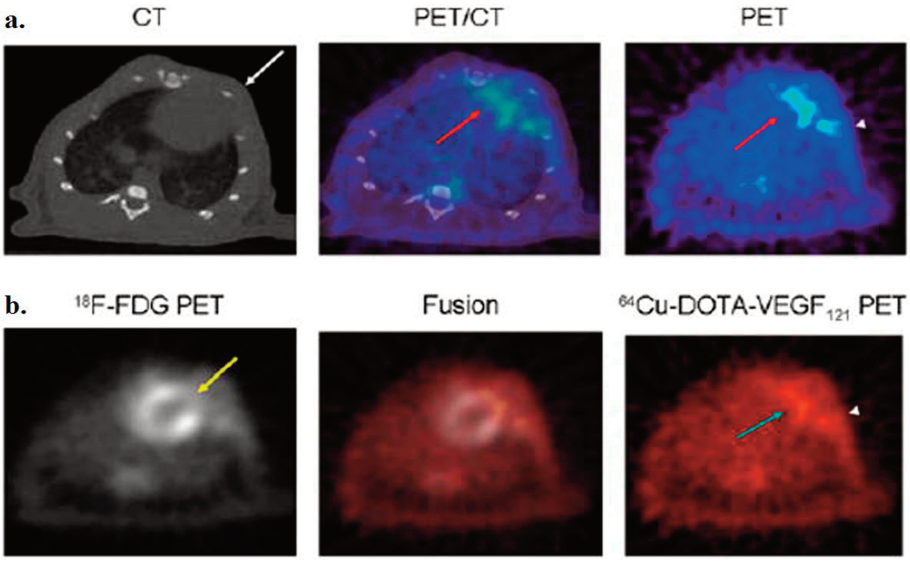

Figure 1. Myocardial origin of ^{64}Cu-DOTA-VEGF$_{121}$ PET signal in a rat model of MI. a) Coregistered images of microCT (left), PET (right), and fused PET/CT image (center) within infarcted region of heart demonstrates that the ^{64}Cu-DOTA-VEGF$_{121}$ signal detected with PET corresponds to anterolateral myocardium (PET and fused images, red arrow) and is clearly separated from intercostal muscle layer (microCT image, white arrow). There is also increased uptake in the surgical wound area (PET image, arrowhead). **b)** Representative images of ^{64}Cu-DOTA-VEGF$_{121}$ (left), ^{18}F-FDG (right), and ^{64}Cu-DOTA-VEGF$_{121}$/^{18}F-FDG fused image (middle). ^{18}F-FDG imaging shows that surgical ligation of the coronary artery resulted in lack of ^{18}F-FDG uptake (yellow arrow) and that uptake of ^{64}Cu-DOTA-VEGF$_{121}$ occurs in areas supplied by ligated coronary artery (turquoise arrow). The fusion of both images results in complementation of ^{18}F-FDG and ^{64}Cu-DOTA-VEGF$_{121}$ signals. There is also increased uptake in the surgical wound area (arrowhead). (reprinted with permission of [13])

Specifically, the αvβ3 integrin, a heterodimeric cell surface receptor, plays a significant role in angiogenesis by allowing cells to interact with the extracellular matrix, contributing to the migration of endothelial cells. Because of their important roles in angiogenesis, the αvβ3 integrin and VEGF have been targeted for multiple molecular imaging studies and remain as a focus of interest [9,24,26,27,29,37].

MOLECULAR IMAGING OF ANGIOGENESIS

Angiogenesis imaging can be categorized as targeted at three major cell types: 1) non-endothelial cell targets (e.g., monocytes, macrophages, and stem cells); 2) endothelial cell targets (e.g., VEGF, growth factor receptors, integrins, CD13, cell adhesion molecules); and 3) extracellular matrix proteins and matrix proteases [1,38]. Recent techniques in nuclear medicine also have focused on the development of tracers targeting the ED-B domain of a fibronectin isoform, as fibronectin plays an important role in the binding of integrins and extracellular matrix components during the process of angiogenesis [39]. Additionally, targeted imaging of stem cell therapy is another emerging technique, which has been performed in MRI [40,41], SPECT [42], and PET [43-45]. The growth of this field should further enhance future angiogenesis cell-based therapy; however, many of the agents used in nuclear imaging for this purpose are not well studied and warrant further investigation. The use of the contrast agents in MRI for targeted imaging of angiogenesis is also gaining interest, as they have been shown to permeate the walls of angiogenic capillaries and assist in targeted imaging of integrins within areas of vascular growth [21,46-48]. Targeted CT contrast agents may

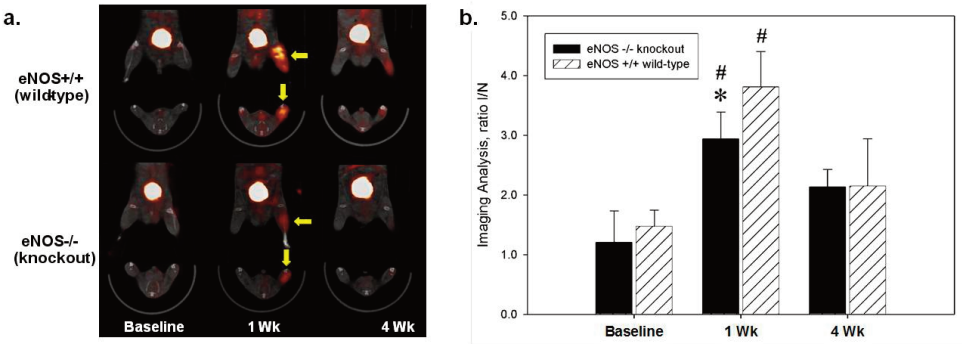

Figure 2. Analysis of wild-type and eNOS-knockout mice. a) Micro-SPECT/CT of mice injected with ^{99m}Tc-NC100692 following right femoral artery ligation. Yellow arrows indicate ischemic regions with increased ^{99m}Tc-NC100692 retention. **b)** Serial images were analyzed and ischemic-to-nonischemic ^{99m}Tc-NC100692 activity ratios calculated. Solid bars represent eNOS -/- knockout. Striped bars represent eNOS +/+ wild-type.
*P < 0.05 vs. wild-type. #P <0.05 vs. baseline. (reprinted with permission of [9]).

offer a similar approach that could be combined with targeted radiotracers using hybrid PET/CT and SPECT/CT.

There has been significant focus on targeted imaging of the αvβ3 integrin with ligand-like peptidomimetics or the RGD (arginine-glycine-aspartate) peptide sequence [9,12,24,26,27,29,49-52]. The RGD binding motif for integrins has led to the development of multiple molecular probes that have advanced the *in vivo* imaging of angiogenesis. Various improvements in integrin-targeted constructs also have increased the optimization of pharmacokinetics and binding characteristics. More recent developments in nanoparticle technology are enhancing the ability to deliver various imaging agents to targeted regions of interest, ultimately leading to improved sensitivity and image quality [1]. This review will focus on more established methods for targeted imaging of the angiogenic process. Most notably, this paper will detail the altered expression of VEGF receptors and αvβ3 integrins.

VEGF RECEPTORS

VEGF has been identified as an important stimulator of the angiogenic process [19]. Because of this, multiple SPECT and PET molecular probes have been developed for targeted imaging of VEGF receptors in tumor, peripheral limb, and myocardial angiogenesis (Table 1). Specifically, many studies have focused on targeted imaging of angiogenesis in animal models of induced ischemia [9,23,24,26,27,29,53]. Human monoclonal anti-VEGF antibodies labeled with radioiodine (^{124}I and ^{123}I) were examined in initial studies of VEGF using PET and SPECT. Unfortunately, the preclinical applications of these studies were limited by slower than usual clearance rates of these antibodies [54,55]. To address this shortcoming, new molecular probes were created. This advancement targeting ligands served as the foundation for more efficient investigation and monitoring of the upregulation of VEGF during angiogenesis. $VEGF_{165}$ and $VEGF_{121}$ were designed for this purpose and implemented in early imaging studies of tumor angiogenesis. These isoforms proved to be successful in patients when radiolabeled with ^{123}I [55].

In addition to targeted imaging of tumor angiogenesis, peripheral angiogenesis also has been examined in ischemia-induced animal models [9,23,24,53]. A murine model of hind limb ischemia-induced angiogenesis has revealed that ^{64}Cu-6DOTA-$VEGF_{121}$ is effective in PET imaging of VEGFR-2 [23]. $VEGF_{121}$ labeled with ^{111}In has also been developed as a targeting ligand for SPECT and was successfully used to image peripheral angiogenesis in a rabbit model of hind limb ischemia [53]. Analysis with immunohistochemistry revealed an increased expression of the VEGF receptors KDR (VEGFR-2)

Table 1. A Summary of Molecular Probes used for Non-invasive Imaging of Angiogenesis.

Marker	Probe	Imaging Modality	Biologic Target
VEGF	^{124}I-VG76e	PET	Tumor angiogenesis
VEGF	^{123}I-VEGF$_{165}$	SPECT	Tumor angiogenesis
VEGF	^{111}In-VEGF$_{121}$	SPECT	Peripheral limb angiogenesis
VEGF	^{64}Cu-VEGF$_{121}$	PET	Tumor angiogenesis
VEGF	^{64}Cu-VEGF$_{121}$	PET	Myocardial angiogenesis
VEGF	^{99m}Tc-scVEGF	SPECT	Peripheral limb angiogenesis
VEGF	^{64}Cu-scVEGF	PET	Tumor angiogenesis
αvβ3	^{111}In-RP748	SPECT	Tumor angiogenesis
αvβ3	^{111}In-RP748	SPECT	Myocardial angiogenesis
αvβ3	^{18}F-AH111585	PET	Tumor angiogenesis

and Flt-1 (VEGFR-1) within skeletal muscle exposed to hypoxia post-femoral artery excision. However, substantially larger biodistribution of the radiotracer (approximately 20-fold higher) in multiple organs systems (liver, kidneys) was evident when compared to the ischemic limb. Possible explanations for this disparity may be associated with VEGF receptor or macrophage density within different regions or may closely correspond to particle size or coating [2,56,57].

A rat model of myocardial infarction (MI) also has revealed enhanced angiogenesis through targeted PET imaging with the tracer ^{64}Cu-6DOTA-VEGF$_{121}$ (Figure 1) [13]. Following left coronary artery ligation, hearts were imaged with microSPECT and microCT at various time points to examine early angiogenesis in ischemic territories of the infarcted heart. Imaging demonstrated that tracer signal was increased within the infarcted region, which was indicated by decreased fluorodeoxyglucose (^{18}F-FDG) uptake, an established marker of cellular viability. The increased signal of ^{64}Cu-6DOTA-VEGF$_{121}$ peaked at three days post-MI and corresponded to post-mortem tissue analysis of VEGF receptor expression, as indicated by immunoflourescence microscopy. In addition to targeted imaging of angiogenesis post-MI, the same VEGF$_{121}$ isoform has been successfully used to image tumor angiogenesis in a mouse model in PET [58] and to develop single-chain technetium-labeled VEGF-based probes that are capable of assessing tumor associated angiogenic vasculature in PET and SPECT [59,60].

The development of a cardiac-specific reporter in microPET imaging of rats may offer insight into an additional therapeutic route via a gene expression system [11,61-63]. This system targets specific sites of the cardiovascular system for the delivery of angiogenic factors, therapeutic gene vectors, and stem cells, with the ultimate goal of stimulating angiogenesis within the region of interest. A previous study has used a similar type of gene technology to identify the expression of VEGF$_{121}$ in porcine myocardium with PET-CT following adenoviral transfer [61]. Ultimately, this system could lead to targeted therapeutic interventions while also incorporating an established imaging modality for clinical assessment.

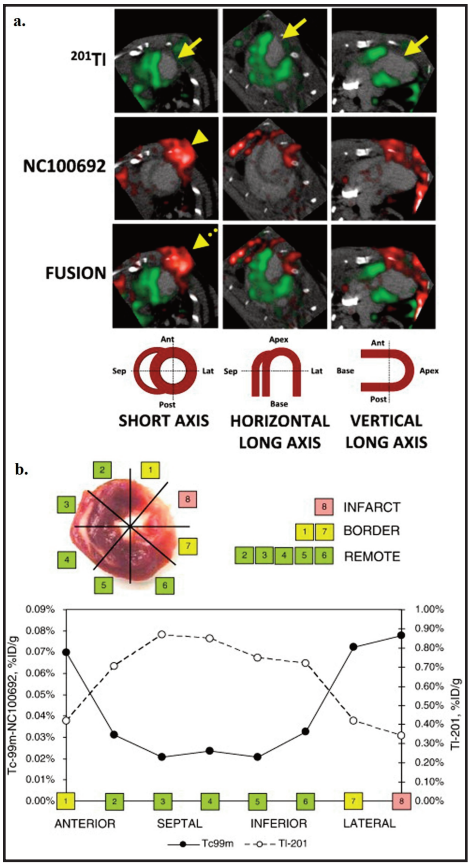

Figure 3. *In vivo* microSPECT-CT imaging and radiotracer quantification post-myocardial infarction. a) Thallium-201 perfusion (top row, green) and αvβ3 integrin targeted imaging (middle row, red) with SPECT-CT in an IGF-1 treated rat at 4 weeks post-MI. Bottom row represents fused image with reference contrast CT image (grayscale). **b)** Gamma count profiles of thallium-201 (open circles) and ^{99m}Tc-NC100692 (solid circles) from middle myocardial sections. (reprinted with permission of [29])

αVβ3 INTEGRIN

The αvβ3 integrin is found in abundance on the surface of proliferating endothelial cells and is a consistent and specific marker of ongoing angiogenesis [4]. For this reason, targeted molecular imaging of the αvβ3 integrin presents a novel noninvasive approach for the assessment of angiogenesis. Early research focused on MRI of the αvβ3 integrin with a paramagnetic-labeled monoclonal antibody to examine angiogenesis in a rabbit model of squamous cell carcinoma [21]. However, poor clearance of this antibody limited its translation into future studies. Since then, development of RGD peptides with high affinities for the αvβ3 integrin has led to the advancement of a variety of radiotracers suitable for the assessment of angiogenesis in PET and SPECT imaging (Table 1) [50,64,65]. Additionally, the peptidomimetic ^{111}In-RP748 has shown a high affinity to the αvβ3 integrin and has been successfully incorporated in multiple studies [26,28]. Specifically, ^{111}In-RP748 has shown increased levels of uptake within localized regions of decreased perfusion in the myocardium of rodent and canine models of myocardial infarction, as assessed by SPECT imaging [26,28]. In these studies, imaging with ^{111}In-RP748 confirmed that increased αvβ3 integrin activity was present within the myocardium at both early (acute) and late (3 weeks) time points post-infarction [26,28]. Given these results, targeted imaging of integrin activation appears to provide a useful technique that can be translated to the clinical setting for the detection of angiogenesis post-myocardial infarction. In support of this argument, the PET imaging tracer ^{18}F-Galakto-RGD targeted at integrin activation previously has been successfully used to image angiogenesis in a patient 2 weeks post-myocardial infarction [51].

^{99m}Tc-NC100692 (maraciclatide®) is a technetium-labeled cyclic RGD peptide that has been used in a variety of SPECT studies to noninvasively assess angiogenesis [2]. The NC100692 compound has a high affinity for the αvβ3 integrin, is metabolically stable, and has a biodistribution and kinetics that are favorable for SPECT imaging. Increased focal activity of ^{99m}Tc-NC100692 has been demonstrated with SPECT at 3 and 7 days post-femoral artery ligation in a murine model of hind limb ischemia [24]. These results were further confirmed by the close correlation of *ex vivo* tissue analysis (gamma counting) and immunofluorescence staining. A similar study using a murine model of hind limb ischemia examined peripheral angiogenesis in wild-type and en-

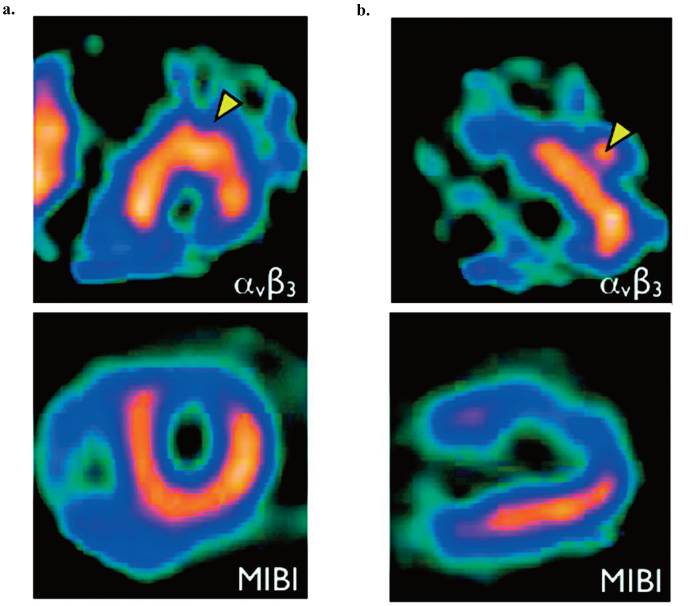

Figure 4. Targeted imaging of angiogenesis with SPECT in a patient 3 weeks post-myocardial infarction. Short **(a)** and long-axis **(b)** views of enhanced αvβ3 integrin signal (arrowheads) within infracted region, with corresponding ^{99m}Tc-MIBI perfusion images. Images courtesy of Drs. Johan Verjans and Leonard Hofstra, University Hospital, Maastricht, the Netherlands. (reprinted with permission of [66])

dothelial nitric oxide synthase (eNOS) deficient animals with microSPECT-CT (Figure 2) [9]. This study, directed at serial quantitative evaluation of angiogenesis, revealed decreased uptake of the compound within ischemic regions of the eNOS deficient mice when compared to wild-type. Both groups, however, exhibited the largest retention of ^{99m}Tc-NC100692 at 7 days post-femoral artery occlusion, as indicated by *ex vivo* gamma counting of ischemic tissue. Other studies using microSPECT-CT have confirmed the uptake of ^{99m}Tc-NC100692 within mice [12] and rats [29,52] following surgically induced myocardial infarction. Dobrucki et al. used ^{99m}Tc-NC100692 microSPECT imaging to evaluate the effects of gene therapy directed at stimulating angiogenesis. In this study, focal uptake of ^{99m}Tc-NC100692 was seen in the peri-infarct region in association with accelerated angiogenesis induced by intramyocardial injection of a viral vector to upregulate expression of insulin-like growth factor (IGF-1) in rats, with further validation provided via gamma counting of heart tissue (Figure 3) [29]. The clinical translation of cardiovascular SPECT imaging with ^{99m}Tc-NC100692 was recently shown (Figure 4) and demonstrated the feasibility and applicability of this targeted imaging approach for broad scale clinical application in ischemic heart disease [66].

The development of an αvβ3 integrin targeted nanoprobe also provides a new technique for the detection of *in vivo* angiogenesis with PET [67]. The nanoprobe allows for labeling with isotopes while also having a shell that is decorated with an RGD peptides to confer specificity to the αvβ3 integrin [4]. This work has demonstrated a more favorable biodistribution of the targeted nanoprobe and retained specificity to regions of ischemia when applied in a murine model of hind limb ischemia (Figure 5) [67]. *Ex vivo* analysis by imaging and histology has confirmed the effectiveness of this nanoprobe for assessment of angiogenesis. This nanoprobe also may allow for targeted drug delivery. Further development and application of this nanotechnology to angiogenesis imaging will offer a promising new approach with the potential for translation of novel theranostics into clinical practice.

CONCLUSIONS

In summary, PET and SPECT imaging can provide molecular targeted approaches for the early *in vivo* assessment of biological processes that precede the physiological or anatomical manifestation of a disease. Research has shown that these molecular imaging approaches offer unique opportunities

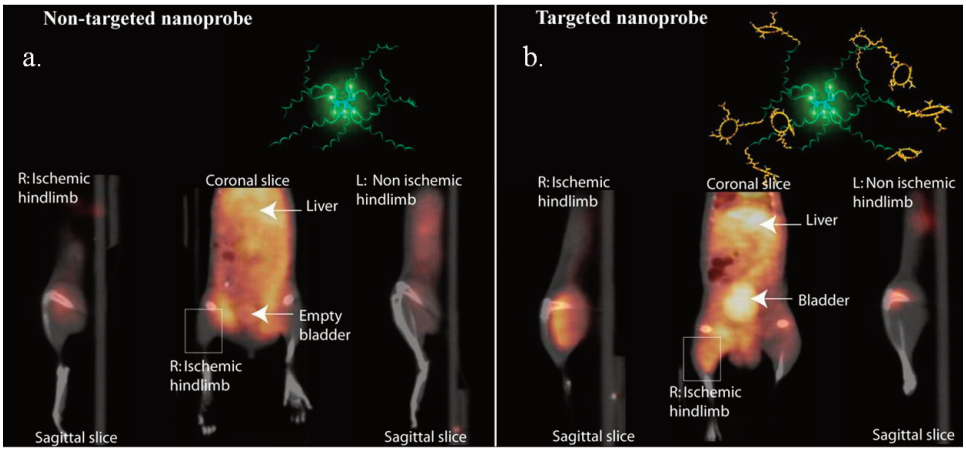

Figure 5. PET/CT imaging of angiogenesis in a murine model of hindlimb ischemia. a) Non-targeted dendritic nanoprobes (bottom center). **b)** Higher uptake of αvβ3 -targeted dendritic nanoprobes in ischemic hindlimb (left side of image) than in control hindlimb (right side of image). (reprinted with permission of [67])

for evaluation of the processes that regulate cardiovascular disease pathogenesis, progression, and therapeutic intervention. Additionally, these imaging modalities have provided methods for the noninvasive assessment of angiogenesis during instances of myocardial and limb ischemia. The development of novel molecular probes and hybrid imaging systems continue to increase the potential for translational research from small and large animal studies into clinical practice. The high sensitivity of PET and SPECT images now can be co-localized with high resolution X-ray CT anatomical images to better identify and quantify radiotracer uptake within vulnerable regions of the heart and vasculature. Although these systems have expanded the possibility for application of molecular imaging into the clinical setting, these systems still need to be optimized and corrected for complicating cardiac and respiratory motion. The potential for additional exposure to radiation from CT imaging is also a cause for concern with these hybrid imaging systems. Despite these imperfections, molecular imaging with new hybrid imaging technology offers a promising, unique approach for translating targeted imaging of biological processes within the cardiovascular system to humans. Pre-clinical animal studies investigating ischemic tissues might offer insight into the future of treatment and targeted imaging. Many clinical trials, to this point in time, have relied on clinical endpoints or the evaluation of the indirect effects of therapy on physiological indices like tissue perfusion and function. The continuing development of more sensitive, noninvasive imaging modalities such as PET and SPECT may someday confirm the delivery of therapeutic angiogenic agents and directly monitor progression of angiogenesis within targeted tissues, providing novel methods for optimizing therapeutic interventions in patients suffering from various forms of cardiovascular disease.

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