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Review

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성균관대학교 의과대학 삼성서울병원 핵의학과
이경한

Cardiovascular Molecular Imaging

Kyung-Han Lee, M.D.
Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Molecular imaging strives to visualize processes in living subjects at the molecular level. Monitoring biochemical processes at this level will allow us to directly track biological processes and signaling events that lead to pathophysiological abnormalities, and help make personalized medicine a reality by allowing evaluation of therapeutic efficacies on an individual basis. Although most molecular imaging techniques emerged from the field of oncology, they have now gradually gained acceptance by the cardiovascular community. Hence, the availability of dedicated high-resolution small animal imaging systems and specific targeting imaging probes is now enhancing our understanding of cardiovascular diseases and expediting the development of newer therapies. Examples include imaging approaches to evaluate and track the progress of recent genetic and cellular therapies for treatment of myocardial ischemia. Other areas include in vivo monitoring of such key molecular processes as angiogenesis and apoptosis. Cardiovascular molecular imaging is already an important research tool in preclinical experiments. The challenge that lies ahead is to implement these techniques into the clinics so that they may help fulfill the promise of molecular therapies and personalized medicine, as well as to resolve disappointments and controversies surrounding the field. (Nucl Med Mol Imaging 2009;43(3):229-239)

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Traditional nuclear cardiology imaging evaluates physiological consequences of disease by relying on changes of blood flow, function, or metabolism. In recent years, the use of targeted markers of key biological processes has become possible owing to advances in molecular biology, genomics and proteomics. In accordance, medical imaging is evolving into a paradigm that noninvasively monitors key molecular biomarkers through imaging probes that specifically interact with important surface epitopes, receptors, enzymes and transporter targets. Some of these approaches are already in use for clinical imaging of cardiovascular disease, including investigation of glycolytic metabolism of hibernating myocardial cells using ^{18}F-FDG PET or sympathetic innervation within the heart using ^{123}I-MIBG SPECT. Others techniques still in developmental or preclinical validation stages include novel approaches for imaging heart failure, thrombosis, apoptosis, atherosclerosis, angiogenesis, and cardiac gene or cell therapies.

This review describes the current status of cardiovascular molecular imaging approaches, and briefly touches upon animal models that are used to validate these new techniques and interrogate disease processes. Together with specific imaging probes and dedicated small animal imaging devices, animal models complete the triad of tools required for molecular imaging experiments. Imaging probes of favorable in vivo kinetics that target molecules of interest with high specificity and affinity are a crucial component for the success of a molecular imaging strategy. Dedicated imaging instruments designed

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• Address for reprints: Kyun-Han Lee, M.D., Departement of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwondong, Kangnamgu, Seoul 135-710, Korea
Tel: 82-2-3410-2630, Fax: 82-2-3410-2639
E-mail: khnm@samsung.com
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for small animals including micro-MRI, micro-CT, micro-PET, and optical imagers are essential to achieve sufficient image resolution in rodents. Finally, the combined effort of researchers from many fields including radiochemistry, analytical chemistry, molecular biology, nanotechnology, animal facility, and imaging sciences is required. Such a multidisciplinary approach will provide the media to facilitate the advancement of cardiac molecular imaging and foster its entrance into clinical application.

Imaging Myocardial Gene Expression

Cardiac gene therapy is an exciting strategy to express therapeutic proteins in the heart via transfer of exogenous DNA. Therapeutic effects of gene transfer has been shown in preclinical studies for promoting angiogenesis in coronary artery disease with vascular endothelial growth factor, fibroblast growth factor, and hypoxia-inducible factor-1 genes, improvement of heart failure with the *SERCA2a gene*, and reduction of ischemia-induced cell death with anti-apoptosis genes. Such encouraging results have led to clinical trials in cardiovascular disease, most of which are currently aimed at testing the safety and efficacy of therapeutic angiogenesis. Unfortunately, many of these trials failed to show consistent results, leaving us with the important lesson that interaction of complex factors in living tissue calls for more sensitive methods to monitor treatment efficacy of gene therapy. Without methods to assess gene expression in vivo, investigators would unable to determine whether poor symptom improvement is due to inadequacy of therapeutic protein or the result of technical problems including poor gene delivery, inefficient gene transfer, and insufficient magnitude or inadequate duration of gene expression. Thus, molecular imaging research can aid in selecting the ideal delivery vector and injection method, and defining pharmacokinetics and pharmacodynamics of transgene expression in the heart.[1)]

Requirements for cardiac gene imaging research include a vector construct that contains your imaging reporter gene, a cognate imaging probe, and an animal model plus a method for target organ gene delivery. Transcription of the reporter gene should produce a protein that interacts with and leads to accumulation of signals from reporter imaging probes. This could be an enzyme that converts a substrate-probe to a metabolite that is trapped, a receptor that binds a specific ligand-probe, or a transporter that mediates intracellular delivery and accumulation of a substrate-probe. Fluorescent reporter proteins such as green fluorescent protein (GFP), although they are generally not applicable for large subjects, are special in that imaging signals are produced without addition of any probe. Enzyme- and transporter-based reporter systems have the advantage of signal amplification via progressive probe accumulation in target cells. Frequently used examples include the herpes simplex virus-1 thymidine kinase (HSV-tk1) gene that phosphorylates and traps radiofluoride- or radioiodide-labeled thymidine analogues, luciferase genes that interact with separately administered substrates (such as D-luciferin) to produce light signals, and the sodium iodide symporter (NIS) gene that transports and accumulates free radioiodide or radiotechnetium (Fig. 1A & 1B). While some genes including GFP and luciferase cDNA are commercially available, others such as HSV-tk1 and NIS cDNA can be obtained through collaboration or by donation from other colleagues and laboratories. These genes are usually contained in plasmid vectors that allow amplification in E. coli and transduction into mammalian cells via liposome-based transfection agents. Sometimes, the genes are obtained in the form of adenovirus, adeno-associated virus, or lentivirus vectors, which offers high efficiency transfection into target cells and tissues. The vector genes are generally controlled by constitutively active viral promoters, virtually ensuring consistent expression at all situations. Therefore, if the experiment requires transcription regulation of the reporter gene by inducible or tissue specific promoters, one would have to perform DNA cloning.

Animal models for cardiac gene imaging may include heart disease models, but most studies to date have used normal rats for proof-of-principle experiments. Cardiac gene delivery is usually accomplished by thoracostomy and direct vector injection into the left ventricular wall of an artificially ventilated anesthesized animal. Alterna-

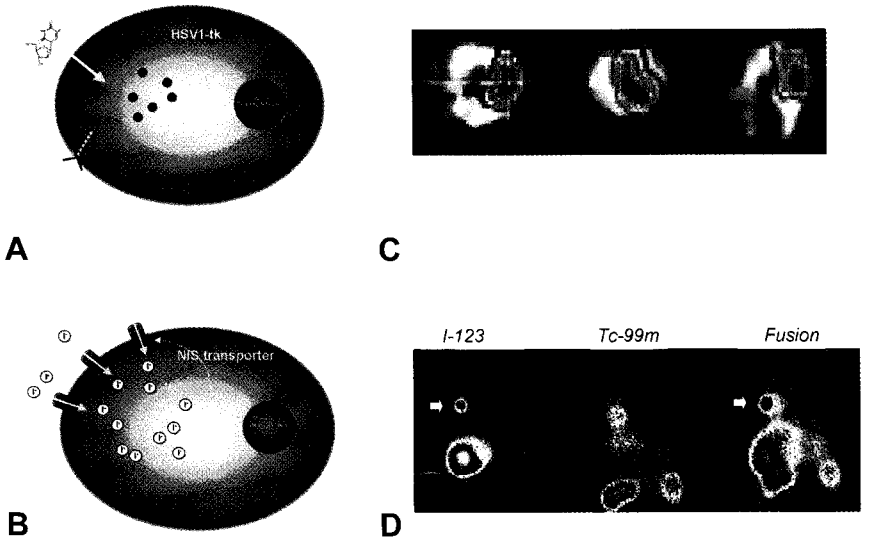

Figure 1. This shows cardiac reporter gene imaging with schemes for reporter imaging using (A) HSV1-thymidine kinase (HSV1-tk) and (B) sodium iodide symporter (NIS) genes, (C) (^{18}F)FPBG micro-PET images of a rat heart injected with an adenovirus expressing HSV1-tk. (^{18}F)FPBG activity expressed in color is overlayed on (13N)ammonia perfusion images (reprinted with permission from ref. 3), and (D) ^{123}I planar scintigraphy of a rat heart injected with an adenovirus expressing NIS. Shown from left to right are ^{123}I images, ^{201}Tl perfusion, and fused images. (Reprinted with permission from Lee et al.[4])

tively, retrograde coronary sinus infusion can be attempted by injection into the left ventricular chamber while briefly clamping the ascending aorta, a method that is more technically demanding but achieves global distribution of gene expression within the myocardia. Other methods include pericardial injection, and for larger animals, intracoronary infusion. After closing of the chest wall, the animals are recovered and housed until imaging studies. Sufficient transcription levels of the reporter protein are usually reached after 3 to 5 days. For DNA sequences that remain episomal in the nucleus, expression levels then gradually decrease with time and become undetectable after a few weeks.

The first report on cardiac gene imaging was by Wu and coworkers using normal rats intra-myocardially injected with an adenovirus expressing firefly luciferase. Optical imaging after administration of the substrate D-luciferin resulted in cardiac activity that peaked by day 5 then decreased over time. In vitro luciferase activity correlated with in vivo imaging signals, demonstrating for the first time the feasibility of in vivo imaging of cardiac reporter gene expression.[2] This was shortly followed by a report of PET imaging of rat heart injected with an adenovirus carrying the HSV1-sr39tk reporter gene. [^{18}F]-FHBG imaging displayed significant uptake in the cardiac wall, and ex-vivo counting showed a 20-fold increase of radioactivity consistent with a 22-fold increase of thymidine kinase enzyme activity (Fig. 1C).[3] Our group has shown that use of NIS and radioiodide is also a promising approach for cardiac gene imaging. The technique was highly sensitive and allowed reliable measurements of gene expression (Fig. 1D).[4] Furthermore, it could be applied safely without causing adverse effects on cardiac function.[5] Cardiac reporter imaging has also been linked to therapeutic gene expression. When rat cardiomyoblasts transduced with the VEGF gene linked to the HSV1-sr39tk gene were injected into the infarcted rat myocardia, [^{18}F]-FHBG PET showed cardiac activity that peak at day 1 and declined over 2 weeks. Image results correlated well with enzyme activity and VEGF level, demonstrating the feasibility of reporter imaging for quantifying therapeutic gene expression levels.[6]

Future cardiac gene imaging research may include such areas as the use of cardiac tissue specific promoters to diminish unwanted extracardiac activity, validation of prolonged transgene expression by less immunogenic

vectors, monitoring of codelivered therapeutic genes with stem cells, and multimodality imaging approaches to monitor the spatial and temporal pattern of gene expressions simultaneously with its downstream effects.

Imaging Cardiac Cell Therapy

Cell therapy is emerging as a potential strategy to heal failed hearts. Patients with myocardial infarction, for instance, can have significant tissue loss that leads to hemodynamic decompensation and progressive refractory heart failure. Following the first report that bone marrow-derived hematopoietic stem cells can transdifferentiate into cardiomyocytes in ischemia-injured hearts of mice, several clinical trials have been initiated to investigate the effect of transplanting various therapeutic cells into myocardial infarction patients. Transplantation of progenitor and bone marrow stem cells have been associated with improvements in ejection fraction and wall motion, and reduced ventricular volume. While several hypotheses are proposed as mechanisms for these improvements, the main limitation of cardiac cell transplantation is the lack of available methods to assess cell survival. Imaging changes in myocardial function, viability, or perfusion by conventional modalites do not provide actual visualization of transplanted cells. This is an important distinction because transplanted cell-mediated improvement is difficult to discern from concurrently performed coronary interventions. Postmortem histologic examination can analyze cell survival in animals, but is invasive and precludes longitudinal monitoring. Thus, the ability to image therapeutic cells in vivo can offer better insights into the underlying biology and physiology of therapeutic cells transplanted into the heart.[7)]

To perform cell transplantation imaging experiments, one needs to have cultured therapeutic cells, a method of labeling of the cells to allow in vivo detection after administration, and an animal model. Some cells including several mouse embryonal stem cells, skeletal myoblasts and cardiomyoblasts can be purchased as cell lines from the American Type Cell Culture (ATCC). Others, such as bone marrow- and adipose-derived stem cells, or progenitor cells would need to be personally isolated or obtained from a collaborating lab. To prepare for injection, several million cells are labeled by your method of choice or transduced with a reporter gene, harvested (preferably without trypsin), and transferred into a syringe in a small volume of phosphate buffered saline or culture media that does not contain serum. The most widely used animal model is the rat (or less often, canine or porcine) myocardial ischemia-reperfusion model induced by temporary ligation of the coronary artery. Injection of the prepared cells is most often done by direct injection into the infarct or peri-infarction zone of the heart, although it seems that cells injected into the remote zone have better survival.[8)] For larger animals and humans, intracoronary delivery via catheterization can be used. Another animal model that can be used is the mouse or rat hindlimb ischemia model, which is much simpler because it does not involve artificial respiration or thoracotomy. It makes sense, therefore, to use the hindlimb model first for setting up new imaging approaches before implementing them to the more difficult myocardial model.

Cell labeling strategies can be categorized into 3 techniques. The most straightforward is to directly label the cells with radioactive tracers to allow tracking by nuclear imaging. The advantages are high detection sensitivity and smoother translation into clinical practice. Examples are ^{18}F-FDG or ^{111}In oxine labeling of bone marrow cells or endothelial progenitor cells to investigate their early in vivo distribution following cardiac transplantation.[9,10)] The usefulness of this technique, however, is substantially rendered by the physical half-lives of the radionuclides that limit the duration of monitoring that is possible. The second approach is to label cells with iron particles and track cell fate by MR imaging. This method has the advantages of very high anatomic resolution that delineates the hypointense appearing cells and the absence of tracer half-life.[11)] However, this approach suffers the problem that ferromagnetic labels will still register an MR signal even after apoptosis or cell death of the injected cells, making it difficult to relate imaging to number of viable cells.

The 3rd labeling approach is to transduce cells with a reporter gene that transcribes a readily detectable reporter protein (Fig. 2A). Reporter signals emit only from living

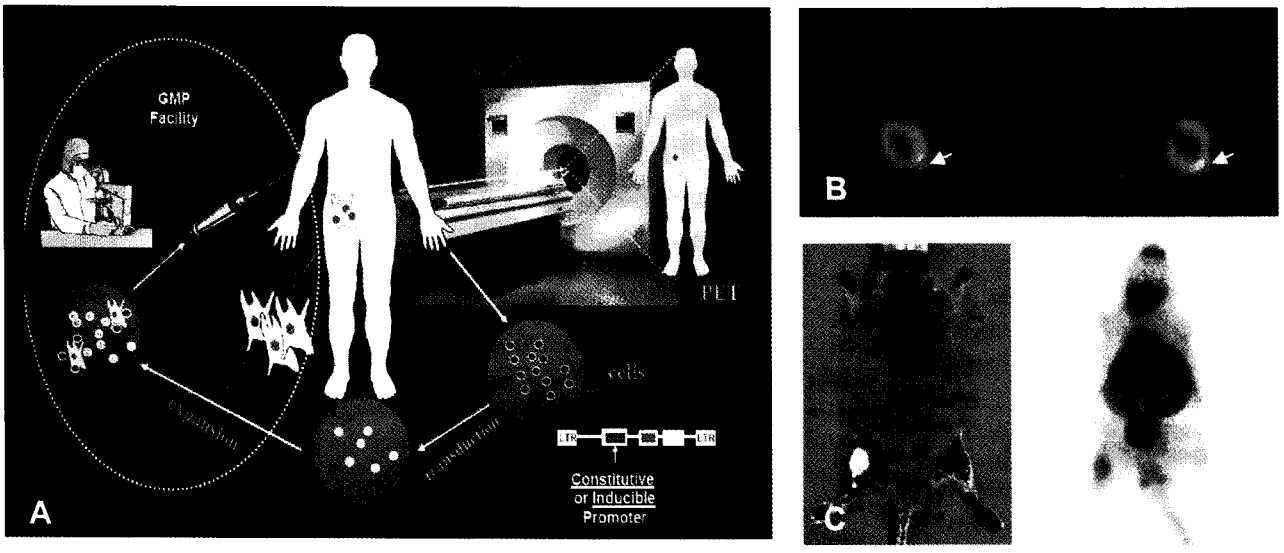

Figure 2. This shows cell therapy imaging with (A) scheme of PET reporter gene-based cell imaging technique, (B) (^{18}F)FPBG PET images of a rat intra-myocardially injected with murine embryonic stem cells expressing HSV1-tk at 2 weeks post transplantation, (^{18}F)FPBG images are overlayed on (^{13}N)ammonia perfusion images (reprinted with permission from ref. 13), and (C) optical images (left) and planar ^{99m}Tc scintigraphy (right) of mouse transplanted with rat cardiomyoblasts expressing the NIS gene in left gluteal muscles on day of injection.

cells, allowing true characterization of the survival, proliferation, and death of transplanted cells. Furthermore, stably transfected cells can be tracked even months after administration. In an early study, it was shown that embryonic cardiomyoblasts transduced with either firefly luciferase or *HSV1-sr39tk* genes could be injected into mouse myocardium and imaged with bioluminescence and micro-PET imaging.[12] Multimodality imaging has also been demonstrated using mouse embryonic stem cells transduced with a tri-fusion reporter gene expressing firefly luciferase, red fluorescent protein, and HSV1-tk. Images taken after delivery of the cells into rodent hearts could provide information on cell survival and proliferation (Fig. 2B).[13] More recently, tri-fusion reporter gene-transfected mesenchymal stem cells were shown to be traceable by PET imaging after injection into pig hearts.[14] The safety of this approach was confirmed by gene transcriptional profiles as well as cell viability, proliferation, and differentiation capacity of mouse embryonic stem cells.[15]

In addition to the HSV1-tk gene, the NIS gene provides an alternative nuclear imaging reporter with the advantages of less immunogenicity and negation of radiochemical probe synthesis (Fig. 2C). In a recent study, NIS gene transduced cardiac-derived stem cells were injected into infarcted rat hearts and imaged with ^{99m}Tc SPECT or ^{124}I PET imaging.[16] This single report of NIS gene-based cell therapy imaging is in contrast to the numerous reports on its use for tumor or organ imaging, and suggests that NIS transporter function in therapeutic cells may be susceptible to various confounding external factors.[17,18]

Molecular imaging may help address many fundamental questions in cardiac cell therapy. These include such issues as the optimal cell type, delivery technique, and cell dosage for therapy; the mechanism of survival, integration, and proliferation of transplanted cells in target organ; and the long-term fate of transplanted cells. Molecular imaging of cardiac cell therapy has the potential to help accelerate research by providing information on such variables as optimal cell type, dosage, or delivery route, particularly in the context of different cell types. It can also aid in investigating methods aimed at preventing acute donor cell death, which may be the most critical determinant for cell therapy efficacy. The final goal would be to contribute to the development of standardized cell transplantation protocols that are safe, quantifiable, and reproducible.

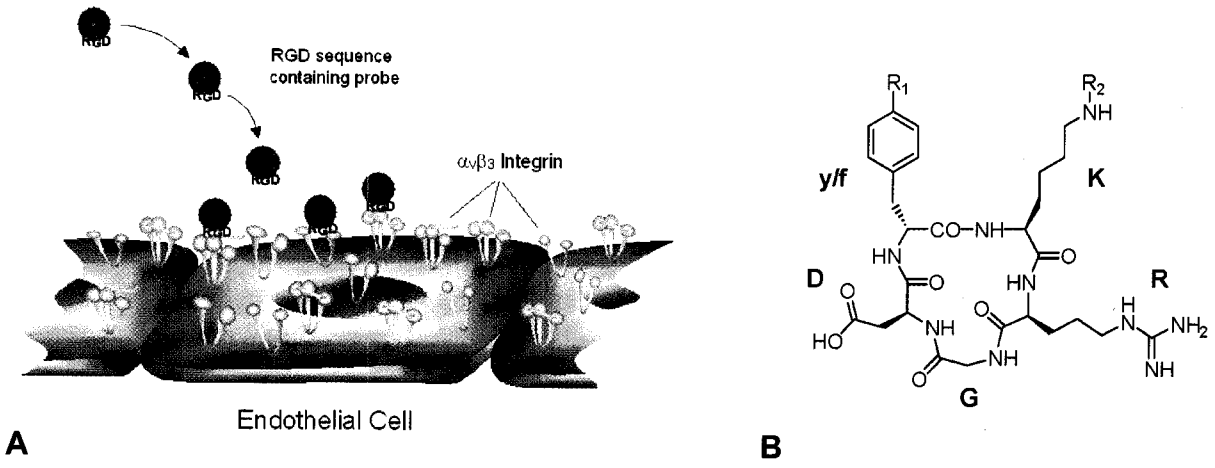

Figure 3. This shows principle of RGD imaging with (A) scheme for targeting αvβ3 integrin receptors overexpressed on angiogenic endothelial cell surface with RGD probes and (B) basic chemical structure of a RGD tripeptide-based angiogenesis imaging probe.

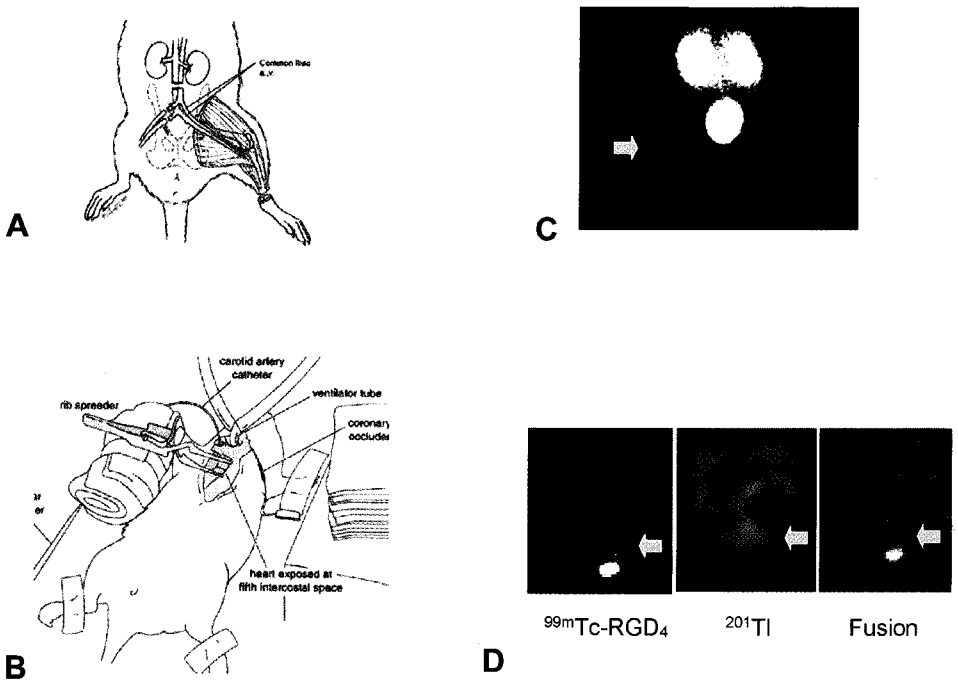

Figure 4. This shows rodent models for imaging of ischemia-induced angiogenesis with cartoons showing (A) induction of mouse hindlimb ischemia via femoral artery ligation/resection, (B) rat myocardial ischemia via left anterior descending coronary artery ligation, (C) planar glucosamine-(^{99m}Tc)-cRGD image of a mouse at 3 days following induction of hindlimb ischemia (arrow), and (D) (^{99m}Tc)-cRGD tetramer and ^{201}Tl SPECT images of a rat heart at day 7 of ischemia-reperfusion injury.

Imaging Therapeutic Angiogenesis

Angiogenesis, the formation of new capillaries by endothelial cell outgrowth from existing microvessels, occurs not only in cancer but also during healing of ischemic injuries. The principal stimuli of ischemia, hypoxia, and inflammation are all prominent in cardiovascular diseases. Particularly important among a large number of angiogenesis modulators are vascular endothelial growth factor (VEGF) receptors and αvβ3

integrins (Fig. 3), both of which serve as promising targets for angiogenesis imaging.[19] Candidate probes for angiogenesis imaging first undergo in vitro experiments to screen for specificity and affinity of binding to these target receptors. Probes that show favorable binding kinetics are then tested in animal models. Most often used are mouse models of hindlimb ischemia or rat models of myocardial ischemia. To induce hindlimb ischemia, anesthesized wild type mice are placed supine and a longitudinal skin incision is applied to the medial thigh of a hindlimb (the contralateral side can be used as a sham operated control). The femoral vascular bundle is separated from surrounding tissue and destruction of all the visible vessels promptly results in severe ischemia of the limb. However, for nuclear imaging experiments, it is the opinion of this author that destruction of venous return can cause increased background blood pool activity. In our lab, we therefore carefully separate the femoral artery from vein and resect only the artery after ligation of the proximal and distal ends (Fig. 4A). To induce myocardial infarction, anesthetized rats are intubated with an angiocatheter and ventilated with a small-animal respirator. A left thoracotomy is performed and the heart apex is sutured to allow traction to the heart. The left anterior descending coronary artery is ligated with a silk suture a few mm from its origin using a slip-knot. After 30 minutes of ischemia, the ligature is released to allow reperfusion and the chest wall is closed. This model produces nontransmural infarction in the anterolateral wall and peri-infarct ischemia resulting in myocardial angiogenesis (Fig. 4B).

VEGF receptors overexpressed in ischemic tissue can be imaged with radiolabeled VEGF probes. VEGF binds to their endothelial cell surface expressed receptors with high specificity and affinity to induce cell proliferation, migration, and survival. Identification of VEGF receptors in peripheral vascular disease could help guide selection of sites for local injection of angiogenic treatments. Although in part limited by receptor density and probe retention in the kidney and liver, ^{111}In-VEGF121 is a promising angiogenesis imaging agent. In a rabbit model of unilateral hindlimb ischemia, ^{111}In-VEGF121 imaging demonstrated increased binding in the ischemic limb that correlated to VEGF receptor expression.[20] A more recent study reports the time course of VEGF receptor expression using ^{64}Cu-DOTA-VEGF121 PET in rats before and serially after myocardial infarction. Cardiac activity increased significantly after infarction and returned to baseline levels after 2 wk.[21] Thus, VEGF receptor imaging is hoped to provide complementary information to routine assessments of flow, and may be useful for providing evidence of therapeutic effect in patients receiving VEGF treatment.

The αvβ3 integrins are selectively and highly expressed in angiogenic vessels, and hence represent another promising target for angiogenesis imaging. Haubner and coworkers were the first to introduce a series of radiolabeled probes based on cyclic Arg-Gly-Asp (RGD) peptides, αvβ3 integrin antagonists that bind the receptor with high specificity and affinity.[22-24] Predominant hepatobiliary clearance of the first generation probes was resolved by glycosylation of RGD peptides that increased hydrophilicity and renal excretion;[23] ^{18}F labeled RGD was synthesized to allow PET imaging.[24] A ^{99m}Tc labeled RGD probe with selective and high affinity binding to integrin has been developed by our group for wide availability and convenient imaging.[25]

Following its initial use for tumor disease, αvβ3 targeted imaging has extended its application for imaging of ischemia-induced angiogenesis. Our group was the first to report that RGD probes are able to image angiogenesis in peripheral ischemia. Mice subjected to femoral artery resection showed increased [^{125}I]c(RGD(I)yV) uptake in hypoperfused limb muscles at days 3 and 8; this was accompanied by immunohistochemical increases in αv integrin staining.[26] The feasibility of RGD imaging to monitor integrin expression in ischemic hearts was determined by Higuchi et al. by performing ^{18}F-Galacto-RGD PET in rats subjected to myocardial-ischemia injury. Focal accumulation in the infarct area started at day 3, and peaked between 1 and 3 weeks; the time course of uptake paralleled vascular density measurements.[27] These studies indicate that RGD imaging is promising for the monitoring of repair processes in ischemic limbs and hearts (Fig. 4C & 4D).

^{111}In-RP748, a quinolone that targets αvβ3 integrin, has

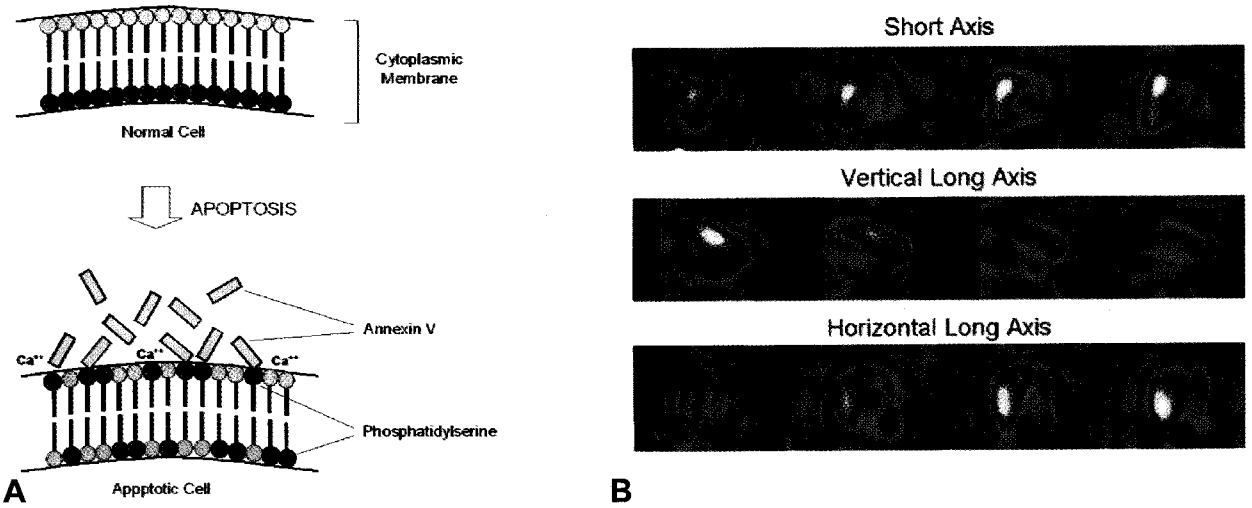

Figure 5. This shows apoptosis imaging with annexin A5 with (A) targeting of externalized phosphatidylserine by ca2+ dependent binding of annexin V and (B) ^{99m}Tc-annexin A5 cardiac SPECT images in a patient after cardiac transplantation showing diffuse myocardial activity indicating apoptosis. (Reprinted with permission from Wolters et al.[31])

also been investigated as a probe for imaging ischemia-related angiogenesis. ^{111}In-RP748 uptake has been shown to increase in infarcted myocardium of canine models,[28] and uptake in infarcted regions has been associated with histological evidence of αvβ3 expression.[29] ^{111}In-RP748 uptake has also been shown to track the proliferative process associated with carotid artery injury in apolipoprotein E knockout mice,[30] although nuclear imaging of vascular lesions presents a unique problem owing to low mass and deep localization.

In the future, additional experiments will be required to define the time course of αvβ3 integrin targeted imaging after ischemic injury-stimulated angiogenesis. In addition, image-based changes will need to be correlated to changes of more functional parameters like mechanical function or regional perfusion, permeability, and hypoxia.

Imaging of Cardiovascular Cell Apoptosis

Apoptosis, a well-organized mode of programmed cell suicide, plays an important role in many cardiovascular diseases. This includes loss of cardiomyocytes after infarction or during congestive heart failure, cardiac allograft rejection, and atherosclerotic plaque instability. Unlike necrotic cells that undergo cellular swelling, plasma membrane rupture and inflammatory activation, apoptosis is characterized by cell shrinkage, formation of membrane-bound apoptotic bodies, maintenance of plasma membrane integrity, and lack of inflammatory responses. Once the apoptotic program is activated, several targets become available for therapeutic interventions, providing the opportunity for pharmaceutical manipulation to limit the extent of cell loss. Hence, exploiting these targets for molecular imaging is important for detecting and quantifying these apoptotic processes. This may allow measurement not only of the extent of reversible damage but also of the efficacy of novel therapies targeting apoptosis.[31]

The most attractive target for apoptosis imaging to date is phosphatidylserine, a negatively charged aminophospholipid highly expressed on the surface of cells undergoing apoptosis but not in normal cells. Phosphatidylserine is a constitutive plasma membrane anionic phospholipid that is normally localized on the cytofacial membrane leaflets of cells, while the exofacial membrane leaflet contains predominantly phosphatidylcholine and sphingomyelin. During apoptosis, the combined inhibition of aminophospholipid translocase and activation of scramblase results in the surface expression of phosphatidylserine, which acts as a flag at the cell surface for phagocytes to recognize and respond by engulfing the dying cell before it induces an inflammatory reaction.

Imaging of phosphatidylserine externalization, one of the earliest events in apoptosis, can be accomplished with the use of annexin A5 based probes.[32] Annexin A5 is an endogenous human protein with high specificity and affinity Ca2+-dependent binding to negative-charged phospholipids (Fig. 5), and has thus been used labeled with fluorophores for in vitro apoptosis assays. Subsequently, radiolabeled annexin A5 probes were developed and evaluated for apoptosis imaging in living subjects. ^{99m}Tc-annexin A5 was first tested to show increased uptake at sites of apoptosis in three models: a mouse fulminant hepatic apoptosis model; a rat model of acute cardiac allograft rejection; and a mouse model of cyclophosphamide treated lymphomas.[33] Apoptosis also occurs in myocardial infarction, where the peripheral zones show mainly apoptotic cell death as opposed to necrotic dominated central zones. Hence a clinical study of apoptosis imaging was performed in patients with acute myocardial infarction using ^{99m}Tc-annexin A5 produced under GMP regulations. As a result, 6 of the 7 patients showed increased uptake in the infarcted region that had perfusion defects.[34] Apoptotic cell death may also contribute to the progression of congestive heart failure, although imaging this disease process is more challenging because of the smaller amount of apoptotic cells present. This possibility, however, was demonstrated in a study of 9 idiopathic dilated cardiomyopathy patients using ^{99m}Tc-Annexin A5. Of these patients, 5 showed focal uptake and 1 showed global uptake in the left ventricle, all of who displayed recent onset or recent worsening of ventricular function and functional class; All 5 cases that did not show Annexin A5 uptake showed stable ventricular function and clinical status.[35]

Other probes for imaging apoptosis includes phosphatidylserine targeting with the C2 domain of synaptotagmin I, and caspase targeting with specific substrates. ^{99m}Tc labeled synaptotagmin has been shown to accumulate in the area at risk of reperfused acutely infracted myocardium of rats.[36] The caspase cascade is activated during apoptosis through death-receptor mediated signal transduction and stress-induced release of cytochrome C from mitochondria, which turn inactive procaspases into proteolytically active enzymes.[37] Although this machinery offers potential targets for apoptosis imaging, previous efforts have largely been hindered by difficulty of imaging probes to permeate through the cell membrane and reach target enzymes.

Another process where molecular imaging may be useful is atherosclerotic plaques. In high-risk lesions, damage to infiltrated macrophages and smooth muscle cells induce apoptosis, which plays a pivotal role in promoting plaque progression and disruption. Since macrophages activation is a key component of high-risk plaques, and ^{18}FDG is a marker for activated macrophages,[38] there is sound logic behind using ^{18}FDG PET for monitoring inflammation of arteries.[39] Meanwhile, direct imaging of apoptosis in atherosclerotic plaques can be approached using ^{99m}Tc-Annexin A5. The first demonstration in a rabbit model of aortic atherosclerosis showed a 9-fold higher binding of the probe compared to control vessels.[40] The possibility of assessing coronary atheroma progression with ^{99m}Tc-Annexin SPECT has also been demonstrated in a porcine model.[41] The first demonstration of the feasibility in human subjects was given in a pilot study of 4 patients: 2 patients who suffered transient ischemia shortly before imaging demonstrated marked Annexin A5 uptake in the culprit carotid vessel, which was histologically localized to apoptotic macrophages.[42]

Annexin A5 imaging of apoptosis in vivo has made a successful transition from the desktop to the bedside, and ^{99m}Tc-labeled Annexin-V is now commercially available (Thesus Imaging, Inc.). In the future, prospective studies are need to be designed to evaluate the clinical meaning of Annexin A5 imaging results in relation to the progression of the underlying disease and risk for clinical events.

Conclusion

In summary, cardiovascular molecular imaging is an exciting field that combines the disciplines of molecular biology, radiochemistry, pharmacology, instrumentation, and clinical medicine into a new imaging paradigm. Cardiovascular molecular imaging can be used to study newer molecular processes that have recently generated much attention including plaque vulnerability, myocardial

and peripheral angiogenesis, as well as gene transfer and cardiac cell therapy. These imaging approaches are already powerful tools for preclinical experiments and have become essential for drug discovery research. Hopefully, many of these new imaging techniques will successfully move forward from translational studies to the clinical arena.

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