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Molecular imaging of atherosclerosis using reporter gene system

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ABSTRACT

Macrophages play a key role in atherosclerotic plaque formation, but their participation has been discerned largely via ex vivo analyses of atherosclerotic lesions. Therefore, we aimed to identify atherosclerosis on noninvasive in vivo imaging using reporter gene system. This study demonstrated that recruitment of macrophages could be detected in atherosclerotic plaques of Apolipoprotein E knockout (ApoE^{-/-}) mice with a sodium iodide symporter (NIS) gene imaging system using ^{99m}Tc-SPECT. This novel approach to tracking macrophages to atherosclerotic plaques in vivo could have applications in studies of arteriosclerotic vascular disease.

Key Word: Atherosclerosis, Click chemistry, Macrophage, Reporter gene, SPECT imaging, PET imaging

Atherosclerosis

Atherosclerotic diseases are a major cause of death and disability worldwide. Preventive strategies currently focus on controlling risk factors, such as smoking, blood pressure, serum glucose and lipid levels (1). Despite the success of these preventive measures, substantial residual risk remains even when treatment goals are fully met (2). In patients that suffered a myocardial infarction, the recurrence risk of an acute coronary syndrome is high, particularly within the first year when recurrence rates are up to 17.4% (3).

A recent study explained this phenomenon by showing that a systemic response to ischemic injury aggravates inflammation in atherosclerotic plaques at a distance, due to increased monocyte recruitment (4). Monocytes that infiltrate the plaque differentiate into macrophages, which produce proteolytic enzymes

that digest extracellular matrix causing plaque rupture (5). The immediate site of plaque rupture contains a high concentration of inflammatory cells (6). Plaque inflammation is therefore pursued as a therapeutic target to prevent atherothrombotic events (7) (Figure 1, 2).

The study of atherosclerosis progression in humans is hindered by the complexity and chronicity of the disease process, by the difficulty to longitudinally monitor the changes of the plaques in an individual patient and by deficiency of the noninvasive detection modalities that provide limited information on the composition of the lesions. The investigation of pathological changes in the arteries of humans is restricted to studies in the cross-sections of autoptic or surgical samples. Therefore, there has been a reliance on animal models, and, since 1992, the mouse has become an excellent system for the study of atherosclerosis, progressively

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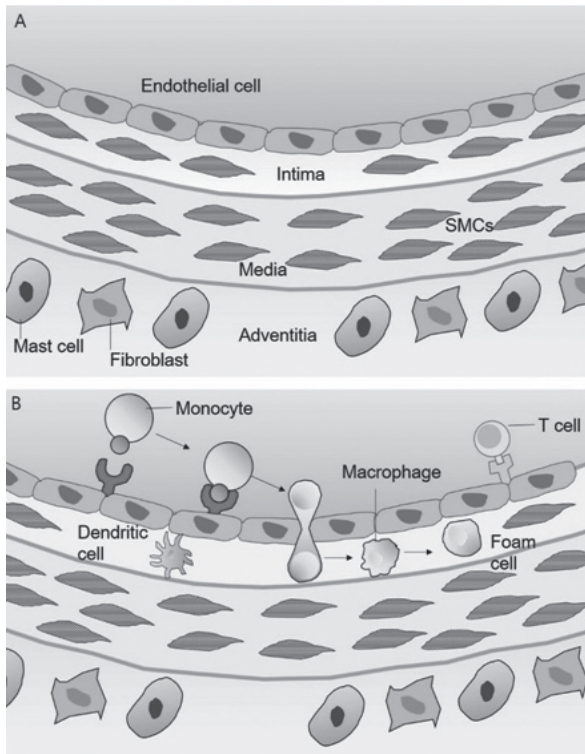


Figure 1. The development of atherosclerotic lesions. (A) There are three layers in the artery, and the inner membrane consists of a monolayer of endothelial cells in contact with blood. (B) In the early stages of atherosclerosis, blood leukocytes accumulate on the activated endothelial layer, and bound white blood cells migrate to the endothelium. When mononuclear cells are macrophage-maturated and lipid-uptake, foam cells are produced.

replacing the use of large animals (8), in particular due to the ability to easily over- or under-express specific genes such as leukocyte adhesion receptors, indicators of macrophage infiltration and angiogenesis.

Molecular imaging using an atherosclerosis model

Recently, studies targeting a specifically expressed gene in atherosclerotic lesions have been actively conducted in mouse models using molecular imaging (9,10). Molecular imaging has become an indispensable tool both in cardiovascular research and clinical care within the last decades. Molecular imaging relies on diverse imaging techniques, which include contrast-

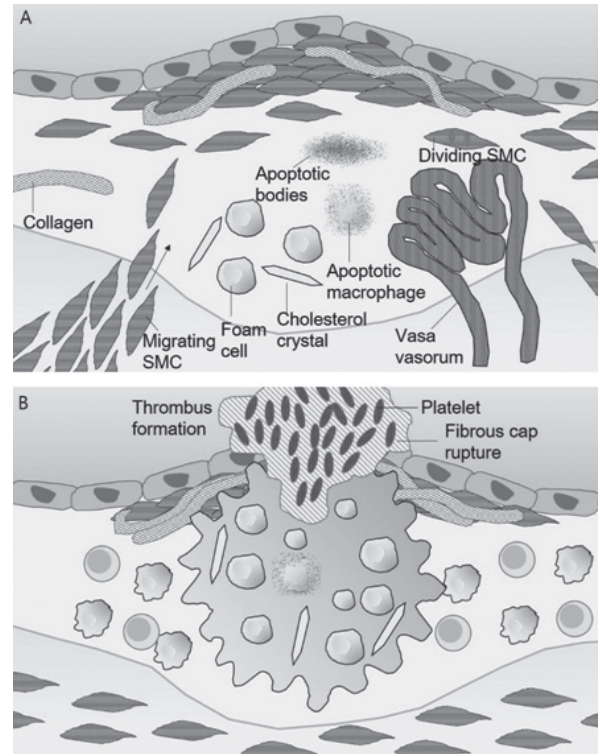


Figure 2. The progress of atherosclerotic lesions. (A) Progression of the lesion is accompanied by proliferation of smooth muscle cells (SMCs) and medium derived SMCs by migration of SMC from the medium to the intima. And increased synthesis of extracellular matrix macromolecules such as collagen, elastin, and proteoglycans. Increased plaque macrophages and SMCs can accumulate in the central region of plaques marked with lipid or necrotic cores. A fibrous cap is formed in the vessel wall and the fat layer in the vessel wall becomes atheroma along with the necrosis process. (B) Thus, the plaque formed causes bleeding, and these processes are repeated, leading to atherosclerosis in which the vessel wall becomes narrower and narrower. The rupture of the fibrous cap of the plaque may cause the blood clotting component to contact the tissue factor inside the plaque and expand into the lumen of the vessel to cause thrombosis, a complication of atherosclerosis

enhanced ultrasound (CEUS), magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), computed tomography (CT), fluorescence molecular tomography (FMT) and photoacoustic imaging (PAI). Various imaging technologies are now available and they have their own strengths and weaknesses (Table 1). Imaging is mostly restricted to depicting anatomy and quantifying the degree of vessel stenosis.

Studies in an atherosclerosis model using nuclear

Table 1. Noninvasive molecular imaging in mouse models of vulnerable atherosclerotic plaques

Imaging modality	Spatial resolution	Sensitivity (mol/L)	Contrast agent / probe concentration	Advantage	limitation
Ultrasound imaging	50~500 μ m	Not well characterized yet	Microbubbles / μ M to nM	real-time, low cost high temporal resolution (0.1-100 s) no ionizing radiation	operator-dependent
Magnetic resonance imaging	10~100 μ m	10^{-3} ~ 10^{-5}	USPIO ^{a)} , SPIO ^{b)} / mM to nM	high tissue contrast and functional parameters no ionizing radiation	high cost, operator-dependent
Nuclear imaging	PET: 1~2 mm SPECT: 0.5~2 mm	PET: 10^{-11} ~ 10^{-12} SPECT: 10^{-10} ~ 10^{-11}	Positron or Y ray emitting radionuclides / pM	molecular and functional parameters high sensitivity	ionizing radiation limited spatial resolution (mm) high-medium cost
Computed tomography imaging	30~400 μ m	10^{-2} ~ 10^{-3}	Iodinated particles, gold nanorods / mM to nM	fast acquisition time high temporal resolution (1-300 s) provides molecular and structural information	ionizing radiation, medium cost low soft tissue contrast resolution
Fluorescence tomographic imaging	1~2 mm	10^{-10} ~ 10^{-11}	NIR fluorophores / nM to pM	high sensitivity, low cost no ionizing radiation	limited depth of penetration (1-20 mm) limited spatial resolution (mm)
Photoacoustic imaging	<100 μ m	< 10^{-12}	NIR fluorophores / nM to pM	high sensitivity, low cost, no ionizing radiation high depth of penetration (<5 cm)	data post-processing and acquisition procedures still being optimized

^{a)} Gd-based contrast agents ^{b)} Iron oxide and other superparamagnetic nanoparticles

techniques such as PET and SPECT potentially provide detection sensitivities in the nanomolar-picomolar range. Such functional imaging enables the investigation of biological events that lead to plaque rupture with high specificity and offers relevant potential results of clinical translatability from basic research to identify high-risk patients. Furthermore, the combination of the nuclear medicine images with the morphological information provided by CT in hybrid scanners, or with the high soft tissue contrast obtained

through MRI, has the potential to map molecular signals with precise anatomic details (11–13). Also, more recently, the association with optical imaging was shown to be particularly helpful for testing innovative probes and for in vivo tracking of targeted cells (14).

Reporter gene in molecular imaging

In a previous study, it was shown that differentiated

thyroid cancers and all anaplastic thyroid cancers fail to concentrate radioiodine due to reduced NIS expression, which prevents the use of radioiodine in metastatic thyroid cancer therapy (15). The recent cloning and characterization of the NIS gene could lead to the development of a novel gene strategy for radioiodine therapy in thyroid cancer (16,17). The transfer of NIS gene and the functional expression of NIS protein in cancer cells would enable these cells to concentrate iodide from plasma and enable radioiodine therapy (18). Previous studies have reported the result for the production of a recombinant NIS (rNIS) (15), and many researchers are interested in the basic and clinical applicability of rNIS. On expression of the NIS protein in the cell membrane following NIS gene transfection, it is possible to perform imaging using ^{99m}Tc -pertechnetate, ^{131}I and ^{123}I , and it is possible to perform therapy using therapeutic radionuclides such as ^{131}I , ^{186}Re , ^{188}Re (19).

Mutant dopamine D2 receptor (D2R) and Herpes simplex virus type 1 thymidine kinase (HSV1-tk) are the most widely used among the reporter genes besides NIS, which was developed for nuclear medicine and molecular imaging (20). Recently, interest has concentrated on the complex molecular image using a multi-reporter system that provides composite gene expression (21).

Especially, the dual reporter gene imaging system expressing a nuclear medical reporter gene (HSV1-TK or NIS) and a reporter gene at the same time for use in optical molecular imaging (luciferase or green fluorescence protein) can be used extensively to assess the progress, challenges of cancer and tracking the treatment progress in vitro and in vivo. Also, an optical imaging reporter gene as a monitoring gene showed that ^{131}I , ^{186}Re or ^{188}Re in the transfected NIS will be able to evaluate the effect of treatment with radionuclides (15,16) (Figure 3).

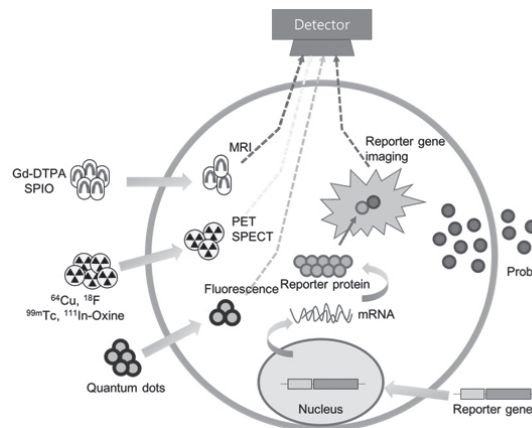


Figure 3. Molecular imaging using reporter gene. A vector containing a DNA reporter construct with the reporter gene(s) driven by a specific promoter. Transcription and translation lead to the expression of mRNA and reporter protein, respectively. After administration of a corresponding reporter probe systemically, the reporter probe will be catalyzed by specific cells that have the reporter proteins. The signals occurred in this amplification process can be detected by a sensitive imaging device.

Detection of atherosclerotic lesion with reporter gene system

Macrophages accumulate in the blood vessel walls where atherosclerosis has developed. Therefore, atherosclerotic plaque can be identified by molecular imaging using macrophages (22).

To detection atherosclerotic lesion with reporter gene system, we sought to establish a noninvasive technique to determine macrophage tracking to atherosclerotic lesions in apolipoprotein E^{-/-} (ApoE^{-/-}) mice with an imaging system based on sodium iodide symporter (NIS) gene coupled with ^{99m}Tc -SPECT imaging. In this study, RAW 264.7 macrophage cells were stably transduced with retrovirus containing NIS gene (RAW-NIS). In RAW-NIS cells, the atherosclerotic ApoE^{-/-} mice were divided into four study groups (Saline, RAW cells, RAW-NIS cells and RAW-NIS cells after atorvastatin treatment). Through ^{99m}Tc -SPECT/CT imaging, in the RAW-NIS cell injected group, the ^{99m}Tc uptake in aorta was higher than other groups, but the injected RAW-

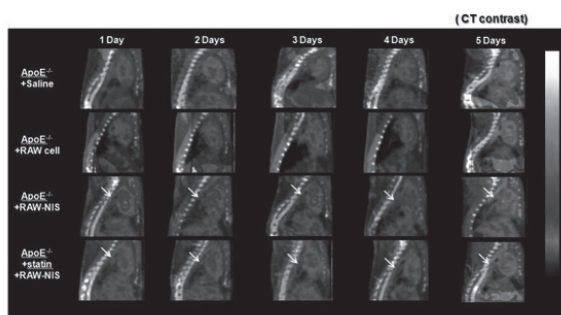


Figure 4. SPECT/CT images of atherosclerotic lesions in ApoE^{-/-} mice on Western diet for 30 weeks. SPECT/CT images of atherosclerotic lesions in ApoE^{-/-} mice confirmed that ^{99m}Tc-pertechnetate uptake of injected saline, RAW264.7 cell, RAW-NIS and atorvastatin-treated RAW-NIS group. Arrows indicate the ^{99m}Tc-pertechnetate uptake in heart and aorta. CT imaging was acquired only on day 5.

NIS cell for the atorvastatin treated group demonstrated reduced recruitment to the aorta (Figure 4). This study confirmed a positive correlation between cell imaging intensity and macrophage marker histological staining of the aorta. Areas of high ^{99m}Tc-pertechnetate uptake correlated with higher immunohistochemistry staining using CD68 antibody.

Conclusion

Generally, atherosclerosis is characterized by intimal plaques of the arterial vessels that develop slowly. Therefore, noninvasive tools for early diagnosis are necessary to screen atherosclerotic lesions at high risk of acute complications.

In the current study, macrophages, known to be activated at the onset of atherosclerosis, trafficking the atherosclerotic lesions of the ApoE^{-/-} model was performed using noninvasive imaging, the NIS reporter gene system. In study, the macrophage cell line which allowed NIS to be stably expressed, the novel approach showed that migration of macrophages to the inflammatory atherosclerotic lesions was effectively monitored by SPECT imaging. In addition, the efficacy of statin could be evaluated by imaging.

An attempt to identify atherosclerosis by molecular imaging using NIS reporter gene was the first in this study. This potential novel approach including the NIS reporter gene system may provide new insights into the role of macrophage tracking monitoring in a living body for making the diagnosis of atherosclerosis and may contribute importantly to enhanced risk assessment.

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