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Monitoring of macrophage accumulation in statin-treated atherosclerotic mouse model using sodium iodide symporter imaging system

Ran Ji Yoo [a,b], Min Hwan Kim [a], Sang-Keun Woo [a], Kwang Il Kim [a], Tae Sup Lee [a], Yang-Kyu Choi [b], Joo Hyun Kang [a], Sang Moo Lim [c], Yong Jin Lee [a,*]

[a] Division of RI-Convergence Research, Korea Institute of Radiological & Medical Sciences, Seoul, Republic of Korea
[b] Department of Laboratory Animal Medicine, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea
[c] Department of Nuclear Medicine, Korea Institute of Radiological & Medical Sciences, Seoul, Republic of Korea

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ABSTRACT

Introduction: Macrophages play a key role in atherosclerotic plaque formation in atherosclerosis, but its detailed understanding has poorly investigated until now. Thus, we sought to demonstrate a noninvasive technique for 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-single-photon emission computed tomography (SPECT).

Methods and results: Macrophage cells (RAW264.7) were stably transduced with retrovirus expressing NIS gene (RAW-NIS). In RAW-NIS cells, uptake of ^{125}I was higher than the parental cells. [^{18}F]FDG signals in the aorta at 30 weeks on an $ApoE^{-/-}$ mice with high cholesterol diet were higher (1.7 ± 0.12% injected dose (ID)) than those in control group (0.84 ± 0.06% ID). Through ^{99m}Tc-SPECT/computed tomography (CT), in the RAW-NIS cell injected group, the ^{99m}Tc-pertechnetate uptake in aorta was higher than control groups. However, according to atorvastatin treatment, RAW-NIS cell recruitment reduced to the aorta. Area of ^{99m}Tc-pertechnetate uptake was positively correlated with immunostaining results against macrophage antigen (CD68). Cholesterol and low-density lipoprotein levels of atorvastatin-treated group showed lower than those of atorvastatin-untreated group, but did not reach statistical difference.

Conclusions: This novel approach to tracking macrophages to atherosclerotic plaques in vivo can be applied for studies of arterosclerotic vascular disease.

1. Introduction

Atherosclerosis is a vascular disease with atheromatous plaque formation seen in the innermost covering of blood vessels. These plaques include deposition of cholesterol along with accumulation of macrophages and calcium buildup for the older, more advanced plaques. Atherosclerotic plaques grow over time and mediators of inflammation, the monocytes/macrophages, enter nascent atherosclerotic lesions, ingest modified lipoprotein particles, and give rise to foam cells, a hallmark of atheromata [1]. Macrophages amplify local inflammation through secretion of cytokines and reactive oxygen species and eventually induce a weakness of the plaque's fibrous cap by secretion of proteases. This destabilizes the atherosclerotic plaques, favoring plaque disruption and increasing the risk of myocardial infarction and stroke [2]. The detection of vulnerable plaques is clinically important for risk stratification and to provide early treatment to affected individuals [3].

With a great medical need to diagnosis atherosclerosis, there are several noninvasive approaches to study the onset of atherosclerosis, such as ultrasonography, multi-slice computed tomography (CT) and magnetic resonance imaging (MRI). However, most of the techniques have inadequate spatial resolution and are unable to detect the nascent plaques and the local inflammation accurately [4,5].

The radiotracer, [^{18}F]fluoro-2-deoxy-d-glucose ([^{18}F]FDG), is a glucose analogue that can be non-invasively imaged using PET. An increased [^{18}F]FDG uptake in the atherosclerotic artery wall has been demonstrated in animal imaging studies and these suggest that plaque macrophages account for most of the FDG uptake [6]. But there is a significant limitation for using [^{18}F]FDG for atherosclerosis imaging. [^{18}F]

Abbreviations: $ApoE^{-/-}$, Apolipoprotein $E^{-/-}$; LDL, low-density lipoprotein; NIS, sodium iodide symporter; PET, Positron Emission Tomography; SPECT, Single-Photon Emission Computerized Tomography; [^{18}F]FDG, [^{18}F]fluoro-2-deoxy-d-glucose; $^{99m}TcO_4^-$, ^{99m}Tc-pertechnetate.

* Corresponding author at: Division of RI-Convergence Research, Korea Institute of Radiological and Medical Sciences (KIRAMS), 75 Nowon-ro, Nowon-gu, Seoul, 01812, Republic of Korea. Tel.: +82 2 970 1364; fax: +82 2 970 1341.
E-mail address: yjlee@kirams.re.kr (Y.J. Lee).

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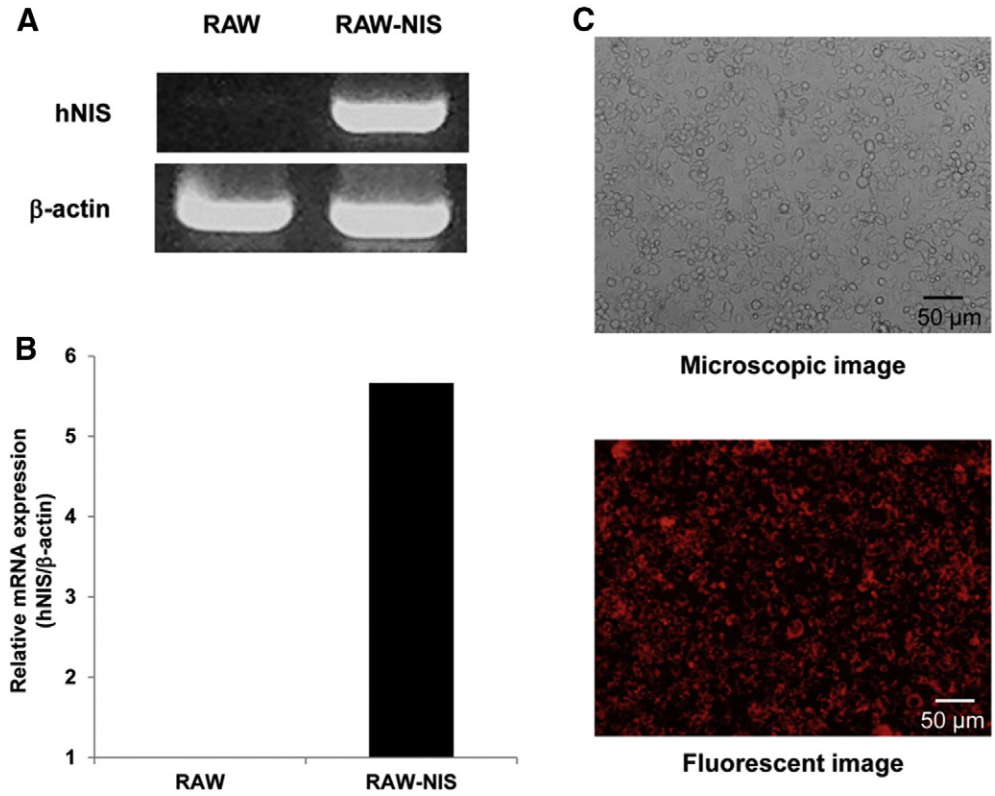

Fig. 1. RT-PCR analysis by parental cells and hNIS transfected cells and labeled cell tracker image of RAW-NIS. (A) RT-PCR analysis of transcripts for hNIS reporter of RAW264.7 or RAW-NIS cells. (B) Relative quantification of hNIS gene expression normalized against ß-actin from (A). (C) RAW-NIS cell was confirmed that striking red fluorescent without or with cell tracker.

FDG has a high myocardial uptake, leading to relatively high background signals and frequently masking the signal from an atherosclerotic plaque [7].

Sodium/iodide symporter, also known as a sodium/iodide cotransporter (NIS), normally expressed in the thyroid gland, can be used as a reporter gene [8]. Recently, NIS reporter gene had received attention as promising reporter genes in preclinical research and clinical trials [9]. They explained that investigational study using NIS was safe and feasible for noninvasive imaging in humans [10]. Multiple studies have been conducted to assess the feasibility of NIS and SPECT imaging systems for in vivo cell tracking [11,12].

As the recruitment of macrophages in atherogenic plaque formation is a dynamic process, a study of the process at various times is required [2]. As such an NIS-enabled imaging may enable study of changes and traffic of target cells with respect to time. In this study using mouse models of atherosclerosis, we compared the [^{18}F]FDG-PET and NIS reporter gene imaging system for noninvasive tracking migration of macrophage populations to atherosclerotic lesions. Furthermore, we demonstrate that ^{99m}Tc-SPECT/CT imaging using NIS reporter gene allows for assessment of the effect of an atorvastatin treatment on macrophage recruitment to atherosclerotic lesion in vivo.

2. Materials and methods

2.1. NIS gene expressing retrovirus production and generation of stable cell lines

The retrovirus containing human NIS (hNIS) was produced using pLNCX retroviral vectors. RAW264.7 cells were stably transduced with retrovirus expressing NIS gene (RAW-NIS). To generate retroviruses expressing human NIS (hNIS), FT-293 cells were plated 10^6 cells/100 mm plates and transfected with the pLNCX (containing Flag-tagged wildtype hNIS), pVSV-G and pGag-pol by the calcium phosphate method. After 8 h transfection, the medium was replaced with fresh DMEM containing 10% FBS, and cells were incubated for an additional 48 h. Then, these supernatants were applied immediately to RAW264.7

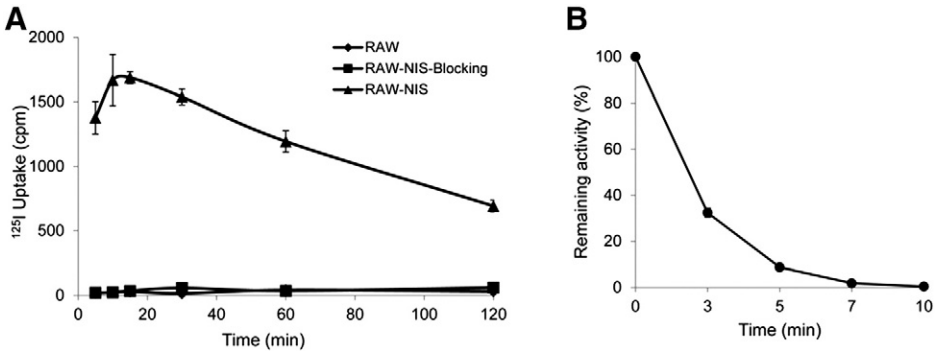

Fig. 2. Radioiodine uptake in RAW264.7 or RAW-NIS and efflux in RAW-NIS. (A) Uptake of ^{125}I by RAW264.7 or RAW-NIS was measured for 120 min. Data presented are means ± S.D. (n = 4). (B) Efflux data of radioiodine from RAW-NIS. Data presented are means ± S.D. (n = 4/each point).