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Adenovirus-mediated expression of human sodium-iodide symporter gene permits *in vivo* tracking of adipose tissue-derived stem cells in a canine myocardial infarction model

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ABSTRACT

Introduction: In vivo tracking of the transplanted stem cells is important in pre-clinical research of stem cell therapy for myocardial infarction. We examined the feasibility of adenovirus-mediated sodium iodide symporter (NIS) gene to cell tracking imaging of transplanted stem cells in a canine infarcted myocardium by clinical single photon emission computed tomography (SPECT).

Methods: Beagle dogs were injected intramyocardially with NIS-expressing adenovirus-transfected canine stem cells (Ad-hNIS-canine ADSCs) a week after myocardial infarction (MI) development. ^{99m}Tc-methoxyisobutylisonitrile (^{99m}Tc-MIBI) and ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) SPECT imaging were performed for assessment of infarcted myocardium and viable stem cell tracking. Transthoracic echocardiography was performed to monitor any functional cardiac changes.

Results: Left ventricular ejection fraction (LVEF) was decreased after LAD ligation. There was no significant difference in EF between the groups with the stem cell or saline injection. ^{125}I uptake was higher in Ad-hNIS-canine ADSCs than in non-transfected ADSCs. Cell proliferation and differentiation were not affected by hNIS-carrying adenovirus transfection. ^{99m}Tc-MIBI myocardial SPECT imaging showed decreased radiotracer uptake in the infarcted apex and mid-anterolateral regions. Ad-hNIS-canine ADSCs were identified as a region of focally increased $^{99m}TcO_4^-$ uptake at the lateral wall and around the apex of the left ventricle, peaked at 2 days and was observed until day 9.

Conclusions: Combination of adenovirus-mediated NIS gene transfection and clinical nuclear imaging modalities enables to trace the fate of transplanted stem cells in infarcted myocardium for translational *in vivo* cell tracking study for prolonged duration.

1. Introduction

Cardiac stem cell therapy for heart diseases has been attempted in various clinical settings for the last 10 years, and still requires significant clinical and laboratory research to optimize therapeutic strategies, including cell type, dose, and route of administration [1]. Adipose tissue-derived stem cells have been proven to differentiate into various types of cells, including cardiomyocytes, and have many advantages over other adult tissue-derived stem cells, without ethical issues [2]. To evaluate the efficacy and feasibility of stem cell therapy in heart disease, *in vivo* tracking of transplanted stem cells is critical.

In the stem cell field, molecular imaging of cell tracking has been studied in a variety of ways, including direct cell labeling methods by using ^{18}F-fluorodeoxyglucose, ^{64}Cu-diacetyl-bis(N4-methylsemicarbazone), or iron oxide, and indirect methods using genetically transduced cells expressing reporter protein such as herpes simplex virus type 1 thymidine

Abbreviations: ADSCs, Adipose-derived stem cells; NIS, Sodium iodide symporter; MOI, Multiplicity of infection; PET, Positron emission tomography; SPECT, Single photon emission computed tomography.

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kinase (HSV1-tk), the sodium iodide symporter (NIS), or the firefly luciferase [3–7]. However, pre-clinical studies using optical imaging systems have limitations when applied to clinical practice [8].

Nuclear imaging modalities, such as single photon emission computed tomography (SPECT) or positron emission tomography (PET), can be used not only for estimating the range of infarcted myocardium, but also for tracking the fate of transplanted stem cells [9,10]. There are several *in vivo* stem cell tracking methods based on coupling different cell labeling techniques and imaging modalities to track the engraftment and survival of transplanted stem cells in diseased hearts [11,12]. The reporter gene system is believed to provide information about the distribution, homing, survival, and functionality of transplanted stem cells for a relatively prolonged duration, and more effectively than direct cell-radiolabeling methods [11–13].

NIS is a reporter gene, which is naturally expressed in the thyroid gland. When the NIS gene is cloned into a vector viral genome and transfected into the target cell, it reveals the location and viability of the target cells *in vivo* by transporting various radioisotopes into the cells, such as ^{123}I, ^{124}I, ^{131}I, and ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$), which can be detected by SPECT or PET [14,15].

In most studies, mainly retro- or lentivirus vectors are used for reporter gene transduction of stem cells through cell tracking imaging. The use of a retro- or lentivirus for stem cell tracking imaging in the clinical field has limitations because of immune reactions and chromosome integrations with the host cell. Early research has confirmed the possibility of NIS gene delivery by using an adenoviral system in normal rat or swine myocardium [13].

In this study, we employed the NIS gene as a reporter gene by using ^{99m}Tc-TcO_4^- SPECT as an imaging modality to trace and quantify transplanted adipose-derived stem cells in infarcted canine myocardium. In addition, the efficacy of adenovirus-mediated transfer of the reporter gene into stem cells was also evaluated.

2. Materials and methods

2.1. Canine adipose-derived stem cell isolation and culture

All procedures for animals used in this study were approved by the Institutional Animal Care and Use Committee at Konkuk University (IACUC No. KU11059). Canine adipose tissue was aseptically isolated from the bilateral rump region of a healthy female adult beagle dog. Canine adipose-derived stem cells (ADSCs) were acquired at RNL (RNL Bio Co., Ltd., Korea) as previously described [16]. Flow cytometry analysis (FACS Calibur®, BD Biosciences, San Jose, CA, USA) was performed for immunophenotyping with CD29, CD44, CD90, CD31 and CD34 antibodies [16].

2.2. Transduction of canine ADSCs with an adenovirus expressing human sodium iodide symporter (hNIS) and green fluorescent protein (GFP)

Approximately a 70–80% confluency of canine ADSCs at passage 2 were transduced with Ad-hNIS-GFP viruses, which is adenovirus (Ad) co-carrying human sodium iodide symporter (hNIS) complementary DNA (cDNA) and green fluorescent protein (GFP) cDNA [21]. The virus was amplified in 293A cells and purified using standard CsCl techniques. To find the optimal conditions for viral transduction, canine ADSCs were transduced with Ad-hNIS-GFP at multiplicities of infection (MOI) of 0, 1, 3, 5, 10, and 20, and GFP expression in transduced cells was assessed with fluorescent microscopy.

Ad-hNIS-GFP viruses were added to canine ADSCs at a multiplicity of infection (MOI) of 5, and suspended in an optimized medium. Cell culture dishes were rotated every 15 min, and the virus was aspirated after 2 h of incubation. Cells were maintained in 5% FBS-containing RKCM medium.

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was prepared from Ad-hNIS-GFP transfected canine ADSCs (Ad-hNIS-GFP-canine ADSCs) using TRI Reagent (Molecular Research Center, Inc., USA). Five microgram of total RNA was reverse-transcribed using SuperScript III Reverse Transcriptase (Invitrogen, USA) and random hexamers to generate cDNA. hNIS and β-actin (an internal control) mRNA were analyzed. The sequence of primers used in PCR reaction was as follows; forward primer (5′-TCTCTCAGTCAACGCCTCT-3′) and reverse primer (5′-ATCCAGGATGGCCACTTCTT-3′) in hNIS, forward primer(5′-CTG GGACGACATGGAGAAAA-3′) and reverse primer (5′-AAGGAAGGCT GGAAGAGTGC-3′) in β-actin. After denaturation phase of 5 min at 94 °C, amplification and elongation process were each performed at an annealing temperature of 54–59 °C and 72 °C for 30 s followed by 25–30 cycles, and an additional elongation was performed at 72 °C for 5 min.

2.4. In vitro radioiodine uptake and efflux assay

Ad-hNIS-GFP-canine ADSCs (10 MOI) were plated at a cell density of 5×10^5 cells per well in a 6-well plate and cultured with optimized medium (RKCM) for canine ADSCs. After a 24-h incubation, the cells were assessed for NIS activity by radioiodine uptake at 37 °C [17,18]. Briefly, the cells were incubated at 37 °C for 120 min in 1 mL of Hank's Balanced Salt Solution (HBSS) containing 0.5% BSA, 3.7 kBq (0.1 μCi) of carrier-free ^{125}I-NaI, and 10 μmol/L of NaI to yield a specific activity of 740 MBq/mmol. For time-dependent iodide uptake evaluation, radioactivity of cell lysis solution was measured at different lengths of time (5, 30, 90, and 120 min). To evaluate specificity of radioiodine uptake, 100 μmol/L of NIS blocker, $KClO_4$, was added to Ad-hNIS-GFP-canine ADSCs incubated in HBSS containing 0.5% BSA, 3.7 kBq (0.1 μCi) of carrier-free ^{125}I-NaI, and 10 μmol/L of NaI. After each incubation, the cells were washed twice with 4 mL of cold PBS and incubated with 1 mL 0.2% SDS solution per well for 5 min at room temperature for cell lysis, and the lysis solution was counted using a γ-counter (480 Wizard 3′, Perkin Elmer, USA). Protein content was determined by a bicinchoninic acid (BCA) protein assay (Pierce, IL). The results are expressed as pmol per milligram of protein. For iodide efflux studies, cells were incubated with 3.7 kBq (0.1 μCi) carrier-free ^{125}I-NaI and 10 μmol/L of NaI in 1 mL of HBSS incubation buffer at 37 °C for 30 min. Cells were then washed twice with HBSS, added to HBSS incubation buffer with 10 μmol/L of NaI kept at 37 °C, and further incubated. At designated time points (5, 10, 15, 20, 25, and 30 min), the buffer HBSS was removed and its radioactivity in the buffer was measured. All data points are displayed as mean ± S.D. ($n = 3$).

2.5. Proliferation and differentiation of Ad-hNIS-GFP transduced canine ADSCs

The proliferation of Ad-hNIS-GFP-canine ADSCs was compared with non-transfected canine ADSCs for 6 days. Ad-hNIS-GFP transduced and non-transduced canine ADSCs were seeded at 5×10^4 cells per dish in a 100 mm dish. Cell proliferation rate was assessed using the trypan blue dye exclusion method once every 24 h for 6 days. All data points are displayed as the mean ± S.D. ($n = 4$). To investigate the multi-lineage differentiation potential of Ad-hNIS transfected ADSCs and non-transfected ADSCs, they were differentiated into osteogenic, chondrogenic, and adipogenic lineages using STEMPRO® Osteogenesis, Chondrogenesis, and Adipogenesis Differentiation Kits (Gibco, USA), respectively. Each differentiation procedure was performed according to the manufacturer's instructions.

2.6. Permanent coronary artery ligation of canine hearts

Five male and 1 female beagle dogs, mean weight 10.09 kg, were prepared for myocardial infarction (MI) procedure. All dogs were sedated with 0.02 mg/kg of medetomidine and anesthesia was