In vitro evaluation of cardiomyogenic differentiation of bone marrow derived mesenchymal stem cells (MSCs)

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1. Introduction

Bone marrow derived mesenchymal stem cells (MSCs) are excellent candidate as therapeutic agent for cell therapy. MSCs can be expanded *in vitro* rapidly (more than 3-5 fold in a weeks), and maintained their stem cell properties for a long culture period. Recently, many investigators have suggested that MSCs have ability to differentiate into cardiomyocytes by given appropriate condition *in vitro* or *in vivo* [1, 2, 3]. Although, MSCs may be useful cell therapeutic agents in heart disease, there are still exist major barriers to track their capacity to differentiate into functional cardiomyocytes. In our previous study, the transgenic mouse model expressing sodium iodide symporter (NIS) driven by α-myosin heavy chain (α-MHC) promoter was developed to image cardiomyocyte with γ-camera and microPET *in vivo* [4]. In this study, we investigate the monitoring availability of α-MHC driven NIS gene of MSCs from the transgenic mouse during cardiomyogenic differentiation *in vitro*.

2. Methods and Results

2.1 *Imaging of single photon emission computed tomography (SPECT)/ small animal computed tomography (CT)*

At the time of imaging, a subset of transgenic mice were injected 370 MBq of ^{99m}Tc-pertechnate into lateral tail vein. 50 min after injection of ^{99m}Tc-pertechnate, the mice were placed in a spread prone position and scanned with single photon emission computed tomography (SPECT)/ small animal computed tomography (CT) scanner (Inveon) for 1 h. The mice were maintained anesthetized condition by isoflurane inhalation during imaging process. The transgenic mouse revealed a higher uptake of ^{99m}Tc-pertechnate in the heart tissue compared to control group (Fig. 1).

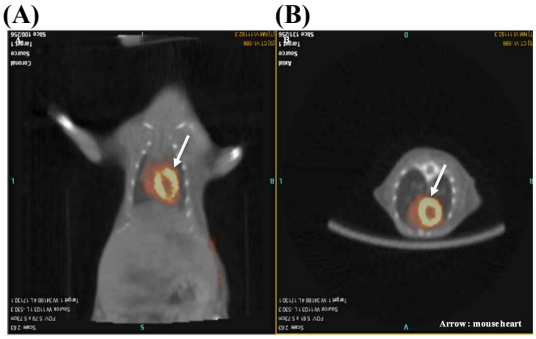

Fig. 1. SPECT/CT image of transgenic mouse using ^{99m}Tc-pertechnate. (A) Coronal section. (B) Transverse section. Transgenic mouse shows high uptake of ^{99m}Tc-pertechnate in heart tissue (arrow).

2.2 *Isolation and cardiomyogenic differentiation of MSCs*

Bone marrow derived MSCs were harvested from the femur and tibia of transgenic mice, and reseeded at $3x10^3/cm^2$ in 100 mm dish. The cells maintained with MSC basal medium. For cardiomyocytes differentiation, 1 μM all trans retinoic acid (ATRA) treated into culture every 3 days for 3 weeks. After 3 weeks, ATRA treated MSCs formed cardiomyocyte phenotype (Fig. 2).

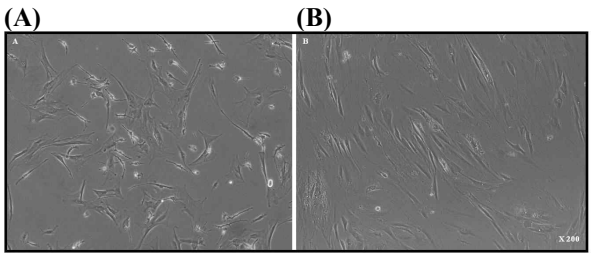

Fig. 2. Microscopic observation of cardiomyogenic differentiation of MSCs by ATRA treatment. ATRA treatment induced morphogenic change of MSCs to cardiomyocytes like cells (B), compared with control (A).

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

Total RNA was extracted from cultures using TRI reagent. 5 μg of total RNA was reverse transcribed using superscript III reverse transcriptase and random hexamer to generate complementary DNA (cDNA).

Stem cell antigen-1 (Sca-1), α-myosin heavy chain (α-MHC), myosin light chain-2 ventricle (MLC-2v) and glyceraldehydes phosphate dehydrogenase (GAPDH; an internal control) mRNA were analyzed. α-MHC and MLC-2v, were up-regulated in ATRA-treated MSCs at 3 weeks post-differentiation. But, according to cardiomyogenic differentiation, the expression of Sca-1 was down-regulated in ATRA treated MSCs (Fig. 3).

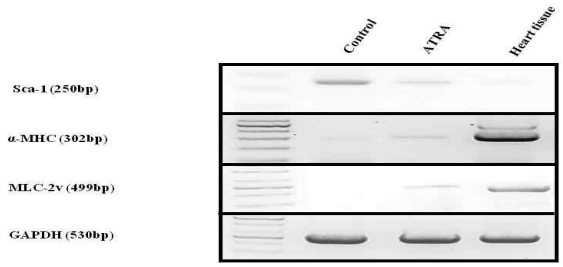

Fig. 3. Expression of cardiac specific genes (α-MHC, MLC-2v) and stem cells specific marker (Sca-1) in cardiomyogenic differentiated MSCs by ATRA treatment. According to cardimyogenic differentiation, cardiac specific genes including α-MHC, MLC-2v were up-regulated, but expression of the stem cells marker, Sca-1, was decreased.

2.4 Radioiodine uptake assay

Radioiodine uptake *in vitro* was measured as described by Nakamoto et al [5]. Briefly, the cells were plated in 6-well plates and cultured until the cells reached confluence (approximately 1.0 x 10^6 cells), ^{125}I uptake was tested. The cells were incubated at 37 ℃ for 60 min in 1 mL of Hank's balanced salt solution (HBSS) containing 0.5 % bovine serum albumin, 3.7 kBq (0.1 μCi) carrier free Na^{125}I, and 10 μmol/L of NaI to yield a specific activity of 740 MBq/mmol. The cells were washed with 4 mL of cold HBSS and the radioactivity of detached cells was counted with γ-counter. Radioiodine uptake was higher in ATRA treated MSCs than control group. The differentiated MSCs accumulated 197.30 pmol/ mg protein at 60 min (Fig. 4).

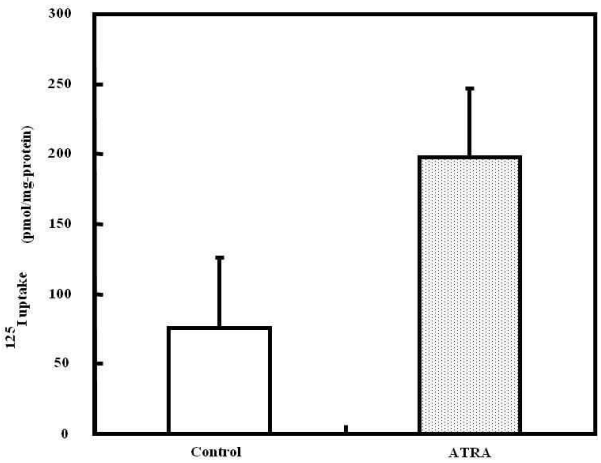

Fig. 4. radioiodine (^{125}I) uptake of cultured MSCs in the presence with or without ATRA in vitro. Cardiomyogenic lineage differentiation by ATRA treatment was shown by higher ^{125}I uptake than control.

3. Conclusions

In this study, the cardiomyocytes differentiation of MSCs from previously established transgenic mice, showed up-regulated expression of cardiac specific gene and increased activity of reporter gene *in vitro*. We demonstrated that NIS under controlled by α-MHC promoter, which showed monitoring capacity of cardiomyogenic differentiation of MSCs *in vitro*, and the reporter gene, NIS, based imaging system may be a useful imaging and monitoring tool for stem cell transplantation *in vivo.*

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