Biochemical and Biophysical Research Communications 424 (2012) 740–746

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Phorbol myristate acetate differentiates human adipose-derived mesenchymal stem cells into functional cardiogenic cells

Woochul Chang [a,1], Soyeon Lim [b,1], Byeong-Wook Song [c,d], Chang Youn Lee [e], Moon-Seo Park [a], Young-An Chung [a], Cheesoon Yoon [a], Se-Yeon Lee [c,d], Onju Ham [c,d], Jun-Hee Park [c,d], Eunhyun Choi [f], Lee-So Maeng [a,*], Ki-Chul Hwang [c,d,f,*]

[a] Institute of Catholic Integrative Medicine, Incheon St. Mary's Hospital, The Catholic University of Korea College of Medicine, Incheon 403-720, Republic of Korea
[b] Severance Integrative Research Institute for Cerebral & Cardiovascular Disease, Yonsei University Health System, 50 Yonsei-ro, Seodamun-gu, Seoul 120-752, Republic of Korea
[c] Cardiovascular Research Institute, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea
[d] Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea
[e] Department of Integrated Omics for Biomedical Sciences, Graduate School, Yonsei University, Republic of Korea
[f] Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea

ARTICLE INFO

Article history:
Received 26 June 2012
Available online 15 July 2012

Keywords:
Adipose-derived mesenchymal stem cells
Cardiac regeneration
Cardiogenic differentiation
Protein kinase C

ABSTRACT

To achieve effective regeneration of injured myocardium, it is important to find physiological way of improving the cardiogenic differentiation of stem cells. Previous studies demonstrated that cardiomyocytes from bone marrow-derived mesenchymal stem cells (BMSCs) activated with phorbolmyristate acetate (PMA), a protein kinase C (PKC) activator, restore electromechanical function in infarcted rat hearts. In this study, we investigated the effect of PMA on cardiogenic differentiation of adipose-derived MSCs (ASCs) for clinical applications. To confirm the effect of PMA, ASCs treated with 1 μM PMA were grown for nine days. The expression of cardiac-specific markers (cardiac troponin T, myosin light chain, myosin heavy chain) in PMA-treated MSCs was demonstrated by immunocytochemistry. Alhough few α_{1A} receptors exist in ASCs, α_1-adrenergic receptor subtypes were preferentially expressed in PMA-treated ASCs. Moreover, expression of the β-adrenergic and muscarinic receptors increased in PMA-treated ASCs compared to normal cells. The mRNA levels of Ca^{2+}-related factors (SERCA 2a; sarcoplasmic reticulum Ca^{2+}-ATPase, LTCC; L-type Ca^{2+} channel) in treated ASCs were similar to the levels in cardiomyocytes. Following the transplantation of chemically activated cardiogenic ASCs into infarcted myocardium, histological analysis showed that infarct size, interstitial fibrosis, and apoptotic index were markedly decreased and cardiac function was restored. In conclusion, PMA might induce the cardiogenic differentiation of human ASCs as well as BMSCs. This result suggests successful use of human ASCs in cardiac regeneration therapy.

1. Introduction

Mesenchymal stem cells (MSCs) found in adult tissues are an attractive source of stem cells for clinical applications in the regeneration of damaged tissues, immunomodulation, and *ex vivo* hematopoietic stem cell expansion [1–4]. MSCs are characterized by plastic adherent growth, specific surface antigen expression and multipotent differentiation potential [5]. When cultured within a specific environment, they are capable of differentiating into several lineages, including bone, adipose, tendon, cartilage, skeletal muscle, marrow stroma, cardiac, neural cells and connective tissue [6–8]. MSCs were originally isolated from bone marrow, but appear to be present in many tissues such as umbilical cord, umbilical cord blood, adipose tissue, dental pulp, periosteum, tendon, skin, synovial membrane, amniotic fluid, limbal tissue and menstrual blood [6,8]. The origin of MSCs is highly important in determining their biological activity; clinical applications might thus differ according to stem cell niche.

Recently, interest has grown in the developmental plasticity and therapeutic potential of stromal cells isolated from adipose tissue, called adipose-derived mesenchymal stem cells (ASCs). The quantity and accessibility of subcutaneous adipose tissue in humans makes it an attractive alternative to bone marrow as a

Abbreviations: ASCs, adipose-derived mesenchymal stem cells; BMSCs, bone marrow-derived mesenchymal stem cells; PMA, phorbolmyristate acetate.

* Corresponding authors. Address: Severance Biomedical Institute, Cardiovascular Research Institute, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, Republic of Korea. Fax: +82 2 365 1878 (K.-C. Hwang). Fax: +82 32 280 6160 (L.-S. Maeng).
E-mail addresses: mls@catholic.ac.kr (L.-S. Maeng), kchwang@yuhs.ac (K.-C. Hwang).

[1] These authors contributed equally to this study.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.022

source of adult stem cells [9–11]. Clinically relevant stem cell numbers can be extracted from isolated adipose tissue because it possesses higher stem cell proliferation rates than bone marrow-derived MSCs (BMSCs). Reports of successful isolation and differentiation of stem cells derived from human adipose tissue have stimulated further studies regarding the ubiquity, similarity, and multipotency of these cells compared to BMSCs [11,12].

Previously, we reported that transplantation of BMSCs to enhance survival rate did not provide a survival benefit compatible with significant improvement in cardiac contractile function because the focal application of MSCs that had not differentiated into electrically functional cardiomyocytes created fixed heterogeneity among host tissues in the engrafted region, possibly predisposing the heart to ventricular arrhythmia. To create a cell type that is able to synchronize with surrounding cardiomyocytes electromechanically, we treated BMSCs with phorbolmyristate acetate (PMA), a protein kinase C (PKC) activator. In previous research for small molecules that induce stem cells to specific lineages, we screened 189 chemicals (135 inhibitors and 54 activators of protein kinases) and found several compounds that induced differentiation of rat BMSCs to myocytes. Of these, PMA upregulated cardiogenic properties in BMSCs. Chemically activated cardiogenic MSCs (ccMSC) prevented sudden death after engraftment into infarcted rats by electromechanically synchronizing with the host myocardium [13,14].

In this study, we examined the effect of PMA on specific differentiation of human ASCs to functional cardiomyocytes. For the present study, we investigated whether PMA induced the expression of cardiac-specific markers and neurohumoral-related receptors. Following the transplantation of chemically activated cardiogenic ASCs into infarcted myocardium, histological analysis showed that infarct size, interstitial fibrosis and apoptotic index were markedly decreased and cardiac function was restored. This finding suggested the successful use of human ADC in cardiac regeneration therapy.

2. Materials and methods

2.1. Culture and characterization of ASCs

ASCs were from Invitrogen (STEMPRO human adipose-derived stem cells; Catalog No. R7788-115). Cells were expanded using MesenPRO RS™ basal medium and growth supplement plus reduced-serum medium (2% fetal bovine serum).

For ASC characterization by immunophenotyping, third-passage ASCs were labeled for various markers including CD34, CD90, CD105, CD106, and intracellular adhesion molecule-1 (ICAM-1) and analyzed by confocal microscopy (LSM700 system; Carl Zeiss) and flow cytometry (FACS Calibur; Becton Dickinson). Cells were harvested, washed with PBS, and labeled with various markers. Fluorescinisothiocyanate (FITC)- and Texas red-conjugated secondary antibody (Jackson Immunoresearch Laboratories) were used at appropriate concentrations.

2.2. Induction of cardiogenic differentiation

ASCs were seeded in 60 mm plates at 2×10^5 cells and PKC activator (PMA, phorbolmyristate acetate; Sigma) was added to a final concentration of 1 μM. At induction of cardiogenic differentiation, we added Dulbecco's modified Eagle's medium (DMEM)-low glucose, supplemented with 10% fetal bovine serum and 1% antibiotic-penicillin and streptomycin solution (Invitrogen). Media were replaced with PMA treatment media every 3 days for a maximum of 9 days.

2.3. Immunofluorescence

Immunocytochemistry of cells is described below. Cells were seeded in a 4-well slide chamber, washed with PBS, and incubated in 4% formaldehyde solution for 10 min. Cells were washed twice with PBS before cell permeabilization in 0.1% Triton X-100 for 7 min. Cell blocking proceeded for 1 h (blocking solution: 3% bovine serum albumin and 10% horse serum in PBS) and stained with primary antibodies. Secondary antibodies were incubated with blocking buffer, and samples were detected by confocal microscopy (Carl Zeiss).

2.4. RT-PCR

Gene expression levels were validated using reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted with Tri-reagent (Sigma) and complementary DNA was generated with the Reverse Transcription System (Promega) according to the manufacturer's instructions. RT-PCR was performed using TaKaRa EX Taq™ at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 49 °C for 30 s, and 72 °C for 1 min before a final extension at 72 °C for 10 min. Human primers were as follows; α_{1A}-adrenergic receptor (AR): 5′-ATGCTCCAGCCAAGAGTTCA-3′, 5′-TCCAAGAAGAGCTGGCCTTC-3′; α_{1B}-AR: 5′-CTGTGCGCCATCTCCA TCGATCGCTAC-3′, 5′-ATGAAGAAGGGTAGCCAGCACAAGATGAA-3′; α_{1D}-AR: 5′-CTCTGCACCATCTCCGTGGA CCGGTAC-3′, 5′-AAAGAA-GAAAGGGAACCAGCAGAGCACGAA-3′; β_1-AR: 5′-CTCTGC TGGCTGC CCTTCTTC-3′, 5′-GGGCTTCGAGTTCACCTGCTATC-3′; β_2-AR: 5′-TGAGTGTGCAGGACGAGTCC-3′, 5′-ATTGGGTGCCAGCAAGAAGG-3′; muscarinic acetylcholine receptor (MR) subtype 1 (MR_1): 5′-CAG-GCAACCTGCTGGTACTC-3′, 5′-CGTGCTCGGTTCTCTGTCTC-3′; MR_2: 5′-CTCCTCTAACAATAGCCTGG-3′, 5′-GGCTCCTTCTTGTCCTTCTT-3′; sarcoplasmic reticulum Ca^{2+} ATPase (SERCA 2a): 5′-ACACCAAAT AAACCAAGCAG-3′, 5′-TTTTAGAC TTGTGGGAGGGT-3′; L-type Ca^{2+} channel (LTCC): 5′-TGGAAGCTCAGCTCCAACAG-3′, 5′-TC CTGGTAG-GAGAGCATCTC-3′; GAPDH: 5′-CATGGGTGTGAACCATGAGAA-3′, 5′-GGTCAT GAGTCCTTCCACGAT-3′. Products were separated by electrophoresis in 1.2% agarose gels, and visualized after staining with ethidium bromide.

2.5. Induction of myocardial infarction and cell transplantation

All experimental procedures for animal studies were approved by the Committee for the Care and Use of Laboratory Animals, Yonsei University College of Medicine, and performed in accordance with the Committee's Guidelines and Regulations for Animal Care. The myocardial infarction (MI) model was male Sprague–Dawley rats (250 g) induced with surgical occlusion of the left anterior descending (LAD) coronary artery, according to previously published procedures [15]. For cell transplantation, ASCs or cardiogenic cells (CGCs) were detached from the plate, suspended in 30 μl of PBS (1×10^6 cells), and transplanted at three injection sites into the viable myocardium bordering the infarction using a Hamilton syringe (Hamilton Co.) with a 30-gauge needle. Animals were ventilated with 95% O_2 and 5% CO_2 using a ventilator (Harvard Apparatus). Nine animals per group were used for morphologic analysis at 1 week and functional analysis at 3 weeks after MI and cell transplantation.

2.6. Measurement of infarct size and fibrosis area

Staining with 2,3,5-triphenyltetrazolium chloride (TTC) was used to measure myocardial infarct size. Tissue slices were incubated in 1% TTC (Sigma–Aldrich) solution, pH 7.4, at 37 °C for 20 min. Hearts were fixed in 10% formalin diluted with PBS overnight at 2–8 °C. Tissues were sectioned horizontally, and MI size

was evaluated as the percentage of unstained tissue of the LV to the sectional area of the whole LV. TTC-stained tissue was photographed with a digital camera.

Fibrosis area was analyzed by Masson's trichrome staining. The interstitial fibrotic area was measured as a percentage of fibrous tissue of the LV to the sectional area of the whole LV using ImageJ 1.45 (NIH).

2.7. Terminal deoxynucleotidyltransferase–mediated dUTP nick-end labeling (TUNEL) assay

TUNEL assay was performed according to the instructions of the manufacturer (Chemicon International Inc.). Mice were sacrificed at 7 days after transplantation and hearts were fixed with 10% formaldehyde for 24 h. After 5 μM of paraffin-embedded slide were deparaffinized, dehydrated, and rinsed with PBS, antigen retrieval was performed with 10 mM sodium citrate (pH 6.0) in a microwave oven for 10 min. Sections were treated with 3.0% H_2O_2, TdT enzyme for 1 h, and digoxigenin-conjugated nucleotide substrate at 37 °C for 30 min. Nuclei exhibiting an apoptotic index were visualized after staining with 3,3-diamino benzidine (DAB) (Vector Laboratories) for 5 min, with sections counterstained with methyl green. The nuclei of apoptotic cardiomyocytes stained dark brown. Sections were observed by light microscopy (Nikon).

2.8. Echocardiography

Transthoracic echocardiography was measured according to previously published procedures [16] and performed by using a GE Vivid Seven ultrasound machine (GE Medical System) with a 10.0 MHz transducer after LAD ligation and cell transplantation. Rats received general anesthesia. The echo transducer was placed on the left hemithorax and short axis views were recorded.

2.9. Statistical analysis

Results are expressed as mean ± SEM. Statistical comparisons between the two groups were performed using the Student's *t*-test. One-way ANOVA using a Bonferroni test was used when comparing more than two groups. A p-value < 0.05 was considered significant.

3. Results

3.1. Morphology and immunophenotype of ASCs

As shown in Fig. 1A, the morphology of third-passage ASCs was observed by microscopy. The ASCs showed a uniform fibroblast-like shape similar to BMSCs [16]. To determine the specific phenotypes of ASCs, we used immunofluorescence. ICAM-1 (CD54), CD105, and CD106 are surface protein markers of human ASCs [17]. CD105 and ICAM-1 were expressed, whereas CD34 expression was not induced in ASCs (Fig. 1B). Other surface markers were examined by flow cytometry. We found high expression of CD 90 (98.57%) and CD106 (99.13%), while CD34 was negative.

3.2. Cardiogenic differentiation of ASCs

We previously induced CGCs from rat MSCs using PMA (a protein kinase C activator) [18]. To investigate whether cardiogenic differentiation was induced in ASCs by regulating PKC signaling, we treated ASCs with PMA for 9 days, as in a previous study [13]. Immunocytochemical staining showed differences between ASCs (Fig. 2A) and CGCs (Fig. 2B). An ASC-positive marker, CD105, had a stable expression level, but cardiac-specific markers such as cTnT, MLC, and MHC were not expressed in ASCs. In CGCs, cTnT, MLC and MHC showed time-dependent expression. CD105 was not expressed. Connexin 43 (Cx43) expression was markedly increased in CGCs but not ASCs in a time-dependent manner.

3.3. Alteration of genes specific for cardiac functional regulation

To address whether receptor-mediated alteration was involved in specific differentiation of rat MSCs into CGCs, RNA was extracted 9 days after cardiogenic induction and analyzed by RT-PCR. Significant increases in expression were seen, by 4.2-fold for α_{1A}- and 2.6-fold for α_{1B}-AR, but not for α_{1D}-AR (Fig. 3A). For β-AR levels, β_1- and β_2-AR were also upregulated, 2.4- and 2.3-fold, respectively

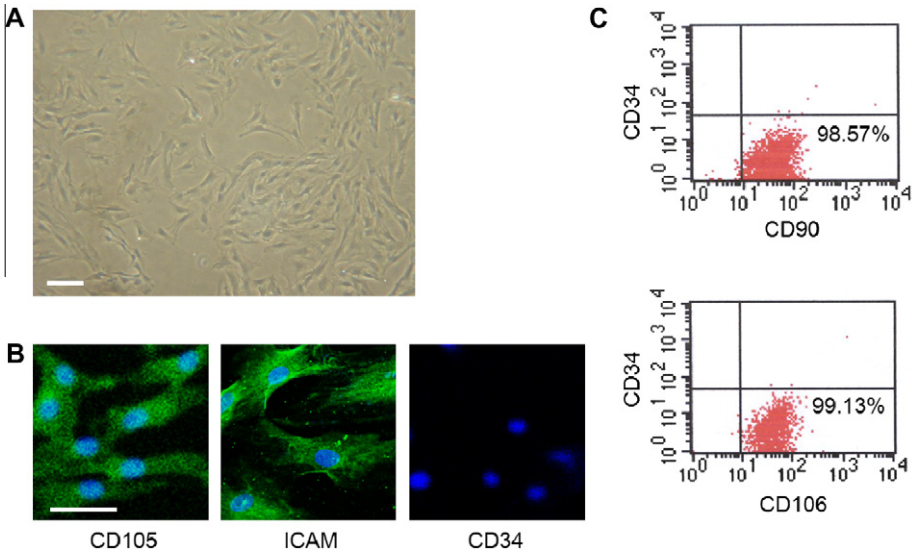

Fig. 1. Characterization of ASCs. (A) Morphology of third-passage ASCs (scale bar = 100 μm; magnification, 100×). (B) Immunophenotype of ASCs using confocal microscopy. ASC-positive or negative markers analyzed by confocal microscopy (scale bar = 50 μm; magnification, 400×). Images of CD105 (green), intracellular adhesion molecule-1 (ICAM; green) or DAPI (blue) were shown. (C) Immunophenotype of ASCs using FACS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

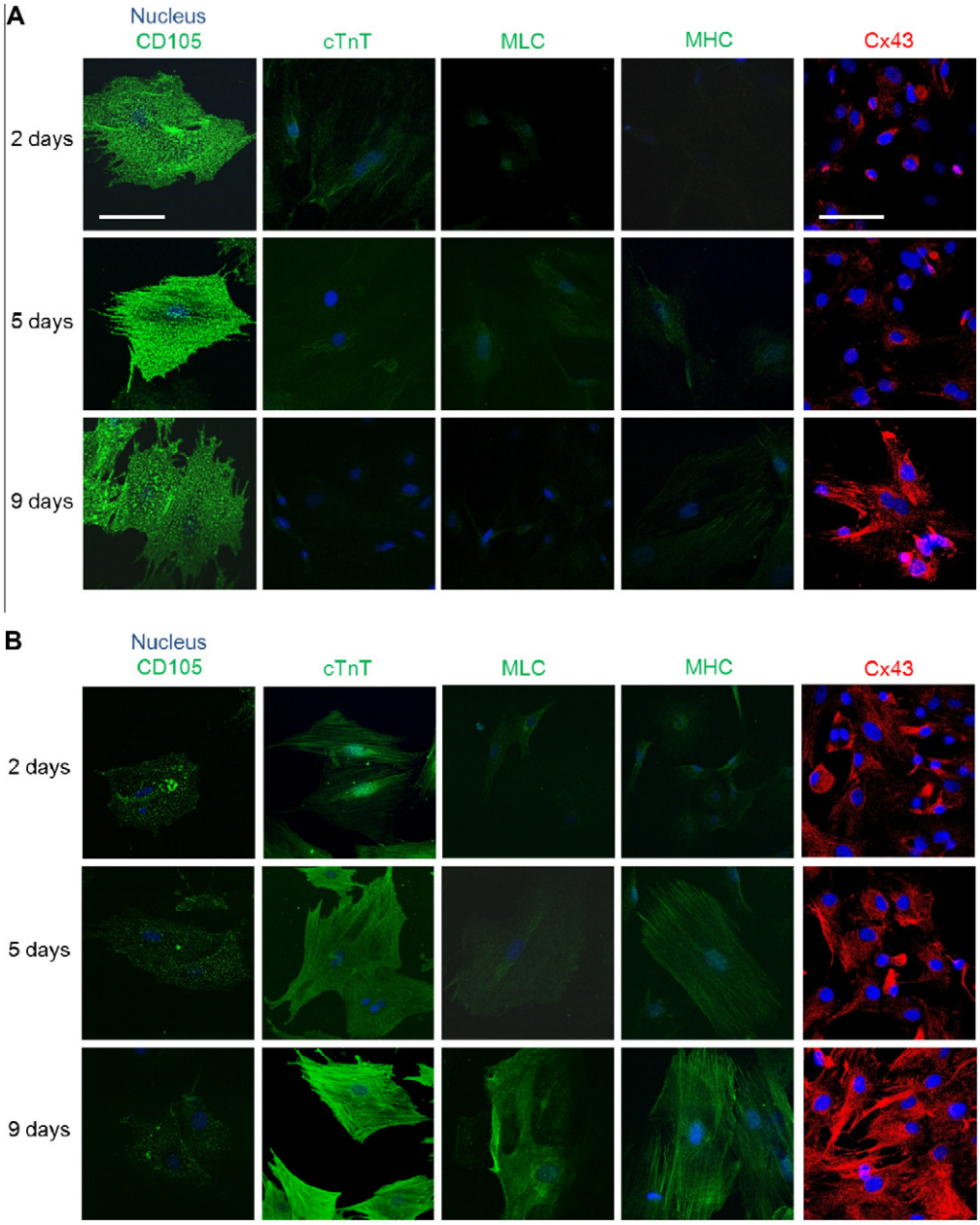

Fig. 2. Time-dependent expression in the cardiac differentiation of ASCs. Immunocytochemical analysis was performed for altered expression of the ASC-specific marker CD105 and cardiac-specific markers cTnT, MLC, MHC, and Cx43 at 9 days after treatment. (A) Expression in normal ASCs. (B) Expression in PMA-treated ASCs. (CD105, cTnT, MLC, and MHC: scale bar = 50 μm; magnification: 400×, Cx43: scale bar = 200 μm; magnification: 100×).

(Fig. 3B). Furthermore, MR associated with individual cardiac function was increased 2.9-fold (M_1-MR) and 2-fold (M_2-MR) (Fig. 3C).

Cardiomyocytes participate in excitation-contraction (EC) coupling. The molecular mechanisms responsible for Ca^{2+}-handling are the closely related SERCA 2a [18] and LTCC [19]. Their mRNA levels were also significantly increased in CGCs, but not in control ASCs (Fig. 3D).

3.4. Improvement of myocardial ischemia in CGC-transplanted rats

Histological changes after cell transplantation in myocardial infarctions were evaluated by TTC, trichrome, and TUNEL staining. TTC and trichrome stain showed that infarct size and interstitial fibrosis areas were significantly decreased in the CGC-injected group compared with the ASC-injected group (Fig. 4A and B). In addition, TUNEL-positive cells (apoptotic index) induced by ischemic conditions in the transplanted region were markedly lower in the CGC-transplanted group than the ASC-transplanted group (Fig. 4C). Results of histological analysis are in Fig. 4D. We investigated the expression pattern of Cx43 in cell-transplanted regions because of its involvement in cell-cell connection [18]. In the CGC-engrafted group, GFP-labeled CGCs and Cx43 were highly co-expressed compared to the ASC-transplanted group (Fig. 4E).

The cardiac performance parameters of the infarcted myocardium after cell transplantation were calculated using transthoracic echocardiography. As Table 1 shows, transplantation of CGCs improved LV systolic performance and systolic function compared with the other groups. Specifically, the peak circumferential and radial strain in the infarct zone and global LV were improved in the CGC-engrafted group compared with MSC group.

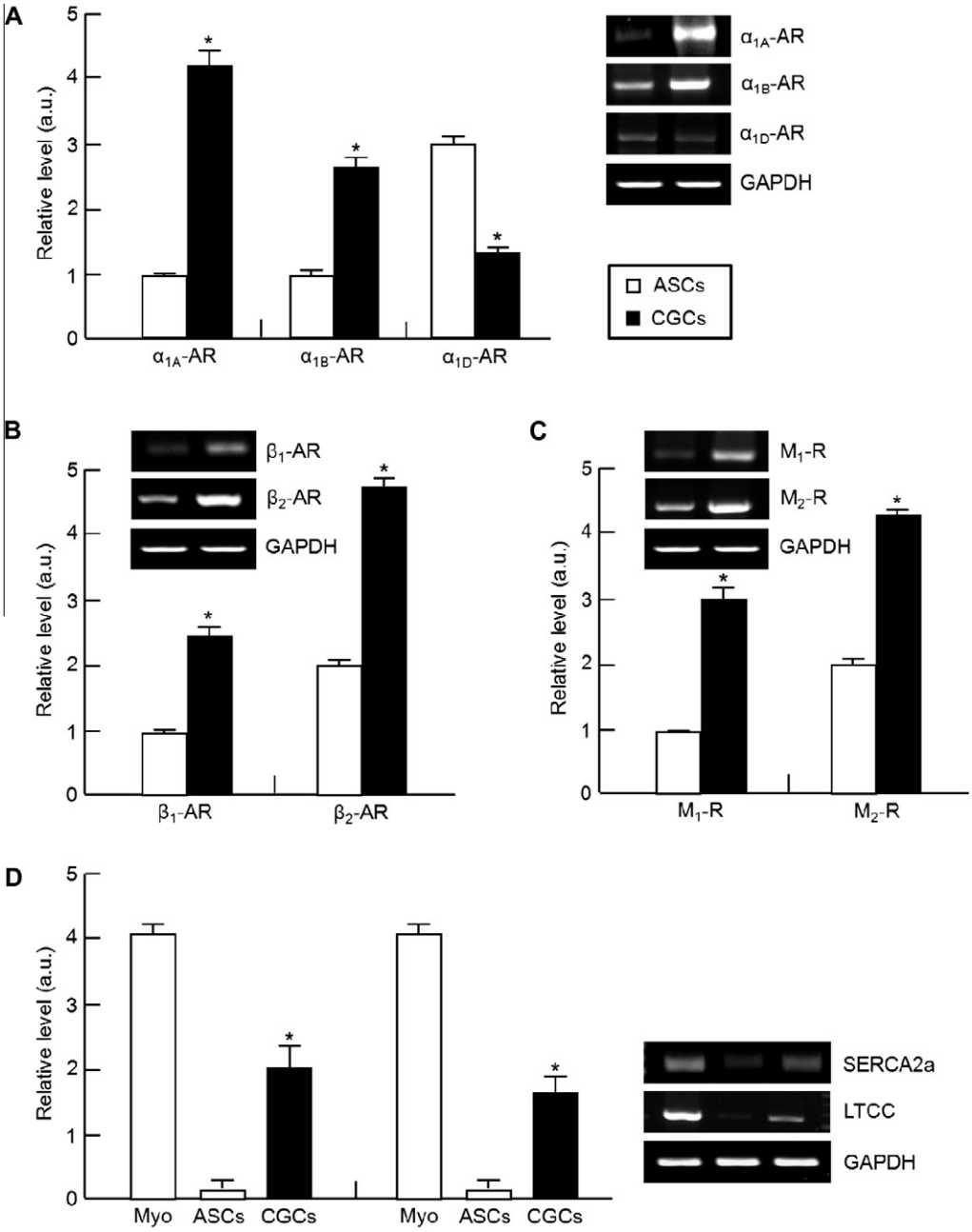

Fig. 3. Altered expression of a cardiac-specific gene in CGCs. Receptors that mediate the function of cardiomyocytes were measured by RT-PCR in ASCs and CGCs. Alteration of α_1-adrenergic receptor (AR) isotypes (A), β-AR isotypes (B), muscarinic receptor isotypes (C), and Ca^{2+} homeostasis-related proteins (D) (*$p < 0.05$ vs. ASCs).

4. Discussion

BMSCs hold promise for cell-based therapy in ischemic heart disease but they still face several challenges such as delivery, survival after transplantation, and electromechanical integration and safety [3]. Although preconditioning of BMSCs, including genetic modification to enhance the survival of transplanted stem cells, has increased their therapeutic potency, the most obvious concern for clinical applications is how engrafted MSCs electromechanically integrate with host tissue [16,20,21]. Previously, we reported that following transplantation, undifferentiated BMSCs contribute to increasing the susceptibility to ventricular arrhythmia, leading to sudden death. To create a cell type that is able to synchronize with surrounding cardiomyocytes electromechanically, we treated BMSCs with PMA. This generated CGCs that prevent sudden death after engraftment into infarcted heart [13]. However, the previous study had limited clinical application because the process of bone marrow harvesting can be painful and yields a limited quantity of aspirate. Recently, MSCs have been isolated from adipose tissue, which would be an ideal source because of the procurement procedure and yield. The major finding of this study was that human ASCs can differentiate into functional cardiomyocytes with PMA treatment.

We found that PMA induced the expression of cardiogenic markers (cTnT, MLC and MHC) compared to normal ASCs (Fig. 2). To confirm whether differentiated ASCs became functional cardiomyocytes through a molecular biological approach, expression levels of neurohumoral factors was determined. Neurohumoral factors control heart rate, myocardial contractility, and cardiac hypertrophy. The most powerful system controlling cardiac func-

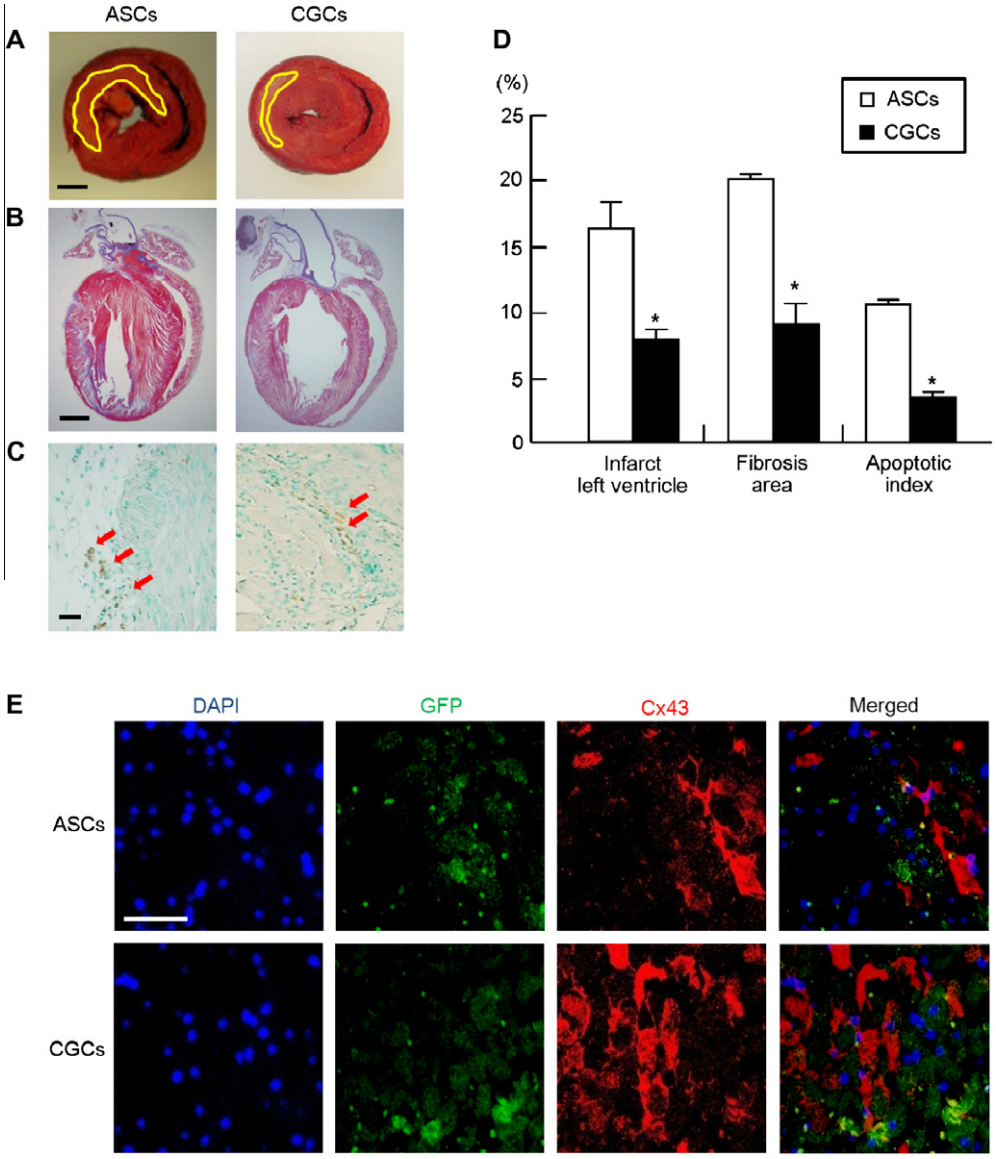

Fig. 4. Immunohistochemical analysis for ischemic myocardium with cell transplantation. (A) TTC staining for determination of left ventricle infarct size (scale bar = 2 mm). (B) Masson's trichrome staining for determination of fibrosis area (scale bar = 2 mm). (C) TUNEL assay for the number of apoptotic cells (scale bar = 200 μm, magnification: 100×). (D) Result of infarct size, fibrosis area, and apoptotic index (*$p < 0.05$ vs. ASCs). (E) Immunostaining for GFP-labeled ASCs or CGCs, and Cx43 (scale bar = 200 μm, magnification: 100×).

tion is the autonomous nervous system, including the sympathetic and parasympathetic nerves acting through adrenergic and muscarinic receptors. The adrenergic receptors are divided into α-adrenergic and β-adrenergic subtypes, and muscarinic receptors have five subtypes, M_1 through M_5 [22]. We explored α_{1A}, α_{1B}, α_{1D}, β_1, and β_2-adrenergic receptors and M_1 and M_2 muscarinic receptors. The characteristics of receptors are critical in modulating cardiac function. The α_{1A}-, α_{1B}-, α_{1D}- receptors cause catecholamine-induced increases in heart rate, conduction velocity and contractility. The β_1- and β_2-adrenergic receptors show expression in murine neonatal and adult cardiomyocytes. M_1 and M_2 are muscarinic receptors [23,24]. Surprisingly, when ASCs that had been treated with PMA for nine days were compared with normal ASCs, the differentiated ASCs had a higher expression of these receptors (Fig. 3).

Cardiomyocytes are linked by EC coupling, which operates by Ca^{2+} influx. LTCC and SERCA 2a are especially meaningful factors in this process. The mRNA of SERCA 2a is a myocardial marker similar to the heart muscle-specific marker gene cTnT. Moreover, a 1000-fold Ca^{2+}-gradient is maintained across the cardiac sarcoplasmic reticulum membrane by these factors, and LTCC is physiologically important in many excitable cells, particularly in the heart. Ca^{2+} entry through these channels not only contributes to impulse generation and conduction but also serves as a second messenger to modulate regulatory protein kinases and the activation of contractile proteins. As seen in Fig. 3, even though cardiomyocytes had a slightly higher expression level of LTCC and SERCA 2a, expression was enhanced in differentiated ASCs.

Histological analysis showed that infarct size and interstitial fibrosis were markedly decreased in CGC-injected rats compared to ASC-injected rats. In addition, the number of apoptotic cells induced by ischemia in the transplanted region was significantly lower in the CGC-injected animals than in ASC-injected animals (Fig. 4). Moreover, our result that Cx43 expression was markedly increased in the CGCs was supported by the result that Cx43-

Table 1
Comparison of cardiac function parameters.

Variables	Control	Sham	ASCs	CGCs
Heart rate (bpm)	236.8 ± 12.30	260 ± 13.47	253 ± 8.98	245.75 ± 7.14
LVEDD (mm)	5.67 ± 0.23	7.64 ± 1.27$^{\int}$	6.82 ± 0.77	6.92 ± 0.28$^{\int\int}$
LVESD (mm)	3.80 ± 0.22	6.90 ± 1.15$^{\int}$	5.92 ± 0.61$^{\int}$	5.62 ± 0.35$^{\int,\#}$
FS (%)	35.99 ± 2.63	9.79 ± 1.05$^{\int}$	13.08 ± 2.11$^{\int}$	20.07 ± 1.29$^{\int,*}$
LVESV (ml)	0.43 ± 0.05	1.03 ± 0.42$^{\int}$	0.74 ± 0.25§	0.79 ± 0.13$^{\int\int,\#}$
LVEDV (ml)	0.13 ± 0.01	0.78 ± 0.32§	0.49 ± 0.15	0.42 ± 0.07
LVEF (%)	70.59 ± 4.41	24.74 ± 2.39$^{\int}$	32.29 ± 4.62$^{\int,\#}$	46.49 ± 2.79$^{\int,*}$
Peak S cir (%; infarct zone)	−14.99 ± 2.41	−2.11 ± 1.86$^{\int}$	−5.02 ± 0.19$^{\int}$	−9.52 ± 2.79§,*
Peak S rad (%; infarct zone)	25.96 ± 0.35	2.19 ± 1.24$^{\int}$	13.54 ± 0.96$^{\int,*}$	21.17 ± 3.83§,*
Global S cir (%)	−15.49 ± 1.43	−5.00 ± 0.62$^{\int}$	−7.50 ± 0.84$^{\int,\#}$	−11.34 ± 1.87$^{\int,*}$
Global S rad (%)	32.75 ± 1.19	4.97 ± 1.48$^{\int}$	19.05 ± 0.97$^{\int,*}$	29.58 ± 1.43§,*

Values are mean ± S.D.
Abbreviations: LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; FS, fractional shortening; LVESV, left ventricular end systolic volume; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; S cir, circumferential strain; S rad, radial strain.
$^{\int}$ $p < 0.001$ vs. control.
$^{\int\int}$ $p < 0.05$ vs. control.
§ $p < 0.01$ vs. control.
* $p < 0.001$ vs. sham.
$^{\#}$ $p < 0.05$ vs. sham.

expressing cells prevent postinfarct arrhythmia [25]. Following transplantation of CGCs, LV systolic performance and systolic function were restored compared with other groups (Table 1).

Ultimately, the application of this cell type might facilitate the prevention of sudden death caused by naive ASCs transplantation, providing clinical strategies for enhancing integration in cell-based therapy for MI. In summary, this study confirmed that PMA induced the specific differentiation of human ASCs as well as BMSCs into functional cardiomyocytes. This finding could represent an important methodology in the clinical application of human ASCs in ischemic heart disease.

Acknowledgments

This research was supported by a Korea Science and Engineering Foundation grant funded by the Korean government (MEST) (2011-0019243, 2011-0019254) and a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A085136).

References

[1] P.K. Mishra, Bone marrow-derived mesenchymal stem cells for treatment of heart failure: is it all paracrine actions and immunomodulation? J. Cardiovasc. Med. 9 (2008) 122–128.
[2] Y. Miyahara, N. Nagaya, M. Kataoka, et al., Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction, Nat. Med. 12 (2006) 459–465.
[3] V.F. Segers, R.T. Lee, Stem-cell therapy for cardiac disease, Nature 451 (2008) 937–942.
[4] S.S. Kelly, C.B.S. Sola, M. de Lima, et al., Ex vivo expansion of cord blood, Bone Marrow Transplant. 44 (2009) 673–681.
[5] M. Dominici, K. Le Blanc, I. Mueller, et al., Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement, Cytotherapy 8 (2006) 315–317.
[6] U. Nekanti, V.B. Rao, A.G. Bahirvani, et al., Long-term expansion and pluripotent marker array analysis of Wharton's jelly-derived mesenchymal stem cells, Stem Cells Dev. 19 (2010) 117–130.
[7] M.F. Pittenger, A.M. Mackay, S.C. Beck, et al., Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[8] Y. Zhu, T. Liu, K. Song, et al., Adipose-derived stem cell: a better stem cell than BMSC, Cell Biochem. Funct. 26 (2008) 664–675.
[9] A.J. Friedenstein, R.K. Chailakhjan, K.S. Lalykina, The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells, Cell Tissue Kinet. 3 (1970) 393–403.
[10] J.K. Fraser, I. Wulur, Z. Alfonso, et al., Fat tissue: an underappreciated source of stem cells for biotechnology, Trend Biotechnol. 24 (2006) 150–154.
[11] P.A. Zuk, M. Zhu, H. Mizuno, et al., Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng. 7 (2001) 211–228.
[12] P.A. Zuk, M. Zhu, P. Ashjian, et al., Human adipose tissue is a source of multipotent stem cells, Mol. Biol. Cell 13 (2002) 4279–4295.
[13] H. Song, H.J. Hwang, W. Chang, et al., Cardiomyocytes from phorbolmyristate acetate-activated mesenchymal stem cells restore electromechanical function in infarcted rat hearts, Proc. Natl. Acad. Sci. 108 (2011) 296–301.
[14] K.C. Hwang, J.Y. Kim, W. Chang, et al., Chemicals that modulate stem cell differentiation, Proc. Natl. Acad. Sci. 105 (2008) 7467–7471.
[15] L.Y. Eun, B.W. Song, M.J. Cha, et al., Overexpression of phosphoinositide-3-kinase class II alpha enhances mesenchymal stem cell survival in infarcted myocardium, Biochem. Biophys. Res. Commun. 402 (2010) 272–279.
[16] H. Song, W. Chang, S. Lim, et al., Tissue transglutaminase is essential for integrin-mediated survival of bone marrow-derived mesenchymal stem cells, Stem Cells 25 (2007) 1431–1438.
[17] J.M. Gimble, Adipose tissue-derived therapeutics, Expert Opin. Biol. Ther. 3 (2003) 705–713.
[18] L. Missiaen, W. Robberecht, L. van den Bosch, et al., Abnormal intracellular ca(2+)homeostasis and disease, Cell Calcium 28 (2000) 1–21.
[19] L.S. Song, A. Guia, J.N. Muth, et al., Ca(2+) signaling in cardiac myocytes overexpressing the alpha(1) subunit of L-type Ca(2+) channel, Circ. Res. 90 (2002) 174–181.
[20] W. Chang, B.W. Song, S. Lim, et al., Mesenchymal stem cells pretreated with delivered Hph-1-Hsp70 protein are protected from hypoxia-mediated cell death and rescue heart functions from myocardial injury, Stem Cells 27 (2009) 2283–2292.
[21] S.W. Song, W. Chang, B.W. Song, et al., Integrin-linked kinase is required in hypoxic mesenchymal stem cells for strengthening cell adhesion to ischemic myocardium, Stem Cells 27 (2009) 1358–1365.
[22] C.C. Felder, Muscarinic acetylcholine receptors: signal transduction through multiple effectors, FASEB J. 9 (1995) 619–625.
[23] O.E. Brodde, H. Bruck, K. Leineweber, et al., Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart, Basic Res. Cardiol. 96 (2001) 528–538.
[24] G.L. Song, M. Tang, C.J. Liu, et al., Developmental changes in functional expression and beta-adrenergic regulation of I(f) in the heart of mouse embryo, Cell Res. 12 (2002) 385–394.
[25] W. Roell, T. Lewalter, P. Sasse, et al., Engraftment of connexin 43-expressing cells prevents postinfarct arrhythmia, Nature 450 (2007) 819–824.