GATA-4 promotes myocardial transdifferentiation of mesenchymal stromal cells via up-regulating IGFBP-4

HONGXIA LI[1,*], **SHI ZUO**[1,†], **ZEESHAN PASHA**[1], **BIN YU**[1], **ZHISONG HE**[1,*], **YIGANG WANG**[1], **XIANGJUN YANG**[2], **MUHAMMAD ASHRAF**[1], and **MEIFENG XU**[1]

[1]Department of Pathology and Laboratory Medicine, University of Cincinnati Medical Center, Cincinnati, USA

[2]Department of Cardiology, the First Affiliated Hospital of Soochow University, Suzhou, P. R. China

Abstract

Background aims—GATA-4 is a cardiac transcription factor and plays an important role in cell lineage differentiation during development. We investigated whether overexpression of GATA-4 increases adult mesenchymal stromal cell (MSC) transdifferentiation into a cardiac phenotype *in vitro.*

Methods—MSC were harvested from rat bone marrow (BM) and transduced with GATA-4 (MSC^GATA-4) using a murine stem cell virus (pMSCV) retroviral expression system. Gene expression in MSC^GATA-4 was analyzed using quantitative reverse transcription–polymerase chain reaction (RT-PCR) and Western blotting. Native cardiomyocytes (CM) were isolated from ventricles of neonatal rats. Myocardial transdifferentiation of MSC was determined by immunostaining and electrophysiologic recording. The transdifferentiation rate was calculated directly from flow cytometery.

Results—The expression of cardiac genes, including brain natriuretic peptide (BNP), Islet-1 and α-sarcomeric actinin (α-SA), was up-regulated in MSC^GATA-4 compared with control cells that were transfected with Green Fluorescent Protein (GFP) only (MSC^Null). At the same time, insulin-like growth factor-binding protein (IGFBP)-4 was significantly up-regulated in MSC^GATA-4. A synchronous beating of MSC with native CM was detected and an action potential was recorded. Some GFP + cells were positive for α-SA staining after MSC were co-cultured with native CM for 7 days. The transdifferentiation rate was significantly higher in MSC^GATA-4. Functional studies indicated that the differentiation potential of MSC^GATA-4 was decreased by knockdown of IGFBP-4.

Conclusions—Overexpression of GATA-4 significantly increases MSC differentiation into a myocardial phenotype, which might be associated with the up-regulation of IGFBP-4.

Keywords

cardiomyocyte; co-culture; GATA-4; genetic engineering; insulin-like growth factor-binding protein-4; stem cell

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Introduction

Regenerative medicine in cardiology is a new field based on the possibility of replacing lost or damaged cardiac cells during ischemia and improving cardiac function by cell transplantation. The bone marrow (BM) is an easily accessible source of autologous adult stem and progenitor cells. BM-derived mesenchymal stromal cells (MSC) are multipotent and have been demonstrated to improve cardiac function following intramyocardial transplantation into infarcted myocardium (1–4). Their ability to repair the myocardium depends on transdifferentiation into myocardial progenitor cells and the protection of the native myocardium under ischemic insult.

Although there is great enthusiasm for repairing the ischemic heart by using cell therapy, simple cell implantation cannot reinstate cardiac function because of the relatively low rate of stem cell differentiation (5,6). Genetic engineering of MSC represents a useful strategy for boosting the therapeutic potency of MSC (7,8). GATA-4 is highly expressed in cardiomyocytes (CM) throughout embryonic development, postnatal growth and adulthood, during which it functions as a critical regulator of cardiac differentiation (9). In postnatal CM, GATA-4 is essential for maintaining the cardiac genetic program (10) and for the adaptive response of the heart to numerous stimuli, including hormones and work overload (11,12). Increased expression of GATA-4 inhibits doxorubicin (DOX)-induced autophagy and reduces CM death, whereas GATA-4 gene silencing triggers autophagy and renders DOX more toxic (13). GATA-4 has also been shown to be essential for cardiac differentiation of pluripotent (P19) stem cells to form beating CM (14,15). Depletion of GATA-4 prevents terminal differentiation and induces apoptosis of the pre-cardiac cells, whilst gain-of-function studies induce ectopic beating CM (15). We have reported that previously overexpression of GATA-4 in MSC significantly increases MSC survival in ischemic myocardium (16). Recently, Rysa *et al.* (17) reported that the reversal of reduced GATA-4 activity prevents adverse post-infarction remodeling through myocardial angiogenesis, anti-apoptosis and stem cell recruitment. However, it is unclear whether and how GATA-4 can increase the adult BM-sourced MSC transdifferentiation into cardiac phenotypes. Our preliminary mRNA microarray study indicated that many mRNA were up-regulated or down-regulated in MSC transduced with GATA-4 (MSC^{GATA-4}) compared with MSC transduced with GFP only (MSC^{Null}). Several mRNA related to anti-oxidative stress and myocardial markers are listed in supplementary Table 1. The expression of insulin-like growth factor-binding protein (IGFBP)-4 was significantly higher in MSC^{GATA-4} than that in MSC^{Null} (9.21 ± 0.60 fold, $P < 0.00091$ versus MSC^{Null}). IGFBP-4 is a member of the IGFBP family (18–20). Recently, several studies have suggested that IGFBP-4 has insulin-like growth factor (IGF)-independent actions on cellular functions (21,22). It has been reported that IGFBP-4 enhances P19CL6 cell differentiation into a cardiac phenotype (23). Our results indicate that GATA-4 promotes MSC transdifferentiation into myocardial phenotypes, which may be associated with up-regulating IGFBP-4.

Methods

All protocols conformed to the *Guidelines for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH) (NIH publication number 85–23, revised 1996) and was approved by the University of Cincinnati (Cincinnati, OH, USA) Animal Care and Use Committee.

MSC culture and transduction with GATA-4 plasmid

MSC were isolated according to a method described previously (24), with some modifications. In brief, femurs and tibias were obtained from Sprague Dawley (SD) rats.

BM cells were flushed and cultured with Iscove's modified Dulbecco's medium (IMDM) supplemented with 20% fetal bovine serum and penicillin (100 U/mL)/streptomycin (100 μg/mL) at 37°C in humid air with 5% CO_2.

The second passage of MSC was used to transduce recombinant GATA-4, as described previously (16). In brief, a retrovirus expressing GATA-4 was constructed using a murine stem cell virus (pMSCV) retroviral expression system (Clontech, Mountain View, CA, USA). IRES-EGFP was cloned into pMSCV vectors at *Xho*I and *Eco*RI sites, and then GATA-4 was excised from pcDNA-GATA-4 (25) with *Hin*dIII and *Xho*I restriction enzymes and cloned into pMSCV-IRES-EGFP at *Bgl*II and *Sal*I sites. GP2-293 cells (Clontech) were co-transfected with pMSCV-GATA-4-IRES-EGFP and pVSVG; control GP2-293 cells were co-transfected with pMSCV-IRES-EGFP and pVSVG. After 48 h, supernatants were filtered and incubated with MSC in the presence of 10 μg/mL polybrene (Sigma, St. Louis, MO, USA) for 12 h. Stable GATA-4- and Green Fluorescent Protein (GFP)-expressing clones were acquired by selecting with puromycin (3 μg/mL; Sigma) for 5 days. The expression of GATA-4 was verified by immunostaining, quantitative real-time–polymerase chain reaction (PCR) and Western blot. pMSCV-IRES-EGFP was transfected into MSC and served as a transfection control (MSC[Null]).

Quantitative reverse transcription–PCR

Total RNA from MSC was isolated using an RNeasy mini kit (Qiagen, Valencia, CA, USA). Quantitative reverse transcription–PCR (RT-PCR) was carried out on an iQ5 real-time system with iQ SYBR Super-mix (Bio-Rad, Hercules, CA, USA) as described previously (26). In brief, complementary DNA was synthesized using SuperScript™ III First-Strand Synthesis for RT-PCR (Invitrogen, Carlsbad, CA, USA) and cDNA was amplified using *Taq* DNA polymerase in the presence of primers (Table I). The expression of each target mRNA relative to Glycer-aldehyde-3-phosphate dehydrogenase (GAPDH) was calculated based on the threshold cycle (C_T) as $r = 2^{-\Delta(\Delta C_T)}$, where $\Delta C_T = C_{T\,target} - C_{T\,GAPDH}$ and $\Delta(\Delta C_T) = \Delta C_{T\,experimental} - \Delta C_{T\,control}$.

Electroimmunoblotting

The protein of different cardiac markers was quantified using Western blotting. Cells were washed in phosphate-buffered saline (PBS) and homogenized in lysis buffer. Protein concentrations were quantified with Bio-Rad DC-Protein Assay Reagent (Bio-Rad). Denatured proteins (25 and 50 μg) were separated using 12% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), transferred to a nitrocellulose membrane (Bio-Rad), and immunoblotted overnight at 4°C with primary antibodies against IGFBP-4 (Abcam, Cambridge, MA, USA), anti-brain natriuretic peptide (BNP; Abcam), Islet-1 (Santa Cruz, CA, USA), α-sarcomeric actinin (α-SA; Sigma) and β-actin (Cell Signaling, Danvers, MA, USA). Membranes were then incubated for 1 h with Horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature, washed and developed with an ECL plus kit (GE Healthcare, Pittsburgh, PA, USA). Blots were analyzed by densitometry with NIH image software (AlphaEase FC, version 6.0.0).

Immunocytochemistry

Cells cultured on glass coverslips were fixed in 4% paraformaldehyde, and incubated with mouse monoclonal anti-α-SA (Sigma) and rabbit polyclonal anti-GATA-4 (Abcam). After thorough washing, secondary antibodies of goat anti-mouse IgG or goat anti-rabbit IgG conjugated with Alexa Fluor-568 (Invitrogen) were applied. Nuclei were stained with 4′,6-diamino-2-phenylindole (DAPI). Confocal images were obtained with a Zeiss LSM 510 confocal/two-photon microscope equipped with Zeiss AIM Version 4.2.

CM culture and co-culture with MSC

Native CM were isolated from ventricles of 1–3-day-old SD rats using a commercially available neonatal CM isolation kit (Worthington Biochemical Co., Lakewood, NJ, USA), in accordance with the supplier's protocol. To investigate transdifferentiation, MSC were plated with CM at a ratio of 1:40 in either mixed co-cultures or a dual-chamber system. In some experiments, neonatal CM were labeled with a red fluorochrome membrane dye, PKH26 (Sigma), before co-culture with MSC. Cell beating was recorded using a charge-coupled device (CCD) camera connected to an inverted phase–contrast microscope equipped with fluorescent filters (Olympus, Center Valley, PA, USA). To quantify the transdifferentiation rate of MSC, co-cultured cells were trypsinized and incubated with anti-α-SA antibody (Sigma) conjugated with allophycocyanin (APC). At least 20 000 cells were analyzed on a BD FACS-Calibur cell sorter (BD Biosciences, San Jose, CA, USA).

Action potential recordings

Intracellular potential changes were measured in cultured cells with glass microelectrodes filled with solution containing (in mM) cesium aspartate 115, CsCl 20, ethylene glycol tetraacetic acid (EGTA 11), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 10, $MgCl_2$ 2.5 and Mg-adenosine triphosphate (ATP 2) (pH 7.2), and had a resistance of 1.5–2.5 MΩ (27,28). Isolated single cells, including GFP$^-$ (native CM) and MSC (GFP$^+$) either cultured alone or co-cultured with CM, were maintained at room temperature (24°C) and perfused with Tyrode's solution containing (in m_M) NaCl 140, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1.8, HEPES 5 and glucose 10 (pH 7.4). The action potential (AP) was recorded using the whole-cell patch clamp technique with an Axon-patch-200B amplifier (Axon Instruments, Foster City, CA, USA).

Statistical analysis

Quantitative data were expressed as mean ± SEM. One-way analysis of variance (SigmaStat 3.1; Systat Software, San Jose, CA, USA) with the Holm–Sidak method and/or Bonferroni correction was used to determine the significance of differences. Differences were considered significant if the *P*-value was less than 0.05.

Results

MSC differentiation into cardiac phenotype

Native myocytes were isolated from neonatal rat ventricles. After being cultured for 24 h, native CM began to beat spontaneously. Immunostaining showed that all CM were positive for α-SA and myofibers were seen with clear Z-lines in sarcomeres. Nuclei were centrally located and homogeneous (Figure 1A).

Both MSC^{GATA-4} and MSC^{Null} were GFP immunopositive; only MSC^{GATA-4} stained intensely for GATA-4 (Figure 1B). Quantitative real-time PCR data indicated that the expression of GATA-4 was significantly increased in MSC^{GATA-4} (Figure 1C). MSC-$^{GATA-4}$ also exhibited higher levels of GATA-4 protein (Figure 1D).

MSC were distinguished from CM on the basis of GFP positivity when they were co-cultured with CM that were pre-labeled with PKH26. We did not observe spontaneous beating from MSC. However, a synchronous contraction of MSC with CM was noted (supplementary video A–D). Furthermore, the beating MSC (GFP$^+$) was connected with rat CM and shown to be PKH26$^+$, which suggested that PKH26 had been transferred from native CM into MSC. To determine whether these cells had a CM-like function, the AP of single isolated cells was recorded. MSC co-cultured with CM showed similar AP to that of neonatal CM (Figure 2A), indicating electrical activity and the existence of functional

sarcolemmal ion channels in these cells. Co-cultured cells were characterized further by immunostaining with α-SA. Some GFP$^+$ MSC were also α-SA$^+$. These cells had GFP$^+$ nuclei, which excluded the possibility of GFP$^+$ cell overlay with α-SA$^+$ CM (Figure 2B). However, compared with native CM, differentiated cells were immature CM with weak α-SA staining.

Cardiac gene expression in MSC was analyzed using quantitative RT-PCR. BNP, α-SA and Islet-1 were significantly up-regulated in MSC^{GATA-4} compared with MSC^{Null}, either cultured alone (Figure 3A) or co-cultured with CM in a dual-chamber system for 7 days (Figure 3B).

GATA-4 up-regulated IGFBP-4 and enhanced MSC myocardial differentiation

It was noted that IGFBP-4 was significantly up-regulated in MSC^{GATA-4} in our microarray study. The up-regulation of IGFBP-4 in MSC^{GATA-4} was further confirmed by Quantitative reverse transcription-PCR (qRT-PCR) and Western blot. IGFBP-4 expression was significantly up-regulated in MSC^{GATA-4} (compared with MSC^{Null}) for mRNA (Figure 4A) and protein (Figure 4B) levels. To study the role of IGFBP-4 in GATA-4-mediated myocardial transdifferentiation, IGFBP-4 activity was knocked down in MSC^{GATA-4} using double-stranded smart pool small interfering RNA (siRNA) (Santa Cruz; sc-39590, CA, USA), designed to target IGFBP-4. Non-silencing, scrambled siRNA (Ambion; 4390843, TX, USA) was used as a negative control. Quantitative real-time PCR showed that the expression of IGFBP-4 in IGFBP-4-siRNA-MSC^{GATA-4} (IG4-siRNA) was only 38% of that in MSC^{GATA-4} ($P < 0.05$). IGFBP-4 protein was reduced in IG4-siRNA cells by 28% compared with MSC^{GATA-4} ($P < 0.05$). No significant change in expression of IGFBP-4 in scrambled siRNA-transfected MSC^{GATA-4} (Sc-siRNA) was detected.

After culturing MSC with native CM in a dual-chamber system, no MSC beating was detected after 7 days. The cardiac proteins α-SA, BNP and Islet-1 in MSC^{Null} were very low (α-SA $8.9 \pm 2.5\%$, BNP $3.6 \pm 1.0\%$, Islet-1 $3.2 \pm 1.2\%$ in CM, respectively) compared with native CM. All of these proteins were significantly increased in MSC^{GATA-4} (α-SA $43.3 \pm 5.4\%$, BNP $14.5 \pm 3.1\%$, and Islet-1 $10.4 \pm 2.3\%$, respectively; $P < 0.05$ versus MSC^{Null}). However, the effect of GATA-4 on expressing cardiac markers in MSC was suppressed when IGFBP-4-siRNA was transfected (Figure 5).

Furthermore, the transdifferentiation of various MSC was quantified using fluorescence-activated cell sorting (FACS) after MSC were co-cultured with native CM for 7 days. The co-cultured cells were stained with α-SA-conjugated with APC. Native CM were α-SA$^+$, MSC were GFP$^+$, and cardiac phenotypes that transdifferentiated from MSC were positive for both α-SA and GFP. The transdifferentiation rate, i.e. the percentage of α-SA$^+$/GFP$^+$ cells among total GFP$^+$ cells, was higher in MSC^{GATA-4} ($34.09 \pm 4.65\%$) than in MSC^{Null} ($14.54 \pm 1.62\%$; $P < 0.05$). However, this effect of MSC^{GATA-4} was abolished by transfecting IGFBP-4-siRNA (Figure 6). No significant difference was detected in Sc-siRNA-transfected MSC^{GATA-4} compared with MSC^{GATA-4}. These results indicated that the up-regulation of IGFBP-4 might play an important role in myocardial transdifferentiation of MSC mediated by GATA-4.

Discussion

The potential of MSC^{GATA-4} differentiation into a cardiac phenotype was investigated *in vitro* not only by analyzing the expression of cardiac markers but also by recording cell beating and AP. Our study indicated that MSC engineering with GATA-4 achieved superior functional and structural benefits in myocardial transdifferentiation, which might be associated with up-regulation of IGFBP-4.

The studies in our laboratory and others have shown that implanted stem cells can repair in part the infarcted heart both structurally and functionally (1,29–31). The contribution of implanted cells to cardiac repair and/or recovery of function can occur through transdifferentiation of implanted cells into myocytes and endothelial cells, and subsequent myocardial regeneration. Rota *et al.* (29) have reported how transplanted BM cells (BMC) regenerated myocardium from engraftment in the infarcted myocardium to form a functional myocyte. Within 12–48 h after infarction and cell implantation, donor cells were integrated structurally with resident cells. Junctional and adhesion complexes were detected between transplanted BMC, and between BMC and adjacent resident myocytes and fibroblasts. Engraftment of BMC shortly after their delivery was followed by myocardial regeneration, which expanded from 5 to 10 and 30 days after infarction. Donor cell-derived myocytes exhibited electrical characteristics similar to spared myocytes but showed a prolongation of the AP and enhanced cell shortening at 15–30 days post-transplantation (29).

In this study, the transdifferentiation of MSC into a cardiac phenotype was evidenced by the expression of cardiac markers, cell contraction and CM-like AP in GFP$^+$ MSC after co-culture with native CM for 7 days. All of these results indicated that MSC had the potential to transdifferentiate and behave as a functional myocardial phenotype.

An alternative possibility for cardiac transdifferentiation involves fusion of MSC with native CM. Some studies have suggested that the phenotypic changes of stem cells are the result of cell fusion (32, 33). It has been reported that cell fusion occurs in approximately 30–40% of cells that acquire a myogenic phenotype (34). We observed directly the contraction of GFP$^+$ MSC following CM beating (supplementary video A–D). The MSC were connected with native CM. The beating MSC showed both green and red fluorescence signals under a fluorescence microscope. We have investigated the possibility of cell fusion and cell communication between MSC and CM in a previous study (26). Approximately 40% MSC were PKH26$^+$ after being co-cultured with PKH26 pre-labeled CM for 7 days. MSC showing double positivity for both PKH26 and GFP were connected with CM by gap junctions expressing Connexin43, or fused with native CM showing multinucleated cells. To investigate further the role of cell connection and fusion in MSC transdifferentiation into cardiac phenotypes, we cultured MSC with CM in a dual-chamber system. The results showed that the expression of cardiac-associated genes was up-regulated (Figure 5), but no beating cells were detected in MSC after 7 days of culture. We did not find α-SA$^+$/GFP$^+$ cells in MSC cultures with native CM in a dual-chamber system. This suggests that specific communication between stem cells and native myocytes is required in the transdifferentiation of stem cells into matured CM. These results indicate that cell connection or fusion is necessary for MSC transdifferentiation into CM-like cells.

The results obtained from quantitative real-time PCR, Western blotting and FACS demonstrated that MSC^{GATA-4} had a higher differentiating potential compared with vector-transduced control MSC (MSC-^{Null}). Our study is consistent with a previous report that GATA-4 enhances MSC transdifferentiation into a cardiac phenotype (14,15,35). It is well known that GATA-4 is a member of the GATA family of zinc finger transcription factors and is an early cardiomyocyte marker. GATA-4 plays an important role in transducing nuclear events that modulate cell lineage differentiation (9,11,12).

We have shown in this study that the expression of IGFBP-4 is up-regulated in MSC^{GATA-4} and transfection of IGFBP-4-siRNA into MSC^{GATA-4} significantly reduces transdifferentiation of MSC^{GATA-4}. Recently, it has been reported that IGFBP-4 enhances P19CL6 cell differentiation into a cardiac phenotype. Treatment with IGF-I and IGF-II or neutralizing antibodies against IGF-I and IGF-II had no effect on the IGFBP-4-induced CM differentiation of P19CL6 cells (23). These observations indicate that IGFBP-4 induces CM

differentiation in an IGF-independent manner. Reporter gene assays and β-catenin stabilization assays revealed that IGFBP-4 was the most potent canonical signaling pathway (Wnt) inhibitor (23). Canonical Wnt proteins bind to Frizzled receptors, which then complex with the co-receptor LRP (Low-density lipoprotein (LDL) receptor-related protein). The signal is transduced by β-catenin, which enters the nucleus to modulate expression of target genes (36). It has been shown that canonical Wnt signals inhibit cardiogenesis. Activation of Wnt/β-catenin signaling in the late phase after embryoid body (EB) formation inhibits CM differentiation (37). Inhibition of canonical Wnt signaling promotes cardiomyocyte differentiation in embryonic stem cells and in chick, *Xenopus* and zebrafish embryos (37–39). To investigate whether IGFBP-4 promotes the differentiation of P19CL6 cells into CM by inhibition of the canonical Wnt pathway, Zhu *et al.* (23) showed that the expression of dominant-negative LRP6 in P19CL6 enhanced CM differentiation of these cells and reversed the inhibitory effect of IGFBP-4 knockdown on cardiomyogenesis. These results therefore collectively suggest that IGFBP-4 promotes cardiogenesis by antagonizing the Wnt/β-catenin pathway through direct interactions with Frizzled and LDL receptor-related proteins LRP-5 or LRP-6 (LRP5/6). Further study is needed to explore how GATA-4 up-regulates IGFBP-4 resulting in MSC myocardial transdifferentiation.

GATA-4 promotes MSC differentiation into myocardial phenotypes after co-culture with native CM. The augmentation of GATA-4 on MSC-mediated myogenesis can be abrogated by transfecting IGFBP-4-siRNA into MSC^{GATA-4}. This suggests that GATA-4 in MSC transdifferentiation into a cardiac phenotype is, at least partially, associated with up-regulating IGFBP-4.

Acknowledgments

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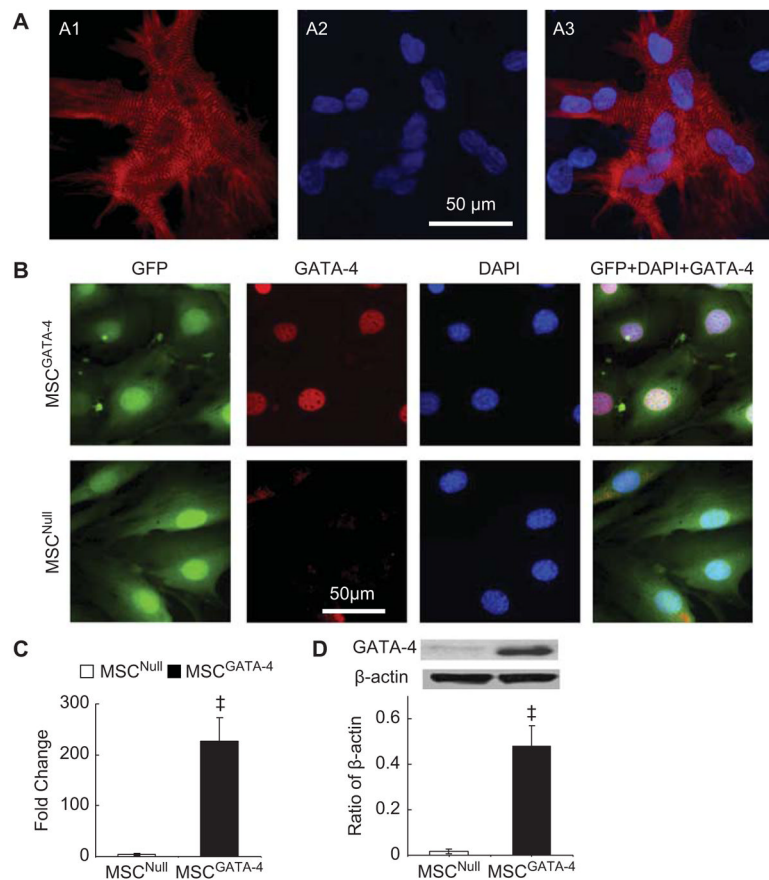
Figure 1.
Characterization of cultured CM and MSC transduced with GATA-4 (MSC^{GATA-4}) or empty vector (MSC^{Null}). (A) Primary cultured CM (5 days) were stained with α-SA antibody. CM were positive for α-SA (red) and myofibers were seen with clear Z-lines in sarcomeres. Nuclei were counterstained with DAPI. (A1) α-SA (red); (A2) DAPI; (A3) merge of A1 and A2. (B) MSC were stained with GATA-4 antibody. Both MSC^{GATA-4} and MSC^{Null} were GFP$^+$, only MSC^{GATA-4} stained intensely for GATA-4 in nuclei (red). (C) Quantitative real-time PCR of GATA-4 expression. (D) Western blot of GATA-4 and corresponding semi-quantitative data. [‡]$P < 0.05$ versus MSC^{Null}.
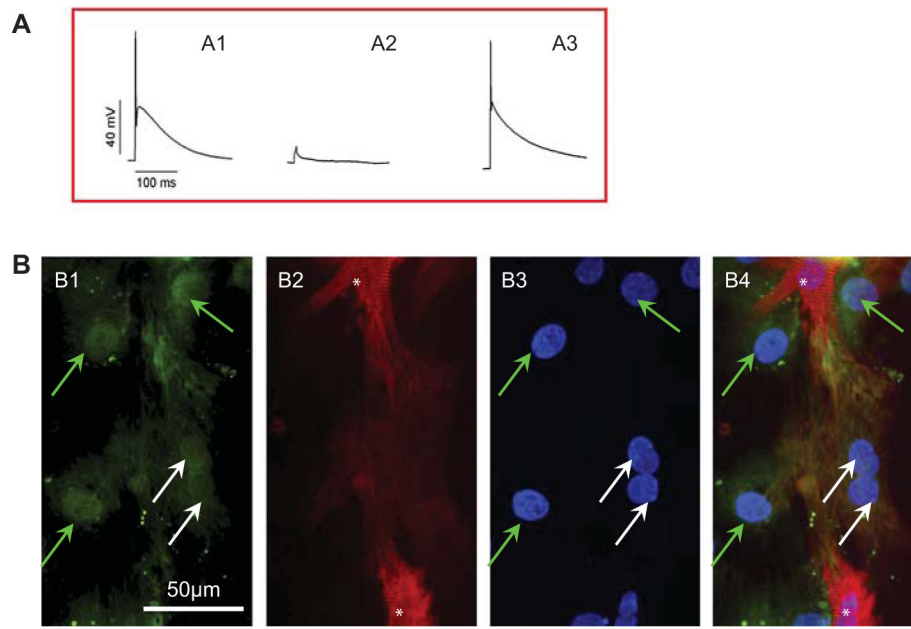
Figure 2.
MSC^{GATA-4} differentiated into cardiac phenotypes after MSC^{GATA-4} were co-cultured with CM for 7 days. (A) AP recorded from various cells: (A1) MSC^{GATA-4} co-cultured with CM; (A2) MSC^{GATA-4} culture alone; (A3) neonatal CM. (B) Representative micrographs showing GFP, α-SA immunolabeling and DAPI staining. (B1) GFP; (B2) α-SA; (B3) DAPI; (B4) merge of B1–B3. White arrows, transdifferentiated MSC^{GATA-4} (GFP$^+$/α-SA$^+$ cell); green arrows, non-differentiated MSC^{GATA-4} (GFP$^+$/α-SA$^-$ cell); white stars, native CM.
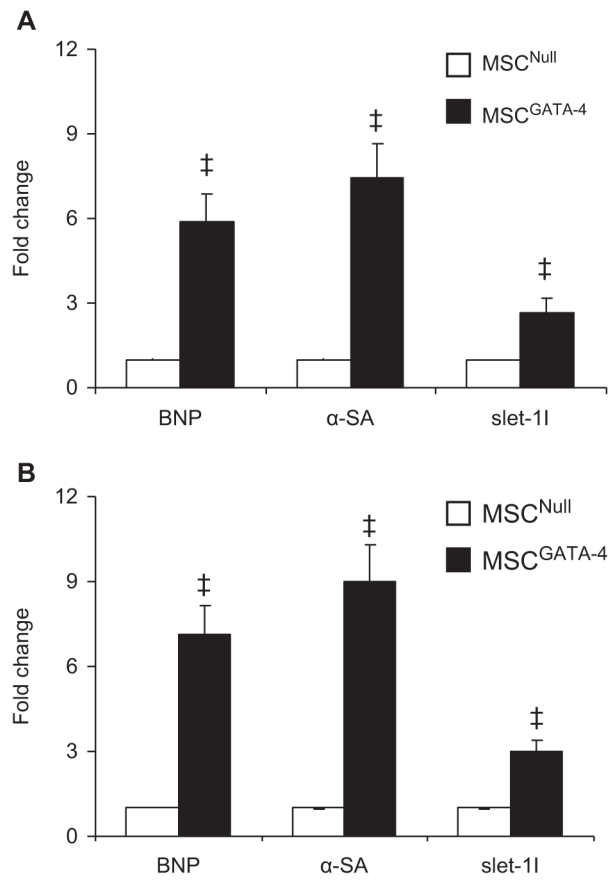
Figure 3.
Expression of cardiac markers in MSC analyzed with quantitative real-time PCR. (A) MSC alone; (B) MSC co-cultured with CM in dual-chamber system for 7 days. $^{\ddagger}P < 0.05$ versus MSC^{Null}.
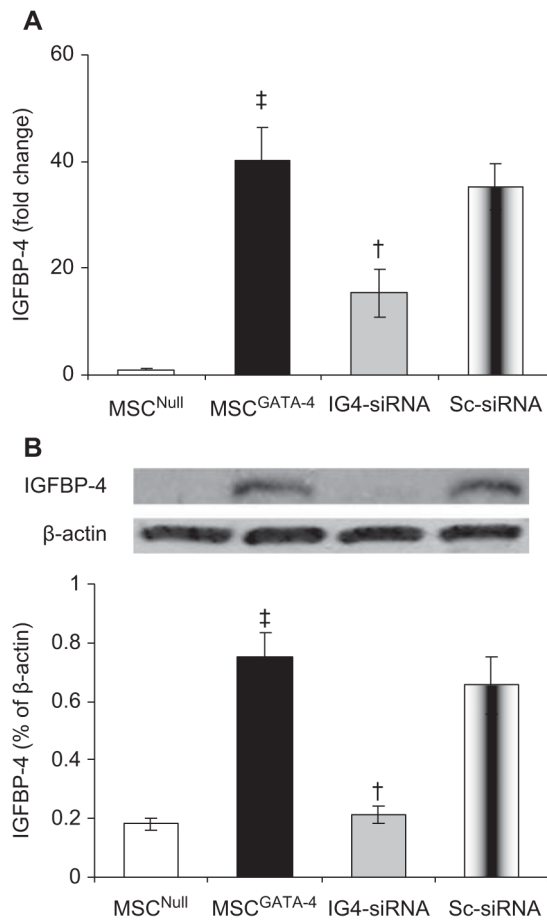
A

B

Figure 4.
Expression of IGFBP-4 in MSC transduced with different genes. (A) Quantitative real-time PCR; (B) Western blot of IGFBP-4 and corresponding semi-quantitative data. $^{\ddagger}P < 0.05$ versus MSC[Null]. $^{\dagger}P < 0.05$ versus MSC[GATA-4]. IG4-siRNA, IGFBP-4-siRNA-MSC[GATA-4]; Sc-siRNA, scrambled siRNA- MSC[GATA-4].
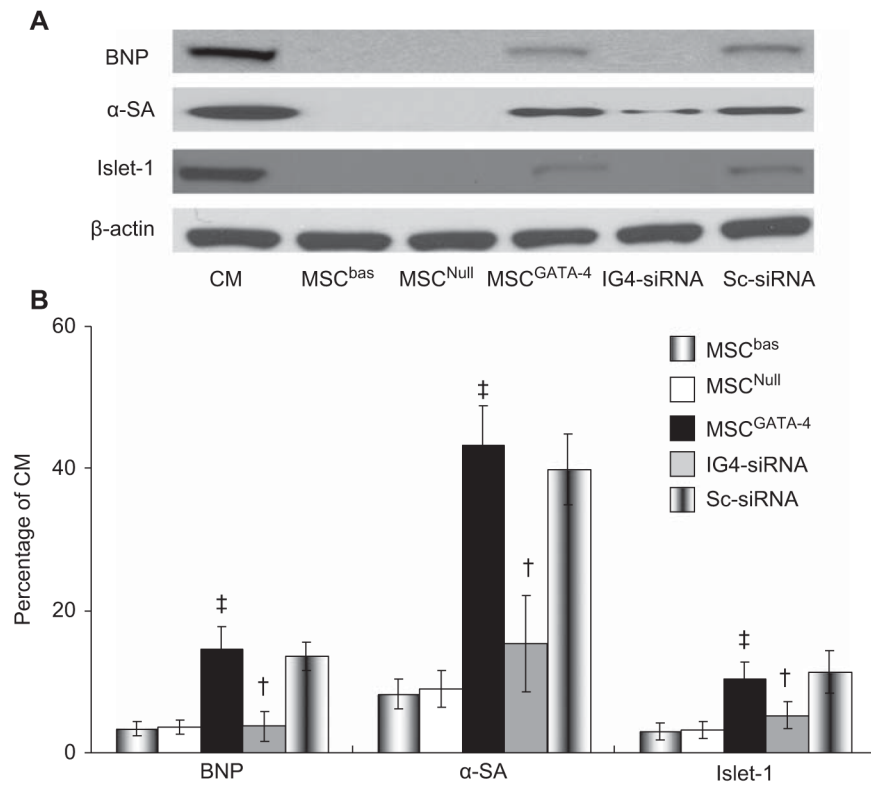
Figure 5.
Western blot analysis of MSC expressing cardiac markers when co-cultured with native CM in dual-chamber system for 7 days. (A) Representative Western blot of cardiac markers; (B) semi-quantitative data of cardiac markers expressed as a ratio to native CM. $\ddagger P < 0.05$ versus MSC^{Null}; $\dagger P < 0.05$ versus MSC^{GATA-4}.
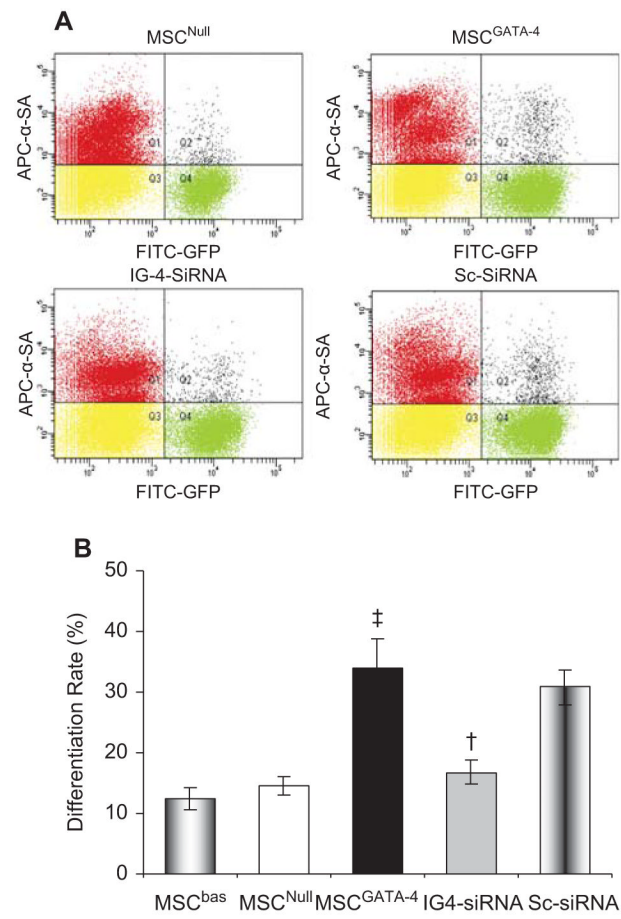
Figure 6.
Transdifferentiation rate of various MSC assessed by flow cytometery following co-culture with native CM for 7 days. (A) Representative flow cytometry data of α-SA$^+$/GFP$^+$ cells. (B) Quantitative assay in various groups. ‡$P < 0.05$ versus MSC^{Null}; †$P < 0.05$ versus MSC^{GATA-4}.

Table I

Sequence for each primer.

Primers	Sense	Anti-sense
IGFBP-4	5′-GAG CCG TAC CCA CGA AGA C	5′-GAC TCA GGC CAA GAC TCC AT
BNP	5′-TTC AGC CTC GGA CTT GGA AAC	5′-CCT TGT GGA ATC AGA AGC AGG
α-SA	5′-TCA TCC TCC TGG GCC ATG T	5′-TAT CAC GCG GCG AAC CA
Islet-1	5′-CAG CTG CAC ACC TTG CGG AC	5′-GTG TAT CTG GGA GCT GCG AG
GAPDH	5′-ATG GGA GCT GGT CAT CAA C	5′-CCA CAG TCT TCT GAG TGG CA