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# *Gata4*, *Tbx5* and *Baf60c* induce differentiation of adipose tissue-derived mesenchymal stem cells into beating cardiomyocytes

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ABSTRACT

The adipose tissue-derived mesenchymal stem cells (ADMSCs) are extensively utilized in tissue engineering, regenerative medicine and cell therapy. ADMSCs can differentiate into cardiomyocytes, and it has been shown that over-expression of a cocktail of factors can induce ectopic heart formation and program cardiogenesis in ESCs. However, which genes are responsible for differentiation of ADMSCs into beating cardiomyocyte-like cells remains unknown. In this study we have shown that the combination of *Gata4, Tbx5* and *Baf60c* is sufficient for inducing ADMSCs to form cardiomyocytes. It also appears that, while *Gata4* and *Baf60c* are key inducers of myocardial differentiation, *Tbx5* is essential for the ability of cardiac cells to contract. These findings provide additional experimental references for myocardial tissue engineering in the emerging field of cell-based therapy of heart diseases.



## 1. Introduction

Q2 Adipose tissue-derived mesenchymal stem cells (ADMSCs) are ideal stem cells in tissue engineering, regenerative medicine and cell therapy. Although many studies have shown that 5-azacytidine (5-aza) could induce ADMSCs to differentiate into cardiomyogenic cells *in vitro* (Fukuda, 2001), 5-aza is cytotoxic and MSCs induced by 5-aza exhibit mixed and disorderly electrical activity without the special changes in myocardial cells (Balana et al., 2006; Liu et al., 2013). It is, therefore, essential to develop safe and effective methods which could induce ADMSCs into differentiated myocardiocytes. Recent studies found that human ADMSCs could differentiate into myocardial cells by using rat myocardial lysate, and the extent of differentiation was concentration dependent, suggesting that the lysates contain some soluble substances with the ability of inducing differentiation (Gaustad et al., 2004; Jumabay et al., 2009). The complexity of lysate composition, however, creates many uncertainties in terms of clinical application. In another study by Robert et al. (Rose et al., 2008), bone marrow-derived MSCs from adult female transgenic mice expressing green fluorescent protein (GFP) under the control of the cardiac-specific-myosin heavy chain promoter were co-cultured with male rat embryonic cardiomyocytes. Their data showed that although MSCs demonstrated cell lineage plasticity by acquiring cardiomyocyte markers, they did not develop the electrical properties of true cardiomyocytes (Rose et al., 2008).

Recent studies have also shown that MSCs could be induced to differentiate into cardiomyocytes-like cells but not functional beating cardiomyocytes (Carvalho et al., 2013; Gao et al., 2014; Raynaud et al., 2013). The molecular regulatory mechanisms governing myocardial differentiation are a focus of a number of recent studies (Dixon et al., 2011; Ieda et al., 2010) However, which genes are, in fact, responsible for reprogramming ADMSCs into beating cardiomyocyte-like cells is still unknown. The present work is aimed to explore the role of *Gata4, Tbx5* and *Baf60c* genes in myocardial differentiation of ADMSCs.

## 2. Materials and methods

### 2.1. Over-expression of Gata4, Tbx5 and Baf60c gene in ADMSCs using lentiviral vectors

ADMSCs were purchased from Guangzhou Cyagen Biosciences, $CD73^+$ADMSCs were isolated using flow cytometry and cultured. P3-P5 cells were used for all subsequent experiments. *Gata4, Baf60c* and *Tbx5* gene over-expression lentivirus was constructed

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**Table 1**
The primer information of *Gata4*, *Baf60c*, *Tbx5*, *cTnT* and *Gapdh* for Real-time PCR.

| Gene | Primer information | Strap |
|---|---|---|
| *Gapdh* -F | TGGTGAAGGTCGGTGTGAAC | 231 bp |
| *Gapdh*-R | GCTCCTGGAAGATGGTGATGG | |
| *Gata4*-F | CGAGGGTGAGCCTGTATGT | 281 bp |
| *Gata4*-R | TGCTGTGCCCATAGTGAGAT | |
| *Baf60c*-F | GACCCATCAGACCAGAAGAAG | 224 bp |
| *Baf60c*-R | TCTTGGACGTAGCCTTTGG | |
| *Tbx5*-F | TCCGCTCATTTCACCTC | 243 bp |
| *Tbx5*-R | GCCGTGTACCGAGTGATA | |
| *cTnT*-F | ACCGGGCGTTGGAAATAG | 180 bp |
| *cTnT*-R | CATAGTGCGGGCATAGGG | |

**Table 2**
The primer information of cardiac relevant genes for RT PCR.

| Gene | Primer information | Strap |
|---|---|---|
| *Gapdh*-F | TGGTGAAGGTCGGTGTGAAC | 231bp |
| *Gapdh* -R | GCTCCTGGAAGATGGTGATGG | |
| *Nanog*-F | GGACTTTCTGCAGCCTTACG | 155 bp |
| *Nanog*-R | TTTCACCTGGTGGAGTCACA | |
| *Gata4*-F | CGAGGGTGAGCCTGTATGT | 281 bp |
| *Gata4*-R | TGCTGTGCCCATAGTGAGAT | |
| *Baf60c*-F | GACCCATCAGACCAGAAGAAG | 224 bp |
| *Baf60c*-R | TCTTGGACGTAGCCTTTGG | |
| *Tbx5*-F | TCCGCTCATTTCACCTC | 243 bp |
| *Tbx5*-R | GCCGTGTACCGAGTGATA | |
| *Nkx2.5*-F | GCTACAAGTGCAAGCGACAG | 184 bp |
| *Nkx2.5*-R | GGGTAGGCGTTGTAGCCATA | |
| *cTnT*-F | ACCGGGCGTTGGAAATAG | 180 bp |
| *cTnT* -R | CATAGTGCGGGCATAGGG | |
| *CX43*-F | ATTGGGGGAAAGGCGTGAGGGA | 175 bp |
| *CX43*-R | CCCATGTCTGGGCACCTCTCTTTCA | |
| *α-Myh6*-F | ATTGGACGACCTTGCCTCA | 427 bp |
| *α-Myh6*-R | TGCCACCTCTGTATTAGC | |
| *α-Actc1*-F | TCAGAGACAAATGGTGACAGC | 239 bp |
| *α-Actc1*-R | GACATTGTTGGCATACAGG | |

by Shanghai Genechem. Experiment groups: CON (Control) – intact normal target cell lines; NC (Negative Control) – normal target cell lines infected with virus; OE1 (Over Expression 1) – *Tbx5*-GFP-LV infected cell lines; OE2 (Over Expression 2) – *Gata4*-LV and *Baf60c*-LV infected cell lines; OE3 (Over Expression 3) – *Tbx5*-GFP-LV, *Gata4*-LV and *Baf60c*-LV infected cell lines. The cells in logarithmic growth phase (amount $5 \times 10^4$) were seeded in a 6-well tissue culture plate in complete culture medium in incubator supplied with 5%$CO_2$ at 37 °C. Upon achieving 30–40% confluence, appropriate amount of medium, virus, Eni.S and Polybrene were added according to the cell MOI value, best infection condition and experimental groups. After 48–72 h infection time, lentivirus reporter gene GFP expression were detected. Cells and their beating conditions were observed using real-time live cell workstation.

### 2.2. Immunocytochemistry

The cells were grown on coverslips in group CON, NC, OE1, OE2 and OE3. ABC method of immunohistochemistry was used and primary antibodies against Gata4 (santa cruz sc-1237, goat polyclonal), Baf60c (santa cruz sc-102120, rabbit polyclonal) and Tbx5(santa cruz sc-17865, goat polyclonal) were applied at 1:200, 1:500 and 1:200 dilutions respectively. For analysis of cTnT (abcom ab8295, mouse monoclonal) expression of target cells infected with lentivirus antibody was used at 1:1000 dilution. The cells were incubated with CY3-conjugated goat anti-mouse IgG secondary antibodies at a dilution of 1:1000 for 1 h at room temperature. Nuclei were stained by 4, 6-diamidino-2-phenylindole (DAPI).

### 2.3. Real-time PCR assay

After target cells were infected with lentivirus, their total RNA were extracted in using Trizol according to manufacturer's manual (Invitrogen, USA); cDNA was obtained from RNA reverse transcription according to M-MLV manual (Promega company); The primer sequences of *Gata4*, *Baf60c*, *Tbx5*, *cTnT* and *Gapdh* are presented in Table 1.

### 2.4. RT-PCR assay.

The expression of genes listed in Table 2 was analyzed one week and two weeks after infection of target cells with lentivirus.

### 2.5. Flow cytometry assay on cTnT expression

After being infected with lentivirus for 14 days, ADMSCs in group OE3 were trypsinized and collected. Then they were centrifuged (1000 rpm) for 5 min and washed three times with PBS (containing 0.3%Triton-100) and again centrifuged (1000 rpm) for 5 min.

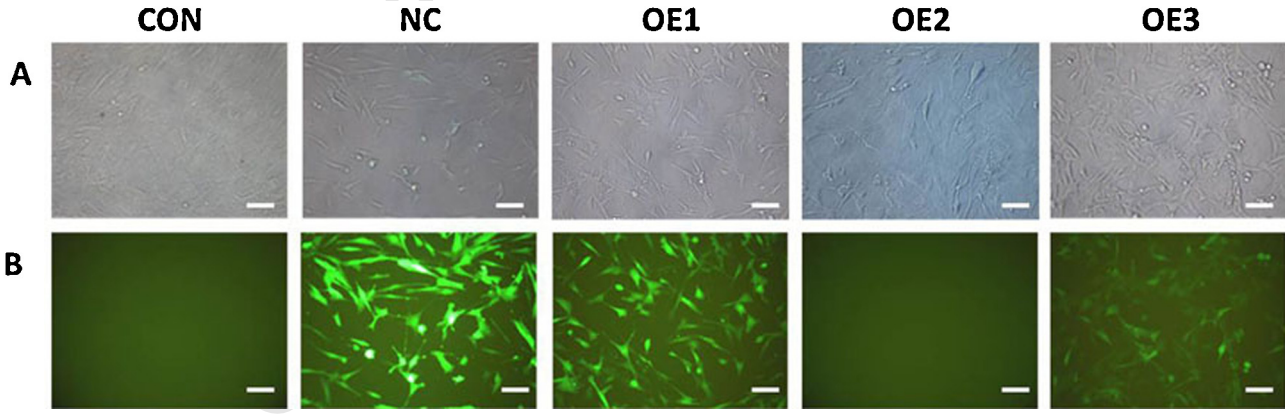


**Fig. 1.** GFP expression in ADMSCs infected with Lentivirus for 3 days. CON (Control), NC (Negative Control) ADMSCs were infected with GFP-LV; OE1 (Over Expression1) ADMSCs were infected with *Tbx5*-GFP-LV; OE2 (Over Expression2) ADMSCs were infected with *Gata4*-LV and *Baf60c*-LV; OE3 (Over Expression3) ADMSCs were infected with *Tbx5*-GFP-LV, *Gata4*-LV and *Baf60c*-LV. (A) morphological change of ADMSCs using phase contrast microscope; (B) GFP expression of ADMSCs using fluorescence microscope. Scale bar: 50 μm.

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