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Original Article

LncRNA MIAT promotes P19 cells differentiation into cardiomyocytes by sponging miR-150

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Abstract: LncRNA MIAT plays an important role in cell proliferation. This study was designed to investigate the effect of MIAT on cardiomyocytes differentiation. Mouse P19 embryonal carcinoma cells were transfected with pcDNA-MIAT, MIAT shRNA, miR-150 inhibitor, miR-150 mimic, scramble or vehicles to induce their differentiation into cardiomyocytes. The transfections with pcDNA-MIAT plasmid or treatment with miR-150 inhibitor enhanced P19 cell proliferation, increased the expression of cardiomyocyte differentiation genes (*GATA4*, *TBX5*, *cTnT*, and *Nkx2.5*), and lead to the inhibition of apoptosis. Conversely, the treatment with MIAT shRNA had the opposite effect. Moreover, the treatment with miR-150 inhibitor attenuated the effects of MIAT shRNA on P19 cell proliferation, apoptosis and the expression of cardiomyocyte differentiation genes. We confirmed that lncRNA MIAT was a competing endogenous RNA (ceRNA) that was acting by sponging miR-150, thereby inducing P19 cells to differentiate into cardiomyocytes. We suggest that lncRNA MIAT expression could be further explored as a therapeutic target of cardiac regeneration.

Keywords: lncRNA MIAT, cardiomyocyte differentiation, competing endogenous RNA, miR-150, cell proliferation and apoptosis

Introduction

Heart development is a complex process involving cardiomyocytes differentiation and cardiovascular development. This is regulated by various factors such as protein coding genes, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and signaling pathways [1-4]. Aberrant expression and abnormal accumulation of these factors contribute to heart development or heart diseases [2, 5].

The role of lncRNAs has been functionally characterized in many cardiovascular diseases, including myocardial infarction and cardiac hypertrophy [6-9]. As reported previously, lncRNAs such as CARMEN [10], Braveheart [11], and CHRF [8] have been associated with cell differentiation. For instance, lncRNA Fendrr was reported to be an essential regulator of heart development in the mouse by binding to the PRC2 and ErxG/MLL complexes, which play pivotal roles in cell differentiation [12]. Further, LncRNA myocardial infarction-associated transcript (MIAT) also plays crucial roles in the pathogenesis of various diseases, such as myocardial infarction and cardiac hypertrophy [13-15]. MIAT also takes great part in endothelial cell proliferation, apoptosis, and migration, in part by acting as a competing endogenous RNA (ceRNA) by sponging miR-150 [13, 16]. Further, it is known that miR-150 regulates the proliferation and differentiation of cardiomyocytes [17], whereas MIAT enhances cardiac hypertrophy by sponging miR-150 [13]. Regardless, there are no reports describing the effect of MIAT on cardiomyocytes differentiation.

This study was specifically designed to investigate the effect of MIAT on cardiomyocytes differentiation. Accordingly, the mouse P19 embryonal carcinoma cells were transfected with MIAT shRNA and overexpression plasmids, and miR-150 inhibitor/mimic to evaluate the cell proliferation, apoptosis, and the expression of cardiomyocytes differentiation factors. It is hoped that this study will help illustrate the effect of MIAT on cardiomyocytes differentiation, as well as its association with miR-150,

Table 1. Primer sequences for qRT-PCR in this study

Gene ID	Primer	Sequences (5'-3')
CTnT	Forward	5'-GGCAGCGGAAGAGGATGCTGAA-3'
	Reverse	5'-GAGGCACCAAGTTGGGCATGAACGA-3'
GATA-4	Forward	5'-TGGCCTGTCATCTCACTACG-3'
	Reverse	5'-TAGCCTTGTGGGGAGAGCTT-3'
Nkx2.5	Forward	5'-CCGCCAACAGCAACTTCGTG-3'
	Reverse	5'-GAGGGTGGGTGTGAAATCTGAGG-3'
TBX5	Forward	5'-AAATGAAACCCAGCATAGGAGCTGGC-3'
	Reverse	5'-ACACTCAGCCTCACATCTTACCCT-3'
LncRNA MIAT	Forward	5'-TTTACTTTAACAGACCAGAA-3'
	Reverse	5'-CTCCTTTGTTGAATCCAT-3'
GAPDH	Forward	5'-TTCCCGTTCAGCTCTGGG-3'
	Reverse	5'-CCCTGCATCCACTGGTGC-3'

thus providing additionaly molecular mechanisms in the pathogenesis of myocardium dysplasia and other cardiovascular diseases.

Materials and methods

Cell culture and induction of cardiomyocyte differentiation

Mouse P19 embryonal carcinoma cells (American Type Culture Collection, ATCC, Rockville, MD, USA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, NY, USA) containing 10% fetal bovine serum (FBS, Gibco), 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C with 5% CO_2. For the induction of cardiomyocyte differentiation, the cells were cultivated as aggregates in bacteriologic dishes with DMEM containing 10% FBS and 1% DMSO (Dimehtylsulfoxide; Sigma-Aldrich) at 37°C in 5% CO_2 for 4 days as described previously [18]. The cell culture media was replaced every third day. On day 5, the cells were transferred into flasks and cultured for another 10 days. The cells were harvested on day 0, 6 and 10 of the differentiation. For cell transfection, the cells were transfected with appropriate vector or vehicle prior to transferring to the flasks.

Construction of MIAT short hairpin RNA (shRNA) expression plasmids

The construction of MIAT shRNA interference plasmid expression was performed as described previously [16]. MIAT shRNA and scrambled shRNA sequence, pcDNA-MIAT and pcDNA-control (pcDNA 3.1 expression vector), miR-150 mimic, miR-150 inhibitor and scrambled oligonucleotide sequences were purchased from GenePharma (Shanghai, China). Lipofectamine 2000 transfection reagent (Invitrogen) was employed for transfection of MIAT shRNA, pcDNA-MIAT plasmids, vehicles, miR-150 mimic, miR-150 inhibitor and NCs into P19 cells.

Cell proliferation analysis

Cell proliferation analysis was performed using MTT (Sigma Aldrich, St Louis, MO, USA) assay [16]. Transfected P19 cells were placed in 96-well plates for 24 h, and 10 μL MTT solution was added to each well followed by incubation for 3 h. Then, optical density at 450 nm absorbance (A450) was determined using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Each experiment was performed in triplicates.

Cell cycle assay

After transfection for 24 h, the cells were harvested, fixed, stained with PI, and kept in the dark for 30 min. For cell cycle analysis and distribution, 10,000 cells at G0/G1, S, and G2/M phase were determined using Becton Dickinson FACS Calibur™ flow cytometry (BD Bioscience). Each experiment was performed in triplicates.

Annexin V apoptosis assay

Cells were seeded in 6-well plates for 24 h followed by appropriate treatments and harvested by trypsin for measuring apoptosis using the Annexin V apoptosis detection kit (Bender MedSystems, Vienna, Austria). The percentage (%) of apoptotic and necrotic cells were determined using the Annexin V-FITC and propidium iodide (PI) staining assay. The cells were labeled with Annexin V and PI and kept in dark for 30 min followed by Becton Dickinson FACS Calibur™ flow cytometry analysis (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Annexin V positive and PI negative (Ann V+/PI-) cells were determined to be early apoptosis cells.

Quantitative real-time PCR (qRT-PCR)

Total RNA from transfected P19 cells was extracted using TRIzol reagent (Invitrogen), and reverse transcription was performed using a

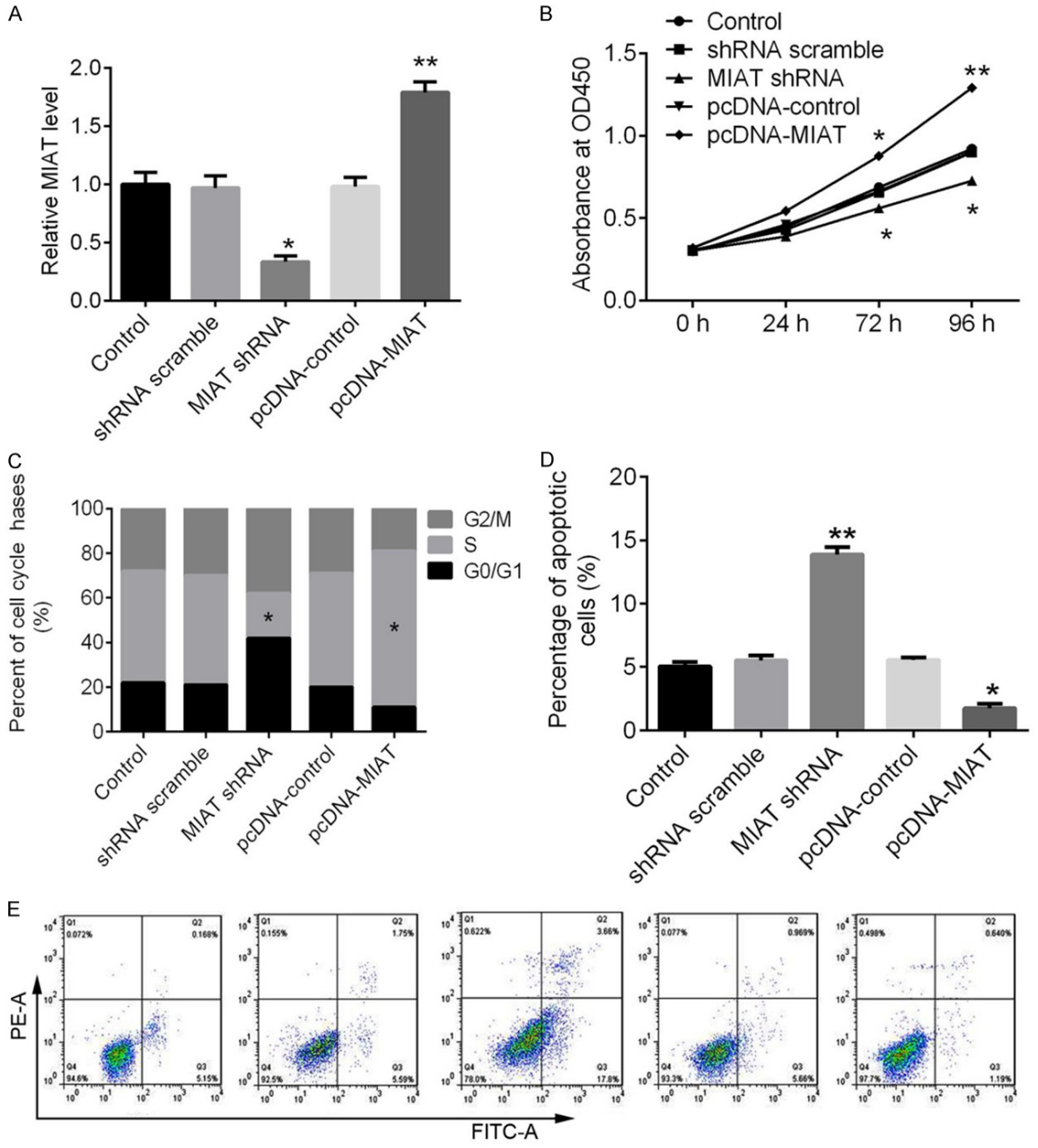

Figure 1. Effect of lncRNA MIAT on cell proliferation, apoptosis, and cell cycle. A. Expression of MIAT was detected using qRT-PCR. B. Cell proliferation was detected using MTT assay. C. Distribution of cell cycle at G0/G1, S, and G2/M phase was determined using Becton Dickinson FACS Calibur™ flow cytometry. D and E. Annexin V apoptosis assay was detected using flow cytometry. * and **indicates significant difference at $P < 0.05$ and $P < 0.01$, respectively, compared with controls.

Gibco/BRL cDNA synthesis kit (Grand Island, NY, USA). qRT-PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems, ABI, Foster City, CA) on a 7500 Fast System Real-Time PCR cycler (Applied Biosystems), according to the manufacturer's instructions. The primers used in the study (**Table 1**) were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The relative gene expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method and normalized to the internal reference gene, GAPDH.

Western blot analysis

Cellular proteins were extracted from transfected cells using the Total Protein Extraction Kit (KeyGen, inc., China), and the protein concentration was determined using a micro bicinchoninicacid (BCA) kit (KeyGen, Nanjing, China). Subsequently, the proteins were separated on 10% SDS-PAGE (Solarbio, Beijing, China) and electro-transferred onto polyvinylidene difluoride (PVDF) membranes (Invitrogen, Carlsbad, CA, USA), which were then blocked with 5% milk (BD Biosciences, San Jose, CA) for 1 h. Then, membranes were probed with the specific primary antibodies for anti GAPDH (1:2000 dilution, Proteintech Group, USA), anti-GATA4 (1:500 dilution, Abcam PLC, Cambridge, UK), anti-TBX5 (1:500 dilution, Abcam), anti-cTnT (1:250 dilution, Abcam), and anti-Nkx 2.5 (1:500 dilution, Abcam) overnight at 4°C, followed by probing with appropriate Cy3-conjugated secondary antibodies for 2 h. The membranes were read using an ECL detection system (Beyotime, Shanghai, China), and band intensity analysis was performed using a densitometry software, QuantiScan (Biosoft Ferguson, MO, USA).

Statistical analysis

All statistical analyses were conducted using SPSS 19.0 (IBM, Armonk, New York, USA) and the data were plotted using GraphPad Prism 6.0 (GraphPad Software Inc. La Jolla, CA). All data were expressed as means ± SE of the triplicate from three separate experiments. The differences among the groups were analyzed using one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

MIAT expression promotes PP19 cell proliferation, inhibits cell apoptosis and arrests cell cycle at the S phase

In order to modulate the expression of MIAT in P19 cells, we constructed a MIAT overexpression plasmid (pcDNA-MIAT) and shRNA plasmid, and transfected P19 cells with the respective plasmids. qRT-PCR results indicated that MIAT expression in P19 cells was significantly reduced by shRNA interference ($P < 0.05$), whereas the transfection with pcDNA-MIAT increased MIAT expression ($P < 0.01$) compared with control or vehicle (**Figure 1A**). We then performed the MTT assay and flow cytometry analyses for the detection of cell proliferation, cell cycle distribution, and apoptosis. Results showed that MIAT shRNA significantly affected P19 cell behavior (**Figure 1B-D**). MIAT shRNA interference inhibited cell proliferation ($P < 0.05$, **Figure 1B**), arrested cells in G0/G1 and G2/M phase ($P < 0.01$, **Figure 1C**), and promoted cell apoptosis ($P < 0.01$, **Figure 1D**), compared with controls. On the contrary, pcDNA-MIAT transfection into P19 cells showed the opposite effect, i.e. enhanced cell proliferation ($P < 0.01$, **Figure 1B**), increased percentage of cells in S phase (**Figure 1C**), and reduced cell apoptosis ($P < 0.01$, **Figure 1D**) in comparison with controls and MIAT shRNA interfered cells. There were no differences in MIAT expression, cell proliferation, apoptosis, and cell cycle distribution among the control groups transfected with scramble or vehicles (**Figure 1**). These data demonstrated that MIAT contribute to cell proliferation, and inhibit cell apoptosis.

MIAT promotes expression of cardiomyocyte differentiation related genes

As reported previously, the expression of genes such as GATA4 and TBX5 promote cardiomyocyte differentiation [19, 20]. Moreover, cTnT and Nkx2.5 are known biomarkers for cardiomyocytes [21]. In order to evaluate the effect of MIAT expression on cardiomyocyte differentiation, we detected the expression of cardiomyocyte differentiation genes (GATA4, TBX5, cTnT, and Nkx2.5) in pcDNA-MIAT and MIAT shRNA transfected P19 cells by performing qRT-PCR and Western blot analysis. Results showed MIAT significantly altered the expression of all the above mentioned differentiation genes (**Figure 2**). We found that both mRNA and protein expression of GATA4, TBX5, cTnT, and Nkx2.5 were significantly reduced by MIAT shRNA treatment, whereas the transfection with pcDNA-MIAT upregulated the four differentiation genes on days 6 and 10 when compared with the corresponding controls ($P < 0.05$). In contrast, vehicle (DMSO) treatment seemed to increase the expression of these four genes on day 10 ($P < 0.05$, **Figure 2**). Taken together, we confirmed that lncRNA MIAT can induce the expression of GATA4, TBX5, cTnT, and Nkx2.5, and promote cardiomyocyte differentiation.

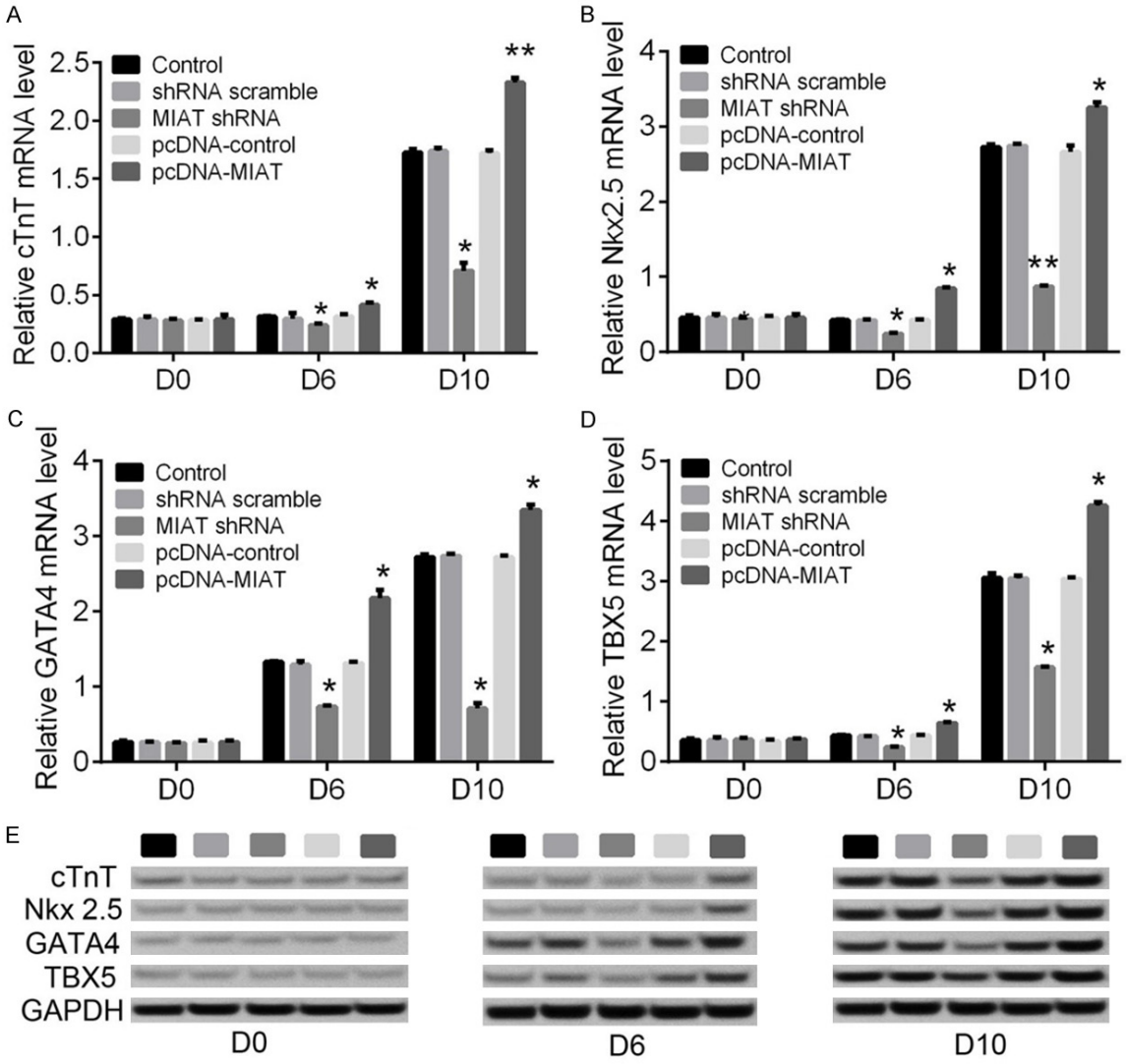

Figure 2. Expression of cardiomyocyte differentiation related genes in transfected P19 cells. A-D. MRNA expression of GATA4, TBX5, cTnT, and Nkx2.5 in transfected cells using qRT-PCR. E. Protein expression of GATA4, TBX5, cTnT, and Nkx2.5 in transfected cells on days 6 and 10 post transfection using Western blot analysis. * and **indicate the significant difference at $P < 0.05$ and $P < 0.01$, respectively, compared with the controls.

MiR-150 inhibitor reversed the effects of MIAT shRNA treatment

Since previous reports have indicated that miR-150 interacts with MIAT expression and cell functions [13, 16], we determined the combined effects of miR-150 and MIAT on P19 cells. Initially, we transfected P19 cells with miR-150 mimic, inhibitor, or corresponding scrambles (NC) and confirmed the expression of MIAT was inhibited or upregulated by miR-150 mimic or inhibitor, respectively (**Figure 3A**). These showed a negative relationship between miR-150 and MIAT expression. Then we co-transfected P19 cells with miR-150 inhibitor and MIAT shRNA to evaluated the cell functions. We confirmed that miR-150 inhibitor upregulated cell proliferation and inhibited cell apoptosis, compared with controls ($P < 0.05$, **Figure 3B** and **3C**). Whereas, cotransfection of the miR-150 inhibitor and MIAT shRNA significantly canceled MIAT shRNA interference-inhibited cell proliferation and -increased cell apoptosis rate ($P < 0.05$). No significant changes were observed in the cells transfected with scrambled sequence or controls. These results indicated that miR-150 is an inhibitor of MIAT.

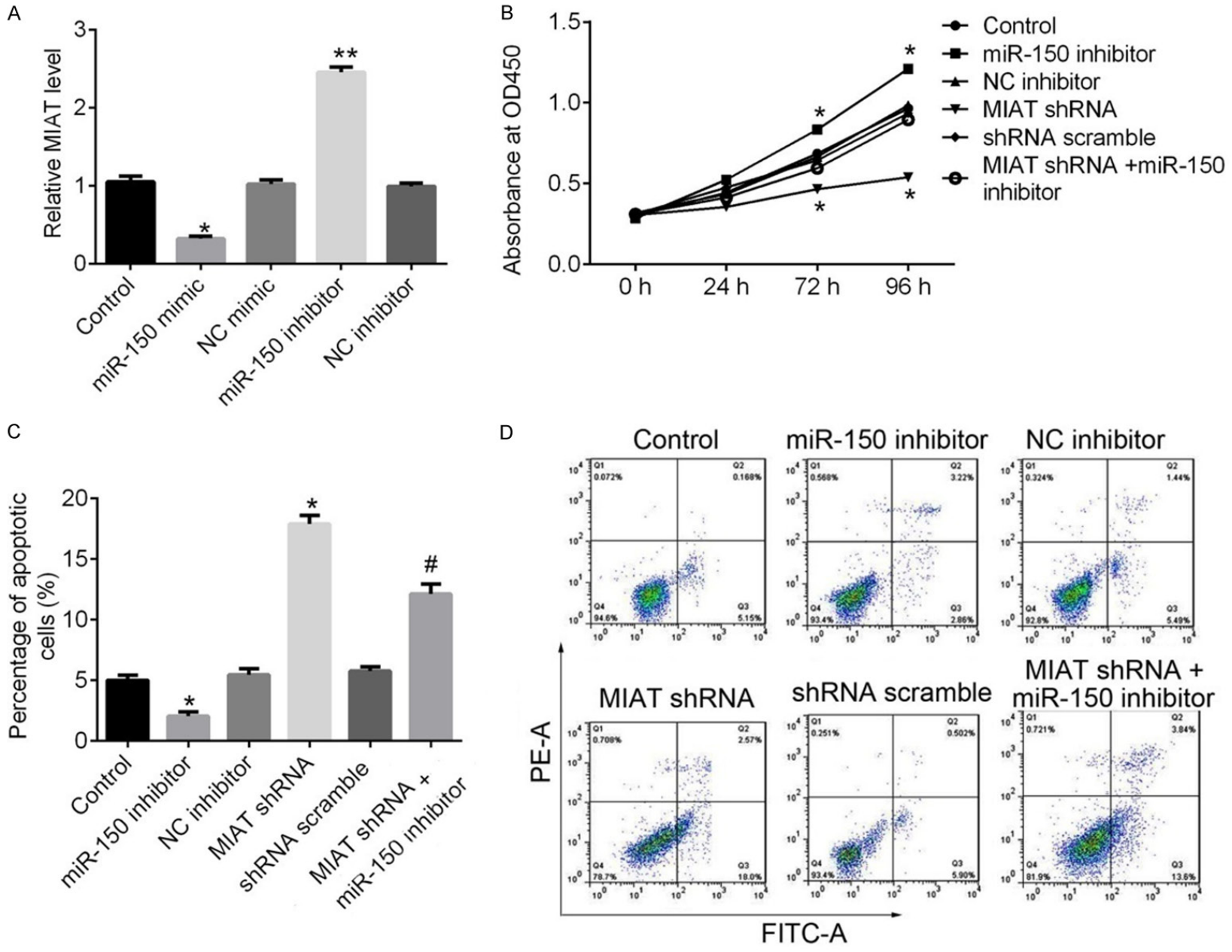
A
Relative MIAT level
Control
miR-150 mimic
NC mimic
miR-150 inhibitor
NC inhibitor
B
Absorbance at OD450
0 h
24 h
72 h
96 h
Control
miR-150 inhibitor
NC inhibitor
MIAT shRNA
shRNA scramble
MIAT shRNA +miR-150 inhibitor
C
Percentage of apoptotic cells (%)
Control
miR-150 inhibitor
NC inhibitor
MIAT shRNA
shRNA scramble
MIAT shRNA + miR-150 inhibitor
D
Control
miR-150 inhibitor
NC inhibitor
MIAT shRNA
shRNA scramble
MIAT shRNA + miR-150 inhibitor
PE-A
FITC-A

Figure 3. Influence of miR-150 on MIAT expression and cell behavior. A. Expression of MIAT was detected using qRT-PCR. B. Cell proliferation was detected using MTT assay. C and D. Annexin V apoptosis assay was detected using flow cytometry. * and **indicates significant difference at $P < 0.05$ and $P < 0.01$, respectively, compared with corresponding controls.

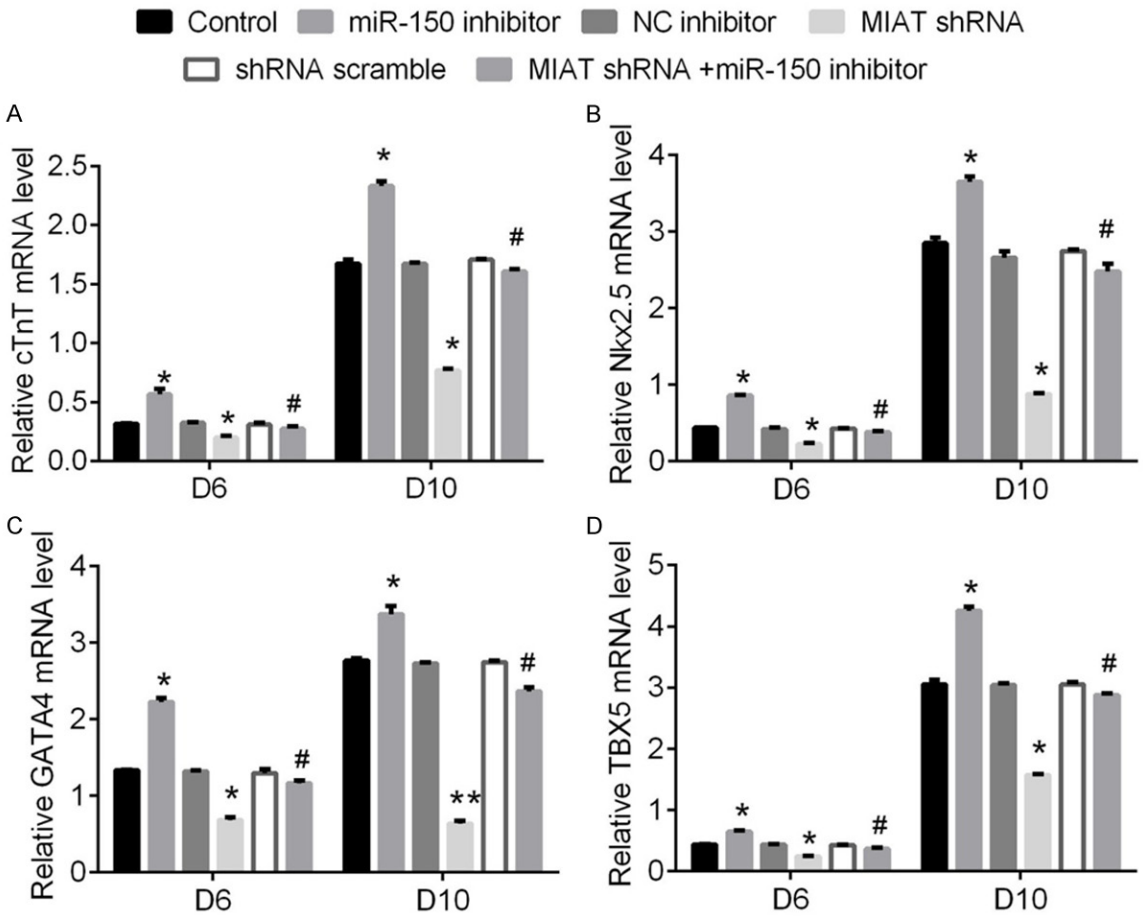

Figure 4. Influence of miR-150 on the expression of cardiomyocyte differentiation related genes. The expression of cardiomyocyte differentiation related genes was detected using qRT-PCR. *indicates significant difference at $P < 0.05$, compared with the corresponding controls. #indicates significant difference at $P < 0.05$, respectively, compared with MIAT shRNA.

MiR-150 inhibitor reverses MIAT shRNA effect on expression of cardiomyocyte differentiation related genes

To evaluate the effect of miR-150 on the expression of cardiomyocyte differentiation genes GATA4, TBX5, cTnT, and Nkx2.5, we detected their expression in cells transfected with miR-150 inhibitor alone or along with MIAT shRNA. **Figure 4** shows that miR-150 inhibitor significantly upregulated GATA4, TBX5, cTnT, and Nkx2.5 mRNA expression on days 6 and 10 post transfection, when compared with control (**Figure 4**), with a higher expression on day 10 compared to day 6. On the contrary, MIAT shRNA treatment significantly reduced the expression of GATA4, TBX5, cTnT, and Nkx2.5 in P19 cells on days 6 and 10 ($P < 0.05$) ($P < 0.05$). Moreover, co-transfection of MIAT shRNA with miR-150 inhibitor into cells significantly reversed influence of MIAT shRNA interference on P19 cells ($P < 0.05$). There were no differences in the mRNA expression of GATA4, TBX5, cTnT, and Nkx2.5 among scramble and control cells, respectively on day 6 and 10 post transfection. These results showed that miR-150 inhibitor reversed influence on MIAT on cardiomyocyte differentiation genes.

Discussion

LncRNAs can act as miRNA sponges thereby regulating the availability of miRNAs that are required to modulate the expression of target mRNAs [13]. Using a luciferase reporter assay, Zhu had demonstrated that miR-150 directly targets MIAT, suggesting that MIAT was a ceRNA via sponging miR-150. In this present study, we confirmed that MIAT was indeed ceRNA *via* sponging miR-150. Further, we demonstrated that MIAT expression can enhance P19 cell proliferation, inhibit apoptosis, and promote the expression of cardiomyocyte differentiation genes. These results clearly imply that MIAT and miR-150 might be responsible for the pathogenesis of cardiovascular diseases, particularly those related to cardiomyocyte differentiation.

As previously reported, MIAT takes part in endothelial cell proliferation, apoptosis, and migration [14]. Further, the knockdown of MIAT in endothelial cells inhibited cell proliferation and enhanced apoptosis [14]. Notably, these results oncur with our findings in the current study. We have demonstrated that MIAT shRNA interference increased apoptosis and decreased cell proliferation in P19 cells. Moreover, MIAT shRNA decreased the percentage of P19 cells in S phase, leading to accumulation of cells in the G2/M and G0/G1 phases. Accordingly, pcDNA-MIAT transfection enhanced cell proliferation, increased the percentage of cells in S phase, and reduced apoptosis (**Figure 1**). Overall, these results demonstrated that MIAT expression can modulate P19 cell proliferation.

Subsequently, we confirmed that MIAT expression negatively correlated with miR-150 expression (**Figure 3**). MiR-150 inhibitor plus MIAT shRNA reversed the effects of MIAT shRNA on cell proliferation and apoptosis. In addition, miR-150 inhibitor enhanced P19 cell proliferation and decreased apoptosis. These data suggested that miR-150 is an inhibitor of MIAT, which behaves like ceRNA by sponging miR-150. Moreover, our findings correlate well with previous reports [14, 16].

Cardiomyocyte differentiation is regulated by various factors, including GATA4 and TBX5 [19, 20]. Previous studies have shown that GATA4 and TBX5 can promote cardiomyocyte differentiation of noncardiomyogenic cells [20, 22, 23]. The overexpression of GATA4 and TBX5, along with Baf60c or Mef2c induced adipose tissue-derived mesenchymal stem cells to form cardiomyocytes and directed the reprogramming of cardiac fibroblasts into cardiomyocytes [20, 23], suggesting that GATA4 and TBX5 could promote non-cardiomyogenic cells to differentiate into cardiomyocytes. In this study, we confirmed that both MIAT expression and miR-150 inhibition promoted the expression of GATA4 and TBX5, as well as the expression of cardiomyocyte markers cTnT and Nkx2.5. This suggestes that MIAT expression contributed to cardiomyocyte differentiation and could be explored as a therapeutic target for heart development and regeneration.

Conclusion

We confirmed that lncRNA MIAT is a kind of ceRNA that worked by sponging miR-150. The expression of MIAT contributed to P19 cell proliferation, and promoted cardiomyocyte differentiation by sponging miR-150. Thus, MIAT expression could be explored as a therapeutic target for hear cardiac regeneration. However, other RNAs might compete with MIAT through miR-150 and other MIAT-related mechanisms that contribute to cardiomyocyte differentiation needs to be further explored in detail.

Disclosure of conflict of interest

None.

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References

[1] Schindler YL, Garske KM, Wang J, Firulli BA, Firulli AB, Poss KD and Yelon D. Hand2 elevates cardiomyocyte production during zebrafish heart development and regeneration. Development 2014; 141: 3112-3122.

[2] Berndt C, Poschmann G, Kai S, Holmgren A and Bräutigam L. Zebrafish heart development is regulated via glutaredoxin 2 dependent migration and survival of neural crest cells. Redox Biol 2014; 2: 673-678.

[3] Kalsotra A, Wang K, Li PF, Cooper TA. MicroRNAs coordinate an alternative splicing network during mouse postnatal heart development. Genes Dev 2010; 24: 653-658.

[4] Garside VC, Chang AC, Karsan A and Hoodless PA. Co-ordinating Notch, BMP, and TGF-β signaling during heart valve development. Cell Mol Life Sci 2013; 70: 2899-2917.
[5] Lescroart F, Chabab S, Lin X, Rulands S, Paulissen C, Rodolosse A, Auer H, Achouri Y, Dubois C, Bondue A, Simons BD, Blanpain C. Early lineage restriction in temporally distinct populations of Mesp1 progenitors during mammalian heart development. Nat Cell Biol 2014; 16: 829-840.
[6] Uchida S and Dimmeler S. Long noncoding RNAs in cardiovascular diseases. Circ Res 2015; 116: 737-750.
[7] Kataoka M and Wang DZ. Non-coding RNAs including miRNAs and lncRNAs in cardiovascular biology and disease. Cells 2014; 3: 883-898.
[8] Wang K, Liu F, Zhou LY, Long B, Yuan SM, Wang Y, Liu CY, Sun T, Zhang XJ and Li PF. The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. Circ Res 2014; 114: 1377-1388.
[9] Ounzain S, Micheletti R, Beckmann T, Schroen B, Alexanian M, Pezzuto I, Crippa S, Nemir M, Sarre A, Johnson R, Dauvillier J, Burdet F, Ibberson M, Guigó R, Xenarios I, Heymans S, Pedrazzini T. Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. Eur Heart J 2015; 36: 353-68a.
[10] Ounzain S, Micheletti R, Arnan C, Plaisance I, Cecchi D, Schroen B, Reverter F, Alexanian M, Gonzales C, Ng SY, Bussotti G, Pezzuto I, Notredame C, Heymans S, Guigó R, Johnson R, Pedrazzini T. CARMEN, a human super enhancer-associated long noncoding RNA controlling cardiac specification, differentiation and homeostasis. J Mol Cell Cardiol 2015; 89: 98-112.
[11] Klattenhoff C, Scheuermann J, Surface L, Bradley R, Fields P, Steinhauser M, Ding H, Butty V, Torrey L, Haas S, Abo R, Tabebordbar M, Lee RT, Burge CB, Boyer LA. Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 2013; 152: 570-583.
[12] Grote P, Wittler L, Hendrix D, Koch F, Währisch S, Beisaw A, Macura K, Bläss G, Kellis M, Werber M, Herrmann BG. The tissue-specific lncRNA fendrr is an essential regulator of heart and body wall development in the mouse. Dev Cell 2013; 24: 206-214.
[13] Zhu XH, Yuan YX, Rao SL and Wang P. LncRNA MIAT enhances cardiac hypertrophy partly through sponging miR-150. Eur Rev Med Pharmacol Sci 2016; 20: 3653-3660.
[14] Yan B, Yao J, Liu JY, Li XM, Wang XQ, Li YJ, Tao ZF, Song YC, Chen Q and Jiang Q. lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA. Circ Res 2015; 116: 1143-56.
[15] Liao J, He Q, Min L, Chen Y, Liu Y and Jie W. LncRNA MIAT: myocardial infarction associated and more. Gene 2015; 578: 158-161.
[16] Shen Y, Dong LF, Zhou RM, Yao J, Song YC, Yang H, Jiang Q and Yan B. Role of long noncoding RNA MIAT in proliferation, apoptosis and migration of lens epithelial cells: a clinical and in vitro study. J Cell Mol Med 2016; 31: 780-785.
[17] Duan Y, Zhou B, Su H, Liu Y and Du C. MiR-150 regulates high glucose-induced cardiomyocyte hypertrophy by targeting the transcriptional coactivator p300. Exp Cell Res 2013; 319: 173-184.
[18] Zhang H, Xu C, Yang R, Chen H, Kong X, Qian L, Sheng Y and Sun W. Silencing of nodal modulator 1 inhibits the differentiation of P19 cells into cardiomyocytes. Exp Cell Res 2015; 331: 369-376.
[19] Chen JX, Krane M, Deutsch MA, Wang L, Rav-Acha M, Gregoire S, Engels MC, Rajarajan K, Karra R and Abel ED. Inefficient reprogramming of fibroblasts into cardiomyocytes using Gata4, Mef2c, and Tbx5. Circ Res 2012; 111: 50-55.
[20] Li Q, Guo ZK, Chang YQ, Yu X, Li CX and Li H. Gata4, Tbx5 and Baf60c induce differentiation of adipose tissue-derived mesenchymal stem cells into beating cardiomyocytes. Int J Biochem Cell Biol 2015; 66: 30-36.
[21] Ding R, Jiang X, Ha Y, Wang Z, Guo J, Jiang H, Zheng S, Shen Z and Wei J. Activation of Notch1 signalling promotes multi-lineage differentiation of c-KitPOS/NKX2.5POS bone marrow stem cells: implication in stem cell translational medicine. Stem Cell Res Ther 2015; 6: 1-15.
[22] Chen W, Zhang L, Shao SX, Wang HP, Cui SJ, Zhang YN, Kong XZ, Yin Q and Zhang JP. Transcription factors GATA4 and TBX5 promote cardiomyogenic differentiation of rat bone marrow mesenchymal stromal cells. Histol Histopathol 2015; 30: 1487-1498.
[23] Inagawa K, Miyamoto K, Yamakawa H, Muraoka N, Sadahiro T, Umei T, Wada R, Katsumata Y, Kaneda R, Nakade K, Kurihara C, Obata Y, Miyake K, Fukuda K, Ieda M. Induction of cardiomyocyte-like cells in infarct hearts by gene transfer of Gata4, Mef2c, and Tbx5. Circ Res 2012; 111: 1147-1156.