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LncRNA myocardial infarction-associated transcript (MIAT) contributed to cardiac hypertrophy by regulating TLR4 via miR-93

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ABSTRACT

It has been reported that lncRNA myocardial infarction-associated transcript (MIAT) facilitated the pathological development in angiotensin II (AngII)-induced cardiac hypertrophy. Nevertheless, the underlying mechanism of MIAT involved in cardiac hypertrophy is largely unknown. In this study, AngII-treated cardiomyocytes were applied as a cardiac hypertrophy model in vitro. The expressions of MIAT and miR-93 were detected by qRT-PCR. The protein levels of toll-like receptor 4 (TLR4), atrial natriuretic factor (ANF), beta-myosin heavy chain (β-MHC), phosphoinositide-3 kinase (PI3K), protein kinase B (Akt), phosphorylated Akt (p-Akt), mammalian target of rapamycin (mTOR), and phosphorylated mTOR (p-mTOR) were determined by western blot. Luciferase reporter assay and RNA immunoprecipitation (RIP) were performed to explore the relationship between MIAT, TLR4 and miR-93. Hypertrophic response was assessed by measuring cell surface area and quantifying the expressions of ANF and β-MHC. The results demonstrated that MIAT was upregulated and miR-93 was downregulated in AngII-treated cardiomyocytes. MIAT functioned as a molecular sponge of miR-93 in cardiomyocytes. Additionally, TLR4 was identified as a target of miR-93 and MIAT promoted TLR4 expression by sponging miR-93. MIAT knockdown decreased cell surface area and the expression levels of ANF and β-MHC in AngII-treated cardiomyocytes by modulating miR-93. Moreover, enforced expression of TLR4 partially reversed the protective effect of miR-93 overexpression on AngII-induced cardiac hypertrophy. Furthermore, MIAT knockdown or miR-93 overexpression inactivated the PI3K/Akt/mTOR pathway via TLR4 in AngII-induced cardiac hypertrophy. Taken together, these data suggested that MIAT knockdown inhibited AngII-induced cardiac hypertrophy by regulating miR-93/TLR4 axis, highlighting a promising therapy target for cardiac hypertrophy.

1. Introduction

Cardiac hypertrophy, characterized by an enlargement of cardiomyocytes and heart mass, is an adaptive reaction of the heart in response to physiological and pathological overload to maintain cardiac function in its initial stage (Oka et al., 2014). However, persistent cardiac hypertrophy is mostly associated with maladaptive cardiac hypertrophy and cardiac remodeling, which may ultimately lead to decreased compliance and increased risk of heart failure as well as sudden death (Dadson et al., 2017). Although numerous pathological stimuli including specific peptide hormones and growth factors have been identified to be involved in the regulation of cardiac hypertrophy, the molecular mechanisms underlying cardiac hypertrophy are still poorly understood (Lyon et al., 2015). To prevent cardiac hypertrophy and heart failure, it is of great significance to identify and characterize key factors that may regulate hypertrophy.

Non-coding RNAs (ncRNAs) are mainly divided into two groups depending on size: long non-coding RNAs (lncRNAs) with more than 200 nucleotides in length, and small non-coding RNAs with less than 200 nucleotides including microRNAs (miRNAs) (Qureshi and Mehler, 2012). LncRNAs, a group of transcribed RNA molecules with no or limited of protein-coding potential, has attracted increasing attention due to their regulation functions in complicated biological events and high abundance in human beings (Da Sacco et al., 2012). There is striking evidence that dysregulated lncRNAs play an essential role in the modulation of heart development and cardiovascular diseases, while only a limited number of lncRNAs are associated with the pathological development of cardiac hypertrophy (Han et al., 2014; Matkovich et al., 2014). LncRNA myocardial infarction-associated transcript (MIAT) is mainly expressed in heart and fetal brain tissues (Papait et al., 2013; Yan et al., 2015). It has been demonstrated that abnormal expression of MIAT was tightly associated with cell proliferation, apoptosis, and migration in various diseases, including myocardial infarction (Ishii et al., 2006; Vausort et al., 2014). Notably,

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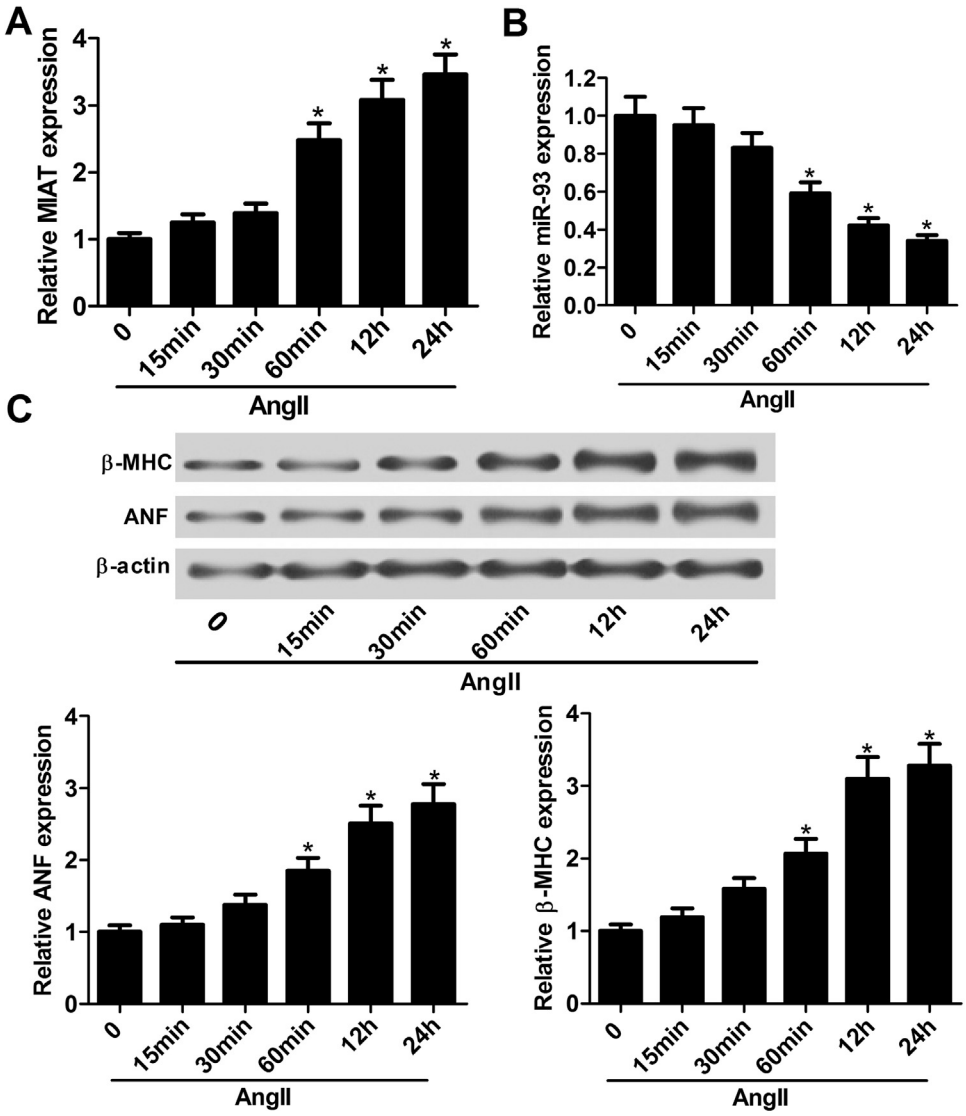

Fig. 1. Expressions of MIAT and miR-93 in AngII-treated cardiomyocytes. Cardiomyocytes were treated with 150 nM AngII for 15 min, 30 min, 60 min, 12 h, and 24 h, qRT-PCR was performed to determine the expressions of MIAT (A) and miR-93 (B). (C) Western blot was conducted to evaluate the protein levels of ANF and β-MHC. *$P < 0.05$.

it has been reported that MIAT was significantly upregulated in angiotensin II (AngII)-induced cardiac hypertrophy and contributed to the pathological development (Zhu et al., 2016). However, the molecular mechanism of MIAT involved in the pathogenesis of cardiac hypertrophy remains to be further explored.

miRNAs are a group of small, highly conserved ncRNAs that negatively regulate gene expression by directly binding to the 3′ untranslated region (UTR) of target mRNA for either translational suppression or mRNA degradation (Gu et al., 2009). It is reported that miRNAs are implicated in a variety of biological processes, including cell proliferation, apoptosis, autophagy, development, and heart disease (Ambros, 2004). Recently, an increasing body of evidence has suggested that miRNAs play key roles in the pathogenesis and development of cardiac diseases, including cardiac hypertrophy (da Costa Martins et al., 2008; Da Costa Martins and De Windt, 2012). It was previously demonstrated that miR-93 was downregulated in cardiac hypertrophy (van Rooij et al., 2006). Nevertheless, it is not known about the detailed function and molecular basis of miR-93 in cardiac hypertrophy. Recently, competitive endogenous RNA (ceRNA) hypothesis has been recently proposed as a novel regulatory mechanism between ncRNA and coding RNA (Salmena et al., 2011). LncRNAs may function as ceRNAs to sponge miRNAs and competitively interact with the miRNA, thus leading to the derepression of target mRNA (Pilyugin and Irminger-Finger, 2014). According to the prediction of potential complementary binding sites between MIAT and miR-93 by our bioinformatics analysis, we hypothesized whether MIAT has a similar function for miR-93 to regulate the pathogenesis of cardiac hypertrophy.

In the present study, we reported for the first time that MIAT knockdown suppressed AngII-induced hypertrophic response by regulating miR-93/TLR4 axis via inactivation of PI3K/Akt/mTOR pathway.

2. Materials and methods

2.1. Cardiomyocyte culture and treatment

Cardiomyocytes were isolated from 1 to 2-day-old Wistar rats as previously described (Tan et al., 2008). Briefly, the dissected hearts were washed, minced into small pieces, and enzymatically digested with 0.25% trypsin (Beyotime, Shanghai, China) at 30 g at 37 °C. After centrifugation, the digested cells were resuspended in Dulbecco's modified Eagle medium/F-12 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 5% heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 0.1 mM ascorbate, insulin-transferring-sodium selenite media supplement (Sigma, St. Louis, MO, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.1 mM bromodeoxyuridine (Gibco, Grand Island, NY, USA) at 37 °C in a humid atmosphere consisting of 5% CO_2 for 1 h. Cells were then diluted to 1×10^6 cells/ml