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Rapid communication

Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy

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Abstract

MicroRNAs (miRNAs) are a recently discovered class of ∼22-nucleotide regulatory RNAs that post-transcriptionally regulate gene expression. We have recently demonstrated that muscle-specific miRNAs miR-1 and miR-133 play an important role in modulating muscle proliferation and differentiation. Here, we investigate the involvement of miRNAs in cardiac hypertrophy. We analyzed the global expression of miRNAs in agonist-induced hypertrophic cardiomyocytes as well as in pressure overload-induced hypertrophic hearts and found the miRNA expression profile altered in those hypertrophic conditions. We further show that inhibition of endogenous miR-21 or miR-18b augments hypertrophic growth. Conversely, introduction of functional miR-21 or miR-18b into cardiomyocytes represses myocyte hypertrophy. Together, our studies point to miRNAs as critical regulators of cardiac hypertrophy.
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1. Introduction

MicroRNAs (miRNAs) are a class of short, non-coding RNA molecules that have recently emerged as important regulators of gene expression [1]. Many miRNAs are highly evolutionarily conserved and more than 400 miRNAs are known to exist in humans alone. miRNAs negatively regulate target gene expression through miRNA complementarity to target sequences within the 3′ untranslated region of target mRNAs through translational repression or by degradation of bound RNA [1–3]. Recently, we and others have demonstrated that a subset of muscle-specific miRNAs, miR-1 and miR-133 in particular, play important roles in muscle cell proliferation and differentiation [4,5]. Given that miRNAs participate in such fundamental processes, we investigated the potential role for miRNAs in regulating the well-documented changes in gene expression that occur during cardiac hypertrophy.

In this report, we show that the expression of a subset of miRNAs is altered in hypertrophic cardiomyocytes. Interestingly, we found more miRNAs that are up-regulated than down-regulated in response to cardiac hypertrophy. We have used both overexpression and knockdown approaches to demonstrate that miR-18b and miR-21 repress hypertrophy in neonatal rat cardiomyocyte in vitro.

2. Materials and methods

2.1. Cardiomyocyte tissue culture and transfection

Preparation of neonatal rat cardiomyocytes was as described [6,7]. LNA oligonucleotides and miRNA duplexes were transfected using Lipofectamine. Hypertrophic stimuli used: PE (100 μM), LIF (1000 units/ml) and fetal bovine serum (FBS) 10%.

2.2. Analysis of microRNA expression by microarray

Total RNA was isolated from rat neonatal cardiomyocytes and mouse heart tissue (Trizol). Microarray production and hybridization methods have been described [8]. Five

Abbreviations: LNA, locked nucleic acid; LIF, leukemia inhibitory factor; miRNA, microRNA; PE, phenylephrine; TAB, thoracic aortic banding.

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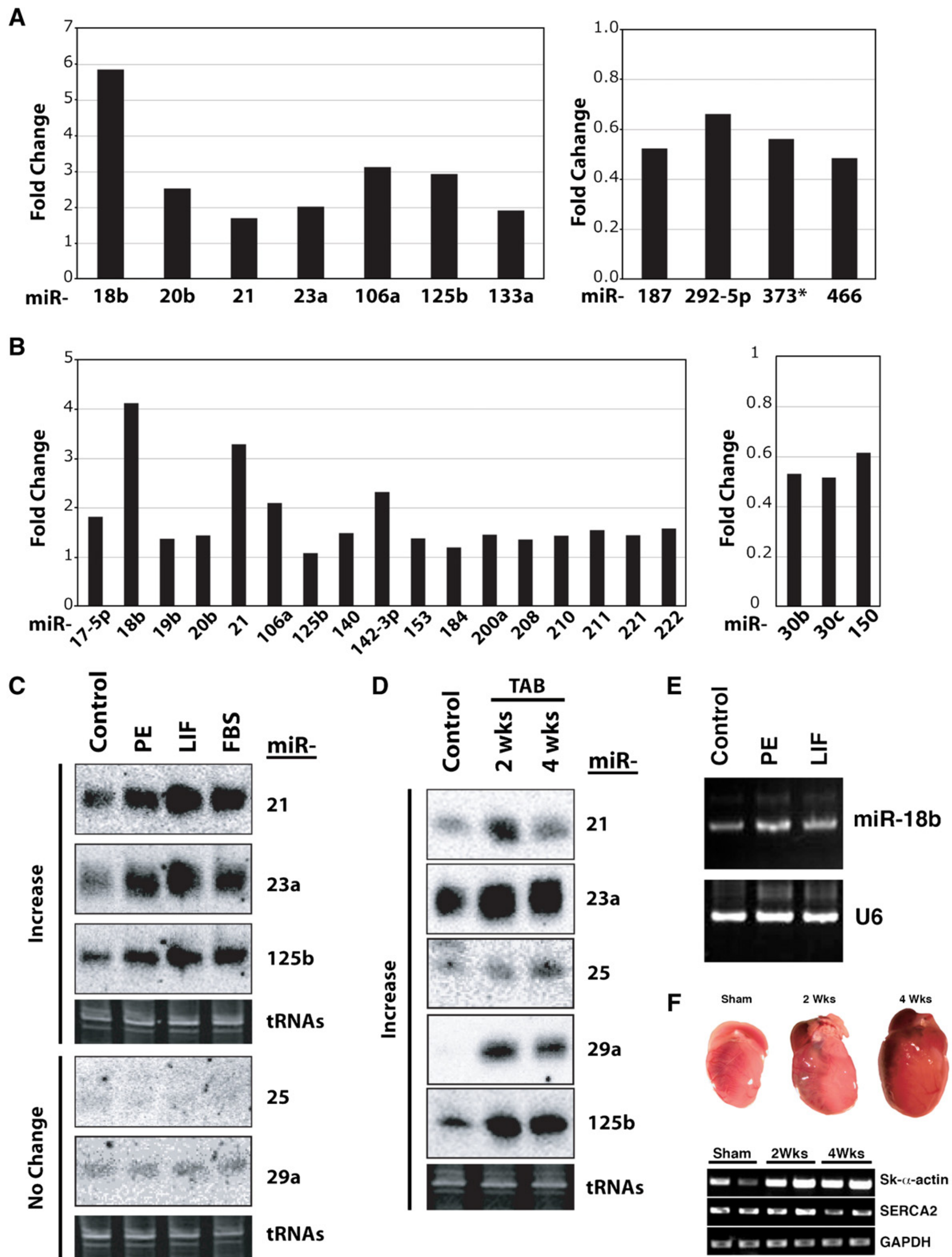

Fig. 1. Microarray screen identifies miRNAs that are regulated during cardiac hypertrophy. (A) Up- and down-regulated miRNAs in phenylephrine (PE) treated neonatal cardiomyocytes. (B) Fold change of miRNA expression in 2 week thoracic aortic banding (TAB) hearts. (C) Northern blot analysis of miRNAs using equal amounts of total RNA isolated from rat neonatal cardiomyocytes treated with various hypertrophic stimuli. PE, phenylephrine; LIF, leukemia inhibitory factor; FBS, fetal bovine serum. (D) Northern blot analysis of miRNAs using equal amounts of total RNA isolated from control, 2 week, or 4 week TAB mice. tRNAs were used as a loading control. (E) RT-PCR analysis for miR-18b expression in PE or LIF treated cardiomyocytes. U6 expression served as control. (F) Gross heart morphology of sham, 2 and 4 week TAB hearts, and RT-PCR analysis for hypertrophic genes using total RNA isolated from those conditions. GAPDH served as control.

micrograms of labeled RNA from each treatment was hybridized to a microRNA microarray with 627 mouse, rat and human miRNAs and analyzed using GenePix Pro (Axon Instruments). Median pixel intensity values were background subtracted and log-transformed (base 2). Significant Analysis of Microarray (SAM, Stanford University) was used to determine statistically significant changes in miRNA expression between treatments (q-value threshold <0.05).

2.3. microRNA Northern blot, RT-PCR analysis and immunocytochemistry

MicroRNA Northern blot analyses were as described [4]. Semi-quantitative RT-PCR and immunocytochemical analyses were essentially as described [7]. For cell size measurement, ~200 cardiomyocytes stained with anti-α-actinin antibody from each treatment were randomly chosen for surface area measurement using a computerized morphometric system (ImageJ). Data were presented as average ± SEM. Student's t test was used for statistical analysis. A PCR method to detect mature miRNAs was adapted with minor modification [9]. Total RNA was DNAse I treated, phenol-chloroform extracted, precipitated and dissolved in DEPC water. RNA was poly-adenylated then reversed-transcribed using an oligo(dT) adapter primer. Resulting cDNA was used in a PCR reaction with oligonucleotide primers complementary to the oligo(dT) adapter primer and to miR-18b. PCR products were resolved on a 15% acrylamide gel with a 10 bp DNA ladder.

2.4. Pressure overload hypertrophy models

Male C57BL6 mice (6–8 weeks old) were subjected to pressure overload by thoracic aortic banding (TAB) [10]. Mice were sacrificed after 2 weeks or 4 weeks banding and hearts were harvested for RNA extraction.

3. Results and discussion

3.1. Global analysis of miRNA expression during cardiac hypertrophy

To better understand the role of miRNAs in cardiac hypertrophy, we sought to identify miRNAs involved in cardiac hypertrophy using a custom miRNA microarray screening approach [4,8]. Using this sensitive and quantitative high-throughput technology, we profiled global miRNA expression in phenylephrine (PE)-treated neonatal cardiomyocytes (Fig. 1A) and in mouse hearts subjected to pressure overload by

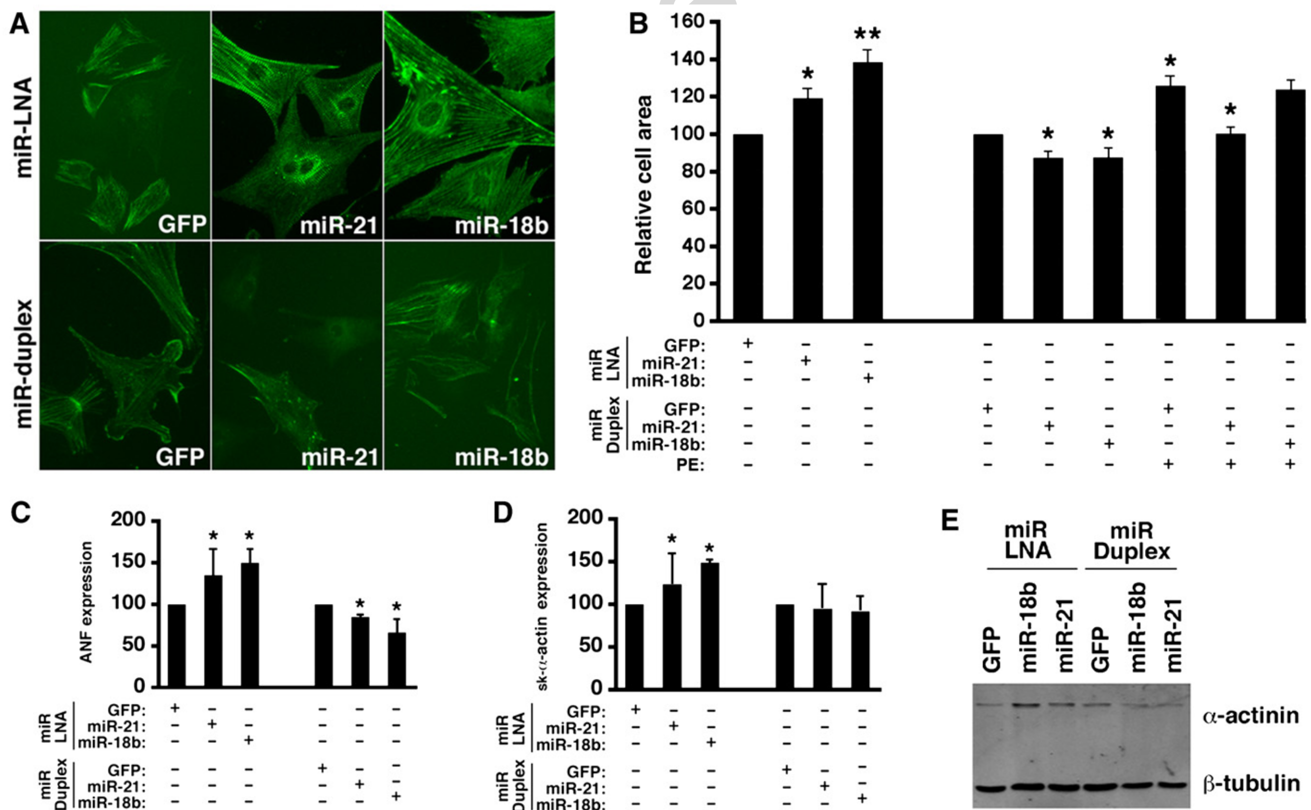

Fig. 2. miR-18b and miR-21 regulate cardiomyocyte hypertrophy. (A) Representative images of α-actinin-stained cardiomyocytes transfected with LNA antisense oligonucleotides against indicated miRNAs (upper panels) or miR-duplexes (lower panels). GFP was used as a control. (B) Quantitative analysis of cardiomyocyte cell size. ~200 cells immunostained with anti-α-actinin antibody from each treatment were randomly chosen for surface area measurement. Results presented as relative cell area compared to controls. (C and D) RT-PCR analysis of hypertrophic marker genes ANF and skeletal muscle α-actin (sk-α-actin) using total RNA isolated from cardiomyocytes treated with indicated LNA oligos, or miRNA duplexes. (E) Western blot analysis of α-actinin expression in cardiomyocytes treated with indicated LNA oligos, miRNA duplexes, or GFP. β-tubulin serves as a loading control. Error bars represent standard deviation; $*p<0.05$; $**p<0.01$.

thoracic aortic banding (TAB) (Figs. 1B and F) and found that the expression signatures of several miRNAs were altered during cardiac hypertrophy.

Northern blot analysis for several miRNAs confirmed the results from the miRNA microarray screening (Figs. 1C and D). The expression of miR-21, miR-23a and miR-125b is increased in agonist-induced hypertrophic cardiomyocytes and in the TAB-induced hypertrophic hearts. Whereas the expression of miR-25 and miR-29a is very low in neonatal cardiomyocytes and appears unchanged during hypertrophy in vitro (Fig. 1C), their expression is drastically up-regulated in TAB-induced hypertrophic hearts (Fig. 1D). Interestingly, miR-18b is among the most highly induced miRNAs in both in vitro and in vivo hypertrophy models (Fig. 1), however, we were unable to confirm its expression by Northern analysis, due to its low expression in cardiomyocytes (data not shown). We applied a technique designed to detect expression of a mature miRNA by PCR [9], which confirmed increased miR-18b expression in response to hypertrophic stimuli (Fig. 1E).

3.2. Inhibition of cardiomyocyte hypertrophy by miR-21 and miR-18b

To explore the potential involvement of miRNAs in the regulation of cardiomyocyte hypertrophy, we specifically inhibited miR-21 and miR-18b in neonatal rat cardiomyocytes using locked nucleic acid (LNA)-modified antisense oligonucleotides [11]. While miR-21 represents an abundantly expressed miRNA (Fig. 1C), miR-18b expression was very low in the cardiomyocytes (Fig. 1E). When LNA oligonucleotides antisense (miR-LNA) to miR-21 or miR-18b were transfected into cardiomyocytes, we consistently observed induction of hypertrophy, including increased cardiomyocyte size (Figs. 2A and B) and induction of hypertrophic markers such as ANF, α-actinin and skeletal muscle α-actin (Figs. 2C–E). Those changes are subtle yet reproducible. The role for miR-18b and miR-21 in hypertrophy is further supported by a gain-of-function approach whereby we transfected cardiomyocytes with short double-stranded RNAs (miR-duplexes), in which a single-strand identical to mature miR-21 or miR-18b is incorporated into the RISC complex [3,12]. Transfection of miR-21 and miR-18b duplexes slightly decreased cardiomyocyte size and decreased expression of hypertrophic markers (Figs. 2A–E). Interestingly, transfection of miR-21 duplexes, but not miR-18b duplexes, inhibited PE-induced cardiomyocyte hypertrophy (Fig. 2B), suggesting distinct functions for these two miRNAs in hypertrophic growth. Collectively, these data suggest that miR-21 and miR-18b may play a causative role in cardiomyocyte hypertrophy.

Emerging evidence has pointed to the critical role of miRNAs in variety of biological processes. As an initial step to explore the potential involvement of miRNAs in the pathophysiology of cardiac function, we profiled the changes of miRNA expression in both in vitro and in vivo cardiac hypertrophy models. Our results demonstrate that many miRNAs are regulated during cardiac hypertrophy and we show that miR-18b and miR-21 play a critical role in the hypertrophic process. Our data are consistent with two recent reports that identified dysregulated miRNA expression during cardiac hypertrophy and documented important roles for miR-1 and miR-195 in cardiac hypertrophy and heart failure [13,14].

We have found that miR-21 expression was induced during hypertrophy and, most importantly, our studies suggest that miR-21 may negatively regulate cardiac hypertrophy. Recent studies have found that miR-21 is likely involved in tumor-related cell growth and apoptosis [4,15,16]. Interestingly, some of those reports appear contradictory: while one study documented that miR-21 inhibition provoked cell growth in HeLa cells [16], others showed that miR-21 inhibition led to activation of apoptosis and decreased cell proliferation [4,15]. Clearly, the identification of the miR-21 regulatory targets and understanding the molecular pathways modulated by miR-21 in different biological systems will be the key to our understanding the biological function of this miRNA.

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References

[1] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004 (Jan 23);116(2):281–97.
[2] Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 2005 (Aug 26);122(4):553–63.
[3] Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. Cell 2003 (Oct 17);115(2):199–208.
[4] Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 2006 (Feb);38(2):228–33.
[5] Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature 2005 (Jul 14);436(7048):214–20.
[6] Nicol RL, Frey N, Pearson G, Cobb M, Richardson J, Olson EN. Activated MEK5 induces serial assembly of sarcomeres and eccentric cardiac hypertrophy. EMBO J 2001 (Jun 1);20(11):2757–67.
[7] Xing W, Zhang TC, Cao D, Wang Z, Antos CL, Li S, et al. Myocardin induces cardiomyocyte hypertrophy. Circ Res 2006 (Apr 28);98 (8):1089–97.
[8] Thomson JM, Parker J, Perou CM, Hammond SM. A custom microarray platform for analysis of microRNA gene expression. Nat Methods 2004 (Oct);1(1):47–53.
[9] Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. Genes Dev 2006 (Aug 15);20 (16):2202–7.
[10] Rockman HA, Ross RS, Harris AN, Knowlton KU, Steinhelper ME, Field LJ, et al. Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in an in vivo murine model of cardiac hypertrophy. Proc Natl Acad Sci U S A 1991 (Sep 15);88(18):8277–81.

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[11] Boutz PL, Chawla G, Stoilov P, Black DL. MicroRNAs regulate the expression of the alternative splicing factor nPTB during muscle development. Genes Dev 2007 (Jan 1);21(1):71–84.
[12] Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. Cell 2003 (Oct 17);115(2):209–16.
[13] Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M. MicroRNAs play an essential role in the development of cardiac hypertrophy. Circ Res 2007 (Feb 16);100(3):416–24.
[14] van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, et al. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc Natl Acad Sci U S A 2006 (Nov 28);103(48):18255–60.
[15] Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005 (Jul 15);65(14): 6029–33.
[16] Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 2005;33(4):1290–7.

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