

microRNA-340-5p Functions Downstream of Cardiotrophin-1 to Regulate Cardiac Eccentric Hypertrophy and Heart Failure via Target Gene Dystrophin

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SUMMARY

Pathological cardiac hypertrophy inevitably leads to the unfavorable outcomes of heart failure (HF) or even sudden death. microRNAs are key regulation factors participating in many pathophysiological processes. Recently, we observed upregulation of microRNA-340-5p (miR-340) in failing human hearts because of dilated cardiomyopathy, but the functional consequence of miR-340 remains to be clarified.

We transfected neonatal cardiomyocytes with miR-340 and found fetal gene expression including *Nppa*, *Nppb* and *Myh7*. We also observed eccentric hypertrophy development upon treatment which was analogous to the phenotype after cardiotrophin-1 (CT-1) stimulation. As a potent inducer of cardiac eccentric hypertrophy, treatment by IL-6 family members CT-1 and leukemia inhibitory factor (LIF) led to the elevation of miR-340. Knockdown of miR-340 using antagomir attenuated fetal gene expression and hypertrophy formation, which means miR-340 could convey the hypertrophic signal of CT-1. To demonstrate the initial factor of miR-340 activation, we constructed a volume overloaded abdominal aorta-inferior vena cava fistula rat HF model. miR-340 and CT-1 were found to be up-regulated in the left ventricle. Dystrophin (DMD), a putative target gene of miR-340 which is eccentric hypertrophy-susceptible, was decreased in this HF model upon Western blotting and immunohistochemistry tests. Luciferase assay constructed in two seed sequence of DMD gene 3'UTR showed decreased luciferase activities, and miR-340 transfected cells resulted in the degradation of DMD.

miR-340 is a pro-eccentric hypertrophy miRNA, and its expression is dependent on volume overload and cytokine CT-1 activation. Cardiomyocyte structure protein DMD is a target of miR-340. (Int Heart J 2015; 56: 454-458)

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Heart failure (HF) is a major public health concern and is the leading cause of mortality worldwide. HF frequently is the unfavorable outcome of pathological cardiac hypertrophy.¹⁾ To increase cardiac output or decrease wall stress, compensatory cardiac hypertrophy phenotype is induced, but prolonged hypertrophy eventually leads to left ventricle dilation, contractile dysfunction, and finally to HF.²⁾ So pro-active intervention based on pathological hypertrophy is thought to be a beneficial strategy for treating or reversing HF.

microRNAs (miRNAs) are small non-protein-coding RNA which negatively control gene expression at the post-transcriptional level by binding to the 3' untranslated region (3'UTR) of target mRNAs by either degradation of the bound mRNA or direct translational inhibition.³⁾ miRNAs could substantially alter the gene expression mode and modulate cell

fate such as proliferation, apoptosis, and migration.^{4,5)} Increasing evidence has demonstrated dysregulated expression patterns of specific miRNAs could alter the cellular response of cardiomyocytes, leading to the pathology of cardiac concentric hypertrophy and heart failure.^{2,6-8)} For example, miR-133 was down-modulated in hypertrophic human heart and played a role in HF via target genes *RhoA*, *Cdc42* and *Nelf-A/WHSC2*;⁹⁾ miR-195 transgenic mice showed pathological cardiac growth and heart failure;³⁾ and the transcription of miR-23a was activated by nuclear factor of activated T cells (NFAT) whose hypertrophic effect was ascribed to miR-23a.¹⁰⁾

Previously we found some dysregulated miRNAs in failing human hearts caused by dilated cardiomyopathy, in which microRNA-340-5p (miR-340) represented the most significant difference (2.6 fold upregulation).¹¹⁾ However, the functional significance of miR-340 is still not fully known. In the present

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study, we have attempted to elucidate whether miR-340 plays a role in the HF process and determine the exact pathway in which it participates.

METHODS

Primary cardiomyocyte culture, transfection, and immunofluorescence: Primary cardiomyocytes were isolated from neonatal C57Bl/6 mice⁷⁾ and cultivated in 6 well plates with Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 110 U/mL penicillin, 100 μ g/mL streptomycin, and 10% fetal bovine serum (Gibco) in humidified air (5% CO₂) at 37°C. For transfection or chemical treatments, cells were seeded into 6-well culture plates (5-10 \times 10⁴ cells/cm²) and serum-starved for 18 hours, and either infected with lentivirus at a multiplicity of infection (MOI) of 100 or with cardiotrophin-1 (CT-1) (1nM), isoprenaline (ISO, 10 μ M) and leukemia inhibitory factor (LIF) (1000 U/mL) for 48-72 hours.

Cells subjected to various treatments were washed, fixed, permeabilized, blocked with goat serum, incubated with primary α -actin antibody and Alexa Fluor 568-conjugated antibody (ZSGB-Bio, Beijing, China), and stained with DAPI (Roche), with each step being followed by a wash procedure in cold PBS.¹²⁾

Lentivirus and antagomir production: Virus packaging was performed in HEK 293T cells after co-transfecting 20 μ g pGCsil-GFP-miR-340 vector with 15 μ g of the packaging plasmid pHler 1.0 vector and 10 μ g of envelope plasmid pHler 2.0 vector using Lipofectamine 2000 (Genchem, Shanghai, China). Antagomir was synthesized to inhibit miR-340 expression. The single-strand RNA analogue sequence complementary to miR-340 was 5'-AAUAUU-UCGUUACUCUGACUAA-3' and the negative control sequence was 5'-AGUGUU-GGAGGAUCUUUCUCAUCU-3', both of which were chemically modified, cholesterol-conjugated and 2'OMe modified. Antagomir and riboFECTTM reagent were produced by Ribobio Co. (Guangzhou, China).

Animals and animal procedures and echocardiography: Sprague-Dawley rats (8 weeks old) were used for the chronic volume overload heart failure model. An abdominal aorta-inferior vena cava fistula was performed as previously described.¹³⁾ Transthoracic echocardiography was performed on lightly anesthetized rats using a Vevo 770 high resolution imaging system (VisualSonics, Canada) at 10-14 weeks postoperatively. M-mode images were obtained and left ventricular parameters including end-diastolic volume (LVEDV), systolic internal diameter (LVIDs), and diastolic posterior wall thickness (LVP-Wd) were measured and fractional shortening (FS) was calculated. The Ethics Committee of Capital Medical University approved the above animal protocols.

Real-time PCR analysis: Total RNA was extracted from cultured cardiomyocytes and heart tissues using an RNeasy Pure Tissue and Cell Kit (Tiangen, Beijing, China) according to the manufacturer's protocol. For quantitative detection of miR-340, mirVanaTM qRT-PCR microRNA Detection Kits were used in conjunction with real-time PCR with an SYBR Green I according to the manufacturer's protocol. U6 rRNA was used as an internal control. For the quantification of DMD, ANP, BNP, MYH7, and CT-1 transcripts levels, cDNAs were synthesized using a Quant cDNA kit (Tiangen, Beijing, Chi-

na). Real-time PCR was performed using a specific set of primers. GAPDH was used for normalization of their expression levels. The results represent at least three experiments. Primer sequences are listed in the Table.

Western blotting, histological examination, and immunohistochemistry: Total proteins were extracted from cardiomyocytes and left ventricle. Samples were placed in RIPA buffer and 1mM PMSF (Solarbio, Beijing, China), homogenized, incubated for 10 minutes and centrifuged at 14,000 g for 10 minutes. A measure of 20-50 μ g protein was loaded, separated by 3% to 12% gradient SDS-polyacrylamide gel electrophoresis, and blotted on PVDF membrane. Blots were incubated with primary antibody Dy4/6D3 (1:250 dilution, Leica Biosystems, Newcastle, UK) which identify the mid rod domain of dystrophin and β actin. HRP-conjugated secondary antibody and luminal/parcumaric acid were used to determine the specific bands.

After dissection, the rat hearts were formalin-fixed and paraffin-embedded for hematoxylin-eosin staining or snap-frozen sectioned for immunohistochemistry to detect DMD at 1:20 of working dilution as described.¹⁴⁾

Luciferase assay: Luciferase assay was carried out in the 293T cell line using a Dual-Luciferase Assay Kit (Promega). The conserved miR-340 binding sequences in the 3' UTR segment of dystrophin were cloned into the luciferase vector (Ambion). Seed mutations for target site 1 or 2 were generated and luciferase activity was assessed with the Luciferase Assay System after 48 hours incubation as described.¹⁵⁾

Evans blue assay: Fluorescent staining of Evans blue assay was performed to analyze the integrity of the sarcolemmal membrane. Evans blue (100 mg/kg, Sigma-Aldrich) was injected into the precava of anesthetized rat models, and after 20 minutes of perfusion, the hearts were removed and washed in cold PBS. The heart tissues were then embedded and cut by cryosection. The cryosections were imaged under a fluorescent microscope.¹⁶⁾

Table. Oligonucleotides Used for Real-Time RT-PCR

Gene	Sequence of primer (5'-3')
mouse-Nppa	Forward: TCCTCGTCTTGGCCTTTTG
	Reverse: CTCATCTTCTACCGGCATCTTC
mouse-Nppb	Forward: GCACAAGATAGACCGGATCG
	Reverse: CCCAGGCAGAGTCAGAAAC
mouse-Myh7	Forward: CCATCTCTGACAACGCCTATC
	Reverse: GGATGACCCTCTTAGTGTTGAC
mouse-Dmd	Forward: TCCTCTCCTTCCACCTCTC
	Reverse: TCTAACCTGTGCTTGCTGTC
rat-Nppa	Forward: AACCTGCTAGACCACCTGGA
	Reverse: GTCAATCTACCCCCGAAGC
rat-Nppb	Forward: CTTTTCCTTAATCTGTCGCCG
	Reverse: GTCTCTGAGCCATTCTCTCTG
rat-Myh7	Forward: ACAACCCCTACGATTATGCG
	Reverse: CGCCTGTGAGCTTGTAATG
rat-CT-1	Forward: TTCCTACCCCAATTGGAGGC
	Reverse: TCCTCCAGCAATTGGTCTGC

Statistical analysis: Group data are expressed as the mean \pm SEM of at least 3 independent experiments. Statistical comparisons (one-way ANOVA) were carried out using SPSS 12.0. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

miR-340 is sufficient to induce cardiac eccentric hypertrophy:

To determine whether miR-340 has functional roles in HF, we transfected neonatal mouse cardiomyocytes with lentiviral vector containing miR-340 precursor sequence (LV340) and scramble sequence (LVss). After 48 hours of transfection, cardiomyocytes expressed significantly increased miR-340 (Supplemental Figure 1A). We observed that miR-340 elevation could induce a secretion of fetal genes including atrial natriuretic factor A, B (Nppa, Nppb), and β -myosin heavy chain (Myh7) (Supplemental Figure 1C). We also observed cells presenting the morphology of hypertrophy where the increased size was more prominent in cell length than in cell width (Supplemental Figure 1B). The assembly of sarcomere units was more likely in series than in parallel, just like that seen after catecholamine stimulation, such as with ISO. We measured the maximum longitudinal extension and compared it with negative control (NC) cells and LVss transfected cells ($n \geq 50$) (Supplemental Figure 1D). The cell length in the LV340 group was significantly greater than that in NC and LVnc ($P < 0.05$). Such a hypertrophy effect of miR-340 is more similar to that of CT-1,¹⁷ and this encouraged us to explore the relationship

between miR-340 and CT-1.

miR-340 up-regulation is induced by CT-1: CT-1, a member of the interleukin-6 (IL-6) superfamily, is a potent inducer of cardiac eccentric hypertrophy whose expression level was reported to be increased in HF patients due to dilated cardiomyopathy.¹⁸ Thus, we attempted to clarify whether miR-340 participated in the hypertrophy process induced by CT-1. Both CT-1 and ISO treated cardiomyocytes showed increased expression of fetal genes (Supplemental Figure 2A), but elevated cell length was only detected in the CT-1 group (Supplemental Figure 2B). When we analyzed the expression of miR-340, we found up-regulation of miR-340 upon CT-1 treatment, while ISO treatment did not induce miR-340 expression (Supplemental Figure 2C). We know that CT-1 functions via a hypertrophic effect through the leukemia inhibitory factor (LIF) receptor/gp130 signal pathway.¹⁹ LIF treated cells showed an induction pattern similar to that of miR-340 and hypertrophic properties similar to those CT-1, which suggest a mediator effect for the gp130/LIF receptor pathway in the induction of miR-340. In view of the effect of mechanical stretch on the induction of CT-1,²⁰ dysregulated hemodynamics may be the initiator of miR-340. In a previous study, upregulated miR-340 was seen in HF patients because of dilated cardiomyopathy,¹¹ so volume-overload may be the initiating factor participating in the activation of miR-340.

Volume overloaded cardiac expression of miR-340 during the development of HF: We next constructed an abdominal aorta–inferior vena cava fistula rat model and assessed miR-340 expression. After 10–14 weeks of compensation stage, cardiac morphology, left ventricle parameters including LVPWd, LVIDd, and LVIDs, and fetal gene expression all demonstrated the pathophysiology of eccentric hypertrophy (Figure 1A–D), although FS was elevated and LVAWd showed no significant difference (data not shown). In left ventricle tissue samples, cardiac hypertrophy induced by volume overload resulted in increased expression of miR-340 as shown by RT-PCR analysis (Figure 1E), which was in accordance with previous human HF results.¹¹ We also detected the expression of CT-1, and its expression was elevated as expected (Figure 1F). This in vivo study further confirmed the induction effect of CT-1 on miR-340.

miR-340 is able to convey hypertrophy signal of CT-1: We next endeavored to clarify whether miR-340 participated in mediating the eccentric hypertrophy effect of CT-1. Chemically modified antagomir was used to inhibit miR-340 expression. After 48 hours of incubation, miR-340 was down-regulated and this attenuated CT-1 induced cell length increase and fetal gene expression (Supplemental Figure 3A–C). These results proved that miR-340 was a key component of the CT-1–gp130/LIF receptor pathway in inducing cardiac eccentric hypertrophy.

Dystrophin is decreased in volume overloaded failing heart: To demonstrate the target gene of miR-340, we attempted to identify potential target genes in silico, especially those having a relationship with eccentric hypertrophy. Interestingly, dystrophin (DMD) was the putative target gene with the highest score in target gene analysis software that included “DIANA TOOLS”, “MICRORNA.ORG”, “MIRDB”, and “TARGET SCAN”. The DMD gene is one of the largest genes that contains different transcripts. DMD (427m) is a key structural protein in muscle, which links cytoskeleton and extracellular ma-

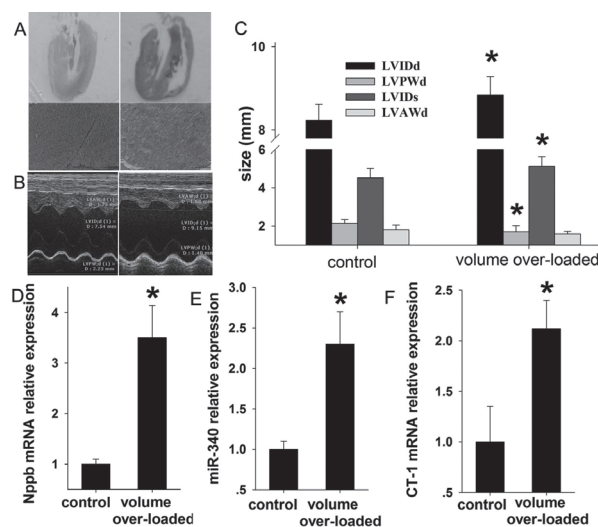


Figure 1. miR-340 is induced by volume overloaded cardiac eccentric hypertrophy. **A–C:** Rat model was prepared through abdominal aorta–inferior vena cava fistula. **A:** Histological examination of hearts and cross-sectional areas analyzed by hematoxylin–eosin staining (scale bar 100 μ m) of control group (left) and hypertrophy group (right). **B and C:** Cardiac size of rat model detected by M-mode echocardiography. **B:** Representative echocardiography pictures of control group (left) and hypertrophy group (right). **C:** Left ventricular parameters including end-diastolic volume (LVEDV), systolic internal diameter (LVIDs), and diastolic posterior wall thickness (LVPWd) of control group and hypertrophy group ($n \geq 5$). **D–F:** Fetal genes of Nppb (**D**), miR-340 (**E**) and CT-1 (**F**) were induced by volume overload ($n \geq 5$). * $P < 0.05$ versus control group.

trix and protects cardiomyocytes from shear stress in the course of contraction and relaxation.²¹⁾ DMD gene mutation is responsible for Duchenne and Becker muscular dystrophies, which present a dilated cardiomyopathy phenotype. DMD is also susceptible to degradation by Coxsackie virus and results in dilated cardiomyopathy phenotype.²²⁾ Such background knowledge encouraged us to determine whether DMD is a target gene of miR-340. We first detected the expression level of DMD in a volume overloaded rat HF model. After about 2-3 months of induction, DMD was significantly decreased in left ventricle (Supplemental Figure 4A). Immunohistochemical results also showed decreased expression of DMD in cell membrane (Supplemental Figure 4B). We also evaluated the sarcolemmal integrity of cardiomyocytes using fluorescent Evans blue staining. We observed some Evans blue staining positive myocytes, especially in interventricular septum (Supplemental Figure 4C), which was most likely ascribed to the down-regulated DMD.

DMD is a target of miR-340: To determine whether DMD is a molecular target of miR-340, luciferase assay was conducted to observe the effect of miR-340 on DMD translation. DMD gene has at least two 'high score' conservative miR-340 seed sequences in its 3'UTR (Figure 2A). Co-transfection of LV340 with luciferase reporter gene linked to wild-type 3'UTR of DMD gene resulted in significantly decreased luciferase activities in both sites, but there were no significant differences in luciferase activities in mutated sites (Figure 2B). In addition, we analyzed DMD expression in LV340 transfected cardiomyocytes. As shown in Figure 2C, the DMD protein expression level was significantly decreased after 48 hours of transfection, but no significant difference in mRNA was detected ($P > 0.05$), suggesting that miR-340 predominantly suppresses DMD translation other than degradation of mRNA.¹⁰⁾ Such results confirmed DMD to be a direct target gene of miR-340.

DISCUSSION

Dysregulated expression of miRNAs in response to external stimuli plays a key role in many types of pathogenesis, including heart diseases. Our present work reveals that volume overload of heart initiates the release of CT-1 which subsequently induces the expression of miR-340. Furthermore, miR-340 plays a key role in the pathology of eccentric hypertrophy and HF via its inhibition effect on target gene cardiac structural protein DMD (427m).

Previously, we found up-regulated miR-340 in heart tissues of HF patients because of dilated cardiomyopathy.¹¹⁾ Function analysis results proved that persistent expression of miR-340 in cardiomyocytes is sufficient to induce HF as shown by fetal gene expression. At the same time, miR-340 is necessary for the induction of eccentric hypertrophy, which is different from concentric hypertrophy like after catecholamine stimulation. Such assembly of sarcomere units in parallel is more analogous to that of a CT-1 induced hypertrophy pattern.¹⁷⁾

CT-1 is one member of the IL-6 family which initiates cardiac hypertrophy through the gp130/LIF receptor signaling pathway, and the activation of gp130/LIF receptor via CT-1 is a pivotal pathway leading to eccentric hypertrophy.^{19,23)} Some studies found increased plasma CT-1 levels in HF patients be-

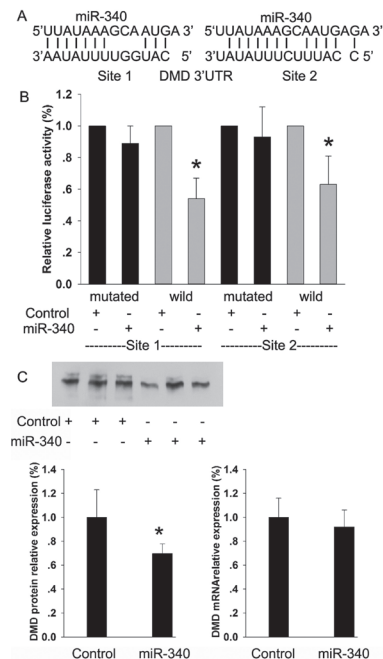


Figure 2. DMD is a target of miR-340. **A:** Two sites of putative miR-340 target in 3'UTR of DMD. **B:** miR-340 inhibits the translation activity of DMD. The luciferase activities in two wild-type sites were decreased, but in mutated sites no significant difference was detected ($n \geq 3$). **C:** miR-340 inhibits the translation of DMD. DMD was decreased in LV340 transfected cardiomyocytes shown by Western blotting, but no significant difference in mRNA was detected ($n \geq 5$). * $P < 0.05$ versus control.

cause of dilated cardiomyopathy,²⁴⁻²⁶⁾ which indicated there was a significant correlation between up-regulation of CT-1 and left ventricle systolic dysfunction.^{27,28)} In our study, miR-340 was up-regulated by the stimulation of CT-1, but was not altered in response to ISO. Furthermore, LIF, another member of the IL-6 family, also exhibited the up-regulation effect of miR-340. Thus, it is apparent that the induction of miR-340 is a downstream factor of the gp130/LIF receptor pathway. In addition, knockdown of miR-340 attenuated the potent hypertrophic signal of CT-1. Thus, miR-340 is indeed a pro-eccentric hypertrophic miRNA participating in the gp130/LIF receptor pathway. A previous in vitro study proved the activating effect of mechanical stretch on the expression of CT-1.²⁰⁾ To further investigate the initiator of cytokine CT-1 and miR-340 in vivo, we constructed a volume overloaded rat HF model. At the decompensation stage, rats displayed significant cardiac eccentric hypertrophy, whose hemodynamics were similar to those of dilated cardiomyopathy.²⁹⁾ The expression of CT-1 in left ventricle was elevated in response to the dysregulated hemodynamics of volume overload. At the same time, miR-340 was increased as expected. Thus, we can conclude CT-1 has an inducing effect on miR-340 both in vitro and in vivo. In addition, the above results demonstrated the key role of external stimuli such as hemodynamic changes in the induction of microRNA, and the contribution of microRNA in the regulation of cell fate, such as cardiac hypertrophy.

Target gene research is a key step to analyze the biological function of a miRNA. In our study, DMD (427m) was proven to be a target of miR-340. DMD is one kind of key

structural protein located in the subsarcolemmal layer of cardiomyocytes and protects cells from shear stress in the course of contraction and relaxation, so a deficiency of DMD could result in the disruption of the sarcolemmal membrane and disorganization of costameric cytoskeleton.²¹⁾ Genetic mutation of DMD underlies cell degeneration in several cardiomyopathies including Duchenne and Becker muscular dystrophies, where dilated cardiomyopathy is the most prominent pathology.³⁰⁾ As one of the largest proteins in humans, DMD is also vulnerable to enteroviral protease 2A, produced by Coxsackie virus, and thus induces dilated cardiomyopathy.^{16,22)} In our study, we found decreased DMD in decompensated failing heart because of volume overload. The sarcolemmal integrity of cardiomyocytes was destroyed because of decreased expression of DMD in cell membrane. Luciferase assay proved that miR-340 indeed can influence the protein translation level of DMD. Following western blot and PCR analysis in transfected cells also proved miR-340 could inhibit the translation of DMD. Such data suggest that DMD is a target gene of miR-340.

Conclusion: Our study reveals an important role for miR-340 in the mediation of the hypertrophy signal. Furthermore, DMD (427m) was found to be a target gene of miR-340 leading to eccentric hypertrophy. Taken together, we provide evidence that miR-340 links the risk factor of heart failure and the pathogenesis of dilated cardiomyopathy.

REFERENCES

- Rosca MG, Tandler B, Hoppel CL. Mitochondria in cardiac hypertrophy and heart failure. *J Mol Cell Cardiol* 2013; 55: 31-41. (Review)
- Ucar A, Gupta SK, Fiedler J, *et al.* The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. *Nat Commun* 2012; 3: 1078.
- van Rooij E, Sutherland LB, Liu N, *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; 103: 18255-60.
- Papageorgiou N, Tousoulis D, Androulakis E, *et al.* The role of microRNAs in cardiovascular disease. *Curr Med Chem* 2012; 19: 2605-10. (Review)
- Orenes-Piñero E, Montoro-García S, Patel JV, Valdés M, Marín F, Lip GY. Role of microRNAs in cardiac remodelling: new insights and future perspectives. *Int J Cardiol* 2013; 167: 1651-9. (Review)
- Jeong MH, Lee JS, Kim do H, Park WJ, Yang DK. Identification of novel microRNAs negatively regulating cardiac hypertrophy. *Biochem Biophys Res Commun* 2012; 428: 191-6.
- Han M, Yang Z, Sayed D, *et al.* GATA4 expression is primarily regulated via a miR-26b-dependent post-transcriptional mechanism during cardiac hypertrophy. *Cardiovasc Res* 2012; 93: 645-54.
- Kelly M, Bagnall RD, Peverill RE, *et al.* A polymorphic miR-155 binding site in AGTR1 is associated with cardiac hypertrophy in Friedreich ataxia. *J Mol Cell Cardiol* 2011; 51: 848-54.
- Carè A, Catalucci D, Felicetti F, *et al.* MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 2007; 13: 613-8.
- Lin Z, Murtaza I, Wang K, Jiao J, Gao J, Li PF. miR-23a functions downstream of NFATc3 to regulate cardiac hypertrophy. *Proc Natl Acad Sci U S A* 2009; 106: 12103-8.
- Zhu X, Wang H, Liu F, *et al.* Identification of micro-RNA networks in end-stage heart failure because of dilated cardiomyopathy. *J Cell Mol Med* 2013; 17: 1173-87.
- Ge Y, Pan S, Guan D, *et al.* MicroRNA-350 induces pathological heart hypertrophy by repressing both p38 and JNK pathways. *Biochim Biophys Acta* 2013; 1832: 1-10.
- Brower GL, Levick SP, Janicki JS. Inhibition of matrix metalloproteinase activity by ACE inhibitors prevents left ventricular remodeling in a rat model of heart failure. *Am J Physiol Heart Circ Physiol* 2007; 292: H3057-64.
- Yanagawa B, Lovren F, Pan Y, *et al.* miRNA-141 is a novel regulator of BMP-2-mediated calcification in aortic stenosis. *J Thorac Cardiovasc Surg* 2012; 144: 256-62.
- van Mil A, Grundmann S, Goumans MJ, *et al.* MicroRNA-214 inhibits angiogenesis by targeting Quaking and reducing angiogenic growth factor release. *Cardiovasc Res* 2012; 93: 655-65.
- Xiong D, Lee GH, Badorff C, *et al.* Dystrophin deficiency markedly increases enterovirus-induced cardiomyopathy: a genetic predisposition to viral heart disease. *Nat Med* 2002; 8: 872-7.
- Wollert KC, Taga T, Saito M, *et al.* Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series VIA gp130/leukemia inhibitory factor receptor-dependent pathways. *J Biol Chem* 1996; 271: 9535-45.
- Zolk O, Ng LL, O'Brien RJ, Weyand M, Eschenhagen T. Augmented expression of cardiotrophin-1 in failing human hearts is accompanied by diminished glycoprotein 130 receptor protein abundance. *Circulation* 2002; 106: 1442-6.
- Calabrò P, Limongelli G, Riegler L, *et al.* Novel insights into the role of cardiotrophin-1 in cardiovascular diseases. *J Mol Cell Cardiol* 2009; 46: 142-8. (Review)
- Pan J, Fukuda K, Saito M, *et al.* Mechanical stretch activates the JAK/STAT pathway in rat cardiomyocytes. *Circ Res* 1999; 84: 1127-36.
- Rybakova IN, Patel JR, Ervasti JM. The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin. *J Cell Biol* 2000; 150: 1209-14.
- Badorff C, Lee GH, Lamphear BJ, *et al.* Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. *Nat Med* 1999; 5: 320-6.
- Bristow MR, Long CS. Cardiotrophin-1 in heart failure. *Circulation* 2002; 106: 1430-2.
- Tsutamoto T, Wada A, Maeda K, *et al.* Relationship between plasma level of cardiotrophin-1 and left ventricular mass index in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 2001; 38: 1485-90.
- Ruiz-Hurtado G, Gómez-Hurtado N, Fernández-Velasco M, *et al.* Cardiotrophin-1 induces sarcoplasmic reticulum Ca(2+) leak and arrhythmogenesis in adult rat ventricular myocytes. *Cardiovasc Res* 2012; 96: 81-9.
- Celik A, Sahin S, Koc F, *et al.* Cardiotrophin-1 plasma levels are increased in patients with diastolic heart failure. *Med Sci Monit* 2012; 18: CR25-31.
- Ravassa S, Beloqui O, Varo N, *et al.* Association of cardiotrophin-1 with left ventricular systolic properties in asymptomatic hypertensive patients. *J Hypertens* 2013; 31: 587-94.
- Stejskal D, Ruzicka V. Cardiotrophin-1. Review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2008; 152: 9-19. (Review)
- Scheuermann-Freestone M, Freestone NS, Langenickel T, Höhnelt K, Dietz R, Willenbrock R. A new model of congestive heart failure in the mouse due to chronic volume overload. *Eur J Heart Fail* 2001; 3: 535-43.
- Jacobs PA, Hunt PA, Mayer M, Bart RD. Duchenne muscular dystrophy (DMD) in a female with an X/autosome translocation: further evidence that the DMD locus is at Xp21. *Am J Hum Genet* 1981; 33: 513-8.

SUPPLEMENTAL FILES

Supplemental Figure 1, 2, 3, 4

Please find supplemental files;

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