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Into molecular mechanism of cardiac hypertrophy: Insights from "miRNA-sponging" non-coding RNAs.

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Abstract

Pathological cardiac hypertrophy is a maladaptive remodelling process in myocardium induced by variable clinical diseases and Heart Failure (HF) comes to the endpoint of myocardium hypertrophy. Non-coding RNAs (ncRNAs), including long non-coding RNA and circular RNA, were identified involving in cardiac hypertrophy remodelling. Competing endogenous RNA (CeRNA) network are the most accepted hypothesis and numerous studies has shown that lncRNA and circRNA can act as miRNA sponges which ultimately regulating the expression of downstream mRNA. Here we summarize the non-coding RNA with miRNA-sponging characteristics and the reported CerRNA networks, as well as the therapeutic value for siRNA drugs in CeRNA regulatory axis in the near future.

Keywords: miRNA-sponging, Cardiac hypertrophy, CeRNA, ncRNA-miRNA

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Introduction

Cardiac hypertrophy, defined as the enlargement of cardiomyocytes both in cell size and cardiac mass [1], is commonly induced by atrial/ventricular wall stress and/or overloading of periphery pressure. According to its primary stimuli, cardiac hypertrophy is generally classified into two types, thus, physical cardiac hypertrophy (like exercise-induced hypertrophy, usually reversible) and pathological cardiac hypertrophy (gradually progress to heart failure). Mechanically, the latter one (pathological hypertrophy) was descripted as maladaptive cardiac remodelling and its inner pathological process is thought to be secondarily induced by variable clinical diseases, including hypertension [2], aortic stenosis [3], mitral or aortic regurgitation [4], Ischemic Cardiac Disease (ICD) [5], cardiomyopathy (caused by genetic mutation [6,7]) and so on. Without any active prevention, progressive deterioration on myocardium would, in turn, lead to the final step-Heart Failure (HF) [8], leaving one of the major public health problems with high morbidity and mortality. Here, we discussed the reported pathological causes for cardiac hypertrophy and focused on the potential clinical value of non-coding RNAs (ncRNAs).

Molecular mechanisms for pathological cardiac hypertrophy

During clinical diagnosis and treatment, contradiction always lies on the unmet need to early detect the process from myocardial hypertrophy to heart failure sensitively and to reverse the enlargement of cardiomyocytes and fibrosis of myocardium effectively [9,10]. Patients are always diagnosed with cardiac hypertrophy only when they appear obvious clinical symptoms. Therefore, it is urgent to find sensitive molecular biomarker or reversible drugs for this knotty problem.

Thanks to the advancement on sequencing techniques (such as next-generation sequencing, single-cell RNA transcriptome sequencing) in recent years, major breakthroughs for seeking potential stimuli and signaling mechanisms have been postulated on genetic (DNA), transcriptional (mRNA) and translational (protein) levels [11-15] in pathological cardiac hypertrophy, such as genetic mutations, impaired Ca^{2+} handling, mitochondrial dysfunction, m6A-methyletation and so on. Notably, researchers are gathering attention into complex mediatory networks between coding and non-coding transcriptome that occurs significant difference both in health heart and hypertrophy heart from animal model or human species, which brings non-coding RNA into our horizon, shedding new lights on the complexity [16].

Non-coding RNAs and CeRNA network

Endogenous non-coding RNAs [17-20], including microRNAs (miRNAs), long non-coding RNAs (lncRNAs)

and circular RNAs (circRNAs), are generally functional sequences without traditional protein-encoding function, which are unlike the "Central Dogma of Gene Expression". Nowadays, scientists are broadly acknowledged that non-coding RNAs exert a diverse repertoire of functions at transcriptional and post-transcriptional levels [21].

Among their various functions, hypothesis of Competing endogenous RNA(CeRNA) [22], is currently well-accepted and attract increasing attention, which mainly engaged with lncRNAs and circRNAs acting as miRNA sponges. Theoretically speaking, miRNAs are a class of short sequencings (characterized as 20-22 nt) which exerting their function by silencing or degrading target specific mRNAs [23]. Meanwhile, lncRNAs and circRNAs contain plenty parts of nucleotide sequences what are identified as miRNA Response Elements (MREs) [24], thus, miRNA recognition and combining site. By interacting with MREs, lncRNAs and circRNAs can sponging miRNA and regulating its activities, thus, to inhibiting or stimulating messenger RNA (mRNA) transcription ultimately [25-27].

Such molecules interactions were defined as Competing endogenous RNA network (CeRNA network). So far, CeRNA networks have been found in various cardiovascular diseases [25], including atherosclerosis, Myocardial Infarction (MI) and, undoubtfully, cardiac hypertrophy. Technically, researchers would use sequencing techniques (usually bulk sequencing or circRNA sequencing) to find Differential Expressing Gene (DEGs) of non-coding RNAs and mRNA in animal or human specimens. Then, down-regulating miRNAs targeted by circRNA and mRNA can be predicted with bioinformation analysis [18] or predictive online software like "miRDB". Construction of CeRNA networks is always built with "cytoscape" software by using Protein-to-Protein Interaction (PPI) function. After that, gain-of-function and loss-of-function experiments *in vivo* and *in vitro* should be applied for repeated verification. What's more, dual-luciferase assay report and RNA pull down experiments are selective for further verification. Around all above procedures, a convincing CeRNA network would be reported and as for therapeutic research, siRNA on CeRNA networks should be designed and injected in mice model with rounds of courses to see the efficacy.

Crosstalk between lncRNA and miRNA on cardiac hypertrophy

LncRNAs are a series of sequences usually ranging a length of more than 200 nt [21,24], characterized by the absence of Open Reading Frame (ORF) with low abundance and/or nuclear localization. Though lack of coding function, plenty of studies have illustrated that lncRNAs act as miRNAs sponges to regulate downstream mRNA on cardiac hypertrophy and heart failure [28].

For example, lncExACT1 was reported over-expressed in Transverse Aortic Constriction (TAC) surgical mice which progressed to heart failure after 2-8 weeks. It is found that lncExACT1 functioned by sponging miRNA-222 and regulating the expression of *DCHS2* gene, then, inducing pathological cardiac hypertrophy by regulating calcineurin and Hippo/Yap1 signaling pathways. These results provide a potentially tractable therapeutic target for clinical methods on cardiomyogensis [29]. What's more, Crosstalk on lncPvt1/miR-196b/OSMR [30], axis also illustrate a novel therapeutic role in cardiac hypertrophy. By knocking down *lncPvt1* expression, miR-196b would increase and attenuate cardiac hypertrophy by targeting 3' Untranslated Region (UTR) of OSMR (major mediator of cardiac remodelling). Same regulation by lncRNA CHRF/miR-93 [31], and lncRNA CYTOR/miR-155 [32], were proven *in vivo* and *in vitro*. The former CeRNA crosstalk regulates the AKT3 signaling pathway which improving cardiac hypertrophy. Knocking-down of CHRF (Cardiac Hypertrophy Related Factor) expression shows an inhibition on ISO-stimulated cardiomyocytes. The latter crosstalk shows the similar regulating mechanism as loss of function experiments shows the opposite trend on IkkB (P65) protein. Sometimes, lncRNAs play a reversible role in cardiac hypertrophy. For instance, lncRNA H19 suppresses cardiac hypertrophy through the microRNA-145-3p/SMAD4 axis [33]. Another research also strengthens the therapeutic role of lncH19 on regulating cardiac CaMKIIδ by sponging miR-675 [34].

Crosstalk between circRNA and miRNA on cardiac hypertrophy

CircRNAs, unlike their linear counterparts, are mainly synthesized by non-canonical mode on RNA splicing-what is, circularized by joining the 3′ and 5′ ends together with back-splicing [35]. As reported, circRNAs contain plenty of miRNA Response Elements (MREs), leading to the inhibition of target miRNA function and subsequently, regulating downstream target mRNAs.

For example, circ_000203 can enhance the expression of fibrosis-associated genes (*Col1a2* and *CTGF*) in cardiac fibroblasts by depressing targeted miR-26b-5p and induce cardiac hypertrophy and fibrosis [36]. While in contrast, Heart-Related circRNA (HRCR) can protect heart from pathological hypertrophy by targeting miR-223/ARC axis [37], since miR-223 acts as a positive regulator of cardiac hypertrophy. Moreover, circNfix/miR-145-5p [38], network could target Activating Transcription Factor 3 (ATF3) in cardiomyocytes and attenuate hypertrophy. Other identified circRNAs were also reported in hypertrophic heart, such as circSlc8a1/miR-133a axis [39], which was identified from pull-down techniques and identified as promising target for clinical drug by inhibiting the expression of Connective Tissue Growth Factor (CTGF). Researchers also try to inhibiting the circular function as silencing circHIPK3 can reverse hypertrophy by sponging miR-185-3p [40], *via CASR* gene expression. Engineered artificial circmiRs [41], were also reported by sponging miR-132 axis as an active attempt for pre-clinical research in reducing the expression of *MYH7* gene (Table 1).

Table 1: *CeRNA networks by lncRNAs or circRNAs in cardiac hypertrophy.*

CeRNA network	Target	Regulation	References
lncRNA ExACT1/ miR-222	Hippo/ Yap1 axis	Stimulation	[29]
lncPvt1/miR-196b	OSMR	Stimulation	[30]
lncRNA CHRF/miR-93	Akt3 axis	Stimulation	[31]
lncRNA CYTOR/ miR-155	NF-κB axis	Stimulation	[32]
lncRNA H19/miR-675	*CaMKIIδ* gene	Inhibition	[34]
lncRNA H19/miR-145-3p	SMAD4 axis	Inhibition	[33]
circ_000203/miR-26b-5p	*Col1a2* and *CTGF*	Stimulation	[36]
circRNA HRCR/miR-223	*ARC* gene	Inhibition	[37]
circNfix/miR-145-5p	*AFT3* gene	Inhibition	[38]
circSlc8a1/miR-133a	*CTGF* gene	Inhibition	[39]
circHIPK3/miR-85-3p	*CASR* gene	Inhibition	[40]

Future perspectives

Recent years, a group of ncRNA-miRNA interactions and construction of CeRNA networks have given scientists brand-new insights for clinical treatment on cardiac hypertrophy. With advancement on sequencing technologies, Gain- and loss of functions are prominent methods to verify the mechanism of CeRNA networks. We can assure that the picture of molecular mechanism on non-coding RNAs was unfolding gradually (Figure 1).

Undoubtedly, ncRNAs have been broadly acknowledged to play a vital role in cardiovascular diseases [20]. As for cardiac hypertrophy, future research should focus on myocardium-associated genes and fibrosis-related genes that interacted by ncRNAs [1,12]. From clinical perspective, all above researches deem ncRNAs and miRNA axis as underlying molecular therapy target for cardiac hypertrophy treatment [25]. Identification of ncRNAs molecules and construction of CeRNAs networks [18,42], both in human and animal models may facilitate the comprehensive regulation relationship on pathological myocardial hypertrophy directly or indirectly [43-45].

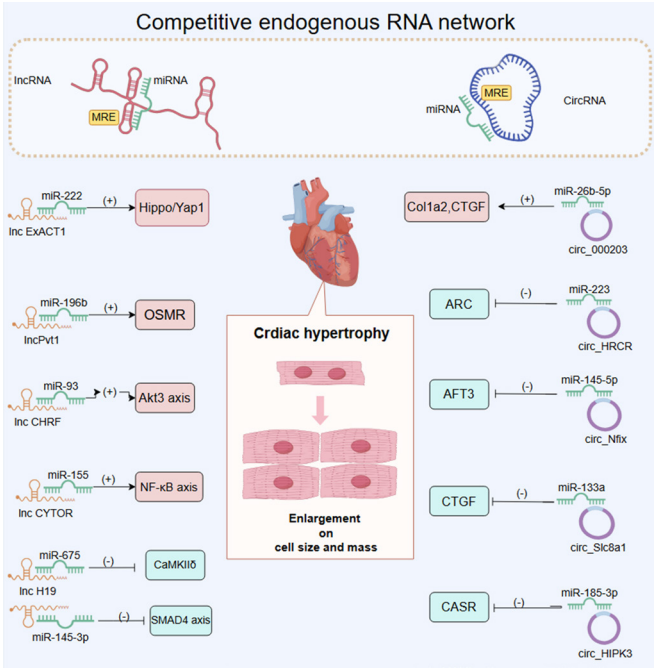

Figure 1. *Graphical abstract for competitive endogenous RNA network.*

Conclusion

In conclude, with the rise of molecule therapy like siRNAs drug in cardiovascular disease (like "inclisiran" in hyperlipidema), future research will focus on strategies on potential targets of ncRNAs. Valuable molecules siRNAs to hold back cardiac hypertrophy on the transcriptional level *via* CeRNA regulating network may prepare for further basic-to-clinic drug translation and clinical application. If so, cardiac hypertrophy treatment may come into a new era.

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