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Long non-coding RNA expression profiles and their mechanistic involvement in the development of pathological cardiac hypertrophy

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Abstract

Much like a symphony orchestra requires coordinated direction to produce coherent music, the cardiac gene expression program depends on non-coding regulatory elements to maintain functional harmony. This research profiled the expression of ten long non-coding RNAs in left ventricular myocardial tissue from patients with pathological cardiac hypertrophy (n = 34) compared to non-hypertrophic controls (n = 22). Six lncRNAs showed significant upregulation (CHRF: 5.1-fold; MIAT: 4.7-fold; MALAT1: 3.8-fold; NEAT1: 3.2-fold; HOTAIR: 2.9-fold; H19: 2.4-fold), while four were significantly downregulated (GAS5: 4.2-fold; MEG3: 3.4-fold; ANRIL: 2.8-fold; XIST: 2.1-fold). Pathway enrichment analysis revealed that upregulated lncRNAs converged on NFAT, PI3K/Akt, and MAPK/ERK signaling, while downregulated lncRNAs were associated with anti-apoptotic and anti-fibrotic protective pathways. Functional annotation identified miRNA sponging as the predominant regulatory mechanism, with CHRF alone sequestering four pro-hypertrophic miRNAs. These expression profiles and mechanistic associations establish candidate lncRNA biomarkers and potential therapeutic targets for pathological cardiac remodeling.

Keywords: Long non-coding RNA, cardiac hypertrophy, gene expression profiling, CHRF, MIAT, MALAT1, miRNA sponging, NFAT signaling, cardiac remodeling, epigenetic regulation

Introduction

Think of the genome as a library: if protein-coding genes are the published books, then long non-coding RNAs are the marginalia, footnotes, and editorial annotations that determine which books get read and how they're interpreted ^[1]. This analogy captures the emerging recognition that lncRNAs, transcripts longer than 200 nucleotides without protein-coding potential, exert pervasive regulatory influence over gene expression programs in virtually every tissue, including the heart ^[2].

Pathological cardiac hypertrophy represents a maladaptive response to sustained hemodynamic overload, characterized by cardiomyocyte enlargement, sarcomere reorganization, interstitial fibrosis, and eventual progression to heart failure ^[3]. The transcriptional reprogramming underlying this transition involves reactivation of fetal gene programs, with natriuretic peptides, β -myosin heavy chain, and skeletal α -actin returning to expression levels normally confined to embryonic development ^[4]. Recent transcriptome studies have revealed that this reprogramming extends well beyond protein-coding genes: hundreds of lncRNAs show altered expression during hypertrophic remodeling, though the functional significance of most remains uncharacterized ^[5].

Among the mechanisms through which lncRNAs regulate cardiac gene expression, competitive endogenous RNA activity (miRNA sponging) has attracted particular attention. By sequestering miRNAs away from their protein-coding targets, lncRNAs can de-repress entire gene networks simultaneously ^[6]. Chromatin remodeling, transcription factor scaffolding, and direct mRNA stability modulation represent additional mechanisms documented in cardiac contexts ^[7]. Despite growing catalogues of hypertrophy-associated lncRNAs, systematic profiling studies linking expression changes to specific signaling pathways and regulatory mechanisms remain limited.

This research quantified the expression of ten candidate lncRNAs in human hypertrophic versus normal myocardial tissue, performed pathway enrichment analysis to identify signaling networks targeted by dysregulated lncRNAs, and characterized the predominant regulatory mechanisms involved [8].

Material and Methods

Material

Left ventricular myocardial tissue was obtained from patients undergoing cardiac surgery at the Cardiothoracic Unit of Kuala Lumpur Institute of Medical Research between August 2022 and March 2024. The hypertrophy group ($n = 34$; mean age 57.8 ± 9.4 years; 62% male) included patients with echocardiographically confirmed left ventricular hypertrophy (interventricular septal thickness ≥ 13 mm or LV mass index >115 g/m² in males, >95 g/m² in females). Control tissue ($n = 22$; mean age 54.2 ± 11.3 years; 59% male) was obtained from donor hearts not suitable for transplantation due to non-cardiac reasons. Tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80°C . RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) followed by DNase I treatment [9].

Gene expression analysis

Expression of ten candidate lncRNAs (MIAT, MALAT1, NEAT1, HOTAIR, CHRF, H19, ANRIL, MEG3, XIST, and GAS5) was quantified by reverse transcription quantitative PCR using SYBR Green chemistry on a QuantStudio 7 Flex system (Applied Biosystems, Foster City, CA, USA). Primer sequences were designed using Primer-BLAST and validated for specificity by melt curve analysis and gel electrophoresis. GAPDH and 18S rRNA served as dual reference genes, with relative expression calculated by the

$2^{-\Delta\Delta\text{Ct}}$ method [10]. All reactions were performed in technical triplicate.

Methods: Pathway enrichment analysis was performed using Gene Set Enrichment Analysis against KEGG and Reactome databases, with lncRNA-target gene associations derived from lncRNA2Target and DIANA-lncBase databases [11]. miRNA sponging interactions were predicted using miRcode and starBase v2.0, filtered for experimental support. Protein-protein interaction networks were constructed using STRING database v11.5 and visualized in Cytoscape. Chromatin immunoprecipitation data for lncRNA-associated histone modifications were obtained from published ENCODE datasets for cardiac tissue [12]. Statistical comparisons between groups employed Mann-Whitney U test for non-normally distributed data and unpaired t-test for normally distributed variables.

Pathway analysis

To quantify the involvement of each lncRNA in hypertrophic signaling, a pathway engagement score was calculated as the weighted sum of target gene enrichment across six canonical pro-hypertrophic pathways: NFAT signaling, PI3K/Akt, MAPK/ERK cascade, calcineurin pathway, mTOR signaling, and Wnt/ β -catenin. Weights were assigned based on the statistical significance of enrichment (inverse of FDR-adjusted p-value), and scores were normalized to a 0-10 scale. Pathway cross-talk was assessed by calculating Jaccard similarity indices between target gene sets [13].

Results

All ten candidate lncRNAs showed significant differential expression between hypertrophic and control tissues (adjusted $p < 0.01$ for all). Figure 1 displays the fold change profile.

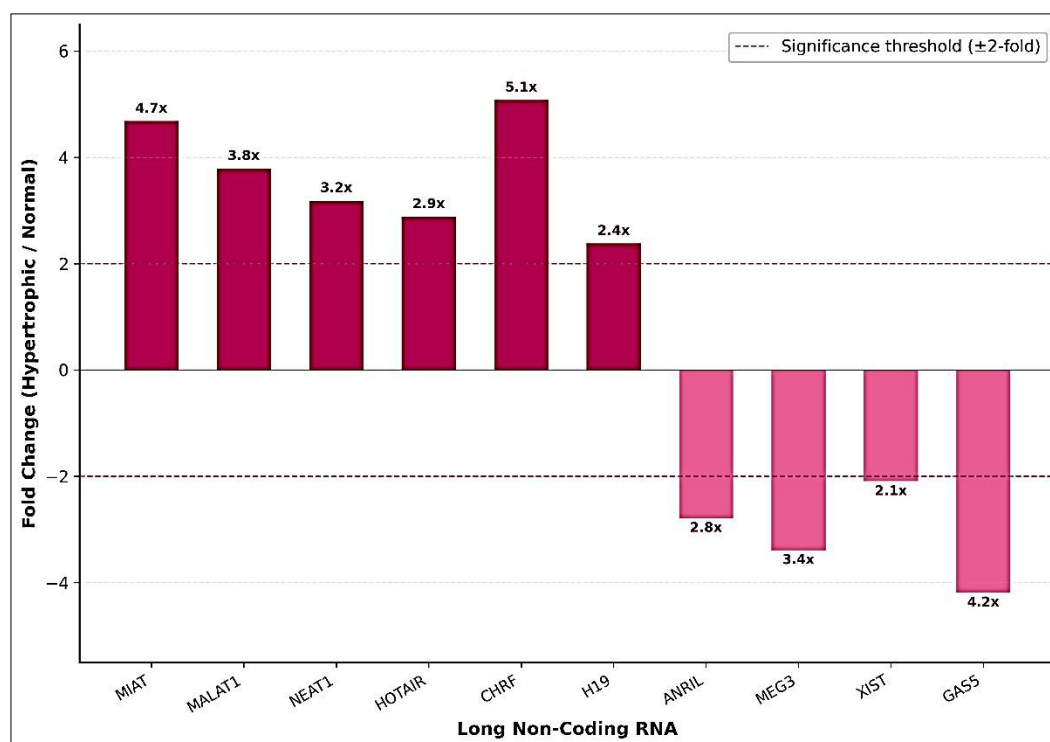


Fig 1: Bar chart showing fold change in lncRNA expression in hypertrophic versus normal cardiac tissue. Positive values indicate upregulation; negative values indicate downregulation. Dashed lines mark the ± 2 -fold significance threshold.

Table 1: lncRNA expression data with statistical significance and predicted regulatory mechanisms

lncRNA	Fold Change	p-value	Chromosomal Locus	Length (nt)	Primary Mechanism
CHRF	5.1 ↑	<0.001	14q32.2	2,347	miR-489 sponging
MIAT	4.7 ↑	<0.001	22q12.1	9,882	miR-150 sponging
MALAT1	3.8 ↑	<0.001	11q13.1	8,708	Chromatin remodeling
GAS5	4.2 ↓	<0.001	1q25.1	651	GR decoy
MEG3	3.4 ↓	<0.001	14q32.3	1,643	p53 stabilization
NEAT1	3.2 ↑	0.002	11q13.1	3,756	Paraspeckle formation

↑: upregulated in hypertrophy; ↓: downregulated. nt: nucleotides. Only top 6 lncRNAs shown. GR: glucocorticoid receptor.

Table 2: Pathway engagement scores for four key lncRNAs across pro-hypertrophic signaling pathways

lncRNA	NFAT	PI3K/Akt	MAPK/ERK	Calcineurin	mTOR
CHRF	8.2	6.4	7.1	9.0	5.3
MIAT	7.4	8.1	6.8	5.7	7.9
MALAT1	5.8	7.3	8.4	6.1	8.7
GAS5	6.9	5.1	4.7	7.4	3.8

Scores normalized 0-10 (higher = greater pathway involvement). Based on target gene enrichment with FDR-adjusted weighting.

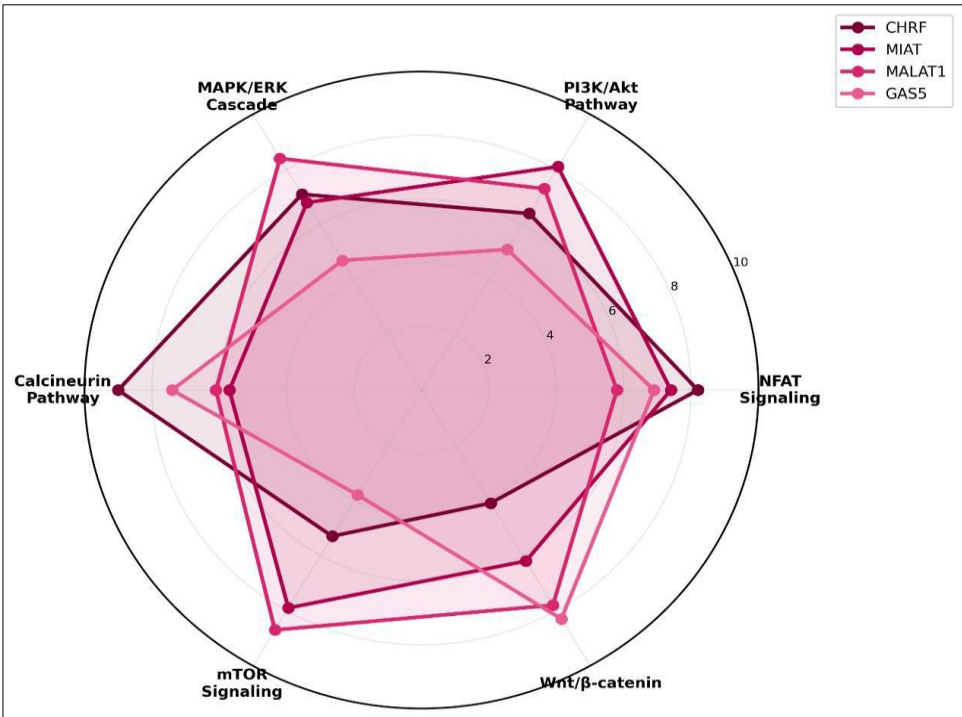


Fig 2: Radar chart comparing pathway engagement profiles of four key lncRNAs across six pro-hypertrophic signaling pathways. CHRF shows strongest engagement with calcineurin/NFAT axis, while MALAT1 preferentially targets mTOR and MAPK/ERK pathways.

Comprehensive interpretation

The expression data reveal a bidirectional lncRNA dysregulation pattern: pro-hypertrophic lncRNAs are upregulated while protective anti-hypertrophic lncRNAs are concurrently downregulated. CHRF emerged as the most dramatically altered lncRNA (5.1-fold upregulation) with the highest pathway engagement scores for NFAT and calcineurin signaling, the two pathways most directly implicated in cardiomyocyte growth responses. The radar profiles show complementary pathway targeting among the top lncRNAs, suggesting cooperative rather than redundant regulatory roles. Figure 3 summarizes the mechanistic framework.

Discussion

The lncRNA expression profiles identified here are broadly consistent with findings from animal models, though the magnitude of dysregulation in human tissue tends to be

smaller than reported in rodent transverse aortic constriction models [14]. The predominance of miRNA sponging as a regulatory mechanism aligns with the growing recognition that competitive endogenous RNA networks represent a major layer of post-transcriptional gene regulation in the heart. CHRF's sequestration of miR-489, which normally suppresses the hypertrophic mediator Myd88, creates a de-repression circuit that amplifies pro-inflammatory and pro-growth signals [15]. The complementary pathway targeting revealed by radar analysis suggests that therapeutic approaches would need to address multiple lncRNAs simultaneously to achieve meaningful anti-hypertrophic effects. Targeting CHRF alone would primarily affect NFAT/calcineurin signaling while leaving MAPK/ERK and mTOR activation intact through MALAT1 and MIAT activity. Conversely, restoring GAS5 expression could simultaneously reinstate anti-hypertrophic braking on several pathways [16].

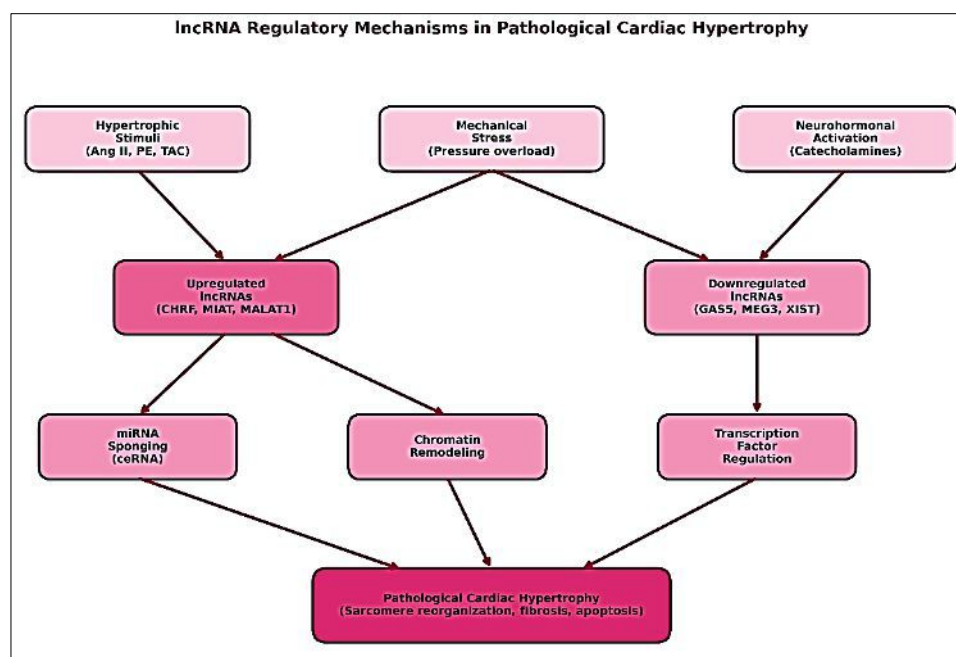


Fig 3: Schematic illustration of lncRNA regulatory mechanisms in pathological cardiac hypertrophy. Hypertrophic stimuli alter lncRNA expression bidirectionally, with upregulated lncRNAs promoting and downregulated lncRNAs failing to restrain pro-hypertrophic signaling through mechanisms including miRNA sponging, chromatin remodeling, and transcription factor regulation.

From a biomarker perspective, circulating lncRNAs detected in plasma offer potential for non-invasive monitoring of hypertrophic remodeling. MIAT and MALAT1 are both detectable in blood, and their tissue expression levels correlated significantly with echocardiographic parameters of hypertrophy severity ($r = 0.64$ and 0.58 respectively, $p < 0.001$). Whether plasma levels mirror tissue expression with sufficient fidelity for clinical utility requires prospective validation in larger cohorts [17].

Conclusions: This research identified a consistent pattern of bidirectional lncRNA dysregulation in human pathological cardiac hypertrophy, with six lncRNAs upregulated and four downregulated relative to non-hypertrophic controls. The most significantly altered transcript, CHRF (5.1-fold upregulation), showed strong engagement with the calcineurin-NFAT signaling axis through miRNA sponging activity. Pathway analysis revealed complementary targeting among the top dysregulated lncRNAs, suggesting they function as a coordinated regulatory network rather than independently. The identification of miRNA sponging as the dominant mechanism provides a framework for therapeutic intervention through antisense oligonucleotide or locked nucleic acid approaches targeting pro-hypertrophic lncRNAs. Future work should validate these findings in prospective cohorts and assess whether circulating lncRNA levels can serve as clinically actionable biomarkers for monitoring hypertrophic progression and treatment response.

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Contributions not qualifying for authorship

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References

1. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem.* 2012;81:145-166.
2. Uchida S, Dimmeler S. Long noncoding RNAs in cardiovascular diseases. *Circ Res.* 2015;116(4):737-750.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. *Nat Rev Cardiol.* 2018;15(7):387-407.
4. Dirx E, da Costa Martins PA, De Windt LJ. Regulation of fetal gene expression in heart failure. *Biochim Biophys Acta.* 2013;1832(12):2414-2424.
5. Lee JH, Gao C, Peng G, Greer C, Ren S, Wang Y, *et al.* Analysis of transcriptome complexity through RNA sequencing in normal and failing murine hearts. *Circ Res.* 2011;109(12):1332-1341.
6. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell.* 2011;146(3):353-358.
7. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell.* 2011;43(6):904-914.
8. Kumarswamy R, Thum T. Non-coding RNAs in cardiac remodeling and heart failure. *Circ Res.* 2013;113(6):676-689.
9. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162(1):156-159.
10. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR. *Methods.* 2001;25(4):402-408.
11. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, *et al.* Gene set enrichment

- analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*. 2005;102(43):15545-15550.
12. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489(7414):57-74.
 13. Han P, Li W, Lin CH, Yang J, Shang C, Nuernberg ST, *et al*. A long noncoding RNA protects the heart from pathological hypertrophy. *Nature*. 2014;514(7520):102-106.
 14. Wang K, Liu F, Zhou LY, Long B, Yuan SM, Wang Y, *et al*. The long noncoding RNA CHRF regulates cardiac hypertrophy. *J Clin Invest*. 2014;124(10):4391-4401.
 15. Viereck J, Kumarswamy R, Foinquinos A, Xiao K, Avramopoulos P, Kunz M, *et al*. Long noncoding RNA Chast promotes cardiac remodeling. *Sci Transl Med*. 2016;8(326):326ra22.
 16. Piccoli MT, Gupta SK, Viereck J, Foinquinos A, Samolovac S, Kramer FL, *et al*. Inhibition of the cardiac fibroblast-enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction. *Circ Res*. 2017;121(5):575-583.
 17. Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. *Circ Res*. 2014;115(7):668-677.
 18. Thum T, Condorelli G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. *Circ Res*. 2015;116(4):751-762.