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ORIGINAL ARTICLE

Abundant and Altered Expression of PIWI-Interacting RNAs during Cardiac Hypertrophy

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Background The discovery of PIWI-interacting RNAs (piRNAs) has fundamentally changed our understanding of post transcriptional regulation of transposons and other genes. Unlike miRNA and siRNA, the piRNAs are the most abundant but least studied RNA species in mammals. Although the expression of PIWI proteins and piRNAs has long been regarded as germline specific, increasing evidences suggest the expression of piRNAs in somatic cells.

Methods In this study, the small RNA sequencing executed during induction of cardiac hypertrophy in both *in vivo* and *in vitro* conditions were annotated for the expression of piRNAs. The expression of piRNAs was validated by qPCR and RNA immunoprecipitation. In addition, the presence of piRNAs in circulation of myocardial infarction patients was studied by qPCR.

Results We identified an abundant and altered expression of piRNAs during cardiac hypertrophy. The differentially expressed piRNAs was validated by qPCR and RNA immunoprecipitation. The significantly and differentially expressed piRNAs were predicted to target different retrotransposons and mRNAs in the rat genome. The detection of specific piRNA in serum of myocardial infarction patients suggests the potential of piRNA for diagnosis.

Conclusion Overall this study is the first to provide a whole-genome analysis of the large repertoire of piRNAs in the cardiac system and this would pave a new path to understanding the molecular aetiology of piRNA and retrotransposons in the physiology and pathology of the cardiac system.

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Keywords Cardiac hypertrophy • Small RNA deep sequencing • Small non-coding RNA • piRNA • Retrotransposons

Introduction

In animalia, three major classes of small non-protein coding regulatory RNAs have been identified: the microRNA (miRNA), the small interfering RNA (siRNA) and the PIWI-interacting RNA (piRNA). Compared with siRNAs and miRNAs, the molecular mechanism behind biogenesis and the role of piRNAs is far less clear. The size of piRNAs is comparatively longer (24-31 nt) than miRNA and evolutionarily less conserved. These piRNAs interact with a group of proteins called PIWI and forms RNA-protein complexes. These complexes are known to silence transposable elements (TEs) at both transcriptional and post-transcriptional level [22]. In addition, piRNA complexes are involved in epigenetic regulations. piRNAs originate from long precursor RNA transcripts that transcribe from distinct genomic regions called piRNA clusters [4]. However, the PIWI-piRNA pathway was initially perceived as specific to germ lines, now the somatic function is also documented [21].

Ground-breaking studies from various groups have proven the expression of piRNA in several somatic tissues [5,12,14,21]. Transposable elements play a vital part in the genome, as they drive evolution by accelerating the translocation of genomic sequences, shuffling of exons and repair of double-stranded disruptions [18]. Retrotransposons constituting $\sim$ 45% of mammalian genome are known to play a vital role in germ lines, neuronal cells and in diseases like diabetes, cancer and multiple sclerosis [8].

Cardiac hypertrophy is an adaptive response of the heart during which the terminally differentiated cardiomyocytes increase in size without undergoing cell division and prolonged hypertrophy can lead to heart failure [1]. The regulation of cardiac hypertrophy is mediated by copious intrinsic and extrinsic molecular signalling mechanisms. Numerous milestones have been achieved in studying the role of protein coding genes and their corresponding regulatory molecules, including miRNA during hypertrophy and heart failure [20]. Whereas, the role of TEs and its regulatory molecules like piRNAs, which are the most abundant small non coding RNA in animal system, is very poorly understood in cardiac tissues. Expression studies of a few selected piRNAs identified in the testes and central nervous system have been reported in the heart [12,14]. But, their relative abundance and altered expression during various stress conditions have not been studied in the cardiac system. In this study, we are reporting the abundant expression of piRNAs in the cardiac system and its altered expression during cardiac hypertrophy in both *in vivo* and *in vitro* systems. In addition, we show the expression of specific piRNAs in serum of patients with myocardial infarction.

Materials and Methods

Small RNA Sequencing Data

The small RNA sequencing data generated in our laboratory from cardiac hypertrophy models and corresponding controls were retrieved from the NCBI-GEO database (Accession numbers: GSE62883 and GSE73597) [20]. The hypertrophy model includes chronic swimming-induced hypertrophied rat heart and control rat heart (*in vivo)* and alpha-2 macroglobulin-induced hypertrophied H9c2 cells and control H9c2 cells (*in vitro*). The induction of hypertrophy was confirmed by studying geometrical parameters and molecular markers [20]. The expression profile of miRNAs also confirmed the induction of cardiac hypertrophy in both *in vivo* and *in vitro* models [20]. The small RNA sequencing was performed in Illumina HiSeq 2000.

Ovarian Small RNA Sequencing

piRNAs are enriched in germline tissues and hence rat ovary is used as a positive control for piRNA analysis. Eight-week old female Albino Wistar rats were anaesthesised with ketamine and subsequently the ovary was harvested. The animal protocol was approved by the Ethical Committee of Madurai Kamaraj University. Total RNA was isolated using TRIzol from the ovary and a small RNA library was constructed from the total RNA using a TruSeq kit (Illumina Inc., USA) as per manufacturer's instructions. Briefly, small RNA was ligated with specific adapters (Illumina RA3 - TGGAATTCTCGGGTGCCAAGG) and a cDNA library was synthesised by RT-PCR using adapter specific primers. Subsequently, the library was run on native PAGE gel and the bands representing the adapter ligated small RNAs of size range 16-33 nt were eluted and purified. Small RNA sequencing was executed at the Centre for Cellular and Molecular Platforms (Government of India) in an Illumina HiSeq and the sequences were deposited in NCBI-GEO database (Accession No. GSE73786)

Processing of Reads

Small RNA sequences from the deep sequencing datasets were initially quality filtered, adapter trimmed and sequences below 16 bp were discarded by fastq-mcf tool of ea-utils.1.1.2-537 package using the parameter -l 16 -q 15 -w 4 -x 10 -u -P 33. FASTQ files were then converted to FASTA file format by the NGS-toolkit package using the parameter Perl. Reads were then mapped to rn5 rat genome by Bowtie2 default parameter -f -x [11]. Mapped reads were retrieved using SAMtools and bam2FastQ [13]. Only those reads having matches within the genome were considered for further