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miRNA editing landscape reveals miR-34c regulated spermatogenesis through structure and target change in pig and mouse

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ABSTRACT

Spermatogenesis has a close relationship with male infertility. MicroRNAs (miRNAs) play crucial roles in their regulation of target genes during spermatogenesis. A huge dataset of high-throughput sequencing all over the world provides the basis to dig the cryptic molecular mechanism. But how to take advantage of the big data and unearth the miRNA regulation is still a challenging problem. Here we integrated transcriptome of spermatogenesis and found miRNA regulate spermatogenesis through miRNA editing. We then compared different species and found that the distributions of miRNA editing site number and editing types among different cell types during spermatogenesis are conservative. Interesting, we further found that nearly half of the editing events occurred in the seed region in both mouse and pig. Finally, we foundmiR-34c, which is edited frequently at all stages during spermatogenesis, regulates its target genes through the RNA structure changing and shows dysfunction when it is edited. Summary, we depicted the overall profile of miRNA editing during spermatogenesis in mouse and pig and reveal miR-34c may play its roles through miRNA editing.

1. Introduction

Spermatogenesis is a complex and dynamic process [1]. Spermatogonia undergo mitosis to form primary spermatocytes. Secondary spermatocytes are generated after the first division and contain duplicate haploid chromosomes. After the second meiotic division, round spermatids with half the number of chromosomes are formed. The round spermatids' morphology is elongated undergo complex and significant changes, and eventually, mature sperm is formed [2–4]. Such a complex process requires different molecules to execute their functions at different stages [5], and a considerable part of these molecules are named of microRNAs (miRNAs), which are recognized as vital regulatory factors to regulate the expression of their target genes [6].

miRNAs are endogenous small non-coding RNAs of about 20–24 nucleotides (nt) and they show important roles in regulating their target genes within different cells. The first 2–8 nt at the 5′-end of miRNAs is the key region to recognize their target and it is called seed region [7]. Each miRNA may have multiple target genes, and the same gene may be regulated by several miRNAssynergistically. It is reported that more than 30% of genes are regulated by miRNAs and they are conserved in different species [8]. Although some miRNAs are ubiquitously expressed in different cells, about 68% of miRNAs are expressed spatiotemporally [9]. Several experiments have revealed that several miRNAs are high, exclusively or preferentially expressed in the testis and in specific testicular cell types [10–12]. Furthermore, miR-34c, miR-21, miRNA-20, and miRNA-106a are preferentially expressed in mouse spermatogonial stem cells (SSCs). In SSCs, the downregulation of miR-21 increased apoptosis and reduced SSC potency [13]. miR-20 and miR-106a were shown to regulate the renewal of SSCs by targeting *STAT3* and *Ccnd1* [14]. miR-34c has been shown to play a role in maintaining stem cell status and regulating meiosis, and overexpression of miR-34c in germ cells triggers apoptosis [15–17]. In summary,

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we can conclude that miRNAs play an important role during spermatogenesis in mouse. But for miRNAs how to control the sperm cells development is still unclear.

Recently, some studies show that miRNA editing may regulate the processing of pre-miRNAs into mature miRNAs [18,19]. As a post-transcriptional regulator, RNA editing can change nucleic acids at the RNA level without affecting the corresponding DNA sequence. In addition, it is well known that miRNAs exert their regulatory functions on target genes no matter perfect or not match with their seed sequences in the 3′-untranslated regions (UTRs). So we hypothesize that miRNA editing, especially that occurs in the miRNA seed region, will affect the binding of the miRNAs to their target genes by changing the arrangement of the seed sequence, thereby changing the miRNA target. So, we want to make it clear whether RNA editing will affect the miRNA regulation and thus affects the entire spermatogenesis process.

Based on the rapid development of second-generation sequencing and the availability of large-scale small RNA sequencing data, it is possible to verify our hypothesis. High-quality bioinformatics tools for miRNA editing calling have been developed in recent years [20,21], providing us the basic experimental and analytical conditions. Here, we collected all the available small RNA sequencing data of mouse spermatogenesis to depict the miRNA editing panorama of mouse spermatogenesis. Then we integrated them with our miRNA sequencing data during pig spermatogenesis and analyzed the results of mouse and pig from a conservative perspective. Interesting, we found that the distributions of miRNA editing site number and editing types among different cell types during spermatogenesis are conservative. Furthermore, we found that nearly half of the editing events occurred in the seed region in both mouse and pig. Finally, we found miR-34c, which is edited frequently at all stages during spermatogenesis, regulates its target genes through the RNA structure changing and shows dysfunction when it is edited. Summary, we depicted the overall profile of miRNA editing during spermatogenesis in mouse and pig and reveal miR-34c may play its roles through miRNA editing.

2. Materials and methods

2.1. Identification of miRNA editing sites

All sequencing reads will be filtered as follows: (1) the quality of each read need to be at least 20 in more than three positions, (2) adaptor sequences will be removed, (3) reads with length longer (>28 bp) or shorter (<15 bp) than the typical length of a mature miRNA will be removed. The filtered reads were trimmed by 2 nt at 3′end because of the 3′end of mature miRNAs undergoes extensive modifications [22,23]. The filtered and trimmed reads were aligned using Bowtie [24] against their corresponding species, mouse (mm10) and pig (susScr11) genomes, allowing up to one mismatch, the best alignment, and no cross-mapping. We focused on the reads aligned against genomic regions of known pre-miRNAs in miRBase (release 21) [25] for mismatch calling based on the binomial test as described in the literature [20,21]. We identified miRNA editing candidates using the cutoff of the Bonferroni-corrected *P*-value of 0.05 and the mismatch base quality score ≥30. In order to eliminate the effect of change in DNA level, we filter out the points marked in dbSNP (142) from the candidates. The scripts used for miRNA editing calling were published by Alon et al., in 2012 and are available at http://www.tau.ac.il/~elieis/miR_editing/. For the sites confirmed as miRNA editing sites, we divided them into two groups: editing sites in seed region and editing sites in the non-seed region.

2.2. Functional analysis of miRNA targets

miRNA targeting relationship, before and after editing, shows significant differences when the RNA editing occurred in the miRNA seed region. Based on the sequence before and after editing, we first used miRanda [26]software to predict the target gene set of WT miRNAs and edited miRNAs.We then performed functional enrichment analysis of the genes to find biological processes that were affected by WT miRNAs and edited miRNAs. The sequence logo is generated by the WebLogo tool [27].

3. Results

3.1. Data collection and classification integration

We detected miRNA editing sites during spermatogenesis in both pig and mouse species. Up to 26 samples of mouse non-coding RNA-seq datasets were derived from the Gene Expression Omnibus (GEO) and they conclude four type cells: spermatogonia (SG), primary spermatocytes (PSC), spermatid and sperm (Table 1). Then we integrated them with our previous miRNAs sequencing dataset with the same types of cells during pig spermatogenesis [28]. We integrated samples from the same period into one item, so we can find consistent editing sites from different studies. After length and quality control on all reads and filtered out ineligible, the reads were aligned to precursor miRNA sequences in miRBasewith release 21 [25] for mismatch calling (see methods). We screened the mismatched bases in the literature (see methods) to obtain candidate miRNA editing sites and then removed the known SNPs to get the final miRNA editing sites. According to the location information of the editing sites, we divided all the miRNA editing sites into two groups, editing sites in the seed region and editing sites in the non-seed region. Then we focused on the editing sites in the seed region. Our analysis process is shown in Fig. 1A.

Table 1
Samples' information.

Reference	GEO ID	Technique	Total Sample	Phase			
				SG	PSC	Spermatid	Sperm
Inoue K et al.,2017	GSE70890	Illumina HiSeq 1500	5	1	3	1	–
Luo M et al.,2017	GSE56522	Illumina HiSeq 2000	2	1	1	–	–
Hilz S et al.,2016	GSE83264	Illumina HiSeq 2000	4	–	4	–	–
Nixon B et al.,2015	GSE70198	Illumina HiSeq 2000	6	–	–	–	6
Gan H et al.,2011	GSE24822	Illumina Genome Analyzer	3	1	1	1	–
Meunier J et al.,2012	GSE40499	Illumina Genome Analyzer IIx	2	–	1	1	–
García-López J et al.,2015	GSE59254	Illumina HiSeq 2000	2	1	–	1	–
Nordstrand LM et al.,2012	GSE37150	Illumina Genome Analyzer IIx	2	–	2	–	–
Total	26	4	12	4	6		

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