Supplementary information, Data S1

RNA extraction, small RNA library preparation and sequencing

Mature sperm were isolated from cauda epididymis of adult male mice (CD1 background, 10-12 weeks), as previously performed [1]. Total RNA was isolated using the TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Before the standard protocol been performed, the sperm was treated with somatic cell lysis buffer (0.1% SDS, 0.5% Triton X in DEPC H_2O) for 20 min on ice to eliminate somatic cell contamination. RNA extraction for adult testis, uterus and elongated spermatids were performed with standard protocols[1]. The prepared RNA for Mature sperm, testis, uterus and elongated spermatids were shipped to the Beijing Genomics Institute (BGI), Shenzhen, China in dry ice for small RNA library construction and Solexa high-throughput sequencing followed their standard protocols[2]. Briefly, 18–40nt fraction was excised and purified, the obtained small RNA was ligated sequentially to 5′ - and 3′ -adapters, followed by RT-PCR to produce the sequencing library. The PCR products were purified and sequenced by Illumina Hi-Seq 2000.

Data processing and analysis

Sequence tags from the Solexa sequencing went through data cleaning by BGI standard protocols, which included filtering out low quality tags and several contaminants, using software developed by BGI. The small RNA clean reads were mapped with mouse genome (mm9) by SOAP (Short Oligonucleotide Analysis Package, developed by BGI) to analyze their expression and distribution on the genome. The small RNA reads that 100% match to genome were used for further analysis. Small RNA annotation was performed by Alignment to Rfam (http://rfam.sanger.ac.uk/) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The genome locations of mature-sperm-enriched small RNA families described in present study were searched by BLAT (https://genome.ucsc.edu/cgi-bin/hgBlat) by mapping to NCBI37/mm9 database. The sequences of mse-tsRNAs were matched with **Genomic tRNA Database** (http://gtrnadb.ucsc.edu/) to obtain their position information from tRNAs' secondary structure and potential cleavage sites. Evolutional conservation analysis of mse-tsRNA family 1-7 and their precursor tRNAs were performed by matching to all species included in **Genomic tRNA Database.** Mse-tsRNA family 1-7 were searched in piRNA database (http://pirnabank.ibab.ac.in/index.shtml)[3] to confirmed their existence in different species. The reads number and percentage of mse-tsRNA family-1 and -2 in purified testicular spermatogenic cells types were performed by analyzing datasets for each cell type, the datasets for type A spermatogonia, pachytene spermatocytes and round spermatids have been published previously (GSM610965, GSM610966, GSM610967)

(http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24822). The small RNA datasets performed in present study were deposited at NCBI: for Mature mouse sperm, testis and uterus (GSE38702), for elongated spermatids (GSE40397).

Purification of testicular spermatogenic cells

Purification of testicular spermatogenic cells (type A spermatogonia, pachytene spermatocytes, round spermatids and elongated spermatids) were performed as we previously described[4].

Purification of sperm heads

Purification of sperm heads from intact sperm were performed as previously described[5].

Collection of oocytes, zygotes and parthenogenetically activated oocytes for quantitative RT-PCR

To collect fertilized eggs (zygotes), CD1 female mice were superovulated by injecting (I.P) 10 IU of PMSG, followed by 10 IU of hCG after 48h, and were then mated with male mice. The fertilized eggs were collected 17h after hCG injection from vaginal plug positive females. The unfertilized oocytes were collected by the same protocols but without mating with male mice. For parthenogenetically activation of oocytes, the oocytes were first collected 13h after hCG injection, then cultured in the parthenogenetically activation media (10 mM $SrCl_2$ CZB) for 5h. For the quantitative examination, 100 embryos were pooled for RNA extraction (Trizol reagent) as an independent sample. We collected 5 samples for oocytes (100 x 5), 6 samples for zygotes (100 x 6) and 5 samples for parthenogenetically activated oocytes (100 x 5). Then the RNAs were extracted for each sample followed by quantitative RT-PCR for mse-tsRNA family-1.

RT-PCR for small RNAs

Reverse Transcription for small RNAs (Testicular spermatogenic cells, mature sperm from mice, rat and human) were performed using QuantiMir RT kit (System Biosciences) following the manufacturer's instruction. Briefly, 2 μg of RNA from each sample was polyadenylated, and then converted to cDNAs with a unique adaptor in the presence of reverse transcriptase, and the cDNAs were amplified with specific small RNA primer in combination with the universal adaptor to examine the expression of a particular small RNA. PCR mixtures contained 12.5 μL of 2× *GoTaq® Green Master Mix,* 0.5μL of forward primer (10 μM) and 0.5μL of universal primer (10 μM), 1 μL of cDNA and 10.5 μL of double-distilled water. For equal loading of each sample, the RNA concentration were measured and calibrated by a MICRO UV-VIS · FLUORESCENCE SPECTROPHOTOMETER (e-spect, Malcom, Japan). The conditions used for PCR were as follows:

For mse-tsRNAs, 95 °C for 10 min, followed by 23 or 28 cycles at 95 °C for 15 s, 60 °C for 15 s and 72 °C for 15 s. For miR-34c, the PCR conditions were 95 °C for 10min, followed by 28 cycles at 95 °C for 15 s, and 60 °C for 1 min. Primers and product sizes were shown in Figure 1H. The amplified products were analyzed by electrophoresis on 4% agarose gels stained with ethidium bromide. PCR products were purified with TaKaRa MiniBEST DNA Fragment Purification Kit and subcloned into pGEM-T easy vector (Promega) for sequencing. SYBR Green based Quantitative PCR examinations of mse-tsRNA family-1 for oocytes, zygotes and parthenogenetically activated oocytes were performed on Roche LightCycler 480 II.

References:

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