YMTHE, Volume *25*

Supplemental Information

Characterization of circRNA-Associated-ceRNA Networks in a Senescence-Accelerated Mouse Prone 8 Brain

Shuai Zhang, Dina Zhu, Hong Li, Hejian Li, Chengqiang Feng, and Wensheng Zhang

Figure S1

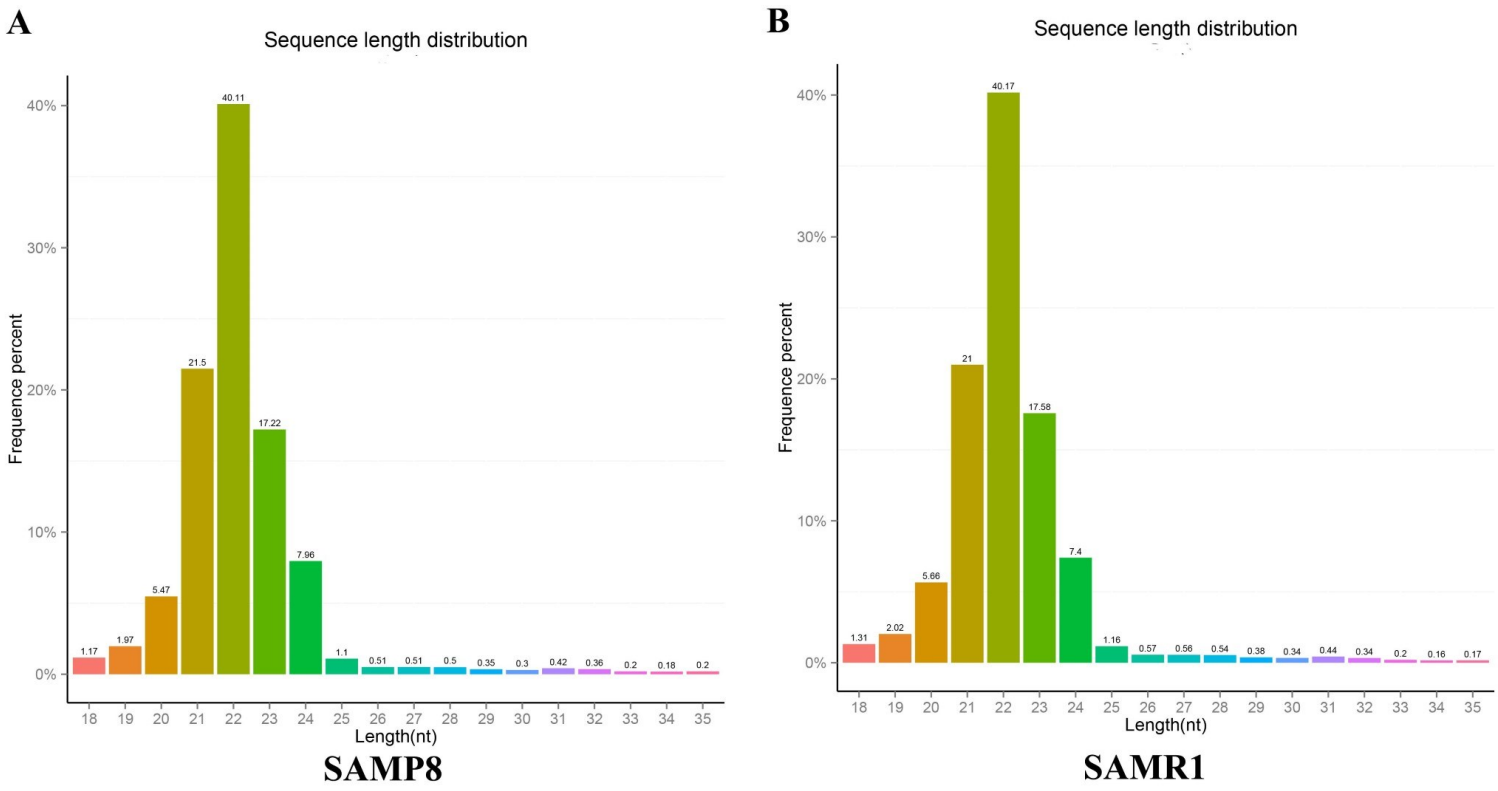

Figure S1. Sequence length distribution of small RNA in SAMP8 and SAMR1 mice. (A) Sequence length distribution in SAMP8 mice. (B) Sequence length distribution in SAMR1 mice.

Table S1. Primers used in qPCR analysis

Acession No.	Primer sequence (5`-3`)	primer direction	product size
ENSMUST00000024823	CGGCTACTGGATTGAGA	forward primer	99
	CTTTGTTCCCGCTGTC	reverse primer	
ENSMUST00000110690	AAACTCCTGTCCTCTGGGCTTCG	forward primer	142
	ATCTTTTACAGCTTCCACGGGAT	reverse primer	
ENSMUST00000167323	CATCGTCTTCTTCGGCTCAC	forward primer	152
	GCTGCTCACTAAGTTCATCCA	reverse primer	
mm10_circ_0011857	ATGGTCTGGTCGGCAAGC	forward primer	106
	GAGCGATGGGTCGTAGGTAA	reverse primer	
mm10_circ_0007422	TGATGATATCCGCCCAGACA	forward primer	106
	CAGGACTAAAACTTCTCGCACTG	reverse primer	
mm10_circ_0013361	AGACAGTGATCCTCCCATTCA	forward primer	136
	TTCTTCAGACATCATCAGTGGC	reverse primer	
mmu-miR-7019-3p	ACATATATCACCTTGGCCGCC	forward primer	74
	TATGGTTGTTCACGACTCCTTCAC	reverse primer	
mmu-miR-466q	GGCAAGTGCACACACACAC	forward primer	71
	TATGGTTTTGACGACTGTGTGAT	reverse primer	
mmu-let-7g-3p	GCACATACATGGTGCACACACA	forward primer	70
	TATGGTTGTTCACGACTCCTTCAC	reverse primer	
U6	CAGCACATATACTAAAATTGGAACG	forward primer	76
	ACGAATTTGCGTGTCATCC	reverse primer	
β-Actin	GCCCATCTACGAGGGCTAT	forward primer	147
	ATGTCACGCACGATTTCC	reverse primer	

Table S3. Significantly and differentially expressed miRNA transcripts between SAMP8 and SAMR1 mice

sRNA	SAMR1_readcount	SAMP8_readcount	$log2^{FoldChange}$	pval
mmu-miR-3086-5p	2.207373438	72.62548772	-3.3761	3.50E-24
mmu-miR-466q	0	23.66608457	-2.6907	5.82E-13
mmu-let-7a-5p	68025.34354	54718.33238	0.31185	6.32E-07
mmu-miR-181d-5p	4965.527081	6006.41333	-0.2723	4.90E-05
mmu-let-7g-5p	448676.6117	368188.6576	0.28232	0.00020599
mmu-miR-344-3p	5177.433908	7226.426387	-0.46674	0.00024286
mmu-miR-221-5p	4562.344165	3809.130442	0.25844	0.00045981
mmu-miR-30e-3p	9940.051867	8161.14345	0.28151	0.00050191
mmu-miR-219b-5p	2155.285501	1208.686225	0.75696	0.0005365
mmu-miR-144-3p	15.74204162	65.87510204	-1.2477	0.00069635
mmu-miR-122-5p	26.55221656	89.89337916	-1.1781	0.00086852
mmu-miR-551b-3p	94.71884185	205.0504622	-0.91206	0.0015702
mmu-miR-138-5p	4130.69484	5307.460187	-0.35343	0.0016526
mmu-miR-744-5p	4227.856839	3460.486003	0.28446	0.0020397
mmu-miR-30b-3p	285.6550283	213.8102253	0.40347	0.0023108
mmu-miR-455-3p	170.2339162	118.0757926	0.49072	0.0024029
mmu-miR-3065-3p	1958.957482	1300.204444	0.5529	0.0028
mmu-miR-338-5p	1997.940091	1329.581848	0.54931	0.0029962
mmu-miR-219a-2-3p	12372.53421	8339.371718	0.53416	0.0032678
mmu-miR-466i-5p	29.37106327	9.308007664	1.0404	0.00423
mmu-miR-7a-1-3p	522.9449924	416.9477258	0.31917	0.0045518
mmu-miR-30c-5p	81923.16111	65829.9945	0.30861	0.0052923
mmu-let-7c-5p	123416.9698	101959.5811	0.2709	0.0054639
mmu-miR-21a-5p	48315.68007	36703.76465	0.38273	0.0058751
mmu-miR-7019-3p	0	3.466885077	-0.92191	0.0061499
mmu-let-7g-3p	14.41850254	31.68328894	-0.86017	0.0071926
mmu-miR-466b-5p	2.820977233	9.738081367	-0.99696	0.0075774
mmu-miR-128-1-5p	568.311237	399.8551909	0.47398	0.0084636
mmu-miR-378c	1429.041877	1786.423398	-0.31289	0.0089111
mmu-miR-1839-5p	5148.845716	4363.856225	0.23522	0.0095727

Supplemental Method 1. The detailed methods of miRNA-seq

Library preparation and sequencing

A total of six cDNA libraries were constructed, i.e., three for SAMP8 mice and another three for SAMR1 mice. We utilized 3 μg RNA per sample as input material for RNA sample preparation. Sequencing libraries were generated using NEBNext® Multiplex Small RNA Library Prep Set for Illumina® (NEB, USA.) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, NEB 3' SR Adaptor was directly, and specifically ligated to 3' end of miRNA, siRNA and piRNA. After the 3' ligation reaction, the SR RT Primer hybridized to the excess of 3' SR Adaptor (that remained free after the 3' ligation reaction) and transformed the single-stranded DNA adaptor into a double-stranded DNA molecule. This step is important to prevent adaptor-dimer formation, besides, dsDNAs are not substrates for ligation mediated by T4 RNA Ligase 1 and therefore do not ligate to the 5´ SR Adaptor in the subsequent ligation step. 5´ends adapter was ligated to 5´ends of miRNAs, siRNA and piRNA. Then first strand cDNA was synthesized using M-MuLV Reverse Transcriptase (RNase H–). PCR amplification was performed using LongAmp Taq 2X Master Mix, SR Primer for illumina and index (X) primer. PCR products were purified on a 8% polyacrylamide gel (100V, 80 min). DNA fragments corresponding to 140~160 bp (the length of small noncoding RNA plus the 3' and 5' adaptors) were recovered and dissolved in 8 μL elution buffer. At last, library quality was assessed on the Agilent Bioanalyzer 2100 system using DNA High Sensitivity Chips.

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq SR Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced at the Novogene Bioinformatics Institute (Beijing, China) on an Illumina Hiseq 2500/2000 platform and 50bp single-end reads were generated.

Data analysis

This section contained Quality control (QC), Reads mapping to the reference sequence, Known miRNA alignment, Remove tags from these sources (protein-coding genes, repeat sequences, rRNA, tRNA, snRNA, and snoRNA), Novel miRNA prediction, Small RNA annotation summary, miRNA editing analysis, miRNA family analysis, and Target gene prediction.

Supplemental Method 2. The detailed methods of circRNA-seq

Library preparation and sequencing

A total of six cDNA libraries were constructed, i.e., three for SAMP8 mice and another three for SAMR1 mice. We utilized 5 μg RNA per sample as input material for RNA sample preparation. Firstly, ribosomal RNAs were depleted by using Epicentre Ribo-zero™ rRNA Removal Kit (Epicentre, USA) to get rRNA-depleted RNAs. rRNA-depleted RNAs were further treated with RNase R (Epicentre, USA) and then were subjected to Trizol extraction. Subsequently, sequencing libraries were generated using the rRNA-depleted and RNase R digested RNAs by NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations. Briefly, fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer. First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNaseH-). Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. In the reaction buffer, dNTPs with dTTP were replaced by dUTP. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 150-200 bp in length, the library fragments were purifed with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37°C for 15 min followed by 5 min at 95°C before PCR. PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primer and Index (X) Primer. Finally, the library was purifed (AMPure XP system) and then qualifed by the Agilent Bioanalyzer 2100 system.

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using HiSeq PE Cluster Kit v4 cBot (Illumina) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced at the Novogene Bioinformatics Institute (Beijing, China) on an Illumina Hiseq 2500 platform and 125bp paired-end reads were generated.

Data analysis

This section contained Quality control (QC), Mapping to reference genome, circRNA identifcation and miRNA binding site prediction.