Comprehensive microRNA-seq transcriptomic profiling across 11 organs, 4 ages, and 2 sexes of Fischer 344 rats

Xintong Yao1,3, Shanyue Sun1,3, Yi Zi1, Yaqing Liu1, Jingcheng Yang2, Luyao Ren1, Guangchun Chen2, Zehui Cao1, Wanwan Hou2, Yueqiang Song2, Jun Shang2, He Jiang1, Zhihui Li1, Haiyan Wang1, Peipei Zhang1, Leming Shi1, Quan-Zhen Li2,3, Ying Yu1,3 & Yuanting Zheng1,3

Rat is one of the most widely-used models in chemical safety evaluation and biomedical research. However, the knowledge about its microRNA (miRNA) expression patterns across multiple organs and various developmental stages is still limited. Here, we constructed a comprehensive rat miRNA expression BodyMap using a diverse collection of 320 RNA samples from 11 organs of both sexes of juvenile, adolescent, adult and aged Fischer 344 rats with four biological replicates per group. Following the Illumina TruSeq Small RNA protocol, an average of 5.1 million 50 bp single-end reads was generated per sample, yielding a total of 1.6 billion reads. The quality of the resulting miRNA-seq data was deemed to be high from raw sequences, mapped sequences, and biological reproducibility. Importantly, aliquots of the same RNA samples have previously been used to construct the mRNA BodyMap. The currently presented miRNA-seq dataset along with the existing mRNA-seq dataset from the same RNA samples provides a unique resource for studying the expression characteristics of existing and novel miRNAs, and for integrative analysis of miRNA-mRNA interactions, thereby facilitating better utilization of rats for biomarker discovery.

Background & Summary

MicroRNAs (miRNAs) are small noncoding RNAs (21–24 nucleotides) that mediate post-transcriptional regulation of gene expression by binding to the 3’ untranslated regions of messenger RNAs (mRNA)1–3. miRNAs play pivotal roles in biological processes such as differentiation, development, tissue growth, and tumor initiation and progression4,5. miRNAs can serve as potential clinical biomarkers for disease diagnosis, prognosis, and treatment selection6–9, as well as therapeutic targets for human diseases10–12. However, the development of miRNA-based biomarkers and therapies is hampered due to incomplete understanding of the characteristics of miRNA profiles across different organs, various developmental stages, or sexes of rat, an extensively used animal model for evaluating drug safety and understanding drug mechanisms of action. Furthermore, the rat miRNA transcriptome is much less well annotated compared to that of mouse and human, with 764, 1915, and 2588 miRNAs annotated in miRBase v.22.1 (http://www.mirbase.org/) for rat, mouse, and human, respectively13,14. Therefore, characterizing rat miRNA expression profiles more comprehensively is warranted, especially with the advancement of high-throughput technologies such as microarrays and next-generation sequencing.

Several efforts for constructing a catalog of rat miRNA expression have been reported. For example, Minami et al.15 reported a dataset of microarray-based expression profiles of 424 miRNAs cross 55 different organs and tissues of 10-week-old male Sprague-Dawley rats. Similarly, Bushel et al.16 developed the RATEmiRs
database consisting of miRNA-seq expression data across 14 organs among 12/13-week-old Sprague Dawley rats of both sexes through six sequencing batches. These studies mainly focused on miRNA expression differences among various organs. However, the diversity and number of expressed miRNAs in animals is not only organ-specific, but also shows age dependence and sex biases. Specifically, the role of miRNAs in the aging process has often been overlooked and is therefore largely unclear.

Ideally, studies on rat transcriptome should simultaneously cover multiple organs across different developmental stages for both sexes. Indeed, in a widely cited previous study, we constructed a comprehensive rat transcriptomic BodyMap with RNA-seq of 320 samples from 11 organs of both sexes of juvenile, adolescence, adult, and aged Fischer 344 rats and identified a large number of transcripts showing organ-specific, age-dependent, or sex-specific expression patterns. The unique rat mRNA dataset has already been widely used by scientific communities to advance our understanding of the dynamics of rat transcriptome, including studies on circRNAs, the development of annotation tools, and enrichment of database resources.

Here, as a follow-up and complementary study, we constructed a comprehensive rat miRNA BodyMap by generating miRNA-seq data from the same 320 samples and described the quality and the dynamic characteristics of miRNA expression profiles across 14 organs, between two sexes, and over the life span of the rats (Fig. 1). We quantified 604 out of the 764 miRNAs annotated in miRbase v22.1, which makes it possible to integrate miRNA data with mRNA data. In addition, reads from all 320 samples were pooled for novel miRNA discovery.
In summary, 320 tissue samples were derived from 16 female and 16 male rats of the Fischer 344 strain, including four rats for each sex under each of the four developmental stages and ten organs (adrenal gland, brain, heart, kidney, liver, lung, muscle, spleen, thymus, and testis or uterus) per rat. That is, four biological replicates per sample group were used in this study. Ground organ tissue was stored at $-80^\circ\text{C}$. Total RNA was extracted from ground tissue by using the miRNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol, including treatment with DNase. RNAs longer than 18 nucleotides were recovered with this method. The quality of each RNA sample was examined with an Agilent 2100 Bioanalyzer.

**Methods**

**Organ collection and RNA isolation.** Female and male Fischer 344 rats (pair-housed under standard conditions) from the National Center for Toxicological Research of the US Food and Drug Administration animal-breeding colony were euthanized by carbon dioxide asphyxiation at 2, 6, 21 and 104 week-of-age as previously described\(^{24,25}\). In summary, 320 tissue samples were derived from 16 female and 16 male rats of the Fischer 344 strain, including four rats for each sex under each of the four developmental stages and ten organs (adrenal gland, brain, heart, kidney, liver, lung, muscle, spleen, thymus, and testis or uterus) per rat. That is, four biological replicates per sample group were used in this study. Ground organ tissue was stored at $-80^\circ\text{C}$. Total RNA was extracted from ground tissue by using the miRNeasy Mini Kit (Qiagen) according to the manufacturer's protocol, including treatment with DNase. RNAs longer than 18 nucleotides were recovered with this method. The quality of each RNA sample was examined with an Agilent 2100 Bioanalyzer.

**Construction and sequencing of miRNA-seq libraries.** Small RNA libraries were constructed using Illumina TruSeq Small RNA Library Prep kit (the sequences of adapters and primers are provided in Table 1) by following the manufacturer's Reference Guide with some modifications. In brief, 1 μg of total RNA (including miRNA) as input was sequentially ligated with 3′ and 5′ adapters, and the ligated RNA fragments were then reverse transcribed to single-stranded cDNAs using Superscript II reverse transcriptase (Invitrogen) and RT primer. The cDNAs were amplified by PCR with 13 cycles, and the resulting products were further purified with 2× Agencourt AMPure XP beads (Beckman), followed by size selection using the Pippin Prep Size Selection System (Sage Science). The final libraries were validated using Agilent High Sensitivity DNA assay, and subjected to 50 bp single-end sequencing (SE50) on an Illumina HiSeq. 2500. No spike-in controls or negative controls were used in the construction of this dataset.

**Pre-processing and processing of the reads.** The adapter sequences were removed with fastp 0.23.2 software (http://opengene.org/fastp/fastp.0.23.2), followed by a quality filter and a length filter. Reads with more than 2 “N” bases were discarded. The clipped reads with length between 16 and 35 nt were retained for alignment to various transcript types, including miRNA (miRBase v22.1, http://www.mirbase.org/), piRNA (piRBase, http://www.regulatoryrna.org/database/piRNA/download.html), tRNA (GtRNAdb, http://gtRNAdb.ucsc.edu/GtRNAdb2 genomes/eukaryota/Rnorv7/Rnorv7 seq.html), other RNA (NCBI transcriptome, https://www.ncbi.nlm.nih.gov/genome/73), and the rat genome (Rn7, https://hgdownload.soe.ucsc.edu/downloads.html#mammals). The alignment was performed with Bowtie 1.3.1 (http://bowtie-bio.sourceforge.net/manual.shtml) with less than 2 mismatched bases allowed.

**Filtering of miRNAs and samples.** The following strategies were used to filter miRNAs and samples of questionable quality: (1) the low-expressed miRNAs less than one count per library on average, were discarded. (2) a sample was removed if it failed to cluster with other samples from the same organ type in a hierarchical clustering analysis. Spl_F_104.4 and Brn_M_006.3 samples were removed, as they clustered to uterus samples instead of spleen and brain, respectively. Ultimately, a miRNA expression matrix with 604 miRNAs and 318 samples was obtained for further analysis.

**Identification of novel miRNA candidates.** First, reads from all 320 samples were pooled for identification of novel miRNA candidates. Using the mapper module provided within miRDeep2.0.1.3, raw reads of the merged samples were subjected to a series of stringent filters (discarding low-quality reads and reads with fewer than 18 nt after clipping the 3′ adapter), and the remaining sequences were then mapped to the rat genome reference (Rn7). Next, the mapped reads were submitted to the miRDeep2 module to detect novel miRNAs with default parameters. Novel miRNAs that have passed the stringent filters (miRDeep2 score $\geq 10$, significant Ranfold $P$-value and no rnaAlert of the possibility of being a rRNA) were selected as the potential novel miRNA candidates.

To validate the organ-specificity of the novel miRNA candidates, we quantified the novel miRNA expressions across 320 samples and performed differential expression analysis between any two organs at each developmental stage. A miRNA is considered ‘organ-specific’ if it is over-expressed by at least 1.5 fold (fold-change $> 1.5$ and adjusted $P$-value $< 0.05$) in one organ over all other organs and across all four developmental stages. Finally, the 12 organ-specific sequences were reported as novel miRNA candidates in Online-only Table 1.

**Data Records**

The miRNA-seq dataset generated in study is available in the NCBI Gene Expression Omnibus (GEO) with series accession number GSE172269\(^{36}\). This accession contains both the raw sequence data files (fq.gz format) and the processed data files (raw counts of mapped sequencing reads) used in this report. All data can be used without restrictions.

### Table 1. Sequence information of the Truseq-small RNA kit.

| Name               | Sequence                                      |
|--------------------|-----------------------------------------------|
| 3′ adapter         | 5′TGAATCTCGGTGTAAGG                           |
| 5′ adapter         | 5′GUCCAGAAGUCUACAGCUCAGACGAUC                |
| PCR primer (RP1)   | 5′AAATGACGCGCACTCACGAGATTTCTACAGTCAGGGA     |
| PCR index primer   | 5′CAACGAGAGACCGCATACGAGAT[6 bases] GTGACTTGGAGTTCTTGCCACCGGAGAATTCCA |

**Note:**
- A miRNA is considered ‘organ-specific’ if it is over-expressed by at least 1.5 fold (fold-change $> 1.5$ and adjusted $P$-value $< 0.05$) in one organ over all other organs and across all four developmental stages. Finally, the 12 organ-specific sequences were reported as novel miRNA candidates in Online-only Table 1.
Technical Validation

Quality of RNA samples. The quality of RNA samples was assessed with an Agilent Bioanalyzer. Most samples showed good RNA Integrity Number (RIN 9.08 ± 1.08, mean ± sd), except for the eight week-2 spleen samples with RIN below 5.2. The RNA integrity numbers of all the 320 samples were listed in Online-only Table 2.

Quality of libraries. The quality of miRNA sequencing data for each sample was checked (Fig. 2). Briefly, a total of 1.6 billion raw reads was obtained, with 5.1 million reads per sample on average, which is sufficient for miRNA quantification. After quality filtering and length filtering, 4.5 ± 2.8 million reads of high quality were retained per sample on average (Fig. 2a). The proportions of various transcript types of these reads were demonstrated in Fig. 2b. The majority of libraries were contributed by miRNA and piRNA sequences, followed by tRNA, rRNA, mRNA and other sequences from the rat transcriptome and genome. Most libraries were dominated by miRNA reads (75.6 ± 8.5%), except for the testis samples from the adolescent and adult rats, which were dominated by piRNA (73.8 ± 0.8%). However, the proportion of miRNA for the Brn_F_104_4 sample was 14.4%, much lower than the average proportion of brain samples.

Reproducibility of biological replicates. To assess the reproducibility of biological replicates, we calculated the Pearson's correlation coefficients between any two of the 320 samples (Fig. 3a–c). The Pearson's correlation coefficients between miRNA expressions of biological replicates from the same group (0.98 ± 0.01, mean ± sd) were much higher than those from different sample groups (0.82 ± 0.09) (Fig. 3a). This result indicated good consistency among biological replicates in which biological variations between animals and technical variations between sample processing were included. Moreover, this conclusion was further confirmed in principal component analysis, from which the majority of biological replicates could be grouped together (Fig. 4a–d).

Validation of organ specific signature. To obtain an overview of miRNA expression profiles, we performed principal component analysis and hierarchical clustering (Fig. 4a–e). The miRNA profiles were discriminating among different organs (Fig. 4e). To identify the miRNAs that contribute the most to organ-specific expression, we set out to identify ‘organ-enriched’ miRNAs. A miRNA is considered ‘organ-enriched’ if it is over-expressed by at least four folds in one organ over any other organs and across all four developmental stages, a quite stringent criterion. Consequently, 67 organ-enriched miRNAs were identified, including a novel miRNA candidate. The z-scaled expression levels of these miRNAs were shown in Fig. 5a.
To independently validate the organ-enriched miRNAs discovered in this study, the literature-reported miRNA profiles of 55 different organs and tissues from normal male rats based on Agilent miRNA microarrays were used. Thirty-one (31) organ-enriched miRNAs identified in our study, including brain-enriched, testis-enriched, liver-enriched, and spleen-enriched and heart-enriched miRNAs, were also profiled in the
miRNA microarray dataset and displayed a pattern of high-level expression in biological systems with functions similar to those of the organs examined in this study (Fig. 5b). This result confirmed the reliability of the organ specific signature presented in our dataset.
Fig. 5 Validation of organ-specific miRNA signatures in a microarray dataset. (a) Expression profiles of 67 organ-enriched miRNAs across 318 samples are arranged by organ type. Expression data were Z-score scaled per gene across all 318 samples. Each row represents a miRNA that is enriched in an organ. (b) Expression profiles of 31 organ-enriched miRNAs across 23 tissues in the microarray miRNA expression dataset of Minami et al. are arranged by biological system. Each row represents a miRNA that is enriched in an organ. Each column represents a sample profiled in the microarray dataset. Abbreviation for organs, Adr, adrenal; Brn, brain; Hrt, heart; Kdn, kidney; Lng, lung; Lvr, liver; Msc, skeletal muscle; Spl, spleen; Thm, thymus; Tst, testis; and Utr, uterus.

Reliability of miRNA-mRNA integration. To examine the reliability of miRNA-mRNA integration using the miRNA dataset presented here and the previously published mRNA dataset, we compared the source of variability in these two datasets. Principal variance component analysis was performed on miRNA and mRNA expression profiles respectively (Fig. 6a). As expected, the ‘organ’ factor accounted for the most total variance in both datasets, with 61.6% for miRNA and 64.2% for mRNA profiles. The variance attributable to each factor was similar in the mRNA and miRNA datasets, except for age. The overall expression difference due to age was significantly higher for miRNA (11.7%) than that for mRNA (1.7%) expression. However, there was a stronger interaction between age and organ type for miRNA (15.7%) compared to that for mRNA (2.5%) expression. It
appeared that there is a more dynamic miRNA expression pattern across the four developmental stages independent of organ types, whereas the temporal expression of mRNA appeared to be more organ dependent than that of miRNA expression. These results highlight the concordance of the two datasets.

Meanwhile, the genomic locus of miRNA genes was used to test the reliability of the association between these miRNA and mRNA datasets. We calculated the correlations between expression levels of miRNAs and those of genes (miRNA-gene pairs). The distributions of correlations between several types of miRNA-gene pairs were demonstrated in Fig. 6b. The correlations between all miRNA-gene pairs were weak (0.01 ± 0.28, median ± sd) as expected. Importantly, the co-transcription events of miRNA and host genes were observed in these datasets, as the expressions of intragenic miRNAs were highly positively correlated with those of their host genes on the same strand (0.60 ± 0.29, N = 179). In addition, the slightly negative correlations (−0.17 ± 0.35) between these miRNAs-host genes (opp) pairs confirmed that co-expression events are rare when intragenic miRNAs and the host genes are located on the opposite strands. In summary, the miRNA dataset along with the mRNA datasets provided a reliable and unique resource for integration.

**Code availability**

The code required for reproducing the figures and tables is freely available on GitHub (https://github.com/XintongYao-96/Rat-microRNA-Bodymap).

Received: 20 May 2021; Accepted: 4 March 2022;
Published online: 12 May 2022

**References**

1. Sun, K. & Lai, E. C. Adult-specific functions of animal microRNAs. Nat Rev Genet 14, 535–548, https://doi.org/10.1038/nrg3471 (2013).
2. Shukla, G. C., Singh, J. & Barik, S. MicroRNAs: Processing, Maturation, Target Recognition and Regulatory Functions. Mol Cell Pharmacol 3, 83–92 (2011).
3. Bartel, D. P. MicroRNAs target recognition and regulatory functions. Cell 136, 215–223, https://doi.org/10.1016/j.cell.2009.01.002 (2009).
4. Ambros, V. The functions of animal microRNAs. Nature 431, 350–355 (2004).
5. Lai, E. C. Micro RNAs are complementary to 3′ UTR sequence motifs that mediate negative post-transcriptional regulation. Nat Genet 30, 363–364, https://doi.org/10.1038/ng865 (2002).
6. Goodall, G. J. & Wickramasinghe, V. O. RNA in cancer. Nat Rev Cancer 21, 22–36, https://doi.org/10.1038/s41568-020-03006-0 (2021).
7. Bertrand-Neagoe, I., Monroig Peld, C., Pasculli, B. & Calin, G. A. MicroRNA-N-gene: a treasure for cancer diagnosis and therapy. Cancer J Clin 64, 311–336, https://doi.org/10.3332/cjc.12144 (2014).
8. Pratama, M. Y., Visintin, A., Croce, L. S., Tiribelli, C. & Pascut, D. Circulatory miRNA as a Biomarker for Therapy Response and Disease-Free Survival in Hepatocellular Carcinoma. Cancers (Basel) 12, https://doi.org/10.3390/cancers12102810 (2020).
9. Wang, X.-H., He, Y., Makiwacki, B. & Gao, B. MicroRNAs as regulators, biomarkers and therapeutic targets in liver diseases. Gut 70, 784–793, https://doi.org/10.1136/gutjnl-2020-322526 (2021).
10. Liu, A. et al. Antagonizing miR-455-3p inhibits chemoresistance and aggressiveness in esophageal squamous cell carcinoma. Mol Cancer 16, 106, https://doi.org/10.1186/s12943-017-0669-9 (2017).
11. Vira, D. et al. Cancer stem cells, microRNAs, and therapeutic strategies including natural products. Cancer Metastasis Rev 31, 733–751, https://doi.org/10.1007/s10555-012-9382-8 (2012).
12. Hayes, I., Peruzzi, P. P. & Lawler, S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 20, 460–469, https://doi.org/10.1016/j.molmed.2014.06.005 (2014).
13. Kozomara, A. & Griffiths-Jones, S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 42, D68–73, https://doi.org/10.1093/nar/gkt1181 (2014).
14. Kozomara, A., Birgaoanu, M. & Griffiths-Jones, S. miRBase: from microRNA sequences to function. Nucleic Acids Res 47, D155–D162, https://doi.org/10.1093/nar/gky1141 (2019).
15. Minami, K. et al. miRNA expression atlas in male rat. Sci Data 1, 140005, https://doi.org/10.1038/data.2014.5 (2014).
16. Bushel, P. R. et al. RATERmiRs: the rat atlas of tissue-specific and enriched miRNAs database. BMC Genomics 19, 825, https://doi.org/10.1186/s12864-018-5220-z (2018).
17. Smith, A. et al. The Rat microRNA body atlas; Evaluation of the microRNA content of rat organs through deep sequencing and characterization of pancreas enriched miRNAs as biomarkers of pancreatic toxicity in the rat and dog. BMC Genomics 17, 694, https://doi.org/10.1186/s12864-016-1958-z (2016).
18. Sun, Y. et al. Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. Nucleic Acids Res 32, e188, https://doi.org/10.1093/nar/gnh186 (2004).
19. Smibert, P. et al. Global patterns of tissue-specific alternative polyadenylation in Drosophila. Cell Rep 1, 277–289, https://doi.org/10.1016/j.celrep.2012.01.091 (2012).
20. Baloun, J. et al. Epilepsy miRNA Profile Depends on the Age of Onset in Humans and Rats. Front Neurosci 14, 924, https://doi.org/10.3389/fnins.2020.00924 (2020).
21. Meder, B. et al. Influence of the confounding factors age and sex on microRNA profiles from peripheral blood. Clin Chem 60, 1200–1208, https://doi.org/10.1373/clinchem.2014.224238 (2014).
22. Veltsos, P., Fang, Y., Cossins, A. R., Snook, R. R. & Ritchie, M. G. Mating system manipulation and the evolution of sex-biased gene expression in Drosophila. Nat Commun 8, 2072, https://doi.org/10.1038/s41467-017-02323-6 (2017).
23. Cui, C. et al. Identification and Analysis of Human Sex-biased MicroRNAs. Genomics Proteomics Bioinformatics 16, 200–211, https://doi.org/10.1007/s19089-018-03004 (2018).
24. Yu, Y. et al. A rat RNA-Seq transcriptomic BodyMap across 11 organs and 4 developmental stages. Nat Commun 5, 3230, https://doi.org/10.1038/ncomms3230 (2014).
25. Yu, Y. et al. Comprehensive RNA-Seq transcriptomic profiling across 11 organs, 4 ages and 2 sexes of Fischer 344 rats. Sci Data 1, 140013, https://doi.org/10.1038/data.2014.13 (2014).
26. Su, Z. et al. A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. Nat. Biotechnol. 32, 903–914, https://doi.org/10.1038/nbt.2957 (2014).
27. Gong, B., Xu, J. & Tong, W. Landscape of circRNAs Across 11 Organs and 4 Ages in Fischer 344 Rats. Chem Res Toxicol https://doi.org/10.1021/acs.chemrestox.0c00144 (2020).
28. Zhou, T. et al. Rat BodyMap transcriptomes reveal unique circular RNA features across tissue types and developmental stages. RNA 24, 1443–1456, https://doi.org/10.1261/rna.067132.118 (2018).
29. You, X. et al. Neuonal circular RNAs are derived from synaptic genes and regulated by development and plasticity. Nat Neurosci 18, 603–610, https://doi.org/10.1038/nrn3975 (2015).
30. Xu, Y. et al. A comprehensive rat transcriptome built from large scale RNA-seq-based annotation. Nucleic Acids Res 48, 8320–8331, https://doi.org/10.1093/nar/gkaa638 (2020).
31. Wen, Z. et al. Expression profiling and functional annotation of oncogenic genes across 11 distinct organs in rat development. Sci Rep 6, 38575, https://doi.org/10.1038/srep38575 (2016).
32. Palascia, O., Santos, A., Stolte, C., Gorodkin, J. & Jensen, L. T. TISSUES 2.0: an integrative web resource on mammalian tissue expression. Database (Oxford) 2018, https://doi.org/10.1093/database/bay028 (2018).
33. Wang, L. et al. MARRVEL: Integration of Human and Model Organism Genetic Resources to Facilitate Functional Annotation of the Human Genome. Am. J. Hum. Genet. 92, 834–853, https://doi.org/10.1016/j.ajhg.2017.04.010 (2017).
34. Friedlander, M. R., Mackowiak, S. D., Li, N., Chen, W. & Rajewsky, N. mirDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Res 40, 37–52, https://doi.org/10.1093/nar/gkr688 (2012).
35. Friedlander, M. R., et al. Discovering microRNAs from deep sequencing data using mirDeep. Nat Biotechnol 26, 407–415, https://doi.org/10.1038/nbt1394 (2008).
36. Gene Expression Omnibus https://identifiers.org/geo:GSE172269 (2021).

Acknowledgements

RNA samples for miRNA sequencing were leftover from an early study for RNA sequencing (Yu Y et al., Nature Communications and Scientific Data 2014) and were obtained by Dr. James C. Fuscoe’s laboratory at the National Center for Toxicological Research of the US Food and Drug Administration, Jefferson, AR, USA. This study was supported in part by the National Key R&D Program of China (2018YFE0201603), the National Natural Science Foundation of China (32170657), and Shanghai Municipal Science and Technology Major Project (2017SHZDJZX01). Figure 1 was created with Biorender.com.

Author contributions

Y.T.Z. and Q.L. conceived the research. miRNA-seq libraries were constructed by G.C. and Q.L. Sequencing data acquisition, data management and scientific support was performed and overseen by J.Y., T.Z.T., Y.Y. and L.S. Data analysis and interpretation were performed by X.Y., S.S., Y.Z., Y.L., J.Y., L.R., Z.C., WH., YS., JS., H.J., Z.L., H.W., P.Z., L.S., Q.L., Y.Y. and Y.T.Z. The manuscript was written and revised by X.Y., S.S., T.Z.T., Y.Y., Q.L. and L.S. All authors reviewed and approved the submitted manuscript.
Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to Q.-Z.L., Y.Y. or Y.Z.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022