

Sequencing and analysis of microRNAs (miRNAs) and mRNAs

Total RNA was extracted from kidney samples using TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was qualified and quantified using NanoDrop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, Inc.).

Analysis of miRNAs

Data filtering. The clean data was filtered from the raw sequencing data by removing low-quality tags, tags with 5' primer contaminants or poly-A, tags without 3' primer or the insert, tags <18 nt. No insert tags and 5' primer contaminants were defined as adaptor contaminants. The oversized insertion manifested as missing 3' primer. Low-quality tags were defined as >4 bases whose quality was <10 or >6 bases whose quality was <13.

Data mapping. The clean data were mapped to the reference genome of mice and other small RNA databases including miRbase version 20 (1) and DeepBase version 2.0 (containing siRNA, piRNA and snoRNA) (2) with Bowtie2 (3) using the software Anchor Alignment-Based Small RNA Annotation version 1.0 (4), except Rfam version 12.0 (5) which was performed using cmsearch version 1.1 (6).

Small RNA classification. The small RNAs were classified into different classes, including miRNAs, Piwi-interacting RNAs, small nucleolar RNAs, RNA family (Rfam) and other small RNAs. Some small RNA tags were mapped to >1 category. To make every unique small RNA mapped to only one annotation, the priority rule was performed as follows: miRNA > piRNA > snoRNA > Rfam > other small RNAs.

miRNA expression. The expression levels of each small RNA were calculated using the Transcripts Per Kilobase Million (TPM) method (7). The use of the TPM method can eliminate the influence of sequencing discrepancy on the calculation of small RNA expression. The difference of miRNA expression between the AA and self-control groups was calculated by log₂ fold change of AA expression/control expression.

Analysis of mRNAs

Data filtering. The clean data was filtered from the raw sequencing data by removing reads with adaptors, reads with >10% unknown bases or reads with a base whose percentage was <15% or >50% in the read.

Genome mapping. The clean data was mapped to the mice genome with annotation using the Hierarchical Indexing for Spliced Alignment of Transcripts software version 2.0.4 (Center for Computational Biology; Johns Hopkins University) (8).

mRNA expression analysis. The expression levels of each gene were calculated using RSEM version 1.2.12, a software package for estimating gene and isoform expression levels from RNA-Seq data (9).

References

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Table SI. Differentially expressed miRNAs induced by AA.

miRNA ID	log ₂ ratio (AA/control)	Up/down regulation	P-value	Q value
mmu-miR-124-5p	4.85	Up	1.26x10 ⁻¹⁵	1.86x10 ⁻¹⁵
mmu-miR-122-3p	4.51	Up	6.44x10 ⁻⁵⁰	1.38x10 ⁻⁴⁹
mmu-miR-124-3p	4.38	Up	<0.001	<0.001
mmu-miR-344d-3p	3.63	Up	7.06x10 ⁻¹²	9.40x10 ⁻¹²
mmu-miR-409-5p	3.52	Up	6.74x10 ⁻¹¹	8.56x10 ⁻¹¹
mmu-miR-129-2-3p	2.82	Up	8.79x10 ⁻³⁶	1.65x10 ⁻³⁵
mmu-miR-496a-3p	2.67	Up	1.66x10 ⁻³⁵	3.10x10 ⁻³⁵
mmu-miR-147-3p	2.60	Up	3.95x10 ⁻⁴⁶	8.15x10 ⁻⁴⁶
mmu-miR-5128	2.52	Up	1.08x10 ⁻²⁸	1.92x10 ⁻²⁸
mmu-miR-7649-3p	2.39	Up	5.95x10 ⁻¹⁴	8.43x10 ⁻¹⁴
mmu-miR-21a-5p	2.29	Up	<0.001	<0.001
mmu-miR-212-5p	2.29	Up	1.57x10 ⁻¹⁶⁸	4.65x10 ⁻¹⁶⁸
mmu-miR-132-3p	2.26	Up	<0.001	<0.001
mmu-miR-34c-5p	2.25	Up	<0.001	<0.001
mmu-miR-146b-5p	2.19	Up	<0.001	<0.001
mmu-miR-5121	2.08	Up	1.80x10 ⁻¹¹	2.34x10 ⁻¹¹
mmu-miR-376a-3p	2.06	Up	4.31x10 ⁻²³³	1.39x10 ⁻²³²
mmu-miR-494-3p	2.05	Up	5.43x10 ⁻¹²	7.28x10 ⁻¹²
mmu-miR-125b-1-3p	2.05	Up	<0.001	<0.001
mmu-miR-34c-3p	2.00	Up	<0.001	<0.001
mmu-miR-701-3p	1.96	Up	7.61x10 ⁻²⁵	1.29x10 ⁻²⁴
mmu-miR-143-5p	1.95	Up	1.05x10 ⁻⁸⁶	2.56x10 ⁻⁸⁶
mmu-miR-2137	1.92	Up	5.66x10 ⁻³⁶	1.07x10 ⁻³⁵
mmu-miR-511-3p	1.91	Up	8.81x10 ⁻¹²⁶	2.45x10 ⁻¹²⁵
mmu-miR-182-3p	1.84	Up	3.29x10 ⁻⁷⁷	7.83x10 ⁻⁷⁷
mmu-miR-298-5p	1.81	Up	1.48x10 ⁻¹²	2.01x10 ⁻¹²
mmu-miR-410-3p	1.81	Up	5.55x10 ⁻⁵³	1.21x10 ⁻⁵²
mmu-miR-3110-5p	1.80	Up	1.64x10 ⁻⁰⁶	1.74x10 ⁻⁰⁶
mmu-miR-21a-3p	1.80	Up	<0.001	0.00E+00
mmu-miR-466p-3p	1.77	Up	1.91x10 ⁻¹⁵	2.79x10 ⁻¹⁵
mmu-miR-206-3p	1.76	Up	1.77x10 ⁻⁴⁸	3.77x10 ⁻⁴⁸
mmu-miR-6994-3p	1.76	Up	8.42x10 ⁻⁰⁸	9.58x10 ⁻⁰⁸
mmu-miR-34b-5p	1.71	Up	<0.001	<0.001
mmu-miR-431-5p	1.70	Up	1.30x10 ⁻¹¹¹	3.47x10 ⁻¹¹¹
mmu-miR-541-5p	1.70	Up	7.53x10 ⁻⁷³	1.75x10 ⁻⁷²
mmu-miR-3071-3p	1.69	Up	2.50x10 ⁻⁰⁶	2.62x10 ⁻⁰⁶
mmu-miR-667-3p	1.68	Up	1.23x10 ⁻¹⁷	1.87x10 ⁻¹⁷
mmu-miR-154-3p	1.67	Up	3.50x10 ⁻⁰⁸	4.10x10 ⁻⁰⁸
mmu-miR-380-3p	1.67	Up	1.50x10 ⁻³⁶	2.87x10 ⁻³⁶
mmu-miR-155-3p	1.64	Up	1.12x10 ⁻¹¹	1.47x10 ⁻¹¹
mmu-miR-434-3p	1.64	Up	1.69x10 ⁻²⁴⁹	5.51x10 ⁻²⁴⁹
mmu-miR-223-5p	1.63	Up	8.03x10 ⁻⁶⁰	1.80x10 ⁻⁵⁹
mmu-miR-34b-3p	1.59	Up	<0.001	<0.001
mmu-miR-299a-5p	1.56	Up	1.41x10 ⁻⁰⁶	1.50x10 ⁻⁰⁶
mmu-miR-511-5p	1.56	Up	1.08x10 ⁻¹²	1.47x10 ⁻¹²
mmu-miR-31-5p	1.55	Up	<0.001	<0.001
mmu-miR-300-3p	1.53	Up	4.83x10 ⁻⁴⁸	1.01x10 ⁻⁴⁷
mmu-miR-433-3p	1.51	Up	4.72x10 ⁻³⁹	9.29x10 ⁻³⁹
mmu-miR-1193-3p	1.51	Up	8.54x10 ⁻¹⁸	1.31x10 ⁻¹⁷
mmu-miR-212-3p	1.50	Up	<0.001	<0.001
mmu-miR-369-3p	1.50	Up	9.50x10 ⁻³⁷	1.83x10 ⁻³⁶
mmu-miR-3073a-5p	-4.70	Down	1.30x10 ⁻⁶³	2.97x10 ⁻⁶³
mmu-miR-3073b-3p	-3.69	Down	8.32x10 ⁻¹⁸⁵	2.55x10 ⁻¹⁸⁴
mmu-miR-3073b-5p	-3.37	Down	4.96x10 ⁻¹⁶⁴	1.44x10 ⁻¹⁶³
mmu-miR-1948-5p	-3.29	Down	2.75x10 ⁻²⁰	4.40x10 ⁻²⁰
mmu-miR-92a-2-5p	-3.13	Down	1.12x10 ⁻¹¹	1.47x10 ⁻¹¹
mmu-miR-3073a-3p	-3.00	Down	1.10x10 ⁻⁶²	2.49x10 ⁻⁶²

Table SI. Continued.

miRNA ID	log ₂ ratio (AA/control)	Up/down regulation	P-value	Q value
mmu-miR-669c-5p	-2.81	Down	1.42x10 ⁻¹⁹⁸	4.39x10 ⁻¹⁹⁸
mmu-miR-7085-5p	-2.54	Down	1.60x10 ⁻²⁹	2.89x10 ⁻²⁹
mmu-miR-1968-5p	-2.42	Down	5.48x10 ⁻²⁵⁴	1.80x10 ⁻²⁵³
mmu-miR-1948-3p	-2.28	Down	1.73x10 ⁻⁸⁹	4.27x10 ⁻⁸⁹
mmu-miR-346-5p	-2.18	Down	<0.001	<0.001
mmu-miR-187-5p	-2.02	Down	<0.001	<0.001
mmu-miR-363-3p	-1.98	Down	<0.001	<0.001
mmu-miR-466b-3p	-1.96	Down	2.63x10 ⁻²¹	4.26x10 ⁻²¹
mmu-miR-486b-3p	-1.93	Down	<0.001	<0.001
mmu-miR-3099-3p	-1.93	Down	2.42x10 ⁻⁰⁷	2.68x10 ⁻⁰⁷
mmu-miR-7240-5p	-1.89	Down	6.95x10 ⁻³⁹	1.36x10 ⁻³⁸
mmu-miR-1968-3p	-1.87	Down	1.32x10 ⁻¹⁵	1.94x10 ⁻¹⁵
mmu-miR-122-5p	-1.85	Down	<0.001	<0.001
mmu-miR-365-2-5p	-1.85	Down	<0.001	<0.001
mmu-miR-190a-5p	-1.74	Down	<0.001	<0.001
mmu-miR-466x103p	-1.74	Down	1.98x10 ⁻⁰⁶	2.09x10 ⁻⁰⁶
mmu-miR-3475-3p	-1.68	Down	6.97x10 ⁻¹²	9.30x10 ⁻¹²
mmu-miR-7085-3p	-1.63	Down	1.46x10 ⁻⁹²	3.68x10 ⁻⁹²
mmu-miR-669o-3p	-1.63	Down	6.03x10 ⁻⁴⁰	1.20x10 ⁻³⁹
mmu-miR-190a-3p	-1.63	Down	<0.001	<0.001
mmu-miR-499-5p	-1.60	Down	8.99x10 ⁻²¹⁰	2.81x10 ⁻²⁰⁹
mmu-miR-187-3p	-1.58	Down	<0.001	<0.001
mmu-miR-547-3p	-1.55	Down	5.64x10 ⁻¹¹	7.19x10 ⁻¹¹
mmu-miR-486a-5p	-1.54	Down	<0.001	<0.001
mmu-let-7a-1-3p	-1.50	Down	<0.001	<0.001

mmu, *Mus musculus*; miRNA/miR, microRNA; AA, aristolochic acid.

Table SII. Opposite direction regulatory pairs of DE miRNA and DE mRNA targets enriched in signaling pathways and extracellular matrix-associated pathways.

miRNA	miRNA regulation	miRNA log ₂ ratio (AA/control)	mRNA	mRNA regulation	mRNA log ₂ ratio (AA/control)
miR-124-5p	Up	4.848444	Lrp2	Down	-4.16085
miR-124-5p	Up	4.848444	Ace2	Down	-2.34235
miR-122-3p	Up	4.505067	Kcnj3	Down	-2.71086
miR-122-3p	Up	4.505067	Adcy9	Down	-1.43472
miR-124-3p	Up	4.380091	Rgl1	Down	-2.33698
miR-344d-3p	Up	3.629804	Abcb6	Down	-1.32081
miR-409-5p	Up	3.520869	Ihh	Down	-1.62485
miR-129-2-3p	Up	2.823353	Col4a3	Down	-1.03832
miR-129-2-3p	Up	2.823353	Sptlc3	Down	-1.18673
miR-129-2-3p	Up	2.823353	Abca13	Down	-2.04054
miR-147-3p	Up	2.603331	Shc3	Down	-2.57836
miR-5128	Up	2.520869	Aph1c	Down	-1.21978
miR-21a-5p	Up	2.294618	Col6a6	Down	-2.48278
miR-21a-5p	Up	2.294618	Grin3a	Down	-2.36071
miR-21a-5p	Up	2.294618	Dgkg	Down	-2.55439
miR-212-5p	Up	2.288143	Pi4ka	Down	-1.69471
miR-132-3p	Up	2.263682	P2rx3	Down	-1.07812
miR-132-3p	Up	2.263682	Il12rb2	Down	-1.21224
miR-5121	Up	2.081105	Esr1	Down	-1.58099
miR-376a-3p	Up	2.059508	Esr1	Down	-1.58099
miR-494-3p	Up	2.047224	Bdnf	Down	-1.19106
miR-125b-1-3p	Up	2.04595	Cadm1	Down	-1.17016
miR-701-3p	Up	1.961902	Acox3	Down	-2.34278
miR-701-3p	Up	1.961902	Egf	Down	-3.17333
miR-701-3p	Up	1.961902	Prlr	Down	-1.38115
miR-182-3p	Up	1.839952	Lpin1	Down	-1.10209
miR-3110-5p	Up	1.799534	Prkcz	Down	-1.54208
miR-21a-3p	Up	1.798831	Pi4ka	Down	-1.69471
miR-21a-3p	Up	1.798831	Lpin1	Down	-1.10209
miR-21a-3p	Up	1.798831	Cd36	Down	-2.76503
miR-466p-3p	Up	1.770907	Rgl1	Down	-2.33698
miR-6994-3p	Up	1.755335	Brap	Down	-1.03307
miR-431-5p	Up	1.702755	Grin3a	Down	-2.36071
miR-431-5p	Up	1.702755	Me1	Down	-2.94505
miR-541-5p	Up	1.699358	Cldn2	Down	-2.03344
miR-541-5p	Up	1.699358	Lpin1	Down	-1.10209
miR-3071-3p	Up	1.691718	Pla2g6	Down	-1.00419
miR-3071-3p	Up	1.691718	Dusp9	Down	-1.29066
miR-667-3p	Up	1.68089	Sufu	Down	-1.29584
miR-154-3p	Up	1.672872	Acvr2b	Down	-1.15883
miR-380-3p	Up	1.672064	Wnt11	Down	-1.32811
miR-223-5p	Up	1.634384	Me1	Down	-2.94505
miR-223-5p	Up	1.634384	Kcnj3	Down	-2.71086
miR-223-5p	Up	1.634384	Smpd2	Down	-1.49458
miR-223-5p	Up	1.634384	Itgae	Down	-1.27005
miR-34b-3p	Up	1.592326	Myh14	Down	-2.01534
miR-34b-3p	Up	1.592326	Abcg8	Down	-3.59383
miR-34b-3p	Up	1.592326	Hmgcr	Down	-1.81721
miR-31-5p	Up	1.554933	Abcc6	Down	-3.17274
miR-1193-3p	Up	1.507407	Ryr2	Down	-2.15789
miR-212-3p	Up	1.504249	Dlg2	Down	-2.96209
miR-212-3p	Up	1.504249	Ace2	Down	-2.34235
miR-369-3p	Up	1.504072	Magi2	Down	-1.12155
miR-369-3p	Up	1.504072	Abcg2	Down	-1.43681
miR-3073a-5p	Down	-4.70335	Flnc	Up	5.688358
miR-3073a-5p	Down	-4.70335	Tcf7l1	Up	1.225878

Table SII. Continued.

miRNA	miRNA regulation	miRNA log ₂ ratio (AA/control)	mRNA	mRNA regulation	mRNA log ₂ ratio (AA/control)
miR-3073a-5p	Down	-4.70335	Adora1	Up	1.687443
miR-3073b-3p	Down	-3.69423	Dusp5	Up	1.796446
miR-3073b-3p	Down	-3.69423	Cers3	Up	1.85976
miR-3073b-5p	Down	-3.37453	Tcf7l1	Up	1.225878
miR-3073b-5p	Down	-3.37453	Lamb2	Up	1.357127
miR-3073b-5p	Down	-3.37453	Il10ra	Up	1.372306
miR-3073b-5p	Down	-3.37453	Fgfr2	Up	1.106133
miR-1948-5p	Down	-3.29204	Cntn1	Up	2.115218
miR-1948-5p	Down	-3.29204	Gng2	Up	1.319333
miR-1948-5p	Down	-3.29204	Pak3	Up	2.307379
miR-1948-5p	Down	-3.29204	Parvg	Up	1.202539
miR-1948-5p	Down	-3.29204	Lama5	Up	1.045592
miR-92a-2-5p	Down	-3.12556	Pik3cd	Up	1.593729
miR-92a-2-5p	Down	-3.12556	Agap2	Up	1.241878
miR-92a-2-5p	Down	-3.12556	Shc2	Up	2.092636
miR-3073a-3p	Down	-3.00122	Itga2	Up	1.386218
miR-669c-5p	Down	-2.80691	Fxyd1	Up	1.592376
miR-669c-5p	Down	-2.80691	Nos3	Up	1.37732
miR-669c-5p	Down	-2.80691	Agap2	Up	1.241878
miR-669c-5p	Down	-2.80691	Gli3	Up	1.966988
miR-669c-5p	Down	-2.80691	Nrcam	Up	3.390697
miR-7085-5p	Down	-2.53622	Ntrk2	Up	1.934301
miR-7085-5p	Down	-2.53622	Lif	Up	5.105977
miR-7085-5p	Down	-2.53622	Il4ra	Up	1.076153
miR-1968-5p	Down	-2.41939	Agap2	Up	1.241878
miR-1948-3p	Down	-2.27923	Cldn19	Up	1.237411
miR-1948-3p	Down	-2.27923	Lamb2	Up	1.357127
miR-1948-3p	Down	-2.27923	Orai2	Up	1.726877
miR-1948-3p	Down	-2.27923	Icam1	Up	1.760257
miR-1948-3p	Down	-2.27923	Jak3	Up	1.255916
miR-346-5p	Down	-2.17607	Acsl4	Up	1.408252
miR-346-5p	Down	-2.17607	Pecam1	Up	1.084648
miR-187-5p	Down	-2.02042	Zak	Up	1.589718
miR-363-3p	Down	-1.98288	Lepr	Up	1.090251
miR-363-3p	Down	-1.98288	Col4a1	Up	2.025393
miR-363-3p	Down	-1.98288	Camk2a	Up	1.538019
miR-466b-3p	Down	-1.95891	Pik3cd	Up	1.593729
miR-466b-3p	Down	-1.95891	Tlr4	Up	2.236069
miR-486b-3p	Down	-1.93435	Pak6	Up	1.659085
miR-486b-3p	Down	-1.93435	Il20rb	Up	1.563282
miR-486b-3p	Down	-1.93435	Pik3cd	Up	1.593729
miR-3099-3p	Down	-1.93292	Tnfrsf9	Up	2.566203
miR-7240-5p	Down	-1.89252	Tcf7l1	Up	1.225878
miR-7240-5p	Down	-1.89252	Smurf1	Up	1.176497
miR-7240-5p	Down	-1.89252	Agap2	Up	1.241878
miR-7240-5p	Down	-1.89252	Csf1	Up	3.189881
miR-1968-3p	Down	-1.86776	Itgb4	Up	2.992973
miR-122-5p	Down	-1.84998	Tgif2	Up	1.245209
miR-122-5p	Down	-1.84998	Camk2d	Up	1.133197
miR-122-5p	Down	-1.84998	Vav3	Up	1.191976
miR-122-5p	Down	-1.84998	Mapkapk3	Up	1.270319
miR-122-5p	Down	-1.84998	Cxcl3	Up	4.794768
miR-122-5p	Down	-1.84998	Ilk1	Up	2.372949
miR-365-2-5p	Down	-1.84646	Pak6	Up	1.659085
miR-365-2-5p	Down	-1.84646	Flnc	Up	5.688358
miR-365-2-5p	Down	-1.84646	Prickle2	Up	1.155501
miR-190a-5p	Down	-1.73766	Cntn1	Up	2.115218

Table SII. Continued.

miRNA	miRNA regulation	miRNA log ₂ ratio (AA/control)	mRNA	mRNA regulation	mRNA log ₂ ratio (AA/control)
miR-190a-5p	Down	-1.73766	Eda2r	Up	5.504216
miR-190a-5p	Down	-1.73766	Pak3	Up	2.307379
miR-466e-3p	Down	-1.73652	Pik3cd	Up	1.593729
miR-466e-3p	Down	-1.73652	Gucy1b3	Up	1.430467
miR-3475-3p	Down	-1.67665	Lif	Up	5.105977
miR-7085-3p	Down	-1.63379	Flnc	Up	5.688358
miR-7085-3p	Down	-1.63379	Dusp5	Up	1.796446
miR-7085-3p	Down	-1.63379	Lif	Up	5.105977
miR-669o-3p	Down	-1.63005	Fzd2	Up	1.638475
miR-190a-3p	Down	-1.62988	Flna	Up	2.873883
miR-190a-3p	Down	-1.62988	Slc2a1	Up	1.40724
miR-190a-3p	Down	-1.62988	Slc27a6	Up	3.602075
miR-499-5p	Down	-1.59606	Cldn7	Up	2.016376
miR-499-5p	Down	-1.59606	Csf1	Up	3.189881
miR-499-5p	Down	-1.59606	Cav2	Up	1.182304
miR-187-3p	Down	-1.57642	Blnk	Up	1.263224
miR-187-3p	Down	-1.57642	Clca3a1	Up	2.145877
miR-486a-5p	Down	-1.53915	Gucy1a2	Up	1.515805
let-7a-1-3p	Down	-1.49626	Ncam1	Up	2.024263
let-7a-1-3p	Down	-1.49626	Gdf15	Up	3.100863
let-7a-1-3p	Down	-1.49626	F3	Up	1.404696

miRNA/miR, microRNA; AA, aristolochic acid; DE, differentially expressed.