

SCIENTIFIC DATA

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miRNA expression atlas in male rat

SUBJECT CATEGORIES

- » Microarrays
- » Toxicology

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MicroRNAs (miRNAs) are small (~ 22 nucleotide) noncoding RNAs that play pivotal roles in regulation of gene expression. The value of miRNAs as circulating biomarkers is now broadly recognized; such tissue-specific biomarkers can be used to monitor tissue injury and several pathophysiological conditions in organs. In addition, miRNA profiles of normal organs and tissues are important for obtaining a better understanding of the source of modulated miRNAs in blood and how those modulations reflect various physiological and toxicological conditions. This work was aimed at creating an miRNA atlas in rats, as part of a collaborative effort with the Toxicogenomics Informatics Project in Japan (TGP2). We analyzed genome-wide miRNA profiles of 55 different organs and tissues obtained from normal male rats using miRNA arrays. The work presented herein represents a comprehensive dataset derived from normal samples profiled in a single study. Here we present the whole dataset with miRNA profiles of multiple organs, as well as precise information on experimental procedures and organ-specific miRNAs identified in this dataset.

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Design Type(s)	microRNA profiling by array design • organism part comparison design
Measurement Type(s)	microRNA profiling assay
Technology Type(s)	DNA microarray
Factor Type(s)	organism part • saline perfusion
Sample Characteristic(s)	Rattus norvegicus • liver • kidney • lung • spleen • pancreas • duodenum • jejunum • ileum • caecum • colon • rectum • ...

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Background & Summary

MicroRNAs (miRNAs) are small (~22 nucleotide) noncoding RNAs that play pivotal roles in regulation of gene expression; miRNAs bind via complementary base-pairing to target transcripts to repress translation or promote mRNA degradation. Major miRNAs are highly conserved across species, allowing translation of biomarkers to the clinic. The machinery for miRNA-mediated gene regulation is conserved from plants to humans, and miRNAs are encoded by their respective genes. As with other regulatory molecules, miRNAs are frequently subject to changes in expression level due to a large range of physiological processes, such as development, immune responses, metabolism and diseases, as well as toxicological outcomes¹. Mounting evidence indicates that miRNAs are frequently overexpressed or downregulated as a result of cancer, obesity, diabetes, inflammation, neurological disorders, cardiovascular diseases or autoimmune diseases^{2–7}. Previous studies have shown that distinct miRNA signatures can be assigned to particular organs and tumor types⁸. For example, miR-21 is ubiquitously expressed and upregulated in various types of cancer, including lymphoma, lung, prostate and colorectal cancers among others⁹. Furthermore, tumor cells have been shown to release tumor-specific miRNAs into systemic circulation. Because of the fundamental roles played by miRNAs in cellular functions, the potential for miRNAs as novel therapeutic targets is now widely recognized. Several candidate therapeutic miRNAs have progressed into clinical and preclinical development; for example, antisense miR-122 is being developed as a treatment for hepatitis C virus, miR-208/499 for chronic heart failure, miR-195 for myocardial infarction and miR-34 and let-7 for cancer^{10,11}. However, serious obstacles obstructing the development of miRNA-based therapies remain, including lack of tissue specificity and risks of systemic toxicity. Characterizing miRNA expression profiles of normal organs and tissues may improve our understanding of the etiology of diseased organs and of organ- and tissue-specificity of miRNAs, and the success rate of development of new miRNA-based therapeutics.

Additionally, miRNAs have the potential to be useful biomarkers for monitoring physiological conditions and tissue injury. Upon tissue injury, miRNAs are released into systemic circulation or other body fluids; upon release, these miRNAs can be detected in small-volume samples via specific and sensitive quantitative real-time PCR. The precise mechanisms underlying the remarkable stability of miRNAs in the RNase-rich environment of blood are not well understood; nevertheless, miRNAs persist and are remarkably stable in blood; and these miRNAs can persist in an encapsulated state in exosomes or in protein complexes with carriers such as Argonaute 2 and Nucleophosmin or the High Density Lipoprotein¹². Moreover, several studies have successfully identified circulating miRNA-based biomarkers; for example, Mitchell *et al.*¹³ found that miR-141 was highly elevated in serum from patients with prostate cancer and hypothesized that this miRNA may be useful as a diagnostic marker. Similarly, Wang *et al.*¹⁴ found that miR-122a, which is specifically expressed in the liver, was circulating systemically in mice with acetaminophen- (APAP-) induced hepatotoxicity; the authors reported that miR-122a and miR-192 were detected in plasma as early as the point when elevated alanine aminotransferase activity was found evident. Laterza *et al.*¹⁵ also reported that plasma miRNA measurements could be useful for monitoring tissue injury in the liver, muscle and brain. Even in clinical research, plasma miR-122a was significantly elevated in APAP-induced hepatitis patients¹⁶. These reports clearly indicate that circulating organ-specific miRNAs can serve as useful biomarkers for tissue injury and disease status in various organs. The organ-specificity of circulating miRNAs, or in other words, the detailed tissue distribution of miRNAs, is important information to be used in obtaining biomarkers for organ toxicity, although desirable properties of biomarkers vary with intended use. In addition, miRNA profiles of normal organs and tissues are important for gaining a better understanding of the source of modulated miRNAs in blood and in gaining an understanding of how those modulations reflect various physiological and toxicological conditions.

To date, organ-specific miRNA profiles have been reported for several animal species, including human, mouse and rat^{17–20}. However, the amount of data currently available is still inadequate, since there are only limited datasets with profiles in a limited number of organs. The main objectives of our data analysis were (i) to establish the validity of this dataset and (ii) to demonstrate organ-specific miRNAs identified in this dataset. In this report, we present a large-scale reference dataset constituting genome-wide miRNA profiles for 55 normal rat organs or tissues. The validity of this dataset was confirmed by comprehensive statistical analysis. We ultimately identified several organ-specific miRNAs in rats. These organ-specific miRNAs can potentially be used as biomarkers for identifying the origin of metastatic tumors and for monitoring toxicity in targeted organs. Furthermore, establishing combinations of organ-specific miRNA measurements may be a novel biomarker for monitoring simultaneous impairment in multiple organs. Our data also support the hypothesis that specificity of miRNA expression is conserved among different species, since the majority of organ-specific miRNAs identified in this rat study were also confirmed as organ-specific in humans. The value of miRNAs as novel therapeutic targets and circulating biomarkers to monitor tissue injury and several pathophysiological conditions in organs is now broadly recognized. In the course of development of miRNAs for practical use as targeted therapeutics and biomarkers, there is no doubt about the importance of open access large-scale datasets for a free miRNA atlas for normal organs/tissues and comparative data analysis. The work presented herein represents a comprehensive dataset derived from normal samples profiled in a single study. We believe that our dataset will be of particular value to both basic and translational scientists in biological and biomedical sciences, especially for novel target discovery and biomarker identification.

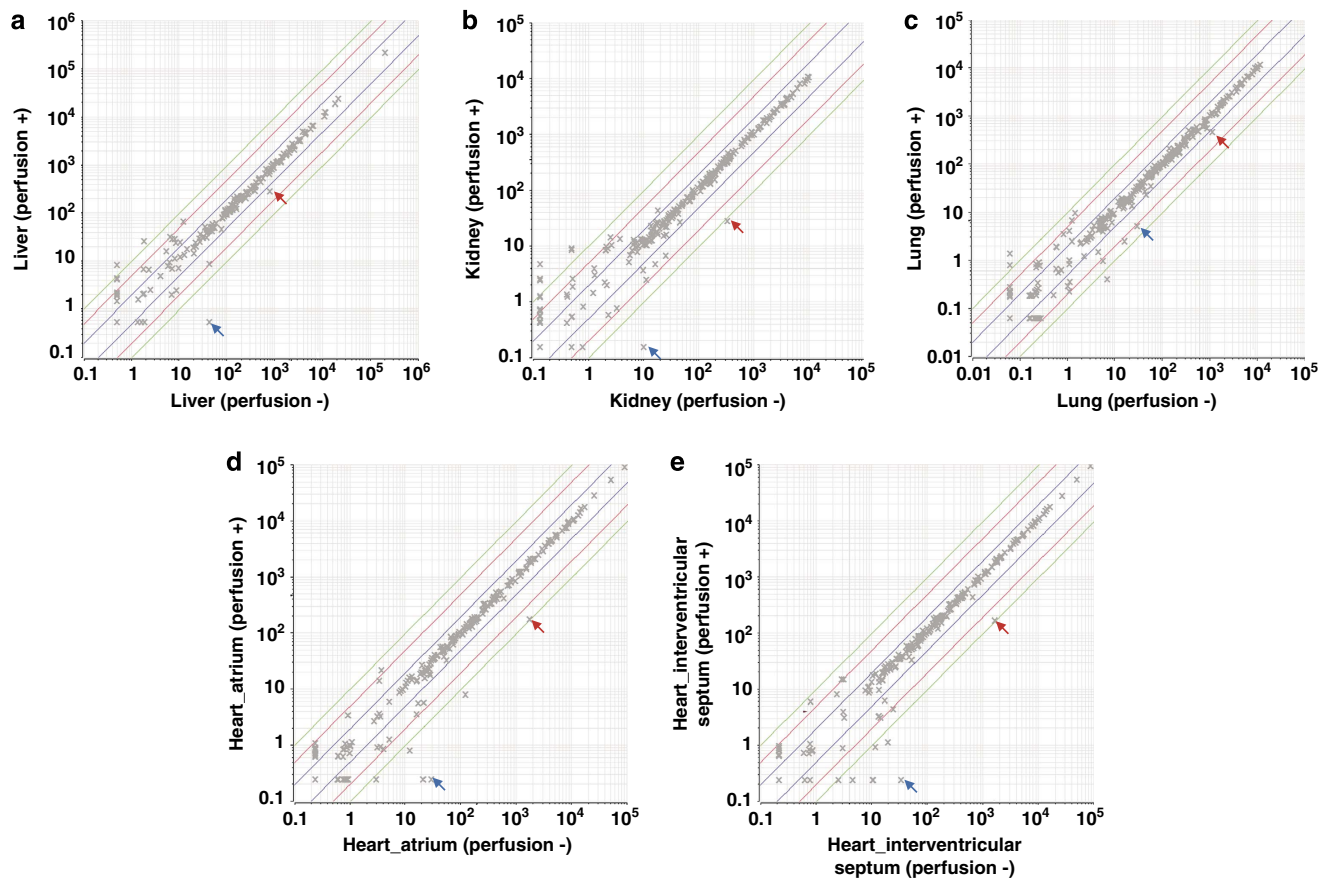


Figure 1. Effect of circulating blood on miRNA expression in organs. Arrows indicate blood specific miRNAs; red arrows: miR-451, blue arrows: miR-144. Horizontal axis: Perfusion (-), vertical axis: Perfusion (+). Correlation coefficient (R^2); (a) Liver: 0.9998, (b) Kidney: 0.9960, (c) Lung: 0.9956, (d) Heart, atrium: 0.9988, (e) Heart, interventricular septum: 0.9994.

Methods

Animal experiments

For each miRNA microarray experiment, 9-week-old male Sprague-Dawley rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan). After a 7-day quarantine and acclimatization period, 10-week-old animals were used ($N=6$). The animals were individually housed in stainless-steel cages in an animal room set to the following conditions: 12 h (7:00–19:00) light phase; ventilation rate, 12/h; temperature, 20 °C–26 °C; and relative humidity, 35–75%. Each animal had free access to water and pellet diet (CRF-1, sterilized by radiation, Oriental Yeast Co., Ltd., Tokyo, Japan). Each animal was anesthetized with ether. The animals were divided into 2 groups to compare the effects of peripheral blood cells in organs; 3 animals were perfused with saline to remove blood from all organs, while the remaining 3 animals that were not perfused were subjected to collection of only the heart (atrium and interventricular septum), kidney, liver and lung. All organs evaluated in this study are listed in Table 1 (available online only). Experimental protocols were reviewed and approved by the Ethics Review Committee for Animal Experimentation of the National Institute of Health Sciences.

RNA extraction and miRNA microarray analysis

RNA was prepared using the miRNeasy kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. RNA was quantified using a DU-7400 spectrophotometer (Beckman Coulter, Brea, CA) and quality was monitored with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Cyanine-3 (Cy-3) labeled RNA was prepared from 0.1 μg RNA using the miRNA Complete Labeling and Hyb Kit (Agilent Technologies), according to the manufacturer's instructions. The entire volume of Cy3-labeled RNA was incubated at 100 °C for 5 min in a 45 μl reaction volume containing 1x Agilent blocking agent, Hybridization Spike-In and Hi-RPM Hybridization Buffer. Upon completion of this incubation, samples were hybridized to an 8 \times 15 k customized Agilent Rat miRNA microarray containing both miRBase 15.0 and 16.0 probes for 20 h at 55 °C on a rotating rack in an Agilent hybridization oven. After hybridization, microarrays were washed for 5 min at room temperature

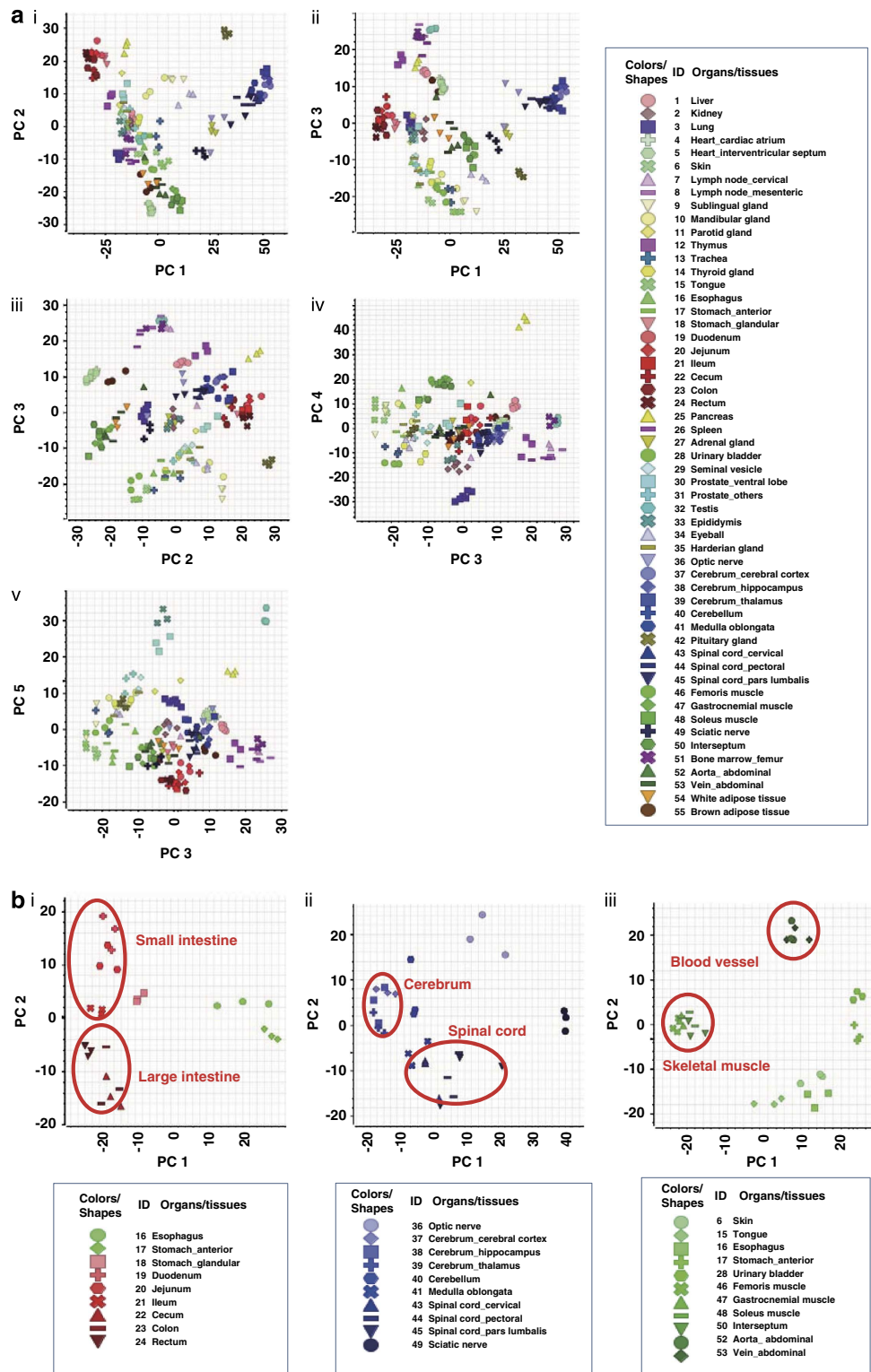


Figure 2. Principal component analysis (PCA). (a) PCA for all organ miRNAs. Spot colors represent broad types of organs; blue: nervous system, red: intestines, green: smooth or skeletal muscles, pink: lymphoid or hematopoietic system, light blue: reproductive system. Eigenvalues for each component: 29.1% (PC₁), 11.4% (PC₂); 8.1% (PC₃), 6.7% (PC₄), 5.0% (PC₅). (b) PCA for digestive organs, nervous system or muscles. Eigenvalues for each component: (i) 37.2% (PC₁), 10.8% (PC₂); (ii) 35.7% (PC₁), 13.2% (PC₂); (iii) 31.0% (PC₁), 16.4% (PC₂).

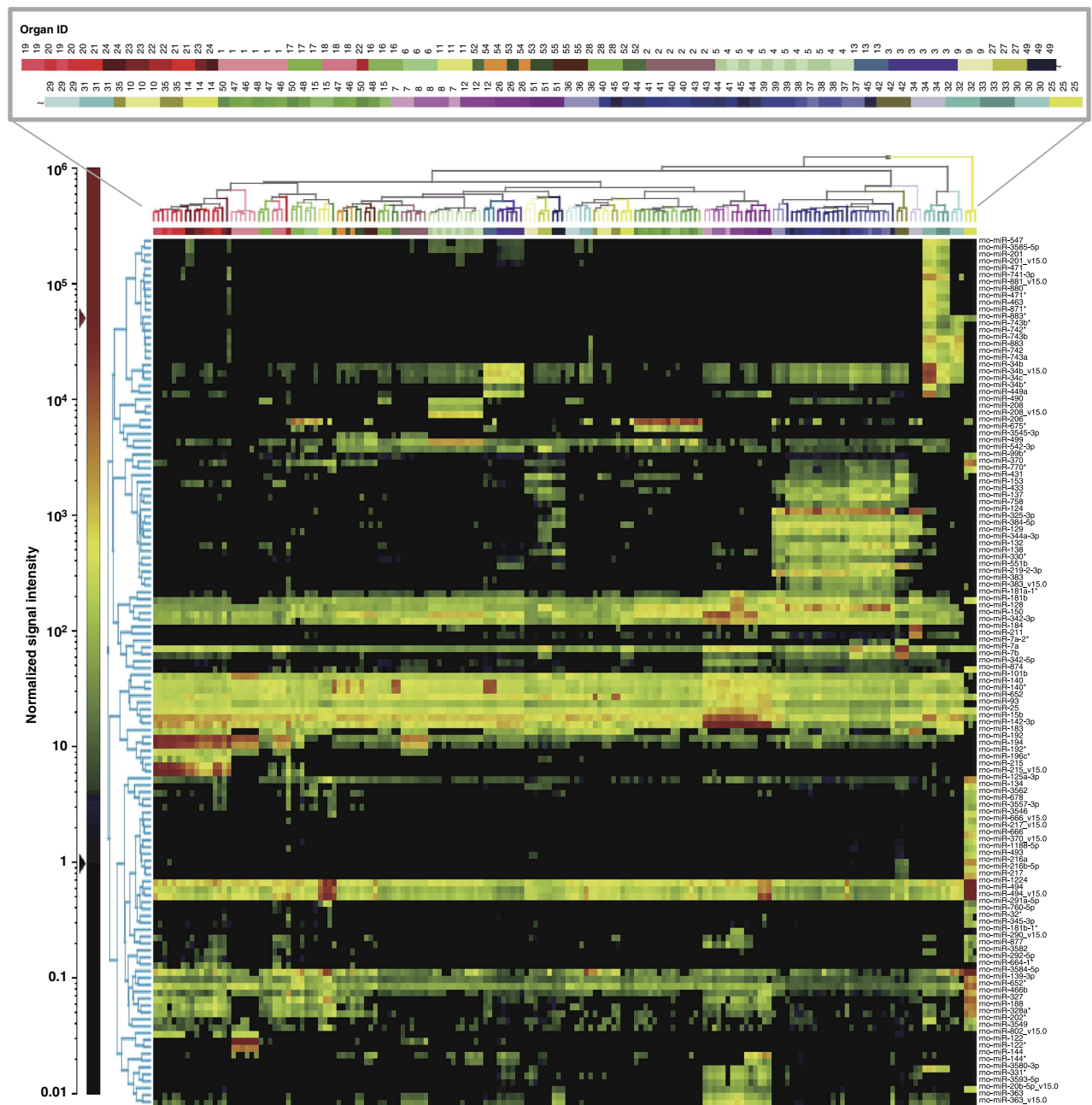


Figure 3. Two-dimensional hierarchical clustering analysis based on the expression profiles of the 128 filtered probes. Horizontal: individual organs, vertical: miRNAs. In this analysis, expression profiles for the 128 probes filtered by model-based clustering were used for clustering.

with GE Wash Buffer 1 (Agilent Technologies) and then for 5 min at 37 °C with GE Wash buffer 2 (Agilent Technologies); slides were then dried immediately. Immediately after this washing and drying step, slides were scanned using an Agilent DNA Microarray Scanner (G2565AA) at the following settings: one-color scan for 8 × 16 k array slides, scan area, 61 × 21.6 mm; scan resolution, 5 μm; dye channel, green; and extended dynamic range scan mode (Hi = 100%, Lo = 5%).

Microarray data analysis

Expressionist analysis software Ver. 7.6 (Genedata AG, Basel, Switzerland) was used for normalization, principal component analysis (PCA) and hierarchical clustering. First, all signal intensities were scaled to the 75th percentile of the median of the dynamic target value for each array using the central tendency normalization method. Next, the following two statistical parameters were calculated for each probe: (i) *P*-value of the Shapiro-Wilk test with Bonferroni correction (R software) and (ii) maximal signal

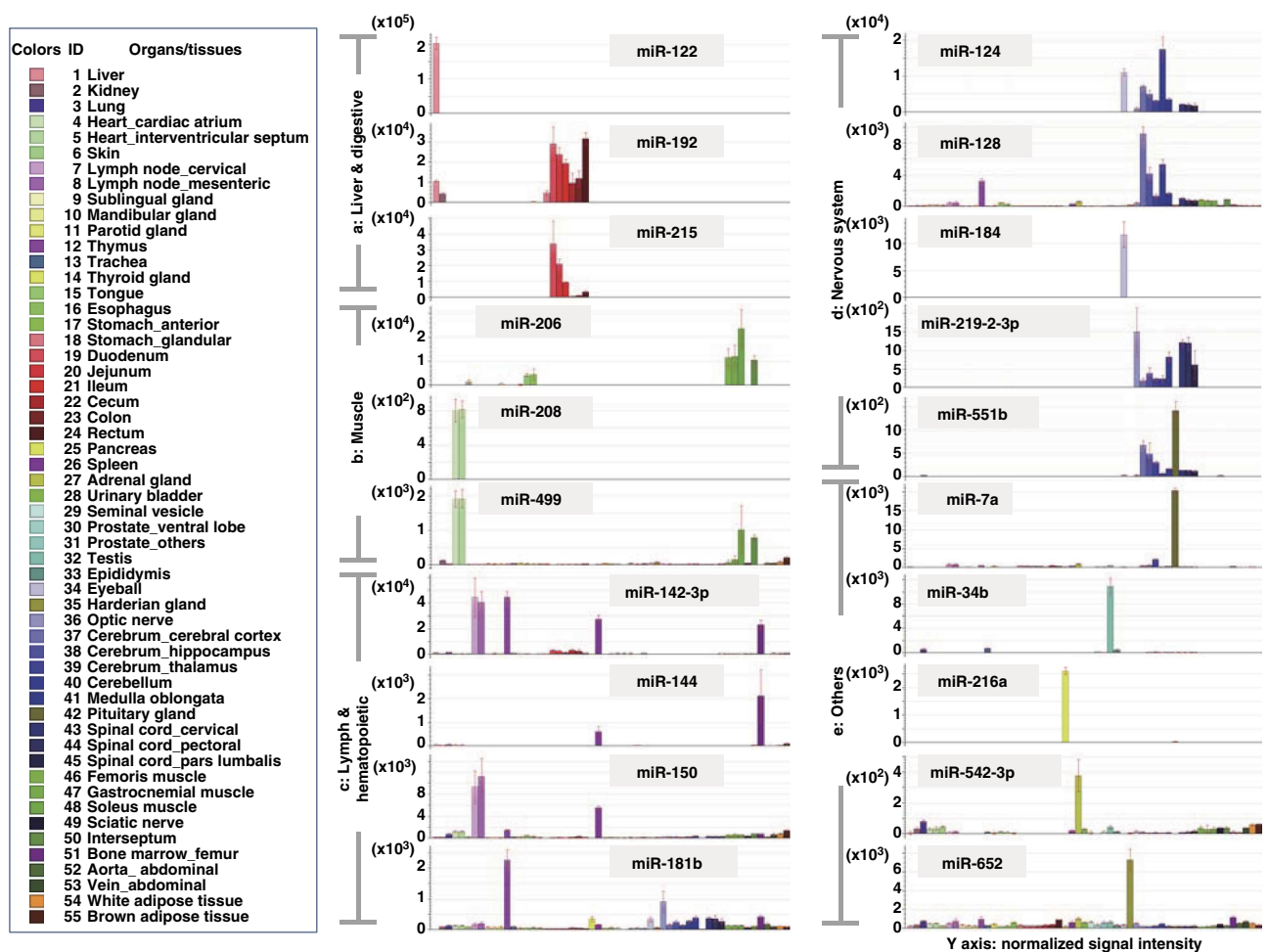


Figure 4. Expression profiles of organ-specific miRNAs identified in this study. Horizontal: individual organs, vertical: normalized signal intensity. Each bar represents mean \pm S.D. (Organ ID 1–5: $N=6$, organ ID 6–55: $N=3$).

intensity. Probes were then filtered by a P -value of < 0.05 and a maximal signal intensity of >100 . To identify organ-specific miRNAs, expression profiles of filtered probes were further analyzed by model-based clustering using the 'mclust' R package (<http://cran.r-project.org/web/packages/mclust/index.html>). For this analysis, a histogram was created to determine the distribution of expression values among different organs for each gene. For multiple distributions, the presence of an isolated peak was considered attributable to organ-specific expression of miRNAs. Genes with isolated peaks consisting of less than 37 microarrays (20% of all microarrays) in the histogram were selected as candidate miRNAs that were specifically expressed in one or a few organs.

Data Records

Microarray data are available in the NCBI Gene Expression Omnibus (GEO), accession GSE52754 (Data Citation 1). This accession contains matrix files of normalized miRNA expression data used in this report and raw data files generated from Agilent microarray systems as .gz files.

Technical Validation

miRNA microarray experiments

In this study, microarray experiments were performed according to the manufacturer's instructions, and the quality of each experiment was assessed in QC reports generated from the Agilent microarray systems.

Possible effects of peripheral blood cell-derived miRNAs

To evaluate the possible effects of peripheral blood cell-derived miRNAs on the miRNA expression profiles of organs, two different sampling conditions, with or without perfusion, were compared to assess effects on miRNA expression profiles in four organs (heart, kidney, liver and lung). As shown in Figure 1, blood-cell-specific miRNAs (e.g., miR-451 and miR-144)²¹ were clearly evident in nonperfused samples,

but not in perfused samples. On the other hand, for each organ, miRNA profiles for perfused samples were highly correlated with those for nonperfused samples ($R^2 > 0.995$). These data support the hypothesis that there are no distinct differences in miRNA profiles between these major organs and peripheral blood cells with some exceptions, such as miR-451 and miR-144, and that expression profiles for the majority of miRNAs were not markedly affected by remaining peripheral blood. Moreover, our data showed that miR-451 and miR-144 were specifically expressed in hematopoietic tissues e.g., spleen and bone marrow (refer to the original dataset for details). Therefore, microarray data obtained from nonperfused samples were analyzed in combination with those from perfused samples when generating the final comprehensive dataset.

Principal component analysis (PCA)

PCA was performed to comprehensively compare miRNA expression profiles among all organs in the entire experimental dataset (Figure 2a). Along the principal component (PC) 1 axis, which accounts for 29.1% of all variation, the clusters of signals from nervous system (brain, spinal cord, optic nerve and sciatic nerve; shown in blue colors), pituitary gland and adrenal gland samples were clearly separated from intestine samples (shown in red), whereas along the PC2 axis (11.4%), muscle (shown in green) samples were clearly separated from nervous system and intestine samples. Along the PC3 axis (8.1%), the clusters of signals from lymph and hematopoietic tissue (shown in pink) samples were clearly separated from those of other samples. Similarly, along the PC4 axis (6.7%) and PC5 axis (5.0%), the clusters of signals from pancreas and lung samples, and those from reproductive organ samples, were clearly separated from others. A tendency towards densely clustered signals from triplicate samples of the same organ was observed in PCA results. Additional information and eigenvalues of PC1 to PC5 axes are summarized in Tables 2 and 3 (available online only).

Furthermore, miRNA microarray data obtained from digestive organs, the nervous system and skeletal/smooth muscles were extracted from the whole dataset and subjected to system-specific PCA (Figure 2b). In the intestinal-specific subanalysis, clusters of signals from upper digestive organ (esophagus and stomach) samples were clearly separated from those of lower digestive organs (small and large intestine) along PC1 (37.2%), whereas along the PC2 axis (10.8%), those from small and large intestine samples were further divided into each organ by functional anatomy of the digestive system. In the nervous system subanalysis, a clear separation of clusters of signals were observed along both PC1 (35.7%) and PC2 (13.2%); those from cerebrum samples were clearly separated from ischial nerve samples along PC1, and those from the spinal cord were separated from optic nerve samples along PC2 (13.2%). Skeletal/smooth muscles were separated into four clusters: skeletal muscles, smooth muscles (blood vessel), smooth muscles (bladder and stomach) and others (esophagus, tongue and skin) (31.0% for PC1 and 16.4% for PC2). Overall, PCA successfully showed the similarity in gene expression profiles among functionally similar organs. These results provided evidence that the microarray data were of high enough quality to assess similarities and differences among miRNA profiles from different organs and tissues. In addition, the global miRNA expression profiles for these 55 normal organs included high-quality, reliable data that reflected the biological function of each organ.

Organ specific miRNA selection

To identify organ-specific miRNAs, all probes with any detectable signal were further filtered by a statistical analysis with criteria described in the Methods section; 296 probes met these criteria and were further analyzed using model-based clustering. In the model-based clustering, 128 probes were identified as miRNAs that were specifically expressed in one or more organs; further details on the statistical parameters calculated are available in Table 4 (available online only). Figure 3 shows a 2-dimensional hierarchical clustering diagram of groups of organs; the clustering was based on the expression profiles of the 128 filtered probes. Triplicate samples of the same organ were densely clustered; moreover, functionally similar organs were closely clustered to each other. Among these clusters, several miRNAs that were specifically expressed in specific organ(s) were successfully identified. Expression profiles of several representative organ-specific miRNAs are summarized (Figure 4 and Table 5 (available online only)). As shown in Table 5, more than half of these miRNAs were expressed in the corresponding human organs/tissues^{22–31}.

Technical validity and limitation of microarray analysis

Microarray technology has become a crucial tool for large-scale and high-throughput measurement. Non-specific hybridization or cross-hybridization is a common concern when interpreting microarrays, particularly with closely related gene family members having highly similar sequences. However, in such cases cross-hybridization is unavoidable since designing appropriate, highly specific, probes is not easy. Advances in microarray technology have successfully reduced, but not completely eliminated, cross-hybridization between the same subfamilies of miRNAs³². Therefore, attention should be paid to interpreting data from genes with highly similar sequences particularly in a microarray dataset similar to the present data. Recently, RNA-sequencing (RNA-seq) technology has emerged as a powerful tool for transcriptomics. This new technology is expected to be a useful tool for miRNA expression analysis.

Usage Notes

Since a large amount of numerical data is produced with microarray analysis, sophisticated bioinformatics approaches are required to analyze and interpret the results. A great number of statistical algorithms for filtering differentially expressed genes are available through the BioConductor project website. In addition, there are several software packages available that combine visualization with statistical analysis, such as Genedata Expressionist (Genedata), GeneSpring (Agilent Technology), Spotfire DecisionSite (Spotfire), TIBCO Spotfire (TIBCO Software Inc.) and ArrayTrack (Tong *et al.*³³).

References

1. Stefani, G. & Slack, F. J. Small noncoding RNAs in animal development. *Nat. Rev. Mol. Cell Biol.* **9**, 219–230 (2008).
2. Farazi, T. A., Hoell, J. I., Morozov, P. & Tuschl, T. MicroRNAs in human cancer. *Adv. Exp. Med. Biol.* **774**, 1–20 (2013).
3. Jordan, S. D. *et al.* Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. *Nat. Cell Biol.* **13**, 434–446 (2011).
4. Lu, T. X., Munitz, A. & Rothenberg, M. E. MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. *J. Immunol.* **182**, 4994–5002 (2009).
5. Nelson, P. T., Wang, W. X. & Rajeev, B. W. MicroRNAs (miRNAs) in neurodegenerative diseases. *Brain Pathol.* **18**, 130–138 (2008).
6. Urbich, C., Kuehnbacher, A. & Dimmeler, S. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc. Res.* **79**, 581–588 (2008).
7. Stanczyk, J. *et al.* Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum.* **58**, 1001–1009 (2008).
8. Lu, J. *et al.* MicroRNA expression profiles classify human cancers. *Nature* **435**, 834–838 (2005).
9. Jazbutyte, V. & Thum, T. MicroRNA-21: from cancer to cardiovascular disease. *Curr. Drug Targets* **11**, 926–935 (2010).
10. Janssen, H. L. *et al.* Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* **368**, 1685–1694 (2013).
11. Srivastava, A., Suy, S., Collins, S. P. & Kumar, D. Circulating MicroRNA as biomarkers: An update in prostate cancer. *Mol. Cell. Pharmacol.* **3**, 115–124 (2011).
12. Cortez, M. A. *et al.* MicroRNAs in body fluids—the mix of hormones and biomarkers. *Nat. Rev. Clin. Oncol.* **8**, 467–477 (2011).
13. Mitchell, P. S. *et al.* Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl Acad. Sci. USA* **105**, 10513–10518 (2008).
14. Wang, K. *et al.* Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc. Natl Acad. Sci. USA* **106**, 4402–4407 (2009).
15. Laterza, O. F. *et al.* Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. *Clin. Chem.* **55**, 1977–1983 (2009).
16. Starkey Lewis, P. J. *et al.* Circulating microRNAs as potential markers of human drug-induced liver injury. *Hepatology* **54**, 1767–1776 (2011).
17. Miska, E. A. *et al.* Microarray analysis of microRNA expression in the developing mammalian brain. *Genome Biol.* **5**, R68 (2004).
18. Landgraf, P. *et al.* A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* **129**, 1401–1414 (2007).
19. Wang, Y. *et al.* Identification of rat lung-specific microRNAs by microRNA microarray: valuable discoveries for the facilitation of lung research. *BMC Genomics* **8**, 29 (2007).
20. Hua, Y. J. *et al.* Identification and target prediction of miRNAs specifically expressed in rat neural tissue. *BMC Genomics* **10**, 214 (2009).
21. Dore, L. C. *et al.* A GATA-1-regulated microRNA locus essential for erythropoiesis. *Proc. Natl Acad. Sci. USA* **105**, 3333–3338 (2008).
22. Bottoni, A. *et al.* Identification of differentially expressed microRNAs by microarray: a possible role for microRNA genes in pituitary adenomas. *J. Cell. Physiol.* **210**, 370–377 (2007).
23. Liang, Y. An expression meta-analysis of predicted microRNA targets identifies a diagnostic signature for lung cancer. *BMC Med. Genomics* **1**, 61 (2008).
24. Smirnova, L. *et al.* Regulation of miRNA expression during neural cell specification. *Eur. J. Neurosci.* **21**, 1469–1477 (2005).
25. Tian, L., Huang, K., DuHadaway, J. B., Prendergast, G. C. & Stambolian, D. Genomic profiling of miRNAs in two human lens cell lines. *Curr. Eye Res.* **35**, 812–818 (2010).
26. Jenkins, R. H., Martin, J., Phillips, A. O., Bowen, T. & Fraser, D. J. Transforming growth factor β 1 represses proximal tubular cell microRNA-192 expression through decreased hepatocyte nuclear factor DNA binding. *Biochem. J.* **443**, 407–416 (2012).
27. Nielsen, S. *et al.* Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. *J. Physiol.* **588**, 4029–4037 (2010).
28. van Rooij, E. *et al.* Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* **316**, 575–579 (2007).
29. Sharbati, S. *et al.* Deciphering the porcine intestinal microRNA transcriptome. *BMC Genomics* **11**, 275 (2010).
30. Szafranska, A. E. *et al.* MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. *Oncogene* **26**, 4442–4452 (2007).
31. Sluijter, J. P. *et al.* MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. *Arterioscler. Thromb. Vasc. Biol.* **30**, 859–868 (2010).
32. Wang, H., Ach, R. A. & Curry, B. Direct and sensitive miRNA profiling from low-input total RNA. *RNA* **13**, 151–159 (2007).
33. Tong, W. *et al.* ArrayTrack—supporting toxicogenomic research at the U.S. Food and Drug Administration National Center for Toxicological Research. *Environ. Health Perspect.* **111**, 1819–1826 (2003).

Data Citation

1. Minami, K. & Uehara, T. Gene Expression Omnibus GSE52754 (2013).

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Author Contributions

K.M. and T.U. designed the study and wrote the paper. Y.M. carried out the statistical data analysis. A.H. conceived the study. K.O., M.K. and A.O. assisted interpretation of analysis results and discussion. H.Y., Y.O. and T.U. supervised the study and project.

Additional information

Tables 1–5 are only available in the online version of this paper.

Competing financial interests: The authors declare no competing financial interests.

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