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Research in Veterinary Science; Short communication

Title: MicroRNA expression profiling of cat and dog kidneys

Running title: miRNAs in feline and canine kidney

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Abstract

MicroRNAs (miRNAs) play a role in the pathogenesis of certain diseases and may serve as biomarkers. Here, we present the first analysis of miRNA expression in the kidneys of healthy cats and dogs. Kidneys were divided into renal cortex (CO) and medulla (MD), and RNA sequence analysis was performed using the mouse genome as a reference. A total of 277, 276, 295, and 297 miRNAs were detected in cat CO, cat MD, dog CO, and dog MD, respectively. By comparing the expression ratio of CO to MD, we identified highly expressed miRNAs in each tissue as follows: 41 miRNAs including miR-192-5p in cat CO; 45 miRNAs including miR-323-3p in dog CO; 78 miRNAs including miR-20a-5p in cat MD; and 11 miRNAs including miR-132-5p in dog MD. Further, the target mRNAs of these miRNAs were identified. These data provide veterinary medicine critical information regarding renal miRNA expression.

Keywords: companion animals, cat, dog, microRNA, kidney, RNA sequencing

MicroRNAs (miRNAs) are small non-coding RNAs with high evolutionary sequence conservation that act as transcriptional and posttranscriptional regulators of target genes by binding to complementary sequences within mRNAs or by regulating promoter activities. miRNAs are also present in body fluids such as blood plasma and urine (Weber et al., 2010). Urinary miRNA is stable because it is included in small vesicles called exosomes (Alvarez et al., 2012), indicating the potential of urinary miRNAs as diagnostic biomarkers for kidney diseases.

Recently, altered miR-192 expression was reported in human kidney diseases such as diabetic nephropathy (Kato et al., 2013). Further, we determined that miR-146a was highly expressed in a murine model of chronic kidney disease (CKD) and its increased expression more closely correlated with the development of tubulointerstitial inflammation (Ichii et al., 2012). Moreover, urinary miRNAs have been evaluated as diagnostic biomarkers, and altered urinary levels of miR-29c (Lv et al., 2013) are present in human patients with CKD. Thus, miRNA levels in kidney and urine correlate significantly with kidney disease progression; however, there are no such data for companion animals.

miRNAs are specifically expressed in certain organs or cell types. For example, in normal rats, the expression of miR-192 and miR-194 is higher in the renal cortex (CO) than in the medulla (MD) (Tian et al., 2008). Although 1908, 291, 326, 783, 360, and 2578 mature miRNAs are respectively listed in the miRBase (http://www.mirbase.org/) for mice, dogs, pigs, cows, horses, and humans, there are no data for cats.

To acquire miRNA expression data for veterinary medicine, we present here a comprehensive investigation of cat and dog kidneys. Kidney tissue from healthy cats

(males, mixed-breed, n = 4, 3–6 months of age) were obtained from The Kitasato Institute (Saitama, Japan, from Dr. Nomoto; n = 3) and Kagoshima University (Kagoshima, Japan; n = 1). Normal dog kidneys, based on the diagnosis of a clinical veterinarian, (male schnauzer, n = 1, 4 years; castrated male shih-tzu, n = 1, 9 years; male beagle, n = 1, 14 years) were obtained at Hokkaido University when they were euthanized and autopsied. These kidney tissues were divided into CO and MD and stored in RNA later (Life Technologies; Carlsbad, CA, USA) until RNA purification.

Total RNA was isolated using an miRNeasy Mini Kit (Qiagen; Venlo, Netherlands). The quality of total RNA was checked using a Bioanalyzer (Agilent; Santa Clara, CA, USA), and samples were pooled as one sample for each tissue. Libraries were prepared using a TruSeq Small Library Preparation Kit (Illumina; San Diego, CA, USA) according to the manufacturer's protocols and sequenced using 50-base reads acquired using a HiSeq 2000 platform. The details of the sequence analysis of small RNAs targeting miRNAs are described in Supplemental Methods. The July 2007 (NCBI37/mm9) mouse (*Mus musculus*) genome data were used as reference (http://genome.ucsc.edu/cgi-bin/hgGateway?db=mm9).

The miRNAs represented the majority of RNAs (>85%) detected in all tissues, indicating the success of miRNA extraction from kidneys (Supplemental Figure 1). Further, cluster analysis revealed that the expression pattern of RNAs depended on species rather than tissue type (Supplemental Figure 1).

Totals of 277, 276, 295, and 297 miRNAs were identified in cat CO, cat MD, dog CO, and dog MD, respectively and are listed in Supplemental Table 1. To determine the

77 miRNAs predominantly expressed in CO or MD, those expressed at greater than a 2.0-fold
78 ratio in CO/MD or MD/CO were selected. Thirty-one and 14 miRNAs were commonly
79 expressed in CO and MD of both animals, respectively. Forty-one and 45 miRNAs were
80 highly expressed in the cat and dog CO, respectively. Seventy-eight and 11 miRNAs were
81 highly expressed in the cat and dog MD, respectively. Table 1 summarizes the miRNAs
82 with higher expression ratios for CO/MD or MD/CO. Further, Table 2 shows the target
83 mRNAs of miRNAs detected in CO or MD. Relevant published findings regarding these
84 miRNAs are presented in the text that follows.

85 Of the miRNAs highly expressed in cat and dog CO, miR-194 and miR-204 are highly
86 expressed in human kidney (Sun et al., 2004), and miR-194 expression is altered in renal
87 ischemic reperfusion injury (IRI) (Godwin et al., 2010). Transforming growth
88 factor-β-induced miR-491-5p expression promotes to disrupt the tight junctions in rat
89 proximal tubular epithelial cells (Zhou et al., 2010). For the miRNAs highly expressed in
90 the cat CO, there are several reports showing a significant correlation between miR-192
91 expression and renal fibrosis progression (Kato et al., 2013). Of the miRNAs highly
92 expressed in the dog CO, miR-377 was upregulated and leads to increased fibronectin
93 production in glucose-exposed mesangial cells (Wang et al., 2008). miR-382 targeting of
94 kallikrein 5 contributes to renal fibrosis in mice (Kriegel et al., 2012).

95 Of the miRNAs expressed in the MD of cat and dogs (Table 1), miR-212 and miR-132
96 expression are highly elevated in the kidneys of rats with hypertension (Eskildsen et al.,
97 2013). The urinary miR-10a level positively correlates with the degree of kidney injury
98 induced by IRI or diabetes (Wang et al., 2012). Of the miRNAs expressed in cat MD, the

level of renal miR-20a is elevated after IRI in mice (Godwin et al., 2010). miR-365 may posttranscriptionally modulate the expression of polycystic kidney and hepatic disease gene 1 (Duan et al., 2012). miR-15b-5p and miR-16-5p are highly expressed in the cat MD, and the miR-15a/16-1 and miR-15b/16-2 clusters play important roles in regulating cell proliferation and apoptosis (Yue and Tigyi, 2010). Of the miRNAs expressed in the dog MD, miR-132 is significantly upregulated in diseased kidneys of PKD/Mhm rats, which serve as a model to study human autosomal polycystic kidney disease (Dweep et al., 2013). The expression of miR-196a is elevated in human hypertensive kidneys (Marques et al., 2011).

Thus, the present analysis shows that miRNAs that regulate renal physiology or are involved in pathologies are expressed in the kidneys of cats and dogs. Other miRNAs, including miR-708, miR-133a, miR-379, miR-135, miR-34a, miR-27a, and miR-203 are expressed in renal cancers (Saini et al., 2011; Kawakami et al., 2012; Laddha et al., 2013; Petillo et al., 2009; Yamada et al., 2013; Yamamura et al., 2012).

Of the miRNA-target mRNAs listed in Table 2, some encode proteins that regulate electrolyte balance and therefore may be required for physiological renal function such as potassium channels, subfamily K, member 3 (*Kcnk3*) in the CO of cats and dogs or potassium voltage-gated channel, subfamily G, member 3 (*Kcng3*) in the dog MD. Further, “ion binding” and “cation binding” were detected as significant gene ontology (GO) terms (Supplemental table 1). Further, we identified genes encoding proteins of the immune system such as CD83 (*Cd83*) in the CO of cat and dog, B cell receptor associated protein 29 (*Bcap29*) in the cat CO, suppressor of cytokine signaling 1 (*Socs1*) in the dog CO and the

121 cat MD, and CD36 antigen (*Cd36*) in the MD of cat and dog. These results suggest
122 important roles of miRNAs in regulating immune responses in the kidneys of cat and dog.
123 Our recent study revealed that the pathogenesis of glomerular and tubulointerstitial
124 lesions differ between cats and dogs (Ichii et al., 2011). Other studies show that the renal
125 expression of molecules associated with the renin-angiotensin system and cyclooxygenase
126 differ between cats and dogs with CKD (Yabuki et al., 2012; Mitani et al., 2013).
127 Interestingly, the gene encoding tubulointerstitial nephritis antigen (*Tinag*) was detected as
128 the target of miRNAs expressed in the cat MD. TINAG is localized to basement
129 membranes underlying the epithelium of Bowman's capsule and renal tubules, and
130 autoantibody formation against this component is associated with primary
131 immune-mediated tubulointerstitial nephritis (Yoshioka et al., 2002). Thus, differences in
132 miRNA expression between cats and dogs may correlate with their respective differences in
133 CKD progression, and our data may help determine the molecular pathogenesis of CKD in
134 cats and dogs.
135
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142 **References**

143 1. Alvarez, M.L., Khosroheidari, M., Kanchi Ravi, R., DiStefano, J.K., 2012. Comparison
144 of protein, microRNA, and mRNA yields using different methods of urinary exosome
145 isolation for the discovery of kidney disease biomarkers. Kidney Int. 82, 1024–32.
146 2. Duan, J., Huang, H., Lv, X., Wang, H., Tang, Z., Sun, H., Li, Q., Ai, J., Tan, R., Liu,
147 Y., Chen, M., Duan, W., Wei, Y., Zhou, Q., 2012. PKHD1 post-transcriptionally
148 modulated by miR-365-1 inhibits cell-cell adhesion. Cell Biochem. Funct. 30, 382–9.
149 3. Dweep, H., Sticht, C., Kharkar, A., Pandey, P., Gretz, N., 2013. Parallel analysis of
150 mRNA and microRNA microarray profiles to explore functional regulatory patterns in
151 polycystic kidney disease: using PKD/Mhm rat model. PLoS One 8, e53780.
152 4. Eskildsen, T. V, Jeppesen, P.L., Schneider, M., Nossent, A.Y., Sandberg, M.B.,
153 Hansen, P.B.L., Jensen, C.H., Hansen, M.L., Marcussen, N., Rasmussen, L.M., Bie, P.,
154 Andersen, D.C., Sheikh, S.P., 2013. Angiotensin II Regulates microRNA-132/-212 in
155 Hypertensive Rats and Humans. Int. J. Mol. Sci. 14, 11190–207.
156 5. Godwin, J.G., Ge, X., Stephan, K., Jurisch, A., Tullius, S.G., Iacomini, J., 2010.
157 Identification of a microRNA signature of renal ischemia reperfusion injury. Proc. Natl.
158 Acad. Sci. U. S. A. 107, 14339–44.
159 6. Ichii, O., Otsuka, S., Sasaki, N., Namiki, Y., Hashimoto, Y., Kon, Y., 2012. Altered
160 expression of microRNA miR-146a correlates with the development of chronic renal
161 inflammation. Kidney Int. 81, 280–92.
162 7. Ichii, O., Yabuki, A., Sasaki, N., Otsuka, S., Ohta, H., Yamasaki, M., Takiguchi, M.,
163 Namiki, Y., Hashimoto, Y., Endoh, D., Kon, Y., 2011. Pathological correlations

between podocyte injuries and renal functions in canine and feline chronic kidney diseases. Histol. Histopathol. 26, 1243–55.

8. Kato, M., Dang, V., Wang, M., Park, J.T., Deshpande, S., Kadam, S., Mardiros, A., Zhan, Y., Oettgen, P., Putta, S., Yuan, H., Lanting, L., Natarajan, R., 2013. TGF-β induces acetylation of chromatin and of Ets-1 to alleviate repression of miR-192 in diabetic nephropathy. Sci. Signal. 6, ra43.
9. Kawakami, K., Enokida, H., Chiyomaru, T., Tatarano, S., Yoshino, H., Kagara, I., Gotanda, T., Tachiwada, T., Nishiyama, K., Nohata, N., Seki, N., Nakagawa, M., 2012. The functional significance of miR-1 and miR-133a in renal cell carcinoma. Eur. J. Cancer 48, 827–36.
10. Kriegel, A.J., Liu, Yong, Cohen, B., Usa, K., Liu, Youhua, Liang, M., 2012. MiR-382 targeting of kallikrein 5 contributes to renal inner medullary interstitial fibrosis. Physiol. Genomics 44, 259–67.
11. Laddha, S. V, Nayak, S., Paul, D., Reddy, R., Sharma, C., Jha, P., Hariharan, M., Agrawal, A., Chowdhury, S., Sarkar, C., Mukhopadhyay, A., 2013. Genome-wide analysis reveals downregulation of miR-379/miR-656 cluster in human cancers. Biol. Direct 8, 10.
12. Lewis, B.P., Burge, C.B., Bartel, D.P., 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20.

184 13. Lohse, M., Bolger, A.M., Nagel, A., Fernie, A.R., Lunn, J.E., Stitt, M., Usadel, B.,
185 2012. RobiNA: a user-friendly, integrated software solution for RNA-Seq-based
186 transcriptomics. Nucleic Acids Res. 40, W622–W627.
187 14. Lv, L.-L., Cao, Y.-H., Ni, H.F., Xu, M., Liu, D., Liu, H., Chen, P.-S., Liu, B.-C., 2013.
188 microRNA-29c in urinary exosome/microvesicle as biomarker of renal fibrosis. Am. J.
189 Physiol. Renal Physiol. [Epub ahead of print]
190 15. Marques, F.Z., Campain, A.E., Tomaszewski, M., Zukowska-Szczechowska, E., Yang,
191 Y.H.J., Charchar, F.J., Morris, B.J., 2011. Gene expression profiling reveals renin
192 mRNA overexpression in human hypertensive kidneys and a role for microRNAs.
193 Hypertension 58, 1093–8.
194 16. Mitani, S., Yabuki, A., Taniguchi, K., Yamato, O., 2013. Association between the
195 intrarenal renin-angiotensin system and renal injury in chronic kidney disease of dogs
196 and cats. J. Vet. Med. Sci. 75, 127–33.
197 17. Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B., 2008. Mapping
198 and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods 5, 621–8.
199 18. Petillo, D., Kort, E.J., Anema, J., Furge, K.A., Yang, X.J., Teh, B.T., 2009. MicroRNA
200 profiling of human kidney cancer subtypes. Int. J. Oncol. 35, 109–14.
201 19. Saini, S., Yamamura, S., Majid, S., Shahryari, V., Hirata, H., Tanaka, Y., Dahiya, R.,
202 2011. MicroRNA-708 induces apoptosis and suppresses tumorigenicity in renal cancer
203 cells. Cancer Res. 71, 6208–19.

204 20. Sun, Y., Koo, S., White, N., Peralta, E., Esau, C., Dean, N.M., Perera, R.J., 2004.
205 Development of a micro-array to detect human and mouse microRNAs and
206 characterization of expression in human organs. Nucleic Acids Res. 32, e188.
207 21. Tian, Z., Greene, A.S., Pietrusz, J.L., Matus, I.R., Liang, M., 2008. MicroRNA-target
208 pairs in the rat kidney identified by microRNA microarray, proteomic, and
209 bioinformatic analysis. Genome Res. 18, 404–11.
210 22. Wang, N., Zhou, Y., Jiang, L., Li, D., Yang, J., Zhang, C.-Y., Zen, K., 2012. Urinary
211 microRNA-10a and microRNA-30d serve as novel, sensitive and specific biomarkers
212 for kidney injury. PLoS One 7, e51140.
213 23. Wang, Q., Wang, Y., Minto, A.W., Wang, J., Shi, Q., Li, X., Quigg, R.J., 2008.
214 MicroRNA-377 is up-regulated and can lead to increased fibronectin production in
215 diabetic nephropathy. FASEB J. 22, 4126–35.
216 24. Weber, J.A., Baxter, D.H., Zhang, S., Huang, D.Y., Huang, K.H., Lee, M.J., Galas,
217 D.J., Wang, K., 2010. The microRNA spectrum in 12 body fluids. Clin. Chem. 56,
218 1733–41.
219 25. Yabuki, A., Mitani, S., Sawa, M., Mizukami, K., Fujiki, M., Yamato, O., 2012. A
220 comparative study of chronic kidney disease in dogs and cats: induction of
221 cyclooxygenases. Res. Vet. Sci. 93, 892–7.
222 26. Yamada, Y., Hidaka, H., Seki, N., Yoshino, H., Yamasaki, T., Itesako, T., Nakagawa,
223 M., Enokida, H., 2013. Tumor-suppressive microRNA-135a inhibits cancer cell
224 proliferation by targeting the c-MYC oncogene in renal cell carcinoma. Cancer Sci.
225 104, 304–12.

226 27. Yamamura, S., Saini, S., Majid, S., Hirata, H., Ueno, K., Chang, I., Tanaka, Y., Gupta,
227 A., Dahiya, R., 2012. MicroRNA-34a suppresses malignant transformation by
228 targeting c-Myc transcriptional complexes in human renal cell carcinoma.
229 Carcinogenesis 33, 294–300.
230 28. Yoshioka, K., Takemura, T., Hattori, S., 2002. Tubulointerstitial nephritis antigen:
231 primary structure, expression and role in health and disease. Nephron 90, 1–7.
232 29. Yue, J., Tigyi, G., 2010. Conservation of miR-15a/16-1 and miR-15b/16-2 clusters.
233 Mamm. Genome 21, 88–94.
234 30. Zhou, Q., Fan, J., Ding, X., Peng, W., Yu, X., Chen, Y., Nie, J., 2010.
235 TGF-{beta}-induced MiR-491-5p expression promotes Par-3 degradation in rat
236 proximal tubular epithelial cells. J. Biol. Chem. 285, 40019–27.