



# MicroRNA Expression Profiling Distinguishes Conventional, Chromophobe, Papillary Types 1 and 2 Renal Cell Carcinomas and Oncocytoma from Normal Renal Parenchyma



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## Abstract

Background: MicroRNAs (miRNA) are small non-coding RNAs that modulate expression of protein-encoding genes by binding and inactivating messenger RNA (mRNA). Renal cell carcinoma (RCC) subtypes are traditionally distinguished by morphologic, immunophenotypic and cytogenetic characteristics. miRNA expression patterns have not been previously reported for all RCC subtypes. The aims of this study are to 1) determine if unique miRNA expression profiles are present among major RCC subtypes (conventional (CON), chromophobe (CHR), papillary type 1 (PAP1), papillary type 2 (PAP2)), oncocytoma (ONC) and normal renal parenchyma (NRP) and 2) identify specific miRNAs that may contribute to underlying mechanisms of renal tumorigenesis.

Design: Five specimens of frozen banked human renal tissue in each group (CON, CHR, PAP1, PAP2, ONC and NRP) that yielded high integrity RNA were labeled without amplification and hybridized on microarrays (Exiqon, Denmark) covering all annotated miRNAs in the miRBase (1168 probes with 4 replicates/array). Statistically significant differential expression was determined by the Significance Analysis of Microarray (SAM) multiclass test (Q=0.03) and post-hoc t-test of individual transcripts.

Results: Differential expression was observed among each specimen class when compared to NRP and between tumor classes.

Conclusion: The expression pattern obtained provided an exclusionary signature for major subtypes of renal tumors (CON, CHR, PAP1, PAP2 and ONC) when compared to NRP and from each other. Our data suggest that post-translational regulation may contribute to RCC tumorigenesis as well as to the molecular differentiation pathways resulting in the major histologic subtypes of RCC. Our future research includes evaluating mRNA profiles in tumors from the corresponding cases to extend the molecular characterization of renal neoplasia.

## Design:

- Utilized frozen tumor banked tissue from CON, CHR, PAP1, PAP2, ONC and NRP.

- RNA extracted with Qiagen spin column.

- RNA quality assessed via

- Nanodrop spectrophotometer: Use 1-1.5 µl of sample to calculate 260/280 λ ratio. Samples with wavelength of 1.8-2.1 were continued to Agilent chip.

- Agilent Bioanalyzer: Samples with RNA Integrity Numbers (RIN) greater than 6 were hybridized.

- 88 samples were attempted which yielded 33 samples of high enough quality RNA for hybridization.

- Yield of quality RNA varied by group from 18 to 60%:

CON 40%	PAP1 50%	ONC 60%
CHR 50%	PAP2 36%	NRP 18%

- Hybridization was performed using 5 samples from the 6 groups (30 samples) and hybridized in 3 batches.

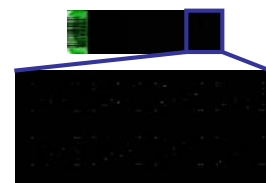
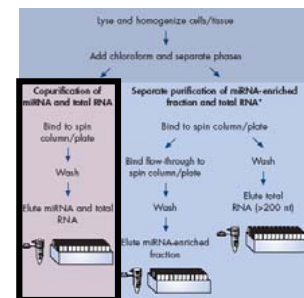
- RNA was labeled with cy3.

- Samples were hybridized to Exiqon array covering all annotated miRNAs in the miRBase (1168 probes with 4 replicates/array).

- Labeling detected using Genepix 4000B scanner and Genepix 6.1 software.

- Mouse synthetic spike-ins were used to normalize hybridization between samples. 1 test sample was used to normalize hybridization between the 3 batches.

- Data analyzed via Statistical Analysis for Microarrays (SAM) (and a post-hoc t-test was also performed).



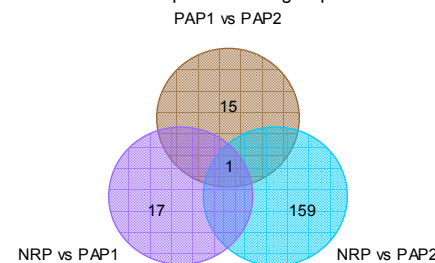
## Tables 1-3 and Figure 1:

Table 1: NRP vs CON					
ID	Name	Average	Average	Fold Change	p-value
17585	hsa-miR-895-5p	1024.379	4180.521	4.081030989	0.0005
17272	hsa-miR-551a	509.8147	286.6079	-1.77878808	0.01
17852	hsa-miR-99a*	338.3917	168.1081	-2.01294106	0.005
10943	hsa-miR-136	467.4043	217.4237	-2.14973955	0.0004
17875	hsa-miR-500	410.0091	179.5362	-2.28371254	0.004
19591	hsa-miR-199b-5p	1230.108	119.961	-10.2542317	0.009

Table 2: NRP vs PAP1					
ID	Name	Average	Average	Fold Change	p-value
19004	hsa-let-7c	41021.91	13654.11	-3.00436462	0.0004
30787	hsa-miR-125b	41916.03	10016.07	-4.18487842	0.009
19008	hsa-SNORD2	3668.437	7932.268	2.162302043	0.008
10925	hsa-miR-10b	24483.02	3886.906	-6.29894535	0.01
29562	hsa-miR-199a-5p	11854.19	1659.087	-7.14500735	0.008
17886	hsa-miR-301b	152.7095	539.538	3.533100115	0.009

Table 3: NRP vs PAP2					
ID	Name	Average	Average	Fold Change	p-value
17272	hsa-miR-551a	509.8147	167.2608	-3.04802307	0.0002
11125	hsa-miR-493*	555.9607	116.7683	-4.76123002	0.002
19004	hsa-let-7c	41021.91	18306.64	-2.24082175	0.002
42776	hsa-miR-938	393.4042	66.20986	-5.94777604	0.0005
10943	hsa-miR-136	467.4043	52.69065	-8.87072646	3E-07
32809	hsa-miR-133a	563.5967	67.60731	-8.33632751	0.006
42454	hsa-miR-138-2*	334.4172	52.47271	-6.3731654	0.002
11091	hsa-miR-377	611.7235	155.0591	-3.94509806	0.003
42529	hsa-miR-939	3694.139	611.8512	-6.03764269	0.005

Figure 1: Intersection of PAP1, PAP2 and NRP: 1 miR separates all 3 groups

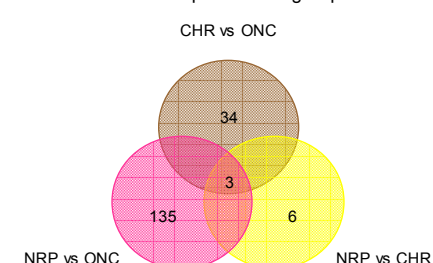


## Tables 4-5 and Figure 2:

Table 4: NRP vs CHR					
ID	Name	Average	Average	Fold Change	p-value
29562	hsa-miR-199a	11854.19	1317.841	-8.99516268	0.006
11023	hsa-miR-222	8027.649	56004.96	6.97650825	9E-10
10943	hsa-miR-136	467.4043	203.3249	-2.29880509	0.0002
10975	hsa-miR-182	1252.649	4946.545	3.94886783	0.009
42735	hsa-miR-371-5p	7174.518	29579.41	4.122843014	0.0004
42808	hsa-miR-874	322.7134	1557.638	4.826691253	2E-06
13147	hsa-miR-96	921.335	4718.494	5.12136631	0.002
28889	hsa-miR-888	166.903	868.0833	5.201123884	0.0004
11022	hsa-miR-221	3990.042	22093.9	5.537260014	5E-06
42760	hsa-miR-557	419.8203	2346.013	5.598135186	0.004

Table 5: NRP vs ONC					
ID	Name	Average	Average	Fold Change	p-value
10946	hsa-miR-141	40200.19	257.1104	-156.353805	0.0007
28889	hsa-miR-888	868.0833	16.45083	-52.7683655	3E-05
42661	hsa-miR-492	746.4993	31.22815	-23.9046894	4E-07
42652	hsa-miR-584	1031.691	265.8333	-3.88097111	0.0005
42711	hsa-miR-887	999.0375	353.3158	-2.82760497	5E-05
42808	hsa-miR-874	1557.638	265.1748	-5.87406003	1E-06
42716	hsa-miR-33a	1437.851	434.6444	-3.30810987	0.006
11023	hsa-miR-222	56004.96	19341.76	-2.89554616	1E-07
42760	hsa-miR-557	2346.013	541.5703	-4.33187131	0.009
42735	hsa-miR-371-5p	29579.41	11322.17	-2.61525094	0.006

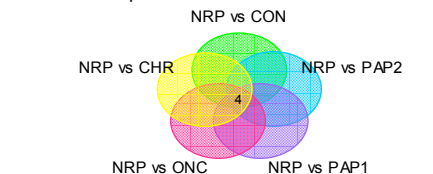
Figure 2: Intersection of CHR, ONC and NRP: 3 miRs separate all 3 groups



## Table 6 and Figure 3:

Table 6: NRP vs all tumor groups (CON, CHR, PAP1, PAP2, ONC)								
ID	Name	NRP	CON	CHR	PAP1	PAP2	ONC	p-value
10943	hsa-miR-136	467.4043	217.4237	203.325	229.6254	52.69065	204.1048	2E-05
42482	hsa-miR-591	111.0377	71.55017	74.0385	18.61712	4.806996	5.042818	4E-06
42512	hsa-miR-136*	261.9128	87.33137	104.39	96.75922	18.83423	32.99911	0.0003
42946	hsa-miR-486-3p	65.89078	65.52801	62.8085	0	0	0	3E-07

Figure 3: Intersection of all tumor groups vs NRP: 4 miRs separate NRP from all tumor classes



## Results:

- Differential expression was observed in each tumor class with the most statistically significant miRs when comparing NRP to each tumor class listed in Tables 1-5.

- miR-21 was increased in CON, consistent with upregulation in numerous other malignancies (data not shown).

- miR-141 was decreased in CON vs CHR and NRP vs ONC. According to Nakada et al, the target of miR-141 is ZFH1B mRNA with increased ZFH1B resulting in
  - Increased expression of vimentin
  - Repression of E-cadherin

- 4 miRs (miR-136, 136\*, 486 and 591) separated NRP from all tumor groups (Table 6 and Figure 3).

- Intersections analysis can be performed to identify subsets of miRs which discriminate between groups (Figures 1-3).

## Conclusion:

- The expression patterns obtained from RCC tumor samples provided an exclusionary signature for each tumor class when compared to NRP and to each other.

- Our data suggest that post-translational regulation may contribute to RCC tumorigenesis as well as to the molecular differentiation pathways resulting in morphologically distinctive histologic subtypes of RCC.

- Future planned research includes evaluating mRNA profiles and single nucleotide polymorphisms (SNPs) in tumors from corresponding cases and comparing data to miRNA expression to extend the molecular characterization of renal neoplasia for both diagnostic and pathogenetic utilization.