Supplementary Material to

Identification of Metastamirs as Metastasis-associated MicroRNAs in Clear Cell Renal Cell Carcinomas by Wotschofsky et al.

CONTENT

Doc S1 of Supplementary Material

RNA Extraction

Tumor tissue samples (between 30-93 mg wet weight) with at least 80% of the tumor cells verified by the two reference pathologists (AE, EK) were selected for RNA isolation [1]. Total RNA, including microRNAs, was extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany), with an additional DNA digestion step on the RNA binding silica gel membrane of the spin column, as previously described [1]. The RNA yield and the A260/280 ratios were determined on a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). A Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) with an RNA 6000 Nano Lab Chip was used for the determination of RNA integrity numbers (RIN), as criteria for the RNA quality and degradation. Similar procedures were used for the isolation and characterization of RNA in the cell culture experiments. We washed the cells twice with PBS (PAA, Pasching, Austria) and lysed them directly with QIAzol®Lysis Reagent (Qiagen, Hilden, Germany).

Microarray-based MiRNA Profiling

The microarray profiling approach has been described in detail previously [1]. Briefly, microarray data of miRNAs from 12 malignant and 12 non-malignant tissue specimens of primary non-metastatic ccRCCs as well as nine samples from ccRCC bone metastases were used (GEO accession number GSE37989). Microarray analyses were performed with one-color hybridizations on human catalog 8-plex 15 K microRNA microarrays (AMADID 016436; Agilent Technologies), encoding probes for 470 human miRNAs from the Sanger database v9.1 [1,2]. The raw data were normalized using GeneSpring GX11 Software (Agilent Technologies) with default parameters (threshold raw signal to 1.0, percent shift to 90th percentile as a normalization algorithm and no baseline transformation). Statistical analysis was performed using the Mann**-**Whitney U test. The Benjamini-Hochberg correction was applied for multiple comparisons. A corrected P-value of <0.05 was set to determine the statistical significance. Fold change differences were calculated by the mean expression values of the different sample groups. Raw values were analyzed to estimate the mean signal strength of the micro-array probesets. Further evaluation of the data to select a candidate metastamir pattern based on these microarray data is described in the Results section.

Quantitative RT-PCR of miRNAs

Mature miRNAs were measured using TaqMan miRNA assays (Applied Biosystems, Foster City, CA, USA) (see Table of "TaqMan MicroRNA Assays" in this DOC S1, page 4) in accordance with the manufacturer’s protocols and the MIQE

guidelines (see Table "MIQE Checklist" in this Doc S1, page 7) [3]. Real-time PCRs were performed on a Light-Cycler 480 Instrument (Roche Applied Science, Mannheim, Germany) in white 96-well plates (cat.no. 04729692001 with sealing foils) [1,2,4]. Briefly, cDNA was synthesized from total RNA (6.67 ng pro 10 µl RT reaction) using miRNA-specific stem-looped primers, 10 nmol dNTP mix, 2.6 U RNase inhibitor, 33.5 U MultiScribe RT enzyme and 1 x RT Buffer (Applied Biosystems). All of the cDNA samples were stored at -20°C until PCR analysis. PCR was performed in 10 µl per well, including 1 µl RNA-specific cDNA, 1x TaqMan Universal PCR Master Mix No AmpErase UNG, and gene-specific TaqMan MicroRNA primer Assay solution. The reactions were incubated at 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 s, and 60°C for 60 s. The samples were measured in triplicate, including a non-template control and two interplate controls in each PCR run. The quantification cycles (Cq values) were calculated automatically using the LightCycler software, release 1.5.0, and the "second derivative maximum" cycle analysis method. The analytical precision of the qPCRs (the standard deviation of the Cq values) was tested by intra-run (n=8) measurements and ranged from 0.051 to 0.109 for mean Cq values between 24.28 and 27.49 for the miRNAs miR-28, miR-103, and miR-106a. The between-run precision (n=18) of the reverse transcription reaction including the intra-run variance of the qPCR determination was controlled by cDNA generation for qPCR measurements of miR-126 in one run and amounted to a standard deviation of 0.20 at mean Cq values of 29.34. Calibration curves were made with dilutions of miRNA-specific cDNAs and were documented, together with further qPCR validation data (see "Information on the qPCR validation experiments" in this Doc S1, page 11). Amplification efficiencies were calculated by the LightCycler software, and a mean efficiency of 1.929 was used for the efficiency correction.

The raw RT-qPCR data were analyzed by the GenEX software (MultiD Analyses AB, Göteborg, Sweden) [5]. Using this software, the correction of amplification efficiencies, the adjustment of between-run variations using the interplate calibrators, and the normalization of the miRNA expressions with the reference gene combination of miR-28, miR-103, and miR-106a were performed [1]. RT-qPCR data in the cell culture experiments were analyzed by the qBase^{PLUS} software (Biogazelle NV, Zwijnaarde, Belgium) and normalized with the reference gene combination of RNU48 and RNU6B [6].

TaqMan MicroRNA Assays (Applied Biosystems; Assay name, Assay ID) for the measurement of mature miRNAs characterized by the permanently assigned miRBase accession number, the miRBase-prescribed ID related to the miRBase version, and the sequence

Assay name	Assay ID	miRBase accession no.	miRBase ID†	Sequence
hsa-miR-10b	002218	MIMAT0000254	hsa-miR-10b (v9.2) hsa-miR-10b-5p (v18)	UACCCUGUAGAACCGAAUUUGUG
hsa-miR-19a	000395	MIMAT0000073	hsa-miR-19a (v9.2) hsa-miR-19a-3p (v18)	UGUGCAAAUCUAUGCAAAACUGA
hsa-miR-19b	000396	MIMAT0000074	hsa-miR-19b (v9.2) hsa-miR-19b-3p (v18)	UGUGCAAAUCCAUGCAAAACUGA
hsa-miR-20a	000580	MIMAT0000075	hsa-miR-20a (v9.2) hsa-miR-20a-5p (v18)	UAAAGUGCUUAUAGUGCAGGUAG
hsa-miR-21	000397	MIMAT0000076	hsa-miR-21 (v9.2) hsa-miR-21-5p (v18)	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-26a	000405	MIMAT0000082	hsa-miR-26a (v9.2) hsa-miR-26a-5p (v18)	UUCAAGUAAUCCAGGAUAGGCU
hsa-miR-28	000411	MIMAT0000085	hsa-miR-28 (v9.2) hsa-miR-28-5p (v18)	AAGGAGCUCACAGUCUAUUGAG
hsa-miR-29a	002112	MIMAT0000086	hsa-miR-29a (v9.2) hsa-miR-29a-3p (v18)	UAGCACCAUCUGAAAUCGGUUA
hsa-miR-29b	000413	MIMAT0000100	hsa-miR-29b (v9.2) hsa-miR-29b-3p (v18)	UAGCACCAUUUGAAAUCAGUGUU
hsa-miR-29c	000587	MIMAT0000681	hsa-miR-29c (v9.2) hsa-miR-29c-3p (v18)	UAGCACCAUUUGAAAUCGGUUA
hsa-miR-100	000437	MIMAT0000098	hsa-miR-100 (v9.2) hsa-miR-100-5p (v18)	AACCCGUAGAUCCGAACUUGUG
hsa-miR-101	002253	MIMAT0000099	hsa-miR-101 (v9.2) hsa-miR-101-3p (v18)	UACAGUACUGUGAUAACUGAA
hsa-miR-103	000439	MIMAT0000101	hsa-miR-103 (v9.2) hsa-miR-103-3p	AGCAGCAUUGUACAGGGCUAUGA
hsa-miR-106a	002169	MIMAT0000103	hsa-miR-106a (v9.2) hsa-miR-106a-5p (v18)	AAAAGUGCUUACAGUGCAGGUAG
hsa-miR-126	002228	MIMAT0000445	hsa-miR-126 (v9.2) hsa-miR-126-3p (v18)	UCGUACCGUGAGUAAUAAUGCG

Assay name	Assay ID	miRBase accession no.	miRBase ID†	Sequence
hsa-miR-127	000452	MIMAT0000446	hsa-miR-127 (v9.2) hsa-miR-127-3p (v18)	UCGGAUCCGUCUGAGCUUGGCU
hsa-miR-130a	000454	MIMAT0000425	hsa-miR-130a (v9.2) hsa-miR-130a-3p(v18)	CAGUGCAAUGUUAAAAGGGCAU
hsa-miR-141	000463	MIMAT0000432	hsa-miR-141 (v9.2) hsa-miR-141-3p (v18)	UAACACUGUCUGGUAAAGAUGG
hsa-miR-143	002249	MIMAT0000435	hsa-miR-143 (v9.2) hsa-miR-143-3p (v18)	UGAGAUGAAGCACUGUAGCUC
hsa-miR-145	002278	MIMAT0000437	hsa-miR-145 (v9.2) hsa-miR-145-5p (v18)	GUCCAGUUUUCCCAGGAAUCCCU
hsa-miR-148a	000470	MIMAT0000243	hsa-miR-148a (v9.2) hsa-miR-148a-3p(v18)	UCAGUGCACUACAGAACUUUGU
hsa-miR-155	002623	MIMAT0000646	hsa-miR-155 (v9.2) hsa-miR-155-5p (v18)	UUAAUGCUAAUCGUGAUAGGGGU
hsa-miR-192	000491	MIMAT0000222	hsa-miR-192 (v9.2) hsa-miR-192-5p (v18)	CUGACCUAUGAAUUGACAGCC
hsa-miR-194	000493	MIMAT0000460	hsa-miR-194 (v9.2) hsa-miR-194-5p (v18)	UGUAACAGCAACUCCAUGUGGA
hsa-miR-195	000494	MIMAT0000461	hsa-miR-195 (v9.2) hsa-miR-195-5p (v18)	UAGCAGCACAGAAAUAUUGGC
hsa-miR-200c	000505	MIMAT0004150	hsa-miR-200c (v9.2) mdo-miR-200c (v18)	UAAUACUGCCGGGUAAUGAUGG
hsa-miR-210	000512	MIMAT0000267	hsa-miR-210 (v9.2, v18)	CUGUGCGUGUGACAGCGGCUGA
hsa-miR-215	000518	MIMAT0000272	hsa-miR-215 (v9.2, v18)	AUGACCUAUGAAUUGACAGAC
hsa-miR-223	002295	MIMAT0000280	hsa-miR-223 (v9.2) hsa-miR-223-3p (v18)	UGUCAGUUUGUCAAAUACCCCA
hsa-miR-224	002099	MIMAT0000281	hsa-miR-224 (v9.2) hsa-miR-224-5p (v18)	CAAGUCACUAGUGGUUCCGUU
hsa-miR-296	000527	MIMAT0000690	hsa-miR-296 (v9.2) hsa-miR-296-5p (v18)	AGGGCCCCCCCUCAAUCCUGU
hsa-miR-370	002275	MIMAT0000722	hsa-miR-370 (v9.2, v18)	GCCUGCUGGGGUGGAACCUGGU
hsa-miR-451	001141	MIMAT0001631	hsa-miR-451 (v9.2) hsa-miR-451a (v18)	AAACCGUUACCAUUACUGAGUU
hsa-miR-494	002365	MIMAT0002816	hsa-miR-494 (v9.2), v18)	UGAAACAUACACGGGAAACCUC
hsa-miR-514	001147	MIMAT0005778	hsa-miR-514 (v9.2)	AUUGACACUUCUGUGAGUAG

Assay name	Assay ID	miRBase accession no.	miRBase ID†	Sequence
			ptr-miR-514 (v18)	
hsa-miR-638	001582	MIMAT0003308	hsa-miR-638 (v9.2, v18)	AGGGAUCGCGGGCGGGUGGCGGCCU

† miRNA ID in the miRBase version 9.2 and 18, respectively.

MIQE Checklist according to Bustin et al., Clin Chem 2009;55:611-22

All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available.

ITEM TO CHECK	IMPORTANCE	CHECKLIST	WHERE IN THE MANUSCRIPT; ADDITIONAL COMMENT
EXPERIMENTAL DESIGN			
Definition of experimental and control groups	**E**	Yes	Materials and Methods: Patients and tissue samples; and in Table 1.
Number within each group	**E**	Yes	Materials and Methods: Patients and tissue samples; Cell culture experiments; Legends to Figure 1 and 2.
Assay carried out by core lab or investigator's lab?	D	Yes	All assays were performed in investigator's lab.
Acknowledgement of authors' contributions	D	Yes	All mentioned authors met the authorship as defined by the journal.
SAMPLE			
Description	**E**	Yes	Materials and Methods
Volume/mass of sample processed	D	Yes	Materials and Methods: RNA extraction; see Doc S1 of Supplementary Materials
Microdissection or macrodissection	**E**	Yes	Materials and Methods: RNA extraction; see Doc S1 of Supplementary Materials, macrodissection with histolological verification.
Processing procedure	**E**	Yes	Materials and Methods: Patients and tissue samples; see Doc S1
If frozen - how and how quickly?	**E**	Yes	Materials and Methods: Patients and tissue samples; see Doc S1
If fixed - with what, how quickly?	**E**	Not applicable	
Sample storage conditions and duration (esp. for FFPE samples)	**E**	Yes	Materials and Methods: Patients and tissue samples; see Doc S1
NUCLEIC ACID EXTRACTION			
Procedure and/or instrumentation	**E**	Yes	Materials and Methods: RNA extraction in Doc S1 of Supplementary Material and references indicated.
Name of kit and details of any modifications	**E**	Yes	Materials and Methods: RNA extraction in Doc S1 of Supplementary Material and references indicated.
Source of additional reagents used	D	Yes	RNase-free DNase set; Qiagen (cat.no. 79254), see subsequent information.
Details of DNase or RNAse treatment	**E**	Yes	Materials and Methods: RNA extraction as in Doc S1 of Supplementary Material; with an optional on-column digestion DNase step.
Contamination assessment (DNA or RNA)	**E**	Yes	See previous comment; according to Chen et al. (Nucleic Acids Res 33 (2005) e179) miRNA measurements by the TaqMan assays are not affected by genomic DNA; see also comment on "Cqs with and without RT".
Nucleic acid quantification	**E**	Yes	Materials and Methods: RNA extraction in Doc S1 of Supplementary Material and references indicated.
Instrument and method	**E**	Yes	Materials and Methods: RNA extraction in Doc S1 of Supplementary Material and references indicated.
Purity (A260/A280)	D	Yes	Results: Characteristics of the isolated total RNA.
Yield	D	Yes	Results: Characteristics of the isolated total RNA
RNA integrity method/instrument	**E**	Yes	Materials and Methods: RNA extraction; in Doc S1 of Supplementary Material: Bioanalyzer 2100 (Agilent)/

ITEM TO CHECK	IMPORTANCE	CHECKLIST	WHERE IN THE MANUSCRIPT; ADDITIONAL COMMENT
RIN/RQI or Cq of 3' and 5' transcripts	**E**	Yes	Results: Characteristics of the isolated total RNA
Electrophoresis traces	D	No	
Inhibition testing (Cq dilutions, spike or other)	**E**	Yes	Dilution experiments were performed; PCR efficiencies were found >90%; see also qPCR validation section. For the three groups of clinical samples, identical isolation procedures were performed.
REVERSE TRANSCRIPTION			
Complete reaction conditions	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Amount of RNA and reaction volume	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Priming oligonucleotide (if using GSP) and concentration	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Reverse transcriptase and concentration	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Temperature and time	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Manufacturer of reagents and catalogue numbers	D	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Cqs with and without RT	D*	Yes	There were no Cqs (<40) in reactions without RT.
Storage conditions of cDNA	D	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs"; -20°C.
qPCR TARGET INFORMATION			
If multiplex, efficiency and LOD of each assay.	**E**	Not applicable	
Sequence accession number	**E**	Yes	See Table "TaqMan assays" in Doc S1 of Supplementary Material, page 4.
Location of amplicon	D	Yes	Use of miRNA specific TaqMan assays; specificity guaranteed by the manufacturer.
Amplicon length	**E**	Yes	Use of miRNA specific TaqMan assays; specificity guaranteed by the manufacturer.
In silico specificity screen (BLAST, etc)	**E**	Yes	Use of miRNA specific TaqMan assays; specificity guaranteed by the manufacturer.
Pseudogenes, retropseudogenes or other homologs?	D	Yes	Use of miRNA specific TaqMan assays; specificity guaranteed by the manufacturer.
Sequence alignment	D	Yes	Use of miRNA specific TaqMan assays; specificity guaranteed by the manufacturer.
Secondary structure analysis of amplicon	D	Yes	Use of miRNA specific TaqMan assays; specificity guaranteed by the manufacturer.
Location of each primer by exon or intron (if applicable)	**E**	Yes	Specificity guaranteed by the manufacturer of the TaqMan assays.
What splice variants are targeted?	**E**	Yes	See Table "TaqMan assays" in Doc S1 of Supplementary Material, page 4; specificity guaranteed by the manufacturer of the TaqMan assay.
qPCR OLIGONUCLEOTIDES			
Primer sequences	**E**	Yes	The manufacturer does not provide this information for miRNAs; see Supplemental Table S2.
RTPrimerDB Identification Number	D	Not applicable	miRNA specific TaqMan assays were used; see Table "TaqMan assays" in Doc S1 of Supplementary Material, page 4.

ITEM TO CHECK	IMPORTANCE	CHECKLIST	WHERE IN THE MANUSCRIPT; ADDITIONAL COMMENT
Probe sequences	D**	Yes	The manufacturer does not provide this information for miRNAs; see Table "TaqMan assays" in Doc S1 of Supplementary Material, page 4.
Location and identity of any modifications	**E**	Yes	The manufacturer does not provide this information for miRNAs; see Table "TaqMan assays" in Doc S1 of Supplementary Material, page 4.
Manufacturer of oligonucleotides	D	Yes	Applied Biosystems as part of Life Technologies.
Purification method	D	Yes	Applied Biosystems does not provide information.
qPCR PROTOCOL			
Complete reaction conditions	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Reaction volume and amount of cDNA/DNA	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Primer, (probe), Mg++ and dNTP concentrations	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Polymerase identity and concentration	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Buffer/kit identity and manufacturer	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Exact chemical constitution of the buffer	D	Yes	The manufacturer does not provide this information
Additives (SYBR Green I, DMSO, etc.)	**E**	Yes	No additional additives
Manufacturer of plates/tubes and catalog number	D	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Complete thermocycling parameters	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Reaction setup (manual/robotic)	D	Yes	Manual setup
Manufacturer of qPCR instrument	**E**	Yes	LightCycler 480; see Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
qPCR VALIDATION			
Evidence of optimisation (from gradients)	D	Yes	Kits from Applied Biosystems (see Table "TaqMan assays" in Doc S1 of Supplementary Material, page 4; optimisation guaranteed by the manufacturer
Specificity (gel, sequence, melt, or digest)	**E**	Yes	Specificity guaranteed by the manufacturer of the TaqMan assays
For SYBR Green I, Cq of the NTC	**E**	Not applicable	miRNA specific TaqMan assays
Calibration curves with slope and y-intercept	**E**	Yes	Material and Methods: see "Information on the qPCR validation experiments" in Doc S1 of Supplementary Material, page 11.
PCR efficiency calculated from slope	**E**	Yes	Material and Methods: see "Information on the qPCR validation experiments" in Doc S1 of Supplementary Material, page 11.
Confidence interval for PCR efficiency or standard error	D	Yes	Material and Methods: see "Information on the qPCR validation experiments" in Doc S1 of Supplementary Material, page 11.
r2 of standard curve	**E**	No	Not provided by the LC480 software.
Linear dynamic range	**E**	Yes	Material and Methods: see "Information on the qPCR validation experiments" in

ITEM TO CHECK	IMPORTANCE	CHECKLIST	WHERE IN THE MANUSCRIPT; ADDITIONAL COMMENT
			Doc S1 of Supplementary Material, page 11. Only 2.7% of all miRNA measurements were outside the linear the dynamic range of the calibration curve.
Cq variation at lowest concentration of the linerar interval of the calibration curve	**E**	Yes	Material and Methods: see "Information on the qPCR validation experiments" in Doc S1 of Supplementary Material, page 11.
Confidence intervals throughout range	D	No	
Evidence for limit of detection	**E**	Yes	See comments in the row "Linear dynamic range" above. Thus, it was not necessary to determine the LOD.
If multiplex, efficiency and LOD of each assay.	**E**	Not applicable	
DATA ANALYSIS			
qPCR analysis program (source, version)	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Cq method determination	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Outlier identification and disposition	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there.
Results of NTCs	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there; NTC did not result in any
Justification of number and choice of reference genes	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there; use of the three reference miRNA miR-28, miR-103, and miR-106a as geometric means as previously shown (see ref. 24 in the main text). RNU48 and RNU6B RNU6B were used as reference genes in cell culture experiments.
Description of normalisation method	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs" and references indicated there; use of the three miRNA reference genes of miR-28, miR-103, and miR-106a as geometric means as previously shown (see ref. 24 in the main text) and RNU48 and RNU6B RNU6B in the cell culture experiments.
Number and concordance of biological replicates	D	Yes	See Legend to Figure 1A-D: n=22 for normal and malignant samples; n=13 for metastatic tissue samples.
Number and stage (RT or qPCR) of technical replicates	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs"; see precision data of RT and qPCR there.
Repeatability (intra-assay variation)	**E**	Yes	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs"; see precision data of RT and qPCR there.
Reproducibility (inter-assay variation, %CV)	D	No	Materials and Methods: see Doc S1 of Supplementary Material "Quantitative RT-PCR of miRNAs"; see precision data here; in addition, biological replicates were
Power analysis	D	Yes	See Doc S2 "Sample size and power calculations" in Supplementary Material, page 13.
Statistical methods for result significance	**E**	Yes	Materials and Methods: Statistical analysis; see legend to Figure 1A-D.
Software (source, version)	**E**	Yes	Materials and Methods: Statistical analysis.
Cq or raw data submission using RDML	D	No	

Information on the qPCR validation experiments according to the MIQE guidelines with respect to the calibration curves and the dynamic range of measurements

Calibration curves were generated with diluted cDNAs. The Cq values were calculated automatically by the LightCycler software, release 1.5.0 using the "second derivative maximum" cycle analysis method. The slopes, intercepts, and errors of the regression lines of the calibration curves from these dilution series and the PCR efficiencies ($E=10^{-1/slope}$) including the dynamic range and the Cq variation at the lower limit (the endpoint of the linear dynamic range) were calculated by the LightCycler 480 software 1.5.0. Validation of the qPCR and calibration curves of the miR-106, miR-145, and miR-192 are exemplarily shown as follows. As efficiencies did only differ in the second decimal place confirming the manufacturer's information that the different TaqMan miRNA assays run with equivalent amplification efficiencies, we used mean efficiency of 1.929 for efficiency correction and the calibration curve of miR-145 for all assays.

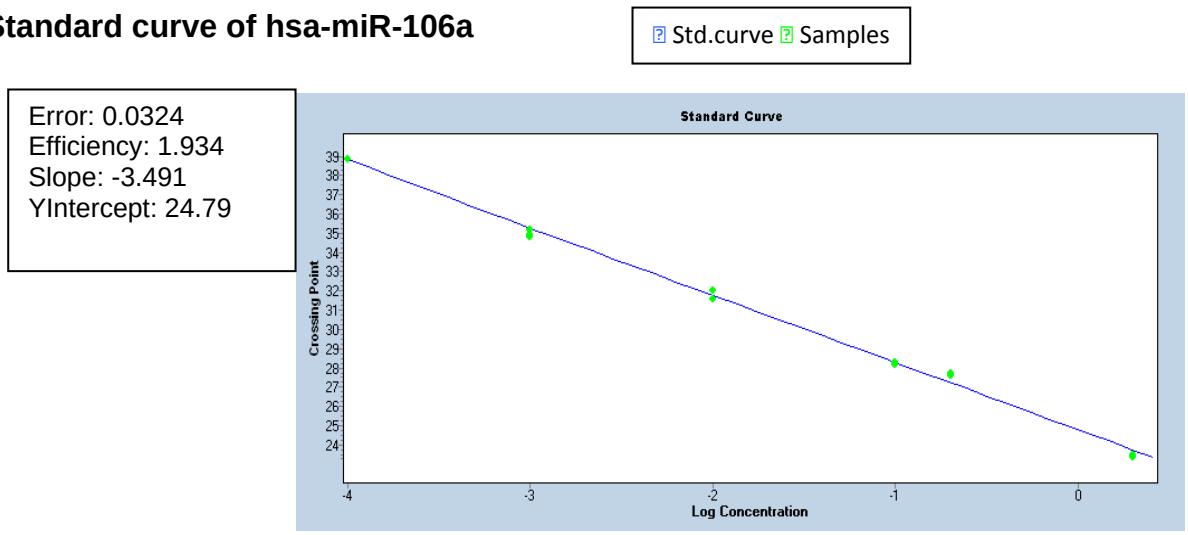
Gene	PCR-Efficiency	Slope	y-Intercept	Error†	Linear dynamic range‡	Cq variation at lowest limit (SD)§
miR-106a	1.934	-3.491	24.79	0.0324	23.35-35.16	0.20
miR-145	1.929	-3.506	19.04	0.0563	18.02-35.42	0.10
miR-192	1.923	-3.523	22.48	0.0363	21.80-36.09	0.14

† The error value is the mean squared error of the single data points fit to the regression line, according to the definition given in the handbook of the LightCycler software.

‡ The linear dynamic range represents the range of the Cq values between the highest and the lowest concentration of linear interval of the calibration curve.

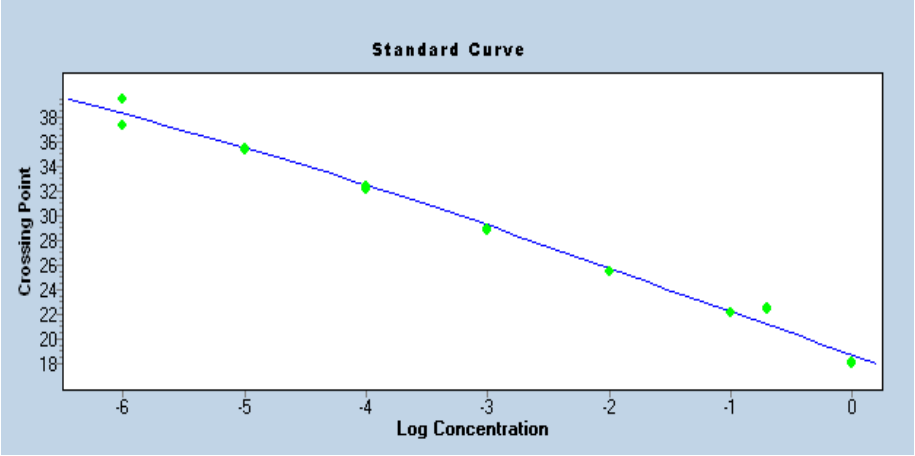
§ Cq variation given as SD at the endpoint of the linear dynamic range that corresponds to the lowest concentration in the linear interval of the calibration curve.

Standard curve of hsa-miR-106a



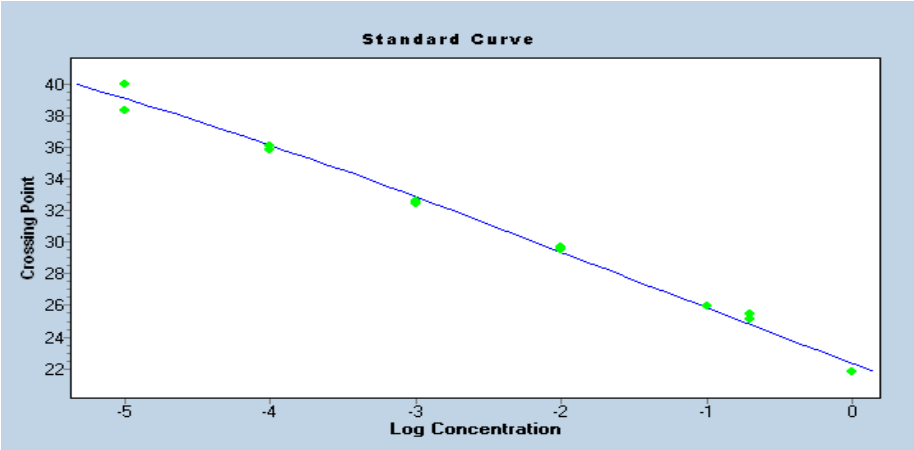
Standard curve of hsa-miR-145

Error: 0.0563
Efficiency: 1.929
Slope: -3.506
YIntercept: 19.04



Standard curve of hsa-miR-192

Error: 0.0363
Efficiency: 1.923
Slope: -3.523
YIntercept: 22.48



References to Doc S1 of Supplementary Material

1. Wotschofsky Z, Meyer HA, Jung M, Fendler A, Wagner I, Stephan C, et al. Reference genes for the relative quantification of microRNAs in renal cell carcinomas and their metastases. Anal Biochem. 2011; 417: 233-41.
2. Jung M, Mollenkopf HJ, Grimm C, Wagner I, Albrecht M, Waller T, et al. MicroRNA profiling of clear cell renal cell cancer identifies a robust signature to define renal malignancy. J Cell Mol Med. 2009; 13: 3918-28.
3. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55: 611-22.
4. Schaefer A, Jung M, Mollenkopf HJ, Wagner I, Stephan C, Jentzmik F, et al. Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma. Int J Cancer. 2010; 126: 1166-76.
5. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonak J, Lind K, et al. The real-time polymerase chain reaction. Mol Aspects Med. 2006; 27: 95-125.
6. Hellemans J, Mortier G, De PA, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 2007; 8: R19.

Doc S2 of Supplementary Material Sample size and power calculations.

Sample size and power calculations for assessing the significances of the expression of the various miRNAs between the three clinical sample groups were performed using the software GraphPad StatMate, version 2.0 (GraphPad Software). The calculation was based on comparing the mean change of expression in two sample groups. To apply a uniform assessment criterion, the change in terms of units of SD was used taking into account the results of our previous expression study [Jung M, Mollenkopf HJ, Grimm C, et al. MicroRNA profiling of clear cell renal cell cancer identifies a robust signature to define renal malignancy. J Cell Mol Med 2009;13:3918-28]. With samples at a ratio of 1:1 or 1:2 in two groups, mean expression differences of one SD between the two groups could be detected with a power of 80% (alpha-error of 5%, one-sided) by studying either 26 (13 each in the two groups) or 30 samples (10 and 20 samples, respectively). Thus, sample sizes calculated for a study power of at least 80 to 90% were selected taking into account the availability of clinical samples, especially metastatic samples as shown in Figure 2A-D (n= 22 for normal and malignant tissue samples from RCC specimens; n=13 from bone metastatic samples). To achieve a correlation coefficient of 0.7 under similar conditions (power 80%, alpha-error of 5%), a sample size of 13 would be necessary.

Table S1 of Supplementary Material
Identification of differentially expressed miRNAs between the sample groups in microarray analysis.

Out of the 28 selected miRNAs highlighted in blue (details are given in Data S1 concerning microarray-based profiling and in the text), 24 miRNAs except for the four miRNAs highlighted in yellow were included in the further validation approach (see Table 2).

Abbreviations: RCC, primary renal cell carcinoma; normal, non-malignant renal tissue samples from specimens after radical nephrectomy; metastases, bone metastases in patients with metastatic renal cell carcinomas.

No.	Systematic Name	RCC to Normal		Metastases to Normal		Metastases to RCC		Selected miRNAs
		P value	Fold-change	P value	Fold-change	P value	Fold-change	
1	hsa-miR-10b_v9.1	0.001	-2.42	0.001	-5.55	0.001	-2.29	**1**
2	hsa-miR-18a_v9.1	0.001	2.31	0.001	1.51	0.004	-1.53	
3	hsa-miR-19a	0.036	1.44	0.001	-1.92	0.001	-2.76	**2**
4	hsa-miR-19b	1.000	1.06	0.001	-1.58	0.003	-1.68	**3**
5	hsa-miR-21	0.002	3.65	0.001	6.03	0.035	1.65	**4**
6	hsa-miR-25	0.001	1.94	0.001	2.95	0.002	1.52	
7	hsa-miR-29a_v9.1	0.090	-1.20	0.001	-2.13	0.003	-1.78	**5**
8	hsa-miR-29b	0.041	-1.24	0.001	-4.19	0.001	-3.36	**6**
9	hsa-miR-29c_v9.1	0.111	-1.42	0.001	-5.14	0.001	-3.63	**7**
10	hsa-miR-30e-5p_v9.1	0.005	-1.33	0.001	-2.65	0.001	-1.99	**8**
11	hsa-miR-32_v9.1	0.101	-1.34	0.001	-2.37	0.009	-1.77	
12	hsa-miR-93	0.001	2.12	0.001	3.33	0.002	1.57	
13	hsa-miR-99a	0.004	-2.04	0.001	-4.41	0.030	-2.16	**9**
14	hsa-miR-100	0.537	-1.19	0.003	-2.85	0.025	-2.40	**10**
15	hsa-miR-101_v9.1	0.720	-1.01	0.001	-2.20	0.001	-2.17	**11**
16	hsa-miR-125a_v9.1	0.001	-1.42	0.001	-2.20	0.001	-1.55	**12**
17	hsa-miR-126*	0.001	2.13	0.795	-1.20	0.003	-2.55	**13**
18	hsa-miR-126_v9.1	0.001	1.80	0.174	-1.53	0.001	-2.76	**14**
19	hsa-miR-128b_v9.1	0.962	1.06	0.007	-1.51	0.006	-1.61	
20	hsa-miR-130a	0.816	-1.07	0.001	-1.89	0.013	-1.76	**15**
21	hsa-miR-130b	0.001	3.16	0.001	5.06	0.015	1.60	
22	hsa-miR-143_v9.1	0.079	1.46	0.222	-1.72	0.015	-2.51	**16**
23	hsa-miR-145_v9.1	0.629	1.28	0.011	-2.19	0.005	-2.80	**17**
24	hsa-miR-148a	0.867	-1.03	0.003	-2.17	0.009	-2.10	**18**
25	hsa-miR-155_v9.1	0.001	6.39	0.001	13.24	0.035	2.07	
26	hsa-miR-185_v9.1	0.015	1.41	0.001	2.13	0.018	1.51	
27	hsa-miR-188_v9.1	0.002	-2.27	0.001	4.13	0.001	9.35	
28	hsa-miR-191*	0.816	-1.01	0.001	2.11	0.001	2.13	
29	hsa-miR-191_v9.1	0.750	-1.13	0.001	1.47	0.001	1.66	
30	hsa-miR-192	0.123	-1.81	0.004	-5.39	0.013	-2.98	**19**
31	hsa-miR-194	0.090	-1.85	0.004	-6.31	0.010	-3.40	**20**
32	hsa-miR-195	0.629	1.24	0.034	-1.86	0.021	-2.31	**21**
33	hsa-miR-212	0.421	1.31	0.001	2.31	0.007	1.76	
34	hsa-miR-223_v9.1	0.023	1.89	0.001	5.37	0.013	2.83	**22**
35	hsa-miR-296-5p	0.449	1.72	0.001	14.15	0.001	8.24	

No.	Systematic Name	RCC to Normal		Metastases to Normal		Metastases to RCC		Selected miRNAs
		P value	Fold-change	P value	Fold-change	P value	Fold-change	
36	hsa-miR-338_v9.1	0.629	1.33	0.222	-1.36	0.007	-1.82	
37	hsa-miR-339_v9.1	0.001	1.95	0.550	1.15	0.013	-1.68	
38	hsa-miR-370_v9.1	0.750	-1.21	0.001	9.45	0.001	11.39	**23**
39	hsa-miR-374a	0.750	1.06	0.001	-1.69	0.002	-1.79	
40	hsa-miR-422b_v9.1	0.111	-1.38	0.843	1.39	0.003	1.91	
41	hsa-miR-425	0.041	1.30	0.001	2.00	0.001	1.54	
42	hsa-miR-451_v9.1	0.750	1.40	0.002	6.71	0.013	4.79	**24**
43	hsa-miR-452_v9.1	0.071	1.68	0.001	8.67	0.001	5.16	
44	hsa-miR-486-5p	0.387	1.55	0.001	6.61	0.013	4.25	
45	hsa-miR-494_v9.1	0.750	-1.03	0.001	5.54	0.001	5.73	**25**
46	hsa-miR-513_v9.1	0.421	-1.63	0.001	8.86	0.001	14.45	
47	hsa-miR-564	0.750	-1.22	0.001	5.62	0.001	6.84	
48	hsa-miR-572	0.629	-1.26	0.003	3.32	0.001	4.18	
49	hsa-miR-575	0.750	-1.06	0.001	7.42	0.001	7.87	**26**
50	hsa-miR-630	0.750	1.11	0.001	16.85	0.001	15.23	**27**
51	hsa-miR-638	0.816	-1.05	0.001	5.62	0.001	5.93	**28**
52	hsa-miR-660	0.001	-3.33	0.001	-5.41	0.041	-1.62	
53	hsa-miR-663	0.123	-2.01	0.001	19.51	0.001	39.24	
54	hsa-miR-671_v9.1	0.123	-1.80	0.001	7.11	0.001	12.79	
55	hsa-miR-765	0.216	-1.56	0.001	16.65	0.001	25.94	
56	hsa-miR-766	0.962	1.01	0.001	3.48	0.001	3.46	
57	hsa-miR-801_v10.1	0.750	-1.15	0.001	18.57	0.001	21.38	

Table S2 of Supplementary Material. Affiliation of the investigated miRNAs to a miRNA gene family or cluster.

miRNA	miRNA gene family[†]	Clustered miRNAs[‡]
miR-10b	miR-10 (miR-10a, miR-10b)	-
miR-19a	miR-19 (miR-19a, miR-19b-1, miR-19b-2)	miR-17, miR-18a, miR-19b-1, miR-20a, miR-92a-1
miR-19b	miR-19 (miR-19a, miR-19b-1, miR-19b-2)	miR-17, miR-18a, miR-19a, miR-20a, miR-92a-1
miR-20a	miR-17 (miR-17, miR-18a, miR-18b, miR-20a, miR-20b, miR-93, miR-106a, miR-106b)	miR-17, miR-18a, miR-19a, miR-19b-1, miR-92a-1
miR-21	miR-21	-
miR-26a	miR-26 (miR-26a-1, miR-26a-2, miR-26b)	-
miR-29a	miR-29 (miR-29a, miR-29b-1, miR-29b-2, miR-29c)	miR-29b-1
miR-29b	miR-29 (miR-29a, miR-29b-1, miR-29b-2, miR-29c)	miR-29a
miR-29c	miR-29 (miR-29a, miR-29b-1, miR-29b-2, miR-29c)	miR-29b-2
miR-100	miR-99(miR-99a, miR-99b, miR-100)	let-7a-2
miR-101	miR-101 (miR-101-1, miR-101-2)	miR-3671
miR-126	miR-126	-
miR-127	miR-127	miR-136, miR-337, miR-431, miR-432, miR-433, miR-665
miR-130a	miR-130 (miR-130a, miR-130b, miR-301a, miR-301b)	-
miR-141	miR-8 (miR-141, miR-200a, miR-200b, miR-200c, miR-429)	miR-200c
miR-143	miR-143	miR-145
miR-145	miR-145	miR-143
miR-148a	miR-148 (miR-148a, miR-148b, miR-152)	-
miR-155	miR-155	-
miR-192	miR-192 (miR-192, miR-215)	miR-194-2
miR-194	miR-194 (miR-194-1, miR-194-2)	miR-215
miR-195	miR-15 (miR-15a, mir-15b, miR-16-1, miR-16-2, miR-195)	miR-497
miR-200c	miR-8 (miR-141, miR-200a, miR-200b, miR-200c, miR-429)	miR-141
miR-210	miR-210	-
miR-215	miR-192 (miR-192, miR-215)	miR-194-1
miR-223	miR-223	-
miR-224	miR-224	miR-452
miR-296	miR-296	miR-298
miR-370	miR-370	-
miR-451	miR-451	miR-144, miR-451b, miR-4732
miR-494	miR-154 (miR-154, miR-300, miR-323a, miR-323b, miR-369, miR-377, miR-381, miR-382, miR-409, miR-410, miR-487a, miR-487b, miR-494, miR-496, miR-539, miR-655, miR-656, miR-1185-1, miR-1185-2	miR-299, miR-323a, miR-329-1, miR-329-2, miR-379, miR-380, miR-411, miR-495, miR-543, miR-758, miR-1193, miR-1197

miRNA	miRNA gene family[†]	Clustered miRNAs[‡]
miR-514	miR-506 (miR-506, miR-507, miR-508, miR-509-1, miR-509-2, miR-509-3, miR-510, miR-511-1, miR-511-2, miR-512-1, miR-512-2, miR-513a-1, miR-513a-2, miR-513b, miR-513c, miR-514a-1, miR-514a-2, miR-514a-3, miR-514b)	miR-510, miR-514-2, miR-514-3, miR-514-4
miR-638	miR-638	-

Data are taken from the miRBase database, release 18.

[†]miRNA gene family represents sequences evolved from a common ancestor and corresponds to miRNAs that have similar sequence which is vertebrate specific.

[‡]miRNAs which are located on the same strand of the chromosome and separated by a distance <10 kb are defined as "clustered miRNAs".

Table S3 of Supplementary Material. Summary statistics of the normalized expression data of miRNAs in the three clinical sample groups. Abbreviations: CI, confidence interval; 10-90 P, range 10-90 percentiles; 25-75 P, range between 25-75 percentiles, the interquartile range; normal distr., test for normal distribution according to D'Agostino-Pearson.

	Normal (non-malignant) samples											
miRNAs	**N**	**Mean**	**95% CI**	**SD**	**SEM**	**Median**	**95% CI**	**Minimum**	**Maximum**	**10 - 90 P**	**25 - 75 P**	**Normal Distr.**
miR-10b	22	3.108	2.598 - 3.618	1.1501	0.2452	2.847	2.545 - 3.699	0.761	6.077	1.965 - 4.433	2.405 - 3.848	0.2236
miR-19a	22	0.515	0.455 - 0.575	0.1353	0.02884	0.566	0.494 - 0.589	0.217	0.685	0.262 - 0.647	0.493 - 0.594	0.0594
miR-19b	22	4.572	4.125 - 5.020	1.0092	0.2152	4.898	4.072 - 5.286	2.479	6.023	2.947 - 5.689	3.980 - 5.304	0.3179
miR-20a	22	2.321	2.106 - 2.536	0.4848	0.1034	2.171	2.076 - 2.588	1.57	3.46	1.767 - 2.996	2.015 - 2.598	0.3743
miR-21	22	22.186	14.29 - 30.09	17.82	3.799	16.408	13.69 - 22.57	2.527	75.12	8.953 - 51.53	12.32 - 22.62	0.0001
miR-26a	22	17.371	16.08 - 18.66	2.9117	0.6208	17.098	16.21 - 19.18	10.341	22.462	14.16 - 21.15	15.86 - 19.88	0.5578
miR-29a	22	18.878	16.31 - 21.45	5.8006	1.2367	20.371	16.49- 21.74	3.675	28.754	11.35 - 25.13	15.99 - 21.87	0.0695
miR-29b	22	0.938	0.677 - 1.198	0.5885	0.1255	0.821	0.704 - 0.981	0.271	3.125	0.406 - 1.497	0.697 - 0.994	<0.0001
miR-29c	22	3.598	3.010 - 4.186	1.3259	0.2827	3.621	3.164 - 4.102	0.627	6.357	2.026 - 5.570	2.656 - 4.273	0.7825
miR-100	22	5.954	5.252 - 6.655	1.5817	0.3372	6.194	5.389 - 6.708	1.806	9.349	4.003 - 7.436	4.929 - 6.934	0.1729
miR-101	22	0.428	0.334 - 0.522	0.2126	0.04533	0.455	0.308 - 0.568	0.0169	0.835	0.133 - 0.635	0.261 - 0.598	0.5693
miR-126	22	12.891	11.744- 14.04	2.5884	0.5519	12.792	11.31 - 13.81	7.918	19.102	9.913- 15.94	11.03 - 13.945	0.25
miR-127	22	0.185	0.148 - 0.222	0.08305	0.01771	0.164	0.155 - 0.188	0.0797	0.432	0.0883 - 0.305	0.139 - 0.206	0.0029
miR-130a	22	1.381	1.098 - 1.664	0.6378	0.136	1.358	1.048 - 1.649	0.0991	2.912	0.613 - 2.074	1.008 - 1.849	0.5199
miR-141	22	0.512	0.381 - 0.643	0.2951	0.06292	0.552	0.269 - 0.629	0.0277	1.159	0.161 - 0.901	0.260 - 0.639	0.5775
miR-143	22	13.872	9.550 - 18.194	9.748	2.0783	12.392	9.256 - 15.462	2.222	46.827	5.141 - 22.575	8.742 - 15.514	<0.0001
miR-145	22	19.645	15.98 - 23.31	8.2569	1.7604	18.876	14.96 - 20.75	10.37	47.681	12.58- 27.72	14.77 - 20.96	<0.0001
miR-148a	22	2.112	1.689 - 2.536	0.9551	0.2036	2.224	1.642 - 2.824	0.163	3.768	0.819 - 3.253	1.396 - 2.888	0.6132
miR-155	22	0.301	0.0155 - 0.587	0.6443	0.1374	0.129	0.0973 - 0.223	0.0675	3.152	0.0830 - 0.399	0.0927 - 0.246	<0.0001
miR-192	22	7.56	5.762 - 9.359	4.0561	0.8648	8.817	5.347 - 10.413	0.235	13.565	1.097 - 12.113	5.272 - 10.601	0.3613
miR-194	22	6.824	5.080 - 8.568	3.9329	0.8385	7.301	4.749 - 10.286	0.206	12.104	0.854 - 11.416	4.437 - 10.500	0.3396
miR-195	22	4.132	3.477 - 4.787	1.4781	0.3151	4.361	3.290 - 4.789	1.47	7.676	2.058 - 5.853	3.274 - 4.879	0.6585
miR-200c	22	2.836	2.315 - 3.356	1.1739	0.2503	2.613	2.441 - 3.246	0.222	6.414	1.794 - 3.908	2.181 - 3.310	0.0066
miR-210	22	0.369	0.240 - 0.499	0.292	0.06225	0.29	0.217 - 0.351	0.149	1.255	0.194 - 0.841	0.205 - 0.352	<0.0001
miR-215	22	0.23	0.168 - 0.293	0.1407	0.03	0.27	0.113 - 0.327	0.000817	0.495	0.0358 - 0.389	0.113 - 0.327	0.5351
miR-223	22	1.268	1.032 - 1.505	0.5336	0.1138	1.155	0.993 - 1.471	0.465	2.68	0.680 - 1.944	0.863 - 1.575	0.1412
miR-224	22	0.0306	0.0188 - 0.0425	0.02672	0.005697	0.0214	0.0185 - 0.0301	0.0113	0.134	0.0121 - 0.0522	0.0182 - 0.0351	<0.0001
miR-296	22	0.0419	0.0359 - 0.0478	0.01334	0.002845	0.0408	0.0374 - 0.0493	0.0165	0.0773	0.0231 - 0.0558	0.0366 - 0.0501	0.2219
miR-370	22	0.0075	0.00521 - 0.0096	0.005053	0.001077	0.00617	0.00414 - 0.00981	0.00185	0.0247	0.00257 - 0.0118	0.00395 - 0.00988	<0.0001
miR-451	22	5.602	3.928 - 7.276	3.7753	0.8049	4.482	3.494 - 7.116	0.195	15.566	1.815 - 11.028	3.392 - 7.642	0.0341
miR-494	22	0.0841	0.0677 - 0.100	0.03704	0.007896	0.0726	0.0636 - 0.105	0.0249	0.162	0.0389 - 0.136	0.0622 - 0.113	0.5443
miR-514	22	0.225	0.150 - 0.299	0.1682	0.03587	0.216	0.116 - 0.284	0.00439	0.685	0.00898 - 0.458	0.111 - 0.305	0.0335
miR-638	22	0.0105	0.00611 - 0.0150	0.009991	0.00213	0.00729	0.00617 - 0.00825	0.00245	0.0392	0.00372 - 0.0263	0.00560 - 0.00903	<0.0001

	Clear cell renal cell carcinoma											
miRNAs	**N**	**Mean**	**95% CI**	**SD**	**SEM**	**Median**	**95% CI**	**Minimum**	**Maximum**	**10 - 90 P**	**25 - 75 P**	**Normal Distr.**
miR-10b	22	1.515	1.144 - 1.886	0.837	0.1785	1.509	1.103 - 1.662	0.219	3.86	0.478 - 2.477	1.010 - 1.686	0.0065
miR-19a	22	0.65	0.578 - 0.721	0.1612	0.03437	0.627	0.580 - 0.658	0.451	1.117	0.454 - 0.848	0.567 - 0.712	0.0016
miR-19b	22	4.583	4.045 - 5.121	1.2131	0.2586	4.442	3.955 - 4.609	3.097	7.999	3.456 - 6.241	3.865 - 4.755	0.0007
miR-20a	22	2.826	2.310 - 3.343	1.1649	0.2484	2.629	2.355 - 2.884	1.513	6.324	1.641 - 4.169	2.231 - 2.921	<0.0001
miR-21	22	67.618	50.188 - 85.049	39.313	8.3815	60.18	40.016 - 82.961	2.744	152.196	24.57 - 131.90	38.453 - 88.591	0.389
miR-26a	22	11.05	9.223 - 12.878	4.1218	0.8788	10.65	9.070 - 11.496	6.002	22.736	6.131 - 17.124	8.185 - 12.043	0.0155
miR-29a	22	16.076	10.922 - 21.231	11.626	2.4787	13.887	11.937 - 15.968	2.837	64.588	9.143 - 20.946	11.688 - 16.150	<0.0001
miR-29b	22	0.893	0.552 - 1.233	0.7679	0.1637	0.723	0.471 - 0.898	0.166	3.678	0.298 - 1.754	0.445 - 0.907	<0.0001
miR-29c	22	2.475	2.004 - 2.945	1.0621	0.2264	2.367	2.128 - 2.694	0.331	4.561	1.290 - 4.205	1.811 - 2.729	0.7945
miR-100	22	4.041	3.240 - 4.843	1.8086	0.3856	3.403	2.762 - 5.743	1.589	7.438	1.968 - 6.506	2.506 - 5.856	0.2939
miR-101	22	0.366	0.269 - 0.463	0.2181	0.04651	0.355	0.266 - 0.478	0.0124	0.821	0.0556 - 0.650	0.222 - 0.481	0.6767
miR-126	22	20.519	16.225 - 24.813	9.6853	2.0649	20.768	13.210 - 27.299	2.812	37.014	8.383 - 32.897	12.162 - 27.601	0.4872
miR-127	22	0.113	0.0835 - 0.143	0.0676	0.01441	0.0881	0.0776 - 0.157	0.0113	0.226	0.0392 - 0.224	0.0671 - 0.168	0.2993
miR-130a	22	1.117	0.853 - 1.381	0.595	0.1269	1.195	0.717 - 1.614	0.0563	1.914	0.256 - 1.821	0.646 - 1.623	0.3205
miR-141	22	0.0504	-0.00207 - 0.103	0.1184	0.02525	0.009	0.00638 - 0.0106	0.00114	0.51	0.00363 - 0.158	0.00611 - 0.0138	<0.0001
miR-143	22	13.855	9.733 - 17.977	9.2967	1.9821	10.813	7.110 - 21.019	0.914	32.282	3.790 - 26.956	5.483 - 21.587	0.3105
miR-145	22	18.596	14.495 - 22.697	9.2501	1.9721	17.919	14.420 - 26.105	0.882	35.684	5.942 - 30.765	14.372 - 26.183	0.6769
miR-148a	22	1.667	1.190 - 2.144	1.0749	0.2292	1.448	0.930 - 2.133	0.15	3.784	0.303 - 3.316	0.850 - 2.350	0.3292
miR-155	22	1.461	0.804 - 2.117	1.4802	0.3156	0.952	0.495 - 1.514	0.101	6.207	0.312 - 3.652	0.487 - 1.828	0.0001
miR-192	22	4.117	3.208 - 5.025	2.0489	0.4368	3.768	2.999 - 5.413	0.0873	7.781	1.334 - 6.974	2.860 - 5.521	0.7108
miR-194	22	3.258	2.490 - 4.025	1.7304	0.3689	2.999	2.174 - 4.955	0.106	5.727	0.986 - 5.314	1.990 - 4.975	0.3891
miR-195	22	4.708	3.549 - 5.867	2.6144	0.5574	4.9	2.433 - 6.630	0.596	9.138	1.447 - 8.067	2.366 - 6.700	0.437
miR-200c	22	0.479	0.267 - 0.691	0.478	0.1019	0.346	0.217 - 0.437	0.0588	2.101	0.124 - 1.148	0.207 - 0.531	<0.0001
miR-210	22	3.95	2.891 - 5.009	2.3884	0.5092	3.687	2.277 - 4.492	0.197	9.491	1.331 - 7.229	2.104 - 5.657	0.4528
miR-215	22	0.14	0.0957 - 0.185	0.1006	0.02144	0.155	0.0563 - 0.201	0.00236	0.377	0.00343 - 0.252	0.0445 - 0.204	0.6394
miR-223	22	2.726	1.431 - 4.022	2.922	0.623	2.142	1.758 - 2.563	1.097	15.476	1.267 - 3.236	1.622 - 2.711	<0.0001
miR-224	22	0.158	0.0951 - 0.220	0.1409	0.03004	0.121	0.0871 - 0.171	0.0361	0.721	0.0612 - 0.244	0.0829 - 0.189	<0.0001
miR-296	22	0.05	0.0314 - 0.0686	0.04194	0.008942	0.0335	0.0192 - 0.0626	0.00735	0.166	0.00839 - 0.101	0.0191 - 0.0891	0.0365
miR-370	22	0.0041	0.00232 - 0.0059	0.003994	0.000872	0.00234	0.00153 - 0.00504	0.000534	0.0142	0.000935 - 0.0106	0.00146 - 0.00663	0.0069
miR-451	22	6.364	3.323 - 9.405	6.8596	1.4625	4.27	3.283 - 5.543	0.0821	24.897	1.629 - 21.046	2.948 - 5.750	0.0002
miR-494	22	0.0632	0.0501 - 0.0764	0.02966	0.006324	0.0668	0.0458 - 0.0745	0.00777	0.126	0.0204 - 0.106	0.0441 - 0.0757	0.9541
miR-514	22	0.0095	0.00484 - 0.0141	0.01043	0.002223	0.00579	0.00226 - 0.0112	0.000582	0.0351	0.00120 - 0.0301	0.00207 - 0.0141	0.0056
miR-638	22	0.0049	0.00284 - 0.0068	0.004447	0.000971	0.00409	0.00344 - 0.00505	0.000359	0.022	0.000814 - 0.00803	0.00311 - 0.00518	<0.0001

	RCC bone metastases											
miRNAs	**N**	**Mean**	**95% CI**	**SD**	**SEM**	**Median**	**95% CI**	**Minimum**	**Maximum**	**10 - 90 P**	**25 - 75 P**	**Normal Distr.**
miR-10b	13	0.474	0.350 - 0.597	0.2044	0.05669	0.439	0.265 - 0.685	0.238	0.768	0.243 - 0.754	0.270 - 0.676	0.3587
miR-19a	13	0.289	0.235 - 0.343	0.0898	0.02491	0.323	0.240 - 0.338	0.104	0.411	0.132 - 0.391	0.245 - 0.334	0.2782
miR-19b	13	2.54	2.148 - 2.932	0.6486	0.1799	2.605	2.124 - 2.966	1.401	3.394	1.461 - 3.381	2.289 - 2.944	0.4733
miR-20a	13	1.898	1.592 - 2.204	0.5062	0.1404	1.884	1.487 - 2.369	1.022	2.593	1.244 - 2.562	1.497 - 2.359	0.5459
miR-21	13	64.616	43.628 - 85.603	34.7301	9.6324	74.263	29.815 - 92.256	8.323	114.761	22.154 - 113.163	31.358 - 90.720	0.474
miR-26a	13	5.571	4.734 - 6.408	1.3856	0.3843	5.057	4.715 - 6.033	3.765	8.352	4.405 - 8.349	4.765 - 5.893	0.0756
miR-29a	13	7.979	5.933 - 10.026	3.3867	0.9393	7.019	5.439 - 10.962	3.196	14.169	4.457 - 12.936	5.453 - 10.340	0.4281
miR-29b	13	0.189	0.131 - 0.247	0.0962	0.02668	0.169	0.119 - 0.260	0.0495	0.372	0.0567 - 0.318	0.125 - 0.250	0.7097
miR-29c	13	0.611	0.460 - 0.761	0.2485	0.06892	0.592	0.385 - 0.879	0.158	0.907	0.318 - 0.894	0.389 - 0.876	0.5315
miR-100	13	2.326	1.360 - 3.292	1.5984	0.4433	1.822	1.118 - 3.126	0.524	5.857	0.669 - 5.057	1.137 - 3.083	0.1403
miR-101	13	0.119	0.0783 - 0.160	0.06781	0.01881	0.0996	0.0769 - 0.145	0.0519	0.303	0.0589 - 0.212	0.0790 - 0.135	0.0011
miR-126	13	9.536	6.092 - 12.980	5.6986	1.5805	7.974	4.875 - 14.092	3.048	21.658	4.040 - 18.431	5.052 - 13.899	0.2729
miR-127	13	0.114	0.0134 - 0.214	0.1662	0.04609	0.0614	0.0263 - 0.0993	0.00465	0.601	0.00698 - 0.371	0.0325 - 0.0963	<0.0001
miR-130a	13	0.63	0.435 - 0.825	0.3224	0.08941	0.671	0.295 - 0.816	0.206	1.358	0.239 - 0.970	0.312 - 0.813	0.4295
miR-141	13	0.0024	0.0015 - 0.0032	0.00144	0.000399	0.0023	0.0017 - 0.0026	0.00087	0.00676	0.000946 - 0.00343	0.00177 - 0.00256	<0.0001
miR-143	13	5.613	3.299 - 7.926	3.8285	1.0618	4.435	2.237 - 10.201	1.094	11.519	1.369 - 10.603	2.352 - 10.147	0.3326
miR-145	13	7.784	4.653 - 10.915	5.1814	1.4371	7.009	3.068 - 12.823	2.001	16.964	2.017 - 14.347	3.198 - 12.616	0.383
miR-148a	13	0.987	0.660 - 1.315	0.5425	0.1505	0.875	0.630 - 1.205	0.241	2.426	0.505 - 1.625	0.643 - 1.205	0.0056
miR-155	13	4.077	-1.248 - 9.402	8.8121	2.444	1.448	0.842 - 2.776	0.29	33.165	0.305 - 9.809	0.849 - 2.717	<0.0001
miR-192	13	1.278	0.348 - 2.207	1.5382	0.4266	0.734	0.146 - 2.058	0.0541	4.808	0.102 - 4.085	0.148 - 1.987	0.0337
miR-194	13	0.84	0.287 - 1.392	0.9145	0.2536	0.426	0.171 - 1.664	0.0597	2.844	0.0804 - 2.201	0.180 - 1.538	0.1351
miR-195	13	2.999	1.703 - 4.295	2.1446	0.5948	1.92	1.242 - 4.795	0.964	7.742	1.081 - 5.924	1.286 - 4.736	0.2529
miR-200c	13	0.173	0.111 - 0.236	0.1033	0.02865	0.132	0.0953 - 0.234	0.0487	0.382	0.0572 - 0.351	0.0978 - 0.232	0.3372
miR-210	13	2.469	1.736 - 3.202	1.2132	0.3365	2.4	1.278 - 3.302	0.901	4.387	1.061 - 4.256	1.348 - 3.287	0.4445
miR-215	13	0.0458	0.0135 - 0.0782	0.05353	0.01485	0.0212	0.00704 - 0.0720	0.00292	0.158	0.00301 - 0.151	0.00708 - 0.0703	0.058
miR-223	13	4.468	1.124 - 7.812	5.5334	1.5347	2.094	1.379 - 4.903	0.637	18.458	0.836 - 15.201	1.393 - 4.861	0.0013
miR-224	13	0.195	0.110 - 0.280	0.1414	0.03921	0.166	0.0811 - 0.324	0.0338	0.49	0.0512 - 0.393	0.0852 - 0.322	0.387
miR-296	13	0.112	0.0797 - 0.144	0.05306	0.01472	0.119	0.0736 - 0.141	0.0212	0.201	0.0326 - 0.192	0.0757 - 0.138	0.9952
miR-370	13	0.0062	0.000782 - 0.012	0.01015	0.002816	0.00327	0.00638 - 0.00486	0.000117	0.0352	0.000133 - 0.0232	0.000678 - 0.0048	<0.0001
miR-451	13	13.217	3.582 - 22.851	15.9434	4.4219	6.816	1.709 - 21.535	0.101	51.831	0.479 - 38.745	2.163 - 18.617	0.0226
miR-494	13	0.14	0.0326 - 0.248	0.1784	0.04948	0.0781	0.0619 - 0.150	0.0147	0.707	0.0288 - 0.301	0.0642 - 0.135	<0.0001
miR-514	13	0.0022	0.00105 - 0.0032	0.001839	0.00051	0.00153	0.00104 - 0.00296	0.000469	0.00741	0.000696 - 0.00435	0.00104 - 0.00284	0.0002
miR-638	13	0.0114	0.00368 - 0.0191	0.01275	0.003536	0.00619	0.00403 - 0.0158	0.000394	0.048	0.000660 - 0.0272	0.00446 - 0.0147	0.0001

Table S4 of Supplementary Material . Spearman rank correlation coefficients >0.70 between the miRNAs in the three groups and in comparison between the groups.

A). Spearman rank correlation coefficients (r_S) between miRNAs in normal (non-malignant) renal tissue samples in comparison to the r_S-values of miRNAs in tissue samples from clear cell renal cell carcinoma (ccRCC) and metastases.

Correlation between miRNA pairs		r_S		
		Non-malignant	ccRCC	Metastases
miR-10b	miR-194	0.721[a,b]	0.161[†,a]	-0.269[†,b]
miR-19a	miR-19b	0.871	0.720	0.945
miR-21	miR-223	0.770[a,b]	0.299[†,a]	0.060[†,b]
miR-29a	miR-130a	0.761[b]	0.447	0.027[†,b]
miR-29a	miR-141	0.730	0.286[†]	0.407[†]
miR-101	miR-194	0.794[b]	0.506	0.203[†,b]
miR-101	miR-215	0.800[b]	0.530	0.275[†,b]
miR-130a	miR-143	0.832[b]	0.496[c]	-0.225[†,b,c]
miR-130a	miR-195	0.840[b]	0.424	0.071[†,b]
miR-143	miR-195	0.780	0.625	0.769
miR-148a	miR-194	0.799[a,b]	0.0224[†,a]	-0.264[†,b]
miR-192	miR-194	0.854	0.835	0.967
miR-192	miR-215	0.768	0.868	0.951
miR-194	miR-215	0.864	0.773	0.940

B). Spearman rank correlation coefficients (r_S) >0.70 between miRNAs in tissue samples from clear cell renal cell carcinoma in comparison to the r_S-values of miRNAs in renal metastatic and normal (non-malignant) renal tissue samples.

Correlation between miRNA pairs		r_S		
		ccRCC	Metastases	Non-malignant
miR-10b	miR-126	0.813	0.808	0.560
miR-19a	miR-19b	0.720	0.945	0.871
miR-19a	miR-20a	0.877[a,c]	0.319[†,c]	0.534[a]
miR-19b	miR-130a	0.870[a,c]	-0.456[†,b,c]	0.375[†,a,b]
miR-29b	miR-29c	0.754	0.462[†]	0.579
miR-127	miR-370	0.906[a]	0.879	0.677[a]
miR-141	miR-210	0.738[c]	0.016[†,c]	0.438
miR-192	miR-194	0.835	0.967	0.854
miR-192	miR-195	0.868[a,c]	-0.423[†,c]	0.001[†,a]
miR-194	miR-215	0.773	0.940	0.864

C). Spearman rank correlation coefficients (r_S) >0.70 between miRNAs in renal bone metastases in comparison to r_S-values of miRNAs in tissue samples of clear cell renal cell carcinoma (ccRCC) and normal (non-malignant) renal tissue samples.

Correlation between miRNA pairs		r_S		
		Metastases	ccRCC	Non-malignant
miR-10b	miR-101	0.709	0.382[†]	0.473
miR-10b	miR-126	0.808	0.813	0.560
miR-10b	miR-143	0.852[b,c]	0.280[†,c]	0.280[†,b]
miR-19a	miR-19b	0.945	0.720	0.871
miR-29b	miR-514	-0.797[b,c]	0.046[†,c]	-0.021[†,b]
miR-126	miR-143	0.907[b,c]	0.144[†,c]	0.247[†,b]
miR-126	miR-145	0.874[b,c]	0.089[†,c]	-0.249[†,b]
miR-126	miR-195	0.835	0.344[†,c]	0.246[†,b]
miR-127	miR-195	0.742	0.235[†]	0.478
miR-127	miR-200c	-0.736	0.073[†,c]	-0.106[†,b]
miR-127	miR-370	0.879	0.906[a]	0.677[a]
miR-143	miR-145	0.934[c]	0.641[c]	0.466[b]
miR-143	miR-195	0.769	0.625	0.780
miR-145	miR-370	0.703	0.522	0.136[†]
miR-192	miR-194	0.967	0.835	0.854
miR-192	miR-215	0.957	0.868	0.768[b]
miR-194	miR-215	0.940	0.773[c]	0.864
miR-451	miR-638	0.775	0.313[†]	0.566
miR-494	miR-638	0.709	0.622	0.353[†]

[†]Correlation coefficients with this superscript indicate non-significant (P>0.05) correlations within the group. Figures without this superscript indicate significant correlation between the corresponding miRNA pair.

[a]Significant difference (at least P <0.05) between the correlation coefficients from non-malignant and primary tumor samples.

[b]Significant difference (at least P <0.05) between the correlation coefficients from non-malignant and metastatic tissue samples.

[c]Significant difference (at least P <0.05) of the correlation coefficients from the primary tumor samples and metastatic tissue samples.

Table S5 of Supplementary Material. Differentially expressed miRNAs described in studies with primary and metastatic tumor tissue of clear cell renal cell carcinoma using microarray and and RT-qPCR analyses.

A. Differentially expressed miRNAs found by microarray analyses in four studies[†]

No.	Heinzelmann et al. [20]	White et al. [21]	Slaby et al. [23]	present study		miRNAs present in		
						all 4 studies	3 of 4 studies	2 of 4 studies
1	let-7a	let-7a						
2	let-7b	let-7b						
3	let-7c	let-7c						
4	let-7d	let-7d	let-7d*					
5	let-7g	let-7e						
6	let-7i	let-7f						
7		let-7g						
8		let-7i						
9			miR-1					
10	miR-10a	miR-10a						
11	miR-10b	miR-10b	miR-10b	miR-10b				
12		miR-15						
13			miR-15b					
14	miR-16							
15		miR-17						
16				miR-18a				
17				miR-19a				
18	miR-19b			miR-19b				
19		miR-20a						
20				miR-21				
21	miR-22							
22	miR-23a							
23	miR-23b	miR-23b						
24	miR-24	miR-24						
25				miR-25				
26	miR-26a	miR-26a	miR-26a					
27	miR-26b	miR-26b						
28		miR-27a						
29	miR-27b	miR-27b						
30		miR-28-5p	miR-28-5p					
31	miR-29a	miR-29a		miR-29a				
32		miR-29b	miR-29b	miR-29b				
33		mir-29c		miR-29c				
34	miR-30a	miR-30a						
35		miR-30a*						
36	miR-30b	miR-30b						
37	miR-30c	miR-30c	miR-30c-1					
38	miR-30d	miR-30d						
39		miR-30e		miR-30e				
40		miR-30e*						

No.	Heinzelmann et al. [20]	White et al. [21]	Slaby et al. [23]	present study		miRNAs present in		
						all 4 studies	3 of 4 studies	2 of 4 studies
41				miR-32				
42			miR-34b*					
43			miR-92a-1*					
44				miR-93				
45		miR-98						
46				miR-99a				
47				miR-100				
48				miR-101				
49		miR-103						
50		miR-106a						
51	miR-106b	miR-106b	miR-106b					
52		miR-107						
53		miR-122						
54			miR-124					
55				miR-125a				
56	miR-125b							
57		miR-126	miR-126	miR-126				
58				miR-126*				
59			miR-127-3p					
60				miR-128b				
61	miR-130a	miR-130a		miR-130a				
62				miR-130b				
63			miR-134					
64			miR-135b					
65			miR-136*					
66			miR-138-1					
67			miR-139-5p					
68			miR-140-3p					
69			miR-140-5p					
70	miR-143	miR-143	miR-143	miR-143				
71			miR-144*					
72		miR-145	miR-145	miR-145				
73			miR-145*					
74			miR-148a	miR-148a				
75		miR-149*						
76			miR-150					
77		miR-151-3p						
78	miR-151-5p	miR-151-5p						
79		miR-152						
80			miR-154*					
81				miR-155				
82	miR-181a	miR-181a						
83		miR-181b						
84				miR-185				

No.	Heinzelmann et al. [20]	White et al. [21]	Slaby et al. [23]	present study		miRNAs present in		
						all 4 studies	3 of 4 studies	2 of 4 studies
85				miR-188				
86	miR-191			miR-191				
87				miR-191*				
88		miR-192		miR-192				
89		miR-194		miR-194				
90		miR-195	miR-195	miR-195				
91		miR-196a						
92		miR-197						
93			miR-198					
94		miR-200a						
95		miR-200b						
96		miR-200c						
97		miR-204						
98				miR-212				
99		miR-215						
100	miR-221							
101	miR-222							
102				miR-223				
103				miR-296-5p				
104			miR-299-3p					
105			miR-299-5p					
106			miR-302c					
107				miR-338				
108				miR-339				
109		miR-361-5p						
110			miR-363					
111				miR-370				
112			miR-374a	miR-374a				
113		miR-374b						
114			miR-376a					
115			miR-376c					
116			miR-382					
117			miR-409-3p					
118			miR-411					
119				miR-422b				
120				miR-425				
121			miR-431					
122	miR-451		miR-451	miR-451				
123				miR-452				
124		miR-455-3p						
125				miR-486-5p				
126			miR-487b					
127			miR-490-3p					
128				miR-494				

No.	Heinzelmann et al. [20]	White et al. [21]	Slaby et al. [23]	present study		miRNAs present in		
						all 4 studies	3 of 4 studies	2 of 4 studies
129			miR-495					
130		miR-498						
131			miR-499-5p					
132			miR-504					
133			miR-511					
134				miR-513				
135			miR-516a-5p					
136			miR-520b+F65					
137			miR-525-3p					
138			miR-539					
139			miR-543					
140			miR-558					
141			miR-561					
142				miR-564				
143				miR-572				
144				miR-575				
145			miR-591					
146			miR-605					
147				miR-630				
148			miR-635					
149		miR-638		miR-638				
150			miR-639					
151			miR-649					
152			miR-655					
153				miR-660				
154		miR-663		miR-663				
155				miR-671				
156		miR-720						
157				miR-765				
158				miR-766				
159	miR-768-3p							
160				miR-801				
161			miR-874					
162			miR-890					
163			miR-935					
164		miR-1469						
165		miR-1915						
No. of miRNAs	**33**	**65**	**64**	**57**		**2**	**13**	**35**

[†]References correspond to citations in the Reference list of the main text.

B. miRNAs associated with metastasis in renal cell carcinoma examined by RT-qPCR in five studies†

miRNAs highlighted in yellow were shown as associated with metastasis

No.	Slaby et al. [19]	Heinzelmann et al. [20]	Khella et al. [22]	Slaby et al. [23]	present study	miRNAs present in all 5 or 4 studies	miRNAs present in 3 of 5 studies	miRNAs present in 2 of 5 studies
1		let-7a						
2		let-7b						
3		let-7c						
4			miR-10b	miR-10b	miR-10b			
5					miR-19a			
6					miR-19b			
7					miR-20a			
8					miR-21 ns			
9		miR-26a		miR-26a	miR-26a			
10					miR-29a			
11					miR-29b			
12					miR-29c			
13		miR-30a						
14		miR-30c						
15					miR-100			
16					miR-101			
17	miR-106a							
18	miR-106b							
19			miR-126	miR-126	miR-126			
20				miR-127	miR-127			
21					miR-130a			
22				miR-136*				
23	miR-141				miR-141			
24				miR-143	miR-143			
25				miR-145	miR-145			
26					miR-148a			
27	miR-155				miR-155			
28	miR-182							
29			miR-192		miR-192			
30			miR-194		miR-194			
31				miR-195	miR-195			
32			miR-196a					
33	miR-200b							
34	miR-200c				miR-200c			
35			miR-204					
36	miR-210				miR-210			
37			miR-215		miR-215			
38					miR-223			
39					miR-224			

No.	Slaby et al. [19]	Heinzelmann et al. [20]	Khella et al. [22]	Slaby et al. [23]	present study		miRNAs present in		
							all 5 or 4 studies	3 of 5 studies	2 of 5 studies
40					miR-296				
41					miR-370				
42				miR-409-3p					
43					miR-451 ns				
44					miR-494 ns				
45					miR-514				
46					miR-638				
No. of miRNAs	**8**	**6**	**7**	**9**	**33**		**0**	**3**	**12**

†References correspond to citations in the Reference list of the main text.