Item S1. Detailed methods, table *a*, and figure *a*.

Detailed Methods

Patients

We prospectively recruited 30 hypertensive patients age ≥18 years, with EH (n=15) or RVH (n=15), and with serum creatinine < 2.5mg/dL to participate in studies[1, 2] between January 2008 and September 2012. Renal artery stenosis in patients with RVH was defined as cross-sectional luminal obstruction >60% by computed tomography (CT) or magnetic resonance (MR) angiography, or Doppler velocities ≥ 300 cm/sec.[3] Exclusion criteria included uncontrolled hypertension [systolic blood pressure > 180 mm Hg despite antihypertensive therapy], diabetes requiring medications, recent cardiovascular events (myocardial infarction, stroke, congestive heart failure within 6 months), pregnancy, and kidney transplant. A 3-day inpatient protocol in the clinical research unit included regulated dietary sodium intake (150 mEq/d), an isocaloric diet, MR imaging studies on Day 2, and CT scanning on Day 3.[1] Antihypertensive medications were continued; for uniformity, all patients were treated with either angiotensin-converting enzyme inhibitors or angiotensin receptors blockers.

Clinical data collection and blood pressure measurement

Clinical data collected by physical examination or via the electrical medical records included age, sex, height, weight, body mass index, and use of concomitant medications. We calculated estimated glomerular filtration rate (eGFR) using the Chronic Kidney Epidemiology Collaboration (CKD-EPI) equation.[4] Blood pressure was measured by automated oscillometric recording (Omron, Kyoto, Japan), averaging 3 measurements at 5, 7, and 9 minutes after a 5-minute rest. Urine samples were collected for 24 hours in all hypertensive patients, while spot urine samples were obtained from HV. Samples were stored at -80°C until measurement.

EV purification

EV were isolated from urine using Total Exosome Isolation reagent® (Invitrogen, Waltham, MA, USA) according to manufacturer's guidelines. In brief, urine samples (1000 µL) were centrifuged at 2000g for 30 minutes at 4℃ to remove cells and debris. Supernatants (800 µL) were mixed with 1 volume of the Total Exosome Isolation reagent® and incubated for 1 hour at room temperature. After incubation, samples were centrifuged at 10,000g for 1 hour at 4℃. Pelleted EVs were re-suspended in PBS.[5]

Measurement of miRs

Total RNA was isolated from urinary exosome by the mirVana PARIS total RNA isolation kit (Life Technologies Cat. # AM1556) following the manufacturer's protocol, Briefly, for endogenous small RNA control, we added Cel-mir-39 (25 fmol, Life Technology, Cat. # 4464066) into each sample, as previously described.[6] A fixed RNA content of 4.8 ng of RNA elute was reverse transcribed by using the TagMan MicroRNA reverse transcription kit (Life Technologies, Cat.# 4366596). For quantitative real-time polymerase chain reaction (qRT-PCR), 1.33 µL of RT product was combined with 10µL of TaqMan universal master mix (Cat.# 4440038), 7.67µL of H2O and 1µL of primers, including miR-21, miR-92a, miR-93, miR-126, miR-148a, miR-192, miR-200b, miR-377 and miR-433 (Life Technologies, Cat. # 4427975, assay ID: 000397, 002137, 001090, 000451, 002134, 002272, 002251, 002181, 001028, respectively), to make up a 20 µL reaction. qRT-PCR was carried out on an Applied Biosystems ViiA7 Real-Time PCR system at 50℃ for 2 min, 95℃ for 10 min and 40 cycles of 95℃ for 15s and 60℃ for 1 min. To generate standard curves, miR mimics were run together with the samples, to calculate absolute numbers of copies of target genes in the samples. These mimics included (all purchased from Life Technology): miR-21 (MC10206), miR-92a (MC12696), miR-

93 (MC10951), miR-126 (MC10401), miR-192 (MC12893), miR-200b (MC10492), miR-148a (MC12683), miR-377 (MC12860) and miR-433 (MC10774). We then normalized these data using a median normalization procedure.[7] Briefly, the median values obtained from cel-mir-39 Ct values of all the samples were used to calculate the normalization factor=$2^{(\text{median cel-mir-39 Ct value-average Ct value of the given sample})}$. The number of the copies of a given miR in each sample was then divided by the normalization factor to generate the normalized copies of genes in each sample.

These miRs were selected because of their postulated involvement in renal fibrosis and/or angiogenesis. miR-92a and miR-126 regulate angiogenesis; miR-21 regulates cell death in ischemia; miR-200 regulates nephrosclerosis; and the remaining miRs regulate renal fibrosis.

References

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Figure *a*: Levels of miRs adjusted for estimated glomerular filtration (eGFR), systolic blood pressure (SBP), triglycerides, high-density lipoprotein, and albuminuria in urinary extracellular vesicles (EVs) of healthy volunteers (HV), renovascular hypertension (RVH) and essential hypertension (EH) patients. Each point represents the relative level of miRs after normalization using Cel-miR-39. Levels of miR-126, 148, 192, 377 and 433 were not significantly different among the groups.

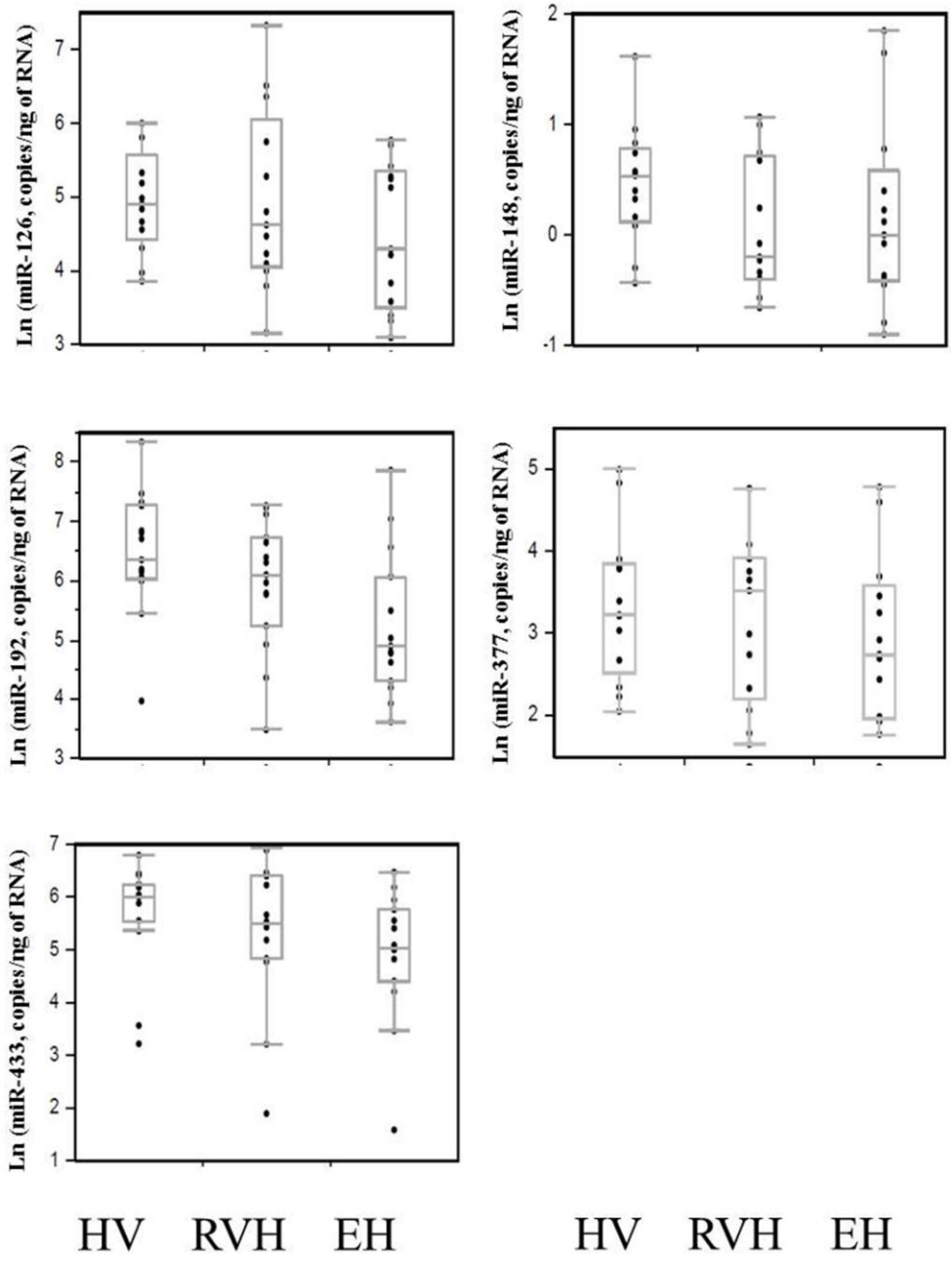

Table *a*. Partial Pearson correlation analysis between miR and clinical variables.

	miR-21		miR-92a		miR-93		miR-126		miR-148		miR-192		miR-200b		miR-377		miR -433	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	p-Value
Age (years)	-0.1	0.1	0.1	0.3	0.6	0.8	-0.1	0.3	0.4	0.3	0.002	0.8	-0.5	0.8	0.1	0.3	0.5	0.4
BMI (kg/m^2)	-0.1	0.6	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.09	0.6	0.04	0.6	0.1	0.3	0.2	0.1
SBP (mmHg)	-0.1	0.4	-0.2	0.3	-0.4	0.1	-0.2	0.4	-0.01	0.6	-0.2	0.1	-0.2	0.3	-0.1	0.4	-0.2	0.2
DBP (mmHg)	-0.07	0.8	0.2	0.1	0.05	0.6	0.3	0.8	0.1	0.4	0.2	0.1	0.03	0.6	0.1	0.5	0.2	0.5
eGFR ($mL/min/1.73m^2$)	-0.3	0.09	-0.2	0.2	-0.3	0.09	-0.3	0.09	0.1	0.2	-0.1	0.3	-0.2	0.1	0.2	0.1	-0.2	0.2
Urinary micro-albumin (μg/mL)	-0.05	0.5	-0.1	0.3	0.08	0.2	0.01	0.7	-0.1	0.7	-0.6	0.1	0.1	0.09	-0.6	0.7	0.03	0.3

Age, BMI, SBP, DBP, total cholesterol, high-density lipoprotein, low-density lipoprotein, eGFR and urinary microalbumin were adjusted in the analysis. Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate