Supplemental methods

Animal Procedures

Induction of RVD

At baseline, unilateral RVD was induced in 13 pigs by placing a local-irritant coil in the main renal artery, as previously described[1, 2]. A telemetry transducer (PhysioTel, Data Sciences) was implanted in the left femoral artery, and mean arterial pressure (MAP) subsequently recorded continuously at 5-minute intervals and averaged for each 24-hour period.

MSC culture and characterization

Subcutaneous adipose tissue (~5g) was harvested from the femoral artery incision of each pig, digested in collagenase H for 1hr, filtered, and cultured in Advanced MEM media supplemented with 5% PLTmax (Mill Creek Life Sciences, Rochester, MN). The culture media is devoid of animal serum, and thus suitable also for clinical applications of MSC. Phenotypic markers for MSC were characterized following the recommendations[3] of the International Society for Cellular Therapy using CD90, CD44, CD105, CD34, CD31, and CD45, and further quantitatively analyzed with fluorescence-activated cell sorting (FACs) for CD44, CD90, and CD105, using standard protocols. For further confirmation of their phenotype, MSC were also transdifferentiated into osteocytes, chondrocytes, and adipocytes[4]. The third passages of MSCs were collected and kept in -80°C for later use.

Renal angiography

Pigs were anesthetized with intra-muscular telazol (5 mg/kg) and xylazine (2 mg/kg), intubated, and mechanically ventilated with room air. Anesthesia was maintained with intravenous ketamine (0.2 mg/kg/min) and xylazine (0.03 mg/kg/min) in normal saline. Under sterile conditions and fluoroscopic guidance, renal angiography was performed to determine the

degree of RVD, as previously described[1, 2], and confirmed using CT angiography. To measure pressure gradients across chronic RVD, arterial pressures were measured in the aorta and distal to the renal artery stenosis using a small catheter (5F) with a transducer. To measure pressure gradients across acute renal arterial stenosis, a 5mm balloon catheter was inflated in the renal artery of 3 normal pigs, and pressures proximal/distal to inflated balloon were then measured using a transducer. The degree of stenosis induced by balloon was subsequently quantified using CT angiography. The pressure drop was expressed as the ratio of distal pressure (Pd) to proximal aortic pressure (Pa), as previously published[5].

In-vivo Studies

Four weeks after MSC infusion *in vivo* functional studies were performed. After angiography, the catheter was positioned in the superior vena cava, and MDCT flow studies were performed for assessment of basal regional-renal perfusion, renal blood flow (RBF), and glomerular filtration rate (GFR), as previously detailed[1, 6]. This involved sequential acquisition of 160 scans after a central venous injection of iopamidol (0.5 cc/kg/2 sec).

Blood Oxygen-dependent Level (BOLD) MRI is sensitive to the concentration of deoxyhemoglobin in blood. Elevated basal levels of the index R2* are indication of hypoxia. In addition, oxygen-dependent tubular transport function can be assessed by the change in medullary oxygenation in response to a loop reabsorption inhibitor such as furosemide. For this purpose, two sets of BOLD images were collected before and 15 minutes after furosemide injection (5mg/kg IV, Hospira, Inc., Lake Forest, IL). The medullary BOLD index, R2*, was calculated in each set, and the difference between the pre and post-furosemide values used as a measure of oxygen-dependent tubular function[7].

Micro-CT procedure

An intravascular Microfil® silicone rubber (MV-122, Flow Tech, Inc) was perfused through the cannulated renal artery at a flow rate of 0.9 ml/min to visualize vascular structure in micro-CT scan. A portion of the kidney (≈2×1×1cm) was then sectioned, prepared, and scanned as previously described[2]. Images were digitized for reconstruction of 3-D volume images, which consisted of cubic voxels of 20μm on-a-side for subsequent analysis. Images analysis was performed with the Analyze software package (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). The kidney cortex was tomographically divided into 3 equal parts, and the data were analyzed in 10 slices obtained at equal intervals from each third. The spatial density of micro vessels (diameters <500μm) was calculated in each region by counting the vessels.

Renin expression was evaluated on kidney section from both the stenotic and contralateral kidney using standard immunohistochemistry protocol (1:500, Abcam, Cambridge, MA).

Procedures in humans

In all patients, clinical and laboratory parameters were collected via the electronic medical records. Dietary intake was regulated at 150 mEq of sodium with an isocaloric diet prepared on site for a 3-day period that all hypertensive patients spent at the Clinical Research Unit. Patients with RVD were identified using criteria similar to those stipulated for recruitment in the Cardiovascular Outcomes in Renal Atherosclerotic Lesions Trial[8] with cross-sectional angiographic luminal occlusion of ≥60%. All RVD patients had a history of refractory stage II hypertension defined as a systolic blood pressure greater than 155 mmHg while taking two or more antihypertensive medications. RVD patients were well-characterized with the clinical syndrome of atherosclerotic renal artery disease and hypertension with hemodynamically significant lesions. Exclusion criteria included uncontrolled hypertension (SBP >180 mmHg, despite antihypertensive therapy), serum creatinine >1.7 mg/dL, diabetes requiring insulin or oral

hypoglycemic medications, recent cardiovascular events (myocardial infarction, stroke, congestive heart failure within 6 months), pregnancy, and kidney transplant. Estimated GFR was calculated using Modification of Diet in Renal Disease study equation. Peripheral blood samples were collected for measurements of cholesterol, triglycerides, and creatinine, levels. Renal vein samples were collected in essential and renovascular hypertensive patients through a 5F Cobra catheter (Cook, Inc) advanced from the common femoral vein to the right, left, and infrarenal inferior vena cava. In healthy volunteers blood samples were collected from an antecubital vein. Systemic and renal vein levels of PRA, TNF-α, IL-10, E-selectin, myeloperoxidase, and granulocyte colony-stimulating factor were detected using luminex assay kits (Millipore, Billerica, MA).

Patient MRI and BOLD Methods: BOLD MR imaging examinations were performed with a 3.0-T system (Twin Speed Signa Excite; GE Medical Systems, Waukesha, Wis). Parametric images of R2* were then generated by fitting signal intensity–versus–echo time data to an exponential function on a voxel-by-voxel basis and solving for R2*. After the first BOLD MR acquisition, 20 mg of furosemide (Lasix; Sanofi-Aventis, Bridgewater, NJ) was administered intravenously and flushed with 20 mL of saline. BOLD MR measurements for each kidney were repeated 15 minutes later. Gadolinium-enhanced MR angiograms were obtained after BOLD MR imaging to confirm the presence or absence of large-vessel renal arterial disease. Analysis of BOLD MR data from axial images was performed by drawing parenchymal ROIs on two to four sections through the midpole hilar region of each kidney on representative T2*-weighted images and then transferring the ROIs to the corresponding R2* parametric image by one author. Two ROIs were traced: In one, the renal cortex (large segment) was selected, and in the other, the entire kidney section, including both cortex and medulla while excluding the renal collecting

system and any incidental renal cysts was selected. The fractional tissue hypoxia was determined as we have recently developed[9] by measuring the percentage of voxels from the whole-kidney ROI with R2* values above 30 sec−1, taking the average of all available sections.

Mir-26a expression was evaluated using RT-PCR in stenotic kidney vein samples in patients with RVD, renal vein samples from patients with essential hypertension, or systemic samples from volunteers. Briefly, total RNA was isolated from 400μl plasma samples by mirVana PARIS total RNA isolation kit (Life Technologies). Due to the lack of established endogenous small RNA controls for plasma samples, we spiked 25fmol of cel-mir-39 (Life Technology) into each sample after adding the 2X denaturing solution supplied by the kit. Real-time PCR (Applied Biosystems ViiA7) used mir26a primer (Life Technologies). Numbers of miR-26a copies were calculated from a standard curve generated by using standard miR-26a.

Cell culture studies

The mature miR-26a sequence used for miR-26a inhibitor design was UUCAAGUAAUCCAGGAUAGGCU, stem loop sequence: GUGGCCUCGUUCAAGUAAUC CAGGAUAGGCUGUGCAGGUC CCAAUGGGCCUAUUCUUGGU UACUUGCACGGGGACGC.


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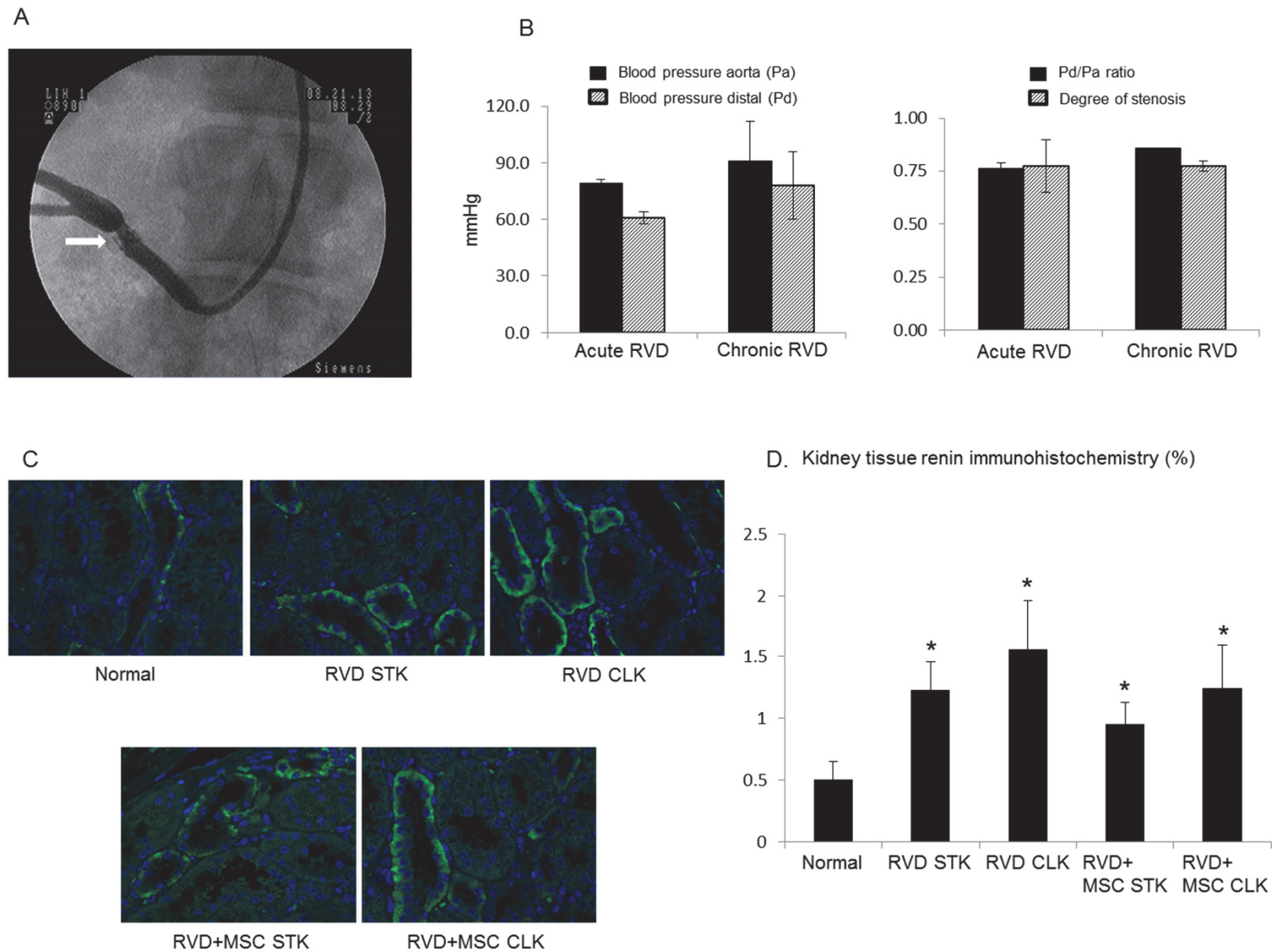
Figure 1s: Renal artery angiography, pressure gradient measurement, and kidney tissue renin immunohistochemistry. A: Representative renal angiography in swine RVD. B: pressure gradient, the ratio between pressures in the proximal aorta and distal to the stenosis, and the degree of stenosis in chronic and acute RVD. C&D: Renin immunohistochemistry images and quantification in normal, RVD, and RVD+MSC pigs, including the stenotic and contralateral kidneys.