Supporting Methods

***In Vitro* Experiments.** Human renal glomerular endothelial cells (ScienCell Research Laboratories) were maintained in Endothelial Cell Medium (ScienCell) in fibronectin (Roche Diagnostics) -coated flasks and used between passages 3 and 6. The cells were serum- starved in Endothelial Cell Medium for 4 h before experimental treatment. Glomerular endothelial cells were incubated with either vandetanib (1 μM in DMSO) or DMSO for another 4 h before the addition of 50 ng/ml VEGF (Chemicon) for 5 min. For immunoprecipitation, cell lysates were incubated with an agarose-conjugated polyclonal antibody directed against VEGFR-2 (Santa Cruz Biotechnology) before SDS/PAGE and transfer to nitrocellulose Hybond ECL membranes (Amersham Pharmacia). After blocking in 3% BSA, membranes were immunoblotted with anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology), washed, and incubated with goat anti-mouse IgG conjugated to horseradish peroxidase (Bio-Rad). Proteins were detected by the ECL system (Amersham Pharmacia). After this, membranes were stripped in 0.2 M glycine (pH 2.2) and immunoblotted with Flk-1 (A-3) (anti-VEGFR-2) mouse monoclonal antibody (Santa Cruz Biotechnology). Densitometry of protein bands was performed by using ImageJ version 1.37 (from the National Institutes of Health and available at http://rsb.info.nih.gov/ij/).

Glomerulosclerosis Index. Eighty glomeruli were examined in PAS-stained kidney sections from each rat. The degree of sclerosis was subjectively graded on a scale of 0-4: grade 0, normal; grade 1, sclerotic area up to 25% (minimal); grade 2, sclerotic area 25-50% (moderate); grade 3, sclerotic area 50-75% (moderate to severe); and grade 4, sclerotic area 75-100% (severe). Glomerulosclerosis was defined as basement membrane thickening, mesangial hypertrophy, and capillary occlusion. A glomerulosclerosis index (GSI) was then calculated by using the formula:

$GSI = \sum_{(i)=0}^{4} Fi$, where Fi is the percentage of glomeruli in the rat with a given score (i).

Quantitative Autoradiography. Film densitometry of *in situ* hybridization autoradiographic images was performed by using a Micro Computer Imaging Device (MCID; Imaging Research, St Catherine's, ON, Canada). With this method, quantitation of transcript is based on the changes in x-ray film density that follows exposure to the radioactive emissions of radio-labeled VEGF, VEGFR-2, or nephrin mRNA. *In situ* hybridization autoradiographic images were placed on a uniformly-illuminating fluorescent light box (Northern Light Precision Luminator Model C60; Image Research) and captured by using a video camera (Sony Video Camera Module CCD) connected to an IBM AT computer with a 512×512 pixel array imaging board with 256 gray levels. After appropriate calibration by constructing a curve of optical density versus radioactivity, quantitation of digitalized autoradiographic images was performed by using MCID software.

Regions of high probe-labeling intensity, confirmed to be glomeruli by light microscopy of emulsion-dipped slides, were targeted by using MCID software. The density in these regions was measured and expressed as average density per unit area of cortex (optical density $\times$ proportional area), normalized to the mean of the values for Sprague–Dawley rats and expressed as optical density per cm^2 relative to control kidneys (Relative Optical Density, ROD). All analyses were performed with the observer masked to the animal study group.

Electron Microscopy. Rats were anesthetized with an i.p. injection of pentobarbital sodium, 60 mg/kg (Boeringer-Ingelheim). The abdominal aorta was then cannulated with an 18-gauge needle. Perfusion-exsanguination commenced at systolic blood pressure via the abdominal aorta with PBS (pH 7.4, 100 to 200 ml) to remove circulating blood. The inferior vena cava adjacent to the renal vein was severed simultaneously, allowing free flow of perfusate. After clearance of circulating blood, 2.5% gluteraldehyde in 0.1M sodium pyrophosphate (pH 7.2) (100-200 ml) was perfused to fix tissues. Kidneys were removed from the animal and decapsulated. Sections of cortex 1 mm^3 were postfixed in the same fixative for 2 h, washed in PBS, and stored in 5% sucrose in PBS (pH 7.4) at 4°C. Tissues were processed in 0.1 M cacodylate buffer (sodium cacodylate-trihydrate in

distilled water, pH 7.4), postfixed in 1% osmium tetroxide (OsO_4), and block-stained in uranyl acetate in maleate buffer (23.6 mM uranyl acetate, 50 mM NaH maleate, 27 mM NaOH, pH 6.0). After dehydration in graded ethanol, tissues were left overnight in 50/50 propylene oxide/epon-araldite [45g agar 100/60 g araldite CY212/75 g dodecanylsuccinic anhydride (DDSA)/3.6 g 2,4,6-Tris(dimethylaminomethyl)phenol (DMP30)] before embedding in molds.

Study 4. ACE inhibition in TGR(mRen-2)27 rats. To determine the role of RAS-activation in glomerular VEGF expression, *in situ* hybridization was performed on historical formalin-fixed kidney sections. Animals were female homozygous TGR(mRen-2)27 rats and had been treated with either the ACE inhibitor perindopril or vehicle for 12 weeks from 6 weeks of age. Animals randomized to perindopril received a daily dose of 0.2 mg/kg administered in drinking water, adjusted for water intake. All rats were maintained in a stable environment (maintained at 22 ± 1°C with a 12-h light-day cycle) and allowed free access to tap water and standard rat chow containing 0.25% Na^+ and 0.76% K^+ (GR2; Clark-King & Co.). Quantitative *in situ* hybridization for VEGF-A and film densitometry of autoradiographic images were performed on 4-μm kidney sections as described in *Methods*. Experimental procedures adhered to the guidelines of the National Health and Medical Research Council of Australia's Code for Care and Use of Animals for Scientific Purposes and were approved by St. Vincent's Hospital Animal Ethics Committee, Melbourne, Australia.

Study 5. Effect of low-dose vandetanib in TGR(mRen-2)27 rats. To investigate whether rise in blood pressure with VEGF inhibition could explain the renal structural and functional changes observed, male heterozygous TGR(mRen-2)27 rats (n = 18) were treated with a lower dose of vandetanib (15 mg/kg) by once daily oral gavage for 24 days.