INVESTIGATION OF THE PATHOMECHANISM OF UREMIC CARDIOMYOPATHY AND THE INFARCT SIZE-LIMITING EFFECT OF ISCHEMIC PRECONDITIONING IN A RAT MODEL OF CHRONIC KIDNEY DISEASE

Summary of the Ph.D. Thesis

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1. Introduction

Chronic kidney disease (CKD) is one of the most rapidly growing non-communicable diseases and an important contributor to morbidity and mortality worldwide. CKD is defined as abnormal renal structure and/or function (glomerular filtration rate [GFR]<60 mL/min/1.73 m^2) present for at least 3 months in patients. In the general population, the global prevalence of CKD varies between 7-12% and is continuously increasing due to the growing incidence of its most common primary causes, including hypertension and diabetes mellitus. The age-standardized global prevalence of early CKD stages (G1-G3, GFR>30mL/min/1.73 m^2) is higher in women than in men. Notably, a higher CKD prevalence among women was found for CKD stages with decreased GFR and albuminuria with normal GFR. Mortality is higher among men in all stages of predialysis CKD, whereas mortality among patients on renal replacement therapy is similar for men and women.

The presence of CKD is an independent risk factor for cardiovascular complications. CKD and end-stage renal disease (ESRD) patients have a 5- to 10-fold higher risk for developing cardiovascular diseases (CVDs) compared to the age-matched control population. Large population studies have reported that all stages of CKD predispose to premature death, mainly from CVDs, and this is not restricted to ESRD. Interestingly, the incidence of cardiovascular mortality is much higher in CKD patients at stages G2 and G3 than in ESRD patients. Cardiovascular events are more commonly fatal in patients with CKD than in individuals without CKD.

Uremic cardiomyopathy (*i.e.*, type 4 cardiorenal syndrome) is defined as the structural, functional, and electrophysiological remodeling of the heart in CKD. It is characterized by left ventricular hypertrophy (LVH) and fibrosis, diastolic and systolic dysfunction, capillary rarefaction, and enhanced susceptibility to further injuries, including acute myocardial infarction (AMI) and arrhythmias. Epidemiological and imaging studies proved that the primary manifestation of uremic cardiomyopathy is LVH, and its prevalence increases with the progression of CKD (stage G3: 31%, G4: 50%, G5 and ESRD: 90%, respectively). The early phase of uremic cardiomyopathy often presents as diastolic dysfunction with LVH. Later, severe cardiac fibrosis and systolic dysfunction develop in uremic cardiomyopathy. Macrovascular disease seems to be more important in the early stages of CKD, and microvascular injury could play a major role in the late stages of CKD. Therefore, AMI is a common cause of death in the early stages of CKD. In contrast, ESRD patients are more prone

to sudden cardiac death due to arrhythmias and heart failure related to LVH, coronary calcification, and electrolyte disturbances.

Both preclinical and clinical studies proved that factors related to CKD (including uremic toxins and renal anemia) provoke the development of LVH beyond the shared risk factors in CKD and CVDs (such as pressure and volume overload with over-activation of the renin-angiotensin-aldosterone system [RAAS] and sympathetic nervous system, hypertension, endothelial injury, inflammation, and increased nitro-oxidative stress). However, the precise molecular mechanisms in the development of uremic cardiomyopathy are still unclear. The severity and persistence of LVH are strongly associated with cardiovascular events and mortality risk in CKD and ESRD patients. Despite the broad availability of standard medications to control the underlying diseases, including hypertension, diabetes mellitus, and hyperlipidemia, cardiovascular morbidity and mortality among CKD patients are still high. Therefore, discovering specific, novel mechanisms in the development of uremic cardiomyopathy and LVH is needed to identify novel therapeutic targets for reducing the burden of CVDs in CKD.

Endogenous microRNAs (miR) are short (approximately 22 bp), non-coding RNA species that are post-transcriptional regulators targeting specific mRNAs, resulting in the suppression of protein synthesis or increased mRNA degradation via complementary binding, thus influencing cellular function. Therefore, miRs have been described as “master switches” in cardiovascular biology. The dysregulation of specific miRs has been implicated as a key pathological factor in many CVDs. The miR-212/132 cluster was identified as a central regulator of the development of pressure-overload-induced LVH and heart failure via the repression of the anti-hypertrophic transcription factor forkhead box protein O3 (FOXO3). Moreover, the overexpression of miR-212 separately from miR-132 was reported to play a role in the development of LVH and heart failure via fetal gene reprogramming in human hearts. Furthermore, the hypertrophic potential of miR-212 was also confirmed in primary neonatal rat cardiomyocytes. These include, for instance, the extracellular signal-regulated kinase 2 (ERK2) and adenosine monophosphate (AMP)-activated protein kinase (AMPK). So far, no literature has been available on the cardiac expression of miR-212 and its cardiac hypertrophy- and fibrosis-associated targets in uremic cardiomyopathy.

One of the most powerful endogenous adaptive mechanisms of the heart is ischemic preconditioning (IPRE), in which short cycles of myocardial ischemia and reperfusion (I/R) periods significantly enhance the ability of the heart to withstand a subsequent prolonged ischemic injury (*i.e.*, AMI). Preinfarction angina, warm-up phenomenon, and transluminal coronary angioplasty are considered clinical equivalents of IPRE. Although IPRE confers

remarkable cardioprotection in various species, including humans, we and others have shown in preclinical and clinical studies that its effectiveness is attenuated by aging and some co-morbidities, such as hypercholesterolemia and diabetes mellitus. However, some preclinical studies on CKD using male animals suggest that despite the complex systemic metabolic changes in CKD, cardioprotection by IPRE is still preserved. It has been reported that IPRE confers its cardioprotective effect via the reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) pathways after 4 weeks of 5/6 nephrectomy or adenine-enriched diet-induced subacute renal failure in male rats. However, experimental models of short-term renal failure may not correctly reflect the clinical situation because CKD frequently remains undiagnosed for a long time. Our research group found that IPRE still reduces the infarct size in prolonged uremia in male rats 30 weeks after 5/6 nephrectomy. Nevertheless, there is no experimental data on ischemia/reperfusion (I/R) injury or the effects of IPRE in CKD in females.

2. Aims of the thesis

In the present thesis, our aims were to

i) investigate the potential role of miR-212 and

ii) its selected targets associated with LVH and fibrosis (*i.e.*, FOXO3 and ERK2) in the development of uremic cardiomyopathy in male rats,

iii) compare the severity of uremic cardiomyopathy, I/R injury, and the potential cardioprotective effects of IPRE in CKD in male and female rats,

iv) furthermore, to investigate the potential role of the RISK and SAFE pathways in the cardioprotection conferred by IPRE in our experimental CKD model in both sexes.

3. Materials and methods

This thesis is based on two different studies. The first one investigated the role of miR-212 and its selected targets, FOXO3 and ERK2, in the development of LVH and fibrosis in CKD in male rats. This thesis will refer to the first study as the miR-212 study. The second study investigated the severity of uremic cardiomyopathy, I/R injury, the cardioprotection conferred by IPRE, and the possible role of RISK and SAFE pathways in IPRE in rats of both sexes in CKD. This second study will be referred to as the sex differences study in this thesis.

3.1. Ethics approval

This investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996) and was approved by the Animal Research Ethics Committee of Csongrád County (XV.1181/2013-2018) and the University of Szeged (MÁB-I-74-2017) in Hungary. All institutional and national guidelines for the care and use of laboratory animals were followed.

3.2. Animals

In the miR-212 study, 66 adult male Wistar rats (250–300 g) were used. 30 animals underwent a sham operation, and 36 animals received 5/6 nephrectomy to induce CKD. In the sex differences study, 196 age-matched female (n=110, 9 weeks old, 180–200 g) and male (n=86, 9 weeks old, 300–350 g) Wistar rats were used. 90 animals (46 males and 44 females) underwent a sham operation, and 106 (40 males and 66 females) received 5/6 nephrectomy to induce CKD.

3.3. Partial (5/6) nephrectomy

Experimental CKD was induced by 5/6 nephrectomy in two phases after pentobarbital anesthesia. First, the 1/3 left kidney on both poles was excised, and one week later, the right kidney was removed. During sham operations, only the renal capsules were removed. After both surgeries, povidone-iodine was applied on the skin's surface, and as a post-operative medication, nalbuphine hydrochloride analgesics and enrofloxacin antibiotics were administered for 4 days.

3.4. Transthoracic echocardiography

Cardiac morphology and function were assessed by transthoracic echocardiography at weeks -1, 4, and 8 in the miR-212 study and week 8 in the sex differences study. Rats were anesthetized with 2% isoflurane, and 2D, M-mode, Doppler, tissue Doppler, and four chamber-view images were performed by the criteria of the American Society of Echocardiography with a Vivid7 (miR-212 study) or a Vivid IQ (sex differences study) ultrasound system (General Electric Medical Systems, New York, NY, USA) using phased array transducers (5.5-12 MHz, GE 10S probe, miR-212 study or 5.0–11 MHz, GE 12S-RS probe, sex differences study). An experienced investigator blindly analyzed data from three consecutive heart cycles. The mean values of three measurements were calculated and used for statistical evaluation.

3.5. Serum and urine laboratory parameters

Blood was collected from the saphenous vein (weeks -1 and 4 in the miR-212 study) and thoracic aorta at week 9 to measure serum parameters. The animals were placed into metabolic cages for 24 h at week 8 to measure urine creatinine and protein levels. Serum urea and creatinine levels were quantified by the kinetic UV method using urease and glutamate dehydrogenase enzymes and Jaffe's method, respectively. Creatinine clearance, an indicator of renal function, was calculated according to the standard formula (urine creatinine concentration [μM]×urine volume for 24 h [mL])/(serum creatinine concentration [μM]×24×60 min) as described previously. The reagents and the platform analyzers were from Roche Diagnostics.

3.6. Ex vivo cardiac perfusions and tissue harvesting

In the miR-212 study, hearts were isolated and perfused for 5 minutes with oxygenated Krebs-Henseleit solution according to Langendorff, as described previously. Then the hearts were weighed, left and right ventricles were separated, and a cross-section of the left ventricle at the ring of the papillae was cut and fixed in 10% buffered formalin for histological analysis. Other parts of the left ventricles were freshly frozen and stored at −80 °C until further biochemical measurements. *In the sex differences study*, isolated hearts were subjected to either a non-conditioning or preconditioning perfusion protocol, respectively. Non-conditioned hearts were subjected to time-matched (45 min) aerobic perfusion followed by 35-min global ischemia. Preconditioned hearts were subjected to 5 min of ischemia and 5 min reperfusion in 3 intermittent cycles before the 35 min of global index ischemia. At the end of the two-hour reperfusion, the hearts were weighed and used for infarct size or biochemical measurements.

3.7. Infarct size determination in the sex differences study

After the end of reperfusions, in a subgroup of hearts, atria were removed, and the total ventricles were used to determine the infarcted area. Briefly, frozen ventricles were cut into 7-8 equal slices and placed into a triphenyl-tetrazolium chloride solution, followed by formaldehyde fixation and phosphate buffer washing steps. As a result, surviving areas were red-stained, while the necrotic area remained pale. Digitalized images from the stained heart slices were evaluated with planimetry, and the myocardial necrosis was expressed as infarct size/area at risk %.

3.8. Determination of the menstrual cycle in female rats

The menstrual cycle of the female animals was tested before heart perfusion protocols because the fluctuation of estrogen and progesterone levels may influence the infarct size and protein expression levels. We aimed to select the female rats in the di-estrus phase, in which the hormone levels are the lowest. The menstrual cycle was determined 12 h before the heart perfusions. Vaginal smear was put on glass slides, stained by Giemsa solution, and examined by light microscopy under 40× magnification. The amount of vaginal epithelial cells, their morphology, and the presence of lymphocytes were evaluated.

3.9. Hematoxylin-eosin (HE) and picrosirius red/fast green (PSFG) staining

In both studies in a subgroup of the animals, 5-μm-thick sections from formalin-fixed paraffin-embedded tissue blocks taken transversely from the subvalvular areas of the left ventricles were stained with HE or PSFG. Histological sections were scanned with a Panoramic Midi II scanner (3DHistech, Budapest, Hungary), and digital images were captured at the magnification of 10×, 40×, and 100×. Cardiomyocyte perimeter was measured in 100 selected, longitudinally oriented, mononucleated cardiomyocytes on digital images from a single LV transverse slide. Cardiac fibrosis was assessed on PSFG slides with an in-house developed program.

3.10. Investigation of the left ventricular miR-212 expression by RT-qPCR

Quantitative RT-PCR was performed with miR-specific primers to monitor miR expression. RNA was isolated from the left ventricles. For quantitative detection of miR-212, TaqMan MicroRNA Reverse Transcription Kit, TaqMan miRNA-212 as well as snoRNA (U64702) Assays, and Absolute Blue qPCR Mix (Abgene, #AB-4136/B) were used according to the manufacturer's instructions. SnoRNA U64702 was used as a control for normalization.

3.11. mRNA expression profiling by RT-qPCR

In the miR-212 study, RNA was isolated using Qiagen RNeasy Fibrous Tissue Mini Kit from heart tissue. Briefly, total RNA was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit, and specific primers for *Foxo3* and FastStart Essential DNA Green Master were used according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), hypoxanthine phosphoribosyl transferase 1 (*Hprt1*), peptidyl prolyl isomerase A (*Ppia*), and ribosomal protein lateral stalk subunit P2 (*Rplp2*) were used as controls for normalization. *In the sex differences study*, total RNA was isolated from left ventricles with 5:1 mixture of Biozol total RNA extraction reagent and chloroform. The RNA containing the

aqueous phase was further precipitated with isopropanol. Then 100 μg of total RNA was reverse transcribed using iScript™ cDNA Synthesis Kit. Specific primers (*Nppa*: A-type natriuretic peptide and *Nppb*: B-type natriuretic peptide) and SsoAdvanced™ Universal SYBR® Green Supermix were used according to the manufacturer's instructions. Peptidyl-prolyl isomerase A (*Ppia*) was a housekeeping control gene for normalization.

3.12. Western blot

In the miR-212 study, a standard Western blot technique was used for the total and phosphorylated (p) forms of FOXO3, protein kinase B (AKT), extracellular signal-regulated kinase 1/2 (ERK1/2) with GAPDH loading background. *In the sex differences study*, total and phosphorylated (p) protein levels of AKT, ERK1/2, and signal transducer and activator of transcription 3 (STAT3) with GAPDH loading background were investigated. In both studies, left ventricular tissue samples were homogenized, and after quantifying the supernatants' protein concentrations, sodium dodecyl-sulfate polyacrylamide gel electrophoresis was performed, followed by the transfer of proteins onto a nitrocellulose membrane. Membranes were blocked and then incubated with primary antibodies overnight. *In the miR-212 study*, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody. After assessing phosphorylated proteins, the membranes were stripped and reassessed for the total proteins. An enhanced chemiluminescence kit was used to develop the membranes. The chemiluminescence signals were analyzed and evaluated by Quantity One Software. *In the sex differences study*, membranes were incubated with IRDye® 800CW Goat Anti-Rabbit and IRDye® 680RD Goat Anti-Mouse secondary antibody (Li-Cor) for 1 h at room temperature in 5% BSA. Fluorescent signals were detected by the Odyssey CLx machine, and digital images were analyzed and evaluated by Quantity One Software.

3.13. Statistical analysis

Statistical analysis was performed using Sigmaplot 12.0 for Windows (Systat Software Inc). All values are presented as mean±SEM. $P<0.05$ was accepted as a statistically significant difference. *In the miR-212 study*, two-sample t-test (in case of normal distribution of the data) or Mann Whitney U test (in case of non-normal distribution of the data) was used to determine the effect of CKD on all measured parameters within each time point. *In the sex differences study*, baseline and different follow-up data, including serum metabolite concentrations and echocardiographic, histologic, and RT-qPCR data, were compared using Two-Way Analysis of

Variance (ANOVA). Infarct size and Western blot measurement data were compared using Three-Way ANOVA.

4. Results

4.1. Results of the miR-212 study

4.1.1. The development of CKD and uremic cardiomyopathy

The serum carbamide and creatinine levels were significantly increased at week 4 and at the endpoint in the 5/6 nephrectomized rats as compared to the baseline values or the values of the sham animals at each time point representing continuously worsening renal function and the development of CKD. Likewise, in the 5/6 nephrectomized rats, urine protein concentration was significantly increased, showing impaired glomerular function at the endpoint. Accordingly, creatinine clearance was significantly decreased in the 5/6 nephrectomized rats both at weeks 4 and 8 compared to the sham rats showing the development of CKD. By contrast, creatinine clearance was significantly increased in the sham animals at the endpoint compared to the baseline values due to the normal growth of healthy animals.

Transthoracic echocardiography showed no difference at week -1 in the measured parameters between the two groups. At week 4, the anterior and septal diastolic wall thicknesses were significantly increased in the CKD group compared to the sham group or baseline values showing starting LVH with mild diastolic dysfunction. At week 8, left ventricular wall thicknesses, including anterior and septal walls in systole and diastole, were significantly increased in CKD rats compared to the sham group and the baseline values, pointing out the presence of LVH. The ejection fraction (EF) remained unchanged in the CKD rats compared to the sham rats or the baseline values both at weeks 4 and 8. The ratio of the early flow velocity (E) and the septal mitral annulus velocity (e′) significantly increased in CKD rats at week 8, indicating diastolic dysfunction. At autopsy, heart weight and heart weight to body weight ratio were significantly increased in CKD animals compared to the controls indicating macroscopic signs of hypertrophy. The weight of the whole left kidney in the sham group was smaller than the remaining one-third of the left kidney in the CKD group, suggesting marked renal hypertrophy in the CKD animals.

Cardiomyocyte diameters verified the development of LVH seen on echocardiographic images and at autopsy. Cross-sectional cardiomyocyte diameters were significantly increased in the CKD group compared to the sham group proving the presence of LVH at the cellular level.

Significant cardiac fibrosis was found with interstitial collagen deposition in the CKD group compared to the sham group.

4.1.2. The left ventricular expression of miR-212 and its targets at the mRNA and protein levels in uremic cardiomyopathy

Left ventricular expression of miR-212 was significantly increased in CKD compared to the sham group. The left ventricular FOXO3 level failed to decrease at the mRNA and protein levels, and the pFOXO3 level and the pFOXO3/FOXO3 ratio did not increase in CKD compared to the sham group. The protein level of AKT did not differ between the two groups. However, the expression of the pAKT showed an increasing tendency, and pAKT/total AKT ratio significantly increased in the CKD group compared to the sham group. In contrast, ERK1 and ERK2 levels, pERK1 and pERK2 levels, pERK1/ERK1 ratio, and pERK2/ERK2 ratio failed to change significantly in CKD compared to the sham group.

4.2. Results of the sex differences study

4.2.1. The effect of sex on the development of CKD and uremic cardiomyopathy

The serum urea and creatinine levels were similar in the sham males and females but showed a significant increase in both sexes in 5/6-nephrectomized rats compared to the sham groups. The serum creatinine levels were significantly higher in male and female 5/6-nephrectomized rats than in the sex-matched sham animals. The urine protein concentration was significantly lower in sham females than in males. Urine protein concentration was significantly increased in male and female 5/6-nephrectomized rats compared to their sex-matched controls.

Transthoracic echocardiography showed that most left ventricular wall thicknesses were not significantly different between males and females in the sham groups. Only the diastolic anterior and septal wall thickness were significantly decreased in the sham females compared to sham males due to their smaller heart size. The left ventricular end-diastolic and end-systolic diameters were significantly reduced in sham females compared to sham males. In response to CKD, systolic and diastolic anterior wall thicknesses were significantly increased in both sexes compared to the sex-matched sham animals. In CKD males, diastolic inferior, posterior, septal, and systolic septal wall thicknesses were significantly increased compared to those of sham males. In contrast, CKD females showed a smaller relative increase in fewer wall thicknesses than CKD males. In CKD females, diastolic inferior, diastolic and systolic septal wall thicknesses were significantly increased compared to sham females. Left ventricular end-

diastolic and end-systolic diameters showed no significant difference in response to CKD in either sex. However, left ventricular end-systolic diameter showed a trend toward a decrease in males in response to CKD, probably due to the more severe LVH. Left ventricular end-diastolic volume, stroke volume, and cardiac output were significantly decreased in CKD males compared to sham males. In contrast, these parameters were not changed significantly in CKD females compared to sham females. The main systolic functional parameter, EF did not change significantly between the groups. The E and e' velocities were significantly increased in sham and CKD females compared to sham and CKD males, respectively. The E velocity did not change significantly in response to CKD in either sex. In contrast, e' was significantly reduced in response to CKD in both sexes, pointing out the presence of diastolic dysfunction in CKD.
Both males and females had significantly increased cardiomyocyte perimeters in CKD compared to the sex-matched sham groups, proving the development of cardiomyocyte hypertrophy at the microscopic level. There was no significant sex-based difference in the cardiomyocyte perimeters within the CKD or sham groups. The male CKD group demonstrated significantly increased left ventricular collagen content compared to the male sham group, proving the development of cardiac fibrosis in CKD. There was no difference in the collagen content between males and females in the sham groups.

4.2.2. Cardioprotective effects of IPRE and the role of the SAFE and RISK pathways in uremic cardiomyopathy

IPRE significantly decreased infarct size in sham-operated and CKD males; however, the presence of CKD did not significantly influence infarct size in either sex. Notably, the infarct size was significantly smaller in both sham-operated and CKD females in the I/R subgroups compared to sham-operated and CKD males in the I/R subgroups, indicating a marked cardioprotective effect of the female sex irrespective of CKD.
Total proteins and their phosphorylated forms related to the cardioprotective SAFE (STAT3) and RISK (AKT, ERK1/2) pathways were measured by Western blot. IPRE significantly increased the pSTAT3/STAT3 ratio in sham males and females compared to sex-matched sham I/R subgroups, respectively. IPRE failed to significantly change the pSTAT3/STAT3 ratio in CKD males and females compared to sex-matched CKD I/R subgroups, respectively. In the female sham I/R and preconditioned subgroups, the pSTAT3/STAT3 ratio was significantly lower than in the sham male I/R and preconditioned sub-groups, respectively. CKD abolished these lowering effects of the female sex on the pSTAT3/STAT3 ratio in the I/R and

preconditioned subgroups compared to male I/R and preconditioned CKD subgroups, respectively. Interestingly, the pSTAT3/STAT3 ratio was significantly higher in CKD males and females in the I/R subgroups compared to the sham sex-matched I/R subgroups, respectively. This increasing effect of CKD on the pSTAT3/STAT3 ratio was not detectable in the preconditioned hearts as compared to sham preconditioned subgroups in both sexes. In pAKT/AKT and pERK1/2/ERK1/2 ratios, there were no significant differences between the groups.

5. Conclusions

From our results, we can conclude that:

i) LVH and fibrosis are accompanied by characteristic left ventricular overexpression of miR-212 in our model of CKD in male rats.

ii) In contrast, the expressions of the selected hypertrophy- and fibrosis-associated miR-212 targets (*i.e.*, FOXO3 and ERK2) failed to change in uremic cardiomyopathy. Therefore, the molecular mechanism of the development of LVH and fibrosis in CKD seems distinct from other cardiac hypertrophy and pathological remodeling forms.

iii) There was no difference in the severity of CKD between male and female rats based on serum urea and creatinine levels as well as creatinine clearance.

iv) As compared to females, males developed more severe uremic cardiomyopathy characterized by left ventricular hypertrophy and fibrosis in CKD based on echocardiography and histology.

v) Both in the sham and CKD groups, the infarct size was significantly smaller in females than in males.

vi) The infarct size-limiting effect of IPRE was preserved in both sexes in CKD despite the more severe uremic cardiomyopathy in male CKD rats.

vii) In both sexes, IPRE significantly increased the pSTAT3/STAT3 ratio in the sham but not in the CKD groups. Therefore, further research is needed to identify the key molecular mechanisms in the cardioprotective effect conferred by IPRE in CKD.

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8. List of abbreviations

AKT: protein kinase B
AMI: acute myocardial infarction
AMPK: adenosine monophosphate-activated protein kinase
ANOVA: analysis of variance
BNP: B-type natriuretic peptide
CKD: chronic kidney disease
CVD: cardiovascular disease
EF: ejection fraction
ERK1/2: extracellular signal-regulated kinase 1/2
ESRD: end-stage renal disease
FOXO3: forkhead box O3
GAPDH: glyceraldehyde 3-phosphate dehydrogenase
GFR: glomerular filtration rate
HE: hematoxylin-eosin
I/R: ischemia/reperfusion
IPRE: ischemic preconditioning
LVH: left ventricular hypertrophy
miR: microRNA
PSFG: picrosirius red and fast green
RISK: reperfusion-induced salvage kinase
RNA: ribonucleic acid
RT-qPCR: real-time quantitative polymerase chain reaction
SAFE: survivor activating factor enhancement
STAT3: signal transducers and activators of transcription 3