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miR-21 Contributes to Xenon-conferred Amelioration of Renal Ischemia–Reperfusion Injury in Mice

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Abstract

Background—MicroRNAs participate in the regulation of numerous physiological and disease processes. The *in vivo* role of microRNAs in anesthetics-conferred organoprotection is unknown.

Methods—Mice were exposed for 2 h to either 70% xenon, or 70% nitrogen, 24 h before the induction of renal ischemia-reperfusion injury. The role of microRNA, miR-21, in renal protection conferred by the delayed xenon preconditioning was examined using *in vivo* knockdown of miR-21 and analysis of miR-21 target pathways.

Results—Xenon preconditioning provided morphologic and functional protection against renal ischemia-reperfusion injury (n = 6), characterized by attenuation of renal tubular damage, apoptosis, and oxidative stress. Xenon preconditioning significantly increased the expression of miR-21 in the mouse kidney. A locked nucleic acid-modified anti-miR-21, given before xenon preconditioning, knocked down miR-21 effectively, and exacerbated subsequent renal ischemia-reperfusion injury. Mice treated with anti-miR-21 and ischemia-reperfusion injury showed significantly higher serum creatinine than antisense oligonucleotides-treated mice, 24 h after ischemia-reperfusion (1.37 ± 0.28 vs. 0.81 ± 0.14 mg/dl; n = 5; $P < 0.05$). Knockdown of miR-21 induced significant up-regulation of programmed cell death protein 4 and phosphatase and tensin homolog deleted on chromosome 10, two proapoptotic target effectors of miR-21, and resulted in significant down-regulation of phosphorylated protein kinase B and increased tubular cell apoptosis. In addition, xenon preconditioning up-regulated hypoxia-inducible factor-1 α and its

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downstream effector vascular endothelial growth factor in a time-dependent manner. Knockdown of miR-21 resulted in a significant decrease of hypoxia-inducible factor-1 α .

Conclusions—These results indicate that miR-21 contributes to the renoprotective effect of xenon preconditioning.

The kidney is highly sensitive to ischemia-reperfusion injury (IRI), which causes renal tubular necrosis, apoptosis, oxidative stress, and inflammation, frequently leading to acute and chronic kidney dysfunction.^{1,2} Renal IRI occurs frequently during the perioperative period, such as cardiac surgery, vascular surgery, or kidney transplantation,^{3–5} due to temporary decrease or cessation of renal blood supply. Renal IRI often results in significant morbidity and mortality.⁶ Current strategies to reduce renal IRI are inadequate and novel therapies are needed.

Preconditioning triggered by ischemic stimulus or pharmacologic agents can protect an organ against IRI. Preconditioning induced by volatile anesthetics, such as isoflurane confers significant protection against renal IRI.^{7,8} The noble gas xenon (Xe) has many properties of an ideal anesthetic.^{9–12} A series of investigations has shown that xenon exhibits organoprotective properties against IRI in the heart, brain, and kidney.^{13–16} Particularly, Ma *et al.*¹⁶ demonstrated that xenon could induce the activation of hypoxia-inducible factor-1 α (HIF-1 α) and provide preconditioning effects on ischemic renal injury in mice. The preconditioning ability could revive the clinical use of xenon as an anesthetic, despite concerns about its cost. It is, therefore, important to fully understand the mechanism underlying xenon preconditioning.

MicroRNAs (miRNAs) are endogenous, small RNA molecules of about 22 nucleotides in length and are involved in regulating gene expression, primarily at the posttranscriptional level. MiRNAs play a ubiquitous role in many vital biological processes, such as development, cell differentiation, proliferation, and apoptosis, and additional action of miRNAs in pain was found recently.¹⁷ Several miRNAs including miR-192,^{18–20} miR-382,^{21,22} and miR-29^{23,24} are involved in the development of kidney injury. miR-21 has been shown to promote proliferation, inhibit apoptosis, regulate oxidative stress, and may play a critical role in the pathogenesis of kidney injury and tissue repair process.^{25–28} We previously showed that up-regulation of miR-21 induced by delayed ischemic preconditioning had a protective effect against renal IRI.²⁹

It is not known whether miRNAs play any role in anesthetic preconditioning in the kidney or the cardiovascular system. We hypothesize that xenon preconditioning could protect against renal IRI, in part, *via* up-regulation of miR-21. Using a mouse model, we investigated the effect of xenon preconditioning on the outcome of renal IRI and on the expression of miR-21. With *in vivo* knockdown of miR-21, we further determined the role of miR-21 and its downstream targets, programmed cell death protein 4 (PDCD4) and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), in the effect of xenon preconditioning on renal IRI.

Materials and Methods

Animals

Experiments were performed on 10-week-old male C57BL/6J mice (Animal Center of Fudan University, Shanghai, China), weighing 20–25 g, housed in temperature- and humidity-controlled cages, with free access to water and rodent food, and a 12-h light/dark cycle. All protocols were approved by the Institutional Animal Care and Use Committee of Fudan University, and adhered strictly to the NIH Guide for the Care and Use of Laboratory Animals. All surgery was performed under sodium pentobarbital anesthesia, and efforts were made to minimize suffering and the number of animals used.

Mouse Model of Gas Exposure and IRI

Mice were exposed to either 70% xenon or 70% nitrogen (N₂) balanced with 30% oxygen for 2 h through a close-loop ventilation system containing a reservoir bag, in which oxygen and xenon or N₂ were mixed and delivered.¹⁶ Gas mixture was delivered into a cage, in which a layer of sodalime and silica gel were placed, to control the carbon dioxide levels and humidity. Twenty-four hours after gas exposure, mice were anesthetized with intraperitoneal sodium pentobarbital (80 mg/kg), and renal IRI was induced by bilateral renal pedicle clamping for 30 min. Sham-operated mice underwent the same surgical procedures but with no occlusion of the renal pedicle. Intrarectal temperature of mice was maintained at 36.5°–37.0°C with a heating pad. The blood samples and kidneys in separate groups of mice were harvested at 12, 24, and 48 h after the surgery.

In a separate experiment, kidneys from additional cohorts of mice were harvested 24 h after gas exposure without the subsequent IRI.

In Vivo Knockdown of miR-21 Using Locked Nucleic Acid-modified Anti-miR

Locked nucleic acid (LNA)-modified antiscrambled or anti-miR-21 oligonucleotides (Exiqon, Woburn, MA) were diluted in saline (5 mg/ml), and administered through intraperitoneal injection (10 mg/kg) within 30 min before xenon exposure, similar to our previous study.²⁹

Assessment of Serum Creatinine and Blood Urea Nitrogen Levels

Serum creatinine and blood urea nitrogen levels were determined in 100-μl serum with an automated analyzer (Vet test 8008; Idexx, Westbrook, ME).

Morphometric Analysis

Kidney slices were embedded in paraffin wax, sectioned at 4 μm and stained with hematoxylin and eosin by standard methods. Histopathological scoring was performed in a blinded manner by a pathologist unaware of the experimental protocol, according to the severity of tubular injury on a semiquantitative scale: no injury (0), mild: less than 25% (1), moderate: less than 50% (2), severe: less than 75%, and (3) very severe: more than 75% (4).³⁰ At least 10 consecutive fields in the corticomedullary junction and outer medulla were examined under ×400 magnification.

TUNEL Staining

We assessed tubular cell apoptosis with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method by using a commercially available *in situ* cell death detection kit (In situ Cell Death Detection kit, Peroxidase; Roche, Mannheim, Germany), according to the manufacturer's protocol. The number of TUNEL-positive cells from 10 areas of randomly selected renal cortex was counted under a light microscope.

Immunohistochemistry

Immunohistochemical staining was performed in 4- μ m paraffinized sections. Briefly, after being dewaxed and dehydrated, the sections were incubated with 3% H₂O₂ to eliminate endogenous peroxidase activity, treated with normal goat serum, and then incubated with primary antibodies against CD68 (ED-1, mouse monoclonal, 1:200; Abcam, Cambridge, MA) or tumor necrosis factor- α (rabbit polyclonal, 1:2,000; Abcam) overnight at 4°C. After being washed, the sections were incubated with horseradish peroxidase-conjugated secondary antibody (anti-rabbit IgG), stained with 3,3'-diaminobenzidine (Sigma, Saint Louis, MO), and counterstained with hematoxylin, then all slides were evaluated under light microscopy. Tumor necrosis factor- α staining was scored according to the following scale: no staining (0), mild staining: less than 25% (1), moderate staining: less than 50% (2), extensive staining: more than 50% (3). At least 10 consecutive fields per section were examined under $\times 400$ magnification.

Examination of Lipid Peroxide

Malondialdehyde was detected using supernatant of the renal cortical homogenate, according to the manufacturer's protocol (TBARS Assay Kit; Cayman Chemical Company, Ann Arbor, MI).

Real-time Reverse Transcription-Polymerase Chain Reaction

Total RNA from harvested kidney tissue was extracted using Trizol (Invitrogen, Carlsbad, CA), followed by quantification. Extracted RNA was reverse-transcribed to complementary DNA (PrimeScript[®] RT reagent Kit; TaKaRa, Kyoto, Japan), and the complementary DNA product was used for real-time polymerase chain reaction (PCR) (SYBR[®] Premix Ex Taq[™] TaKaRa). PCR primers (Sangon, Shanghai, China) were designed with sequences as follows: PDCD4 forward: 5'-ACCCTGACAATTTAAGCGACTCTC-3', reverse: 5'-CTTTTGGCTTTTGCATTGATTCTA-3'. PTEN forward: 5'-GGTCTGCCAGCTAAAGGTGAAG-3', reverse: 5'-AGGCAATGGCTGAGGGAACT-3'. β -actin forward: 5'-GATTACTGCCCTGGCTCCTA-3', reverse: 5'-TCATCGTACTCCTGCTTGCT-3'. Expression level of miR-21 was quantified using real-time reverse transcription-PCR with the Taqman chemistry (Applied Biosystems, Hayward, CA), as described previously.²³ 5S ribosomal RNA and β -actin mRNA were used as endogenous control for miRNAs and mRNAs, respectively. The relative gene expressions were calculated in accordance with the 2^{-C_t} method. Relative mRNA levels were expressed as 2^{-C_t} (Tn) and ratios to control.

Protein Isolation and Western Blot Assay

The dissected renal tissues were homogenized in icecold lysis buffer, including 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and protease inhibitors (25 mM Na-HEPES, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride). After being centrifuged at 12,000g for 15 min at 4°C, the supernatant was collected, and protein concentrations were determined. Samples (50 µg per Lane) were loaded and separated on a sodium dodecyl sulfate-polyacrylamide gel, and then transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% nonfat milk and incubated with the primary antibodies against PDCD4 (rabbit polyclonal 1:1,000; Novus, Littleton, CO), PTEN (rabbit monoclonal 1:500; Abcam), total AKT (rabbit monoclonal 1:500; Cell Signaling Technology, Danvers, MA), phospho-AKT (rabbit monoclonal 1:2,000; Cell Signaling Technology), HIF-1α (rabbit polyclonal 1:500; Bioworld, Saint Louis, MO), and vascular endothelial growth factor (polyclonal 1:1,000; Abcam) overnight at 4°C, then incubated with horseradish peroxidase-conjugated secondary antibodies, and developed by chemiluminescent Horseradish Peroxidase Substrate (Millipore, Billerica, MA). Results were normalized with glyceraldehyde-3-phosphate dehydrogenase and expressed as ratios to control.

Statistical Analysis

Statistical analysis was performed using the statistical software SPSS Version 16.0 (SPSS Inc., Chicago, IL). Data were presented as a mean ± SD for continuous variables. Multiple comparison was by ANOVA followed by *post hoc* Student and Newman-Keuls test where appropriate, and two-way ANOVA for time and treatment data. The values of score were presented as a class variable, analyzed by the Mann-Whitney or Kruskal-Wallis nonparametric test. All comparisons were two tailed, and a *P* value less than 0.05 was considered significant.

Results

Xenon Preconditioning Protected the Kidney from IRI

We demonstrated that a 2 h xenon preconditioning provided morphologic and functional renoprotection against renal IRI in mice, in a model of 30-min bilateral renal ischemia. Compared with the ischemia-reperfusion (IR) group and N₂ + IR group, mice in the Xe + IR group showed a significant decrease in serum creatinine (Scr) and blood urea nitrogen (fig. 1, A and B). Morphologic changes were assessed using histopathologic scoring of cortical tubular damage, which integrated tubular cell necrosis or swelling, tubular casts, brush border loss, and interstitial inflammatory cell infiltration. The histologic score in the IR group and N₂ pretreatment group was also significantly higher than that in the xenon pretreatment group (fig. 1, C and D).

TUNEL staining was used to analyze tubular cell apoptosis. Quantitatively, TUNEL-positive cells in the kidneys were significantly fewer in the xenon pretreatment group (4.8 ± 1.2 per high power field) than the IRI group (11.1 ± 2.3 per high power field) and N₂ pretreatment group (10.1 ± 1.2 per HPF; *P* < 0.05; fig. 1, E and F).

To determine the effect of xenon treatment on inflammatory response in the kidney, we examined the protein expression of a macrophage or monocyte marker (ED-1) and tumor necrosis factor- α , with immunohistochemical staining assay. Expression of both proteins was significantly higher in the three experimental groups than the sham group, but there were no significant differences between the experimental groups (fig. 1, G–J). This indicated that xenon preconditioning did not attenuate inflammation in renal IRI.

The *in vivo* lipid peroxidation status was examined by measuring malondialdehyde in the kidney, which is used as an indicator of oxidative stress injury.³¹ Thiobarbituric acid reactive substances assay was used to detect malondialdehyde content in renal tissues. We found that malondialdehyde concentration was significantly reduced in xenon pretreatment group compared with the IRI group and N₂ pretreatment group (fig. 1K).

miR-21 Contributed to Xenon Preconditioning of the Kidney

We measured expression of miR-21 by Taqman real-time reverse transcription-PCR, and found that compared with the absence of xenon preconditioning, xenon pretreatment significantly up-regulated miR-21 expression in mouse kidneys at 24 h after IRI (fig. 2A). Time-course analysis indicated that miR-21 was up-regulated 2 h after xenon exposure, and remained significantly higher 24 h after xenon preconditioning, and decreased to baseline levels at 48 h after xenon exposure (fig. 2B).

To examine the functional role of miR-21 in the renal protection conferred by xenon preconditioning, LNA-modified antisense or anti-miR-21 oligonucleotides were administered to mice through intraperitoneal injection before xenon exposure. As shown in figure 2, C and D, renal levels of miR-21 expression were substantially reduced in mice receiving LNA anti-miR-21 at 24 h after xenon exposure, or at 24 h after IRI, following xenon preconditioning ($P < 0.05$). We measured serum creatinine levels at 12-, 24-, and 48-h time points following the IRI. Mice treated with anti-miR-21 and xenon preconditioning showed significant increases in serum creatinine levels 24 and 48 h after IRI, whereas mice treated with the antisense oligonucleotides and xenon preconditioning showed clear renal protection against IRI (fig. 2E). Consistent with the Scr results, morphologic damages occurred in the kidneys of mice receiving anti-miR-21, including tubular cell necrosis or swelling, and tubular casts. Mice receiving the scrambled anti-miR only exhibited mild-to-moderate renal histologic damage (fig. 2, F and G). These results suggested that knockdown of miR-21 attenuated the renal protection conferred by xenon preconditioning.

Mechanisms Underlying the Role of miR-21

To determine the possible mechanisms mediating the protective role of miR-21 in xenon preconditioning, we measured PDCD4 and PTEN, two proapoptotic target effectors of miR-21, at mRNA and protein levels in Xe + IR mice receiving LNA anti-miR-21 or scrambled anti-miR. Quantitative real-time reverse transcription-PCR analysis showed that PDCD4 and PTEN mRNA expression was not significantly different between the two groups (fig. 3A). However, mice receiving LNA anti-miR-21 exhibited significantly higher protein expressions of PDCD4 and PTEN, compared with mice receiving scrambled anti-miR (fig. 3, B and C).

Activated protein kinase B (p-Akt) is a key activator of antiapoptotic survival to protect cells from IRI,³² and the activation of Akt is regulated by phosphatidylinositol 3-kinase (PI3K). PTEN is an antagonist of PI3K by degrading phosphatidylinositol-3,4,5-trisphosphate (PIP3) to phosphatidylinositol-4,5-bisphosphate (PIP2) and is a major negative regulator of the PI3K/Akt signaling pathway. We examined the abundance of Akt and p-Akt in mouse kidney, and found that mice receiving anti-miR-21 treatment showed a significant down-regulation of p-Akt when compared with mice receiving scrambled anti-miR treatment (fig. 3D). There was no significant difference in the abundance of total Akt between the two groups. The data indicated that knockdown of miR-21 inhibited the activation of Akt.

Concomitantly, mice receiving the anti-miR-21 treatment showed a substantial increase of apoptotic cells in kidneys compared with mice receiving the scrambled anti-miR (fig. 3, E and F). Collectively, these results suggested that miR-21 might contribute to xenon preconditioning in part *via* down-regulation of PDCD4 and PTEN, activation of Akt, and subsequent attenuation of tubular cell apoptosis.

Knockdown of miR-21 Led to a Reduction in HIF-1 α

It is well established that activation of the HIF-1 α pathway is organ protective against hypoxic or ischemic injury. We have shown previously that activation of HIF-1 α in renal epithelial cells up-regulates miR-21.²⁹ As shown in figure 4A, HIF-1 α levels gradually increased 2 h after xenon exposure, reached the peak at 24 h, and declined at 48 h. The protein level of vascular endothelial growth factor was consistent with the pattern of the HIF-1 α expression. The alteration of HIF-1 α expression paralleled the expression of miR-21 induced by xenon preconditioning. To test whether knockdown of miR-21 affects HIF-1 α expression, we measured HIF-1 α protein expression in Xe + IRI mice receiving LNA anti-miR-21 or scrambled anti-miR. Mice receiving scrambled anti-miR showed higher HIF-1 α protein expression in kidneys compared with mice receiving anti-miR-21 (fig. 4B). The data suggest that miR-21 contributes to the up-regulation of HIF-1 α following xenon preconditioning, probably forming a positive feedback loop.

Discussion

The current study shows that miRNAs contribute to anesthetic preconditioning *in vivo*. A growing body of evidence shows an organ protective effect against IRI *in vivo* by volatile anesthetics, such as isoflurane and xenon.^{7-9,16} miRNAs are known to be involved in the development of IRI in several organs and in the protective effect of ischemic preconditioning.^{25,26,29} Data obtained from cultured neuron-like cells suggest miR-203 might contribute to neuroprotection conferred by isoflurane preconditioning.³³ This study demonstrates xenon preconditioning provides renal protection against IRI by attenuation of renal tubular damage, apoptosis, and reducing lipid peroxidation estimated by malondialdehyde. The protective effect of xenon could be at least in part attributed to miR-21.

MiR-21 was highly up-regulated in rat kidneys following IRI,^{25,27} and urinary level of miR-21 increased 1.2-fold in patients with acute kidney injury. Our previous study showed delayed ischemic preconditioning increased the expression of miR-21 in mouse kidney and

contributed to renoprotection.²⁹ Yin *et al.*³⁴ demonstrated that injection of chemically synthesized exogenous miR-21 could protect the heart against IRI in mice. When mice were cotreated with antagomir-21, the miR-21-induced protection was abolished. In the current study, we demonstrated that miR-21 contributed to protection against renal IRI conferred by xenon preconditioning. In addition, miR-21 appears to contribute to the development of renal fibrosis.^{35,36} Taken together, these studies support an important role of miR-21 in the development of acute and chronic renal injury or the protection against it.

Acute kidney injury from IRI is associated with tubule cell apoptosis, and apoptosis may represent an important mechanism for the loss of tubule cells, following IRI.^{37,38} MiR-21, acting as a strong antiapoptotic factor,^{25,26} inversely regulates several proapoptotic target genes, including PDCD4³⁹ and PTEN.⁴⁰ PDCD4 is a critical mediator for cancer cell apoptosis. Recent studies have demonstrated that miR-21 inhibited cell apoptosis by down-regulating PDCD4 in cultured cardiac myocytes,²⁶ in cultured mouse tubular epithelial cells,²⁵ and in mouse kidneys.²⁹

PTEN is an antagonist of PI3K by removing the 3'-phosphate from PIP3, and the activation of Akt is regulated by PI3K. As an antiapoptotic protein kinase,^{41,42} activated Akt (p-AKT) phosphorylates Bad (a proapoptotic Bcl-2 family protein), resulting in tolerance to cellular apoptotic signals.⁴³ PTEN is a major negative regulator of the PI3K/AKT signaling pathway. Sayed *et al.*⁴⁴ found overexpression of miR-21 in a transgenic mouse heart resulted in a decrease of PTEN and FasL expression, an increase in p-Akt, and inhibition of apoptosis. Overexpression of miR-21 also led to the activation of Akt in human tumor cells.⁴⁵ Jang *et al.*³² demonstrated that ischemic preconditioning increased the levels of phosphorylated Akt and Bad in mouse kidney, and the activation of Akt signaling pathway contributed to renal resistance to IRI-induced apoptosis. Our data suggest that knockdown of miR-21 in mouse kidney induces significant up-regulation of PDCD4 and PTEN, and results in down-regulation of p-Akt, leading to an increase of tubular cell apoptosis and exacerbation of renal IRI (fig. 5).

Recent studies have demonstrated that xenon could induce the activation of HIF-1 α and its target gene responses, which is associated with the preconditioning effect of xenon on hypoxic or ischemic renal injury.^{16,46} Ma *et al.*¹⁶ showed xenon preconditioning increased expression of HIF-1 α and its downstream effectors through the mammalian target of rapamycin pathway. In the current study, we also found xenon preconditioning induced up-regulation of HIF-1 α in a time-dependent manner. The alteration of HIF-1 α expression paralleled the pattern of miR-21 expression, and knockdown of miR-21 resulted in down-regulation of HIF-1 α . It suggests the presence of a positive feedback loop between HIF-1 α activation and miR-21 expression, in which HIF-1 α activation up-regulates miR-21, as we demonstrated previously,²⁹ and miR-21 further up-regulates HIF-1 α (fig. 5). It remains to be determined how miR-21 feeds back to affect HIF-1 α in the kidney and how much of the effect of miR-21 on IRI is mediated by its effect on HIF-1 α . Liu *et al.*⁴⁵ demonstrated that miR-21 enhanced HIF-1 α and vascular endothelial growth factor expression through targeting PTEN, activating Akt and ERK1/2 signaling pathways in tumor, and HIF-1 α is a key downstream target of miR-21 in regulating tumor angiogenesis.

The current study had several limitations. The study was performed in mice with complete interruption of renal blood flow. Clinically, it often involves a reduction (not complete absence) in renal blood flow. Clinical studies will be needed to examine whether the findings of the current study can be extrapolated to humans. It has been reported that anesthetics regulate the expression of several miRNAs, and the effect may be specific for different anesthetics.⁴⁷ It remains to be determined whether miRNAs, other than miR-21, contribute to any functional effects of anesthetics. In addition, the functional data were obtained up to 48 h after IR in the current study, which showed clear renoprotective effects. However, it remains to be determined how long the effect of xenon can last.

In conclusion, our results suggest that xenon preconditioning is an efficient inducer of miR-21, which in turn provides a renal protective effect against IRI by inhibiting apoptosis *via* its proapoptotic targets PDCD4, PTEN, and the Akt signaling pathway. A better understanding of the molecular mechanisms that mediate renoprotection by xenon preconditioning might help to facilitate the application of this inhalational anesthetic for organoprotective purpose in patients at high risk of IRI.

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What We Already Know about This Topic

- MicroRNAs are endogenous small RNA molecules and participate in the regulation of numerous physiological and physiopathological processes, but their role in anesthetic-induced preconditioning remains unknown

What This Article Tells Us That Is New

- In mice, miR-21 contributes to the renoprotective effect (attenuation of tubular damage, apoptosis, and lipid peroxidation) of xenon *in vivo* by inhibiting apoptosis and the Akt signaling pathway

Fig. 1.

Xenon (Xe) preconditioning protected mouse kidneys from ischemia-reperfusion (IR) injury and attenuated tubular cell damage, apoptosis, and oxidative stress, but not inflammation.

(A) Serum creatinine concentration. Mice were exposed to xenon or nitrogen (N₂) control for 2 h. IR injury surgery was performed 24 h after the gas exposure, and serum creatinine was measured 24 h after IR injury. (B) Blood urea nitrogen (BUN) concentration. (C and D) Tubular cell injury and quantification of histologic scoring 24 h after IR injury. Kidney sections were stained with hematoxylin and eosin and the outer medulla photographed at

×200 magnification. (*E* and *F*) Mean values of terminal deoxynucleotidyl transferase-mediated digoxigenin deoxyuridine nick-end labeling (TUNEL)-positive cells in renal sections. TUNEL staining was conducted and the outer medulla was photographed at ×200 magnification. (*G–J*) Immunohistochemical staining of ED-1 and tumor necrosis factor- α (TNF- α) in the renal sections. (*G* and *I*) Representative photomicrographs, ×100 magnification, with *brown color* indicating positive staining. (*H*) Scores of TNF- α staining-positive area in renal sections. (*J*) Mean value of ED-1 positive cells in renal sections. (*K*) Malondialdehyde (MDA) concentration in renal tissues. * $P < 0.05$ versus Xe + IR group; n = 6. Data are means $\pm$ SD. ED-1 = anti-CD68 antibody.

Fig. 2.

Xenon (Xe) preconditioning up-regulated miR-21 and knockdown of miR-21 exacerbated renal ischemia-reperfusion (IR) injury following xenon exposure. (A) Xenon significantly increased miR-21 expression 24 h after IR injury following xenon preconditioning. $*P < 0.05$ versus IR group, $n = 5$. (B) Xenon significantly increased miR-21 expression from 2 h after exposure compared with N₂ control. $*P < 0.05$ versus control, $n = 5$ at each time point. (C) Locked nucleic acid anti-miR-21 (10 mg/kg), administered before xenon preconditioning inhibited miR-21 expression effectively in kidneys 24 h after xenon exposure. $*P < 0.05$ versus Xe + scramble, $n = 5$. (D) Locked nucleic acid anti-miR-21 administered before xenon preconditioning, inhibited miR-21 expression effectively in kidneys 24 h after IR injury. $*P < 0.05$ versus Xe + scramble + IR, $n = 5$. (E) Knockdown of miR-21 in mice exposed to xenon significantly increased serum creatinine at 24 and 48 h after IR injury. $*P < 0.05$ versus Xe + scramble + IR group, $n = 5$ at each time point. (F and G) Tubular cell injury and quantification of histologic scoring 24 h after IR injury in mice receiving anti-miR-21 or scrambled anti-miR. $*P < 0.05$ versus Xe + scramble + IR group, $n = 6$. Data are means $\pm$ SD. N₂ = nitrogen.

Fig. 3.

Knockdown of miR-21 up-regulated programmed cell death protein 4 (PDCD4) and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), decreased p-AKT, and increased apoptosis. (A) Quantitative reverse transcription-polymerase chain reaction analysis of mRNA expressions of PDCD4 and PTEN in mouse kidneys. (B) Locked nucleic acid anti-miR-21 administered before xenon (Xe) preconditioning significantly increased PDCD4 levels in mouse kidneys 24 h after ischemia-reperfusion (IR) injury. (C) Locked nucleic acid anti-miR-21 administered before xenon preconditioning significantly increased PTEN levels 24 h after IR injury. (D) Ratio of p-Akt to total Akt. Locked nucleic acid anti-miR-21 administered before xenon preconditioning inactivated Akt 24 h after IR injury. (E and F) Knockdown of miR-21 in mice before xenon preconditioning significantly increased tubular cell apoptosis 24 h after IR injury. * $P < 0.05$, ** $P < 0.01$ versus Xe + scramble + IR group, $n = 6$. Data are means $\pm$ SD. GAPDH = glyceraldehydes-3-phosphate dehydrogenase; Akt = protein kinase B; p-Akt = phospho-Akt; mRNA = messenger ribonucleic acid.

Fig. 4.

Xenon (Xe) preconditioning activated hypoxia-inducible factor-1α (HIF-1α) and knockdown of miR-21 reduced the abundance of HIF-1α. (A) Protein levels of HIF-1α and vascular endothelial growth factor (VEGF) in mouse kidneys were significantly increased from 12 h after xenon preconditioning compared with N₂ control. * $P < 0.05$, ** $P < 0.01$, $n = 5$ at each time point. (B) Locked nucleic acid anti-miR-21 administered before xenon preconditioning reduced HIF-α levels in kidneys 24 h after ischemia-reperfusion (IR) injury. * $P < 0.05$ versus Xe + scramble + IR, $n = 5$. Data are means $\pm$ SD. GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

Fig. 5.

Schema of the pathways underlying renal protection conferred by xenon (Xe) preconditioning. Xenon preconditioning up-regulates miR-21 expression likely *via* hypoxia-inducible factor 1α (HIF-1α). miR-21 down-regulates two of its proapoptotic target effectors programmed cell death protein 4 (PDCD4) and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), resulting in activation of Akt, reduction in apoptosis, and renoprotection. MiR-21 further up-regulates HIF-1α in a positive feedback loop, reinforcing the protective effect of miR-21. Other HIF-1α target genes, such as vascular endothelial growth factor (VEGF) may also contribute to renoprotection conferred by xenon. Akt = protein kinase B; p-Akt = phospho-Akt. Vertical arrows: “↓” means decrease, “↑” means increase.