Selective renal overexpression of human heat shock protein 27 reduces renal ischemia-reperfusion injury in mice

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Kim M, Park SW, Kim M, Chen SW, Gerthoffer WT, D’Agati VD, Lee HT. Selective renal overexpression of human heat shock protein 27 reduces renal ischemia-reperfusion injury in mice. Am J Physiol Renal Physiol 299:F347–F358, 2010. First published May 19, 2010; doi:10.1152/ajprenal.00194.2010.—We have previously shown that exogenous and endogenous A1 adenosine receptor (A1AR) activation protects against renal ischemia-reperfusion (IR) injury in mice by induction and phosphorylation of heat shock protein 27 (HSP27). With global overexpression of HSP27 in mice, however, there was a paradoxical increase in systemic inflammation with increased renal injury after an ischemic insult due to increased NK1.1 cytotoxicity. In this study, we hypothesized that selective renal expression of HSP27 in mice would improve renal function and reduce injury after IR. Mice were subjected to renal IR injury 2 days after intrarenal injection of saline or a lentiviral construct encoding enhanced green fluorescent protein (EGFP) or human HSP27 coexpressing EGFP (EGFP-huHSP27). Mice with kidney-specific reconstitution of huHSP27 had signiﬁcantly lower plasma creatinine, renal necrosis, apoptosis, and inflammation as demonstrated by decreased proinflammatory cytokine mRNA induction and neutrophil inﬁltration. In addition, there was better preservation of the proximal tubule epithelial ﬁlamentous (F)-actin cytoskeleton in the huHSP27-reconstituted groups than in the control groups. Furthermore, huHSP27 overexpression led to increased colocalization with F-actin in renal proximal tubules. Taken together, these ﬁndings have important clinical implications, as they imply that kidney-speciﬁc expression of HSP27 through lentiviral delivery is a viable therapeutic option in attenuating the effects of renal IR.

acute kidney injury; acute renal failure; apoptosis; inflammation; lentivirus; necrosis

ACUTE KIDNEY INJURY (AKI) remains a major clinical problem during the perioperative period (17, 19). Development of AKI frequently leads to multiorgan failure and sepsis, leading to increased morbidity and mortality in hospitalized patients. In the perioperative period, hemodynamic changes and decreased renal blood flow often cause ischemia-reperfusion (IR) injury that leads to AKI. Despite continued advances in understanding the pathophysiology of AKI, there is no conclusive evidence that any of the currently available treatments offers renal protection during surgery (17, 43).

We previously reported that endogenous and exogenous A1 adenosine receptor (A1AR) activation protected both mice (24, 28) and rats (22, 23) against renal IR injury. Mechanistically, activation or overexpression of the A1AR led to upregulation of heat shock protein 27 (HSP27) in both cultured human proximal tubule cells as well as in the mouse kidney. Overexpression of HSP27 was also associated with resistance to peroxide-induced necrosis in vitro (4). However, global upregulation of HSP27 led to a paradoxical increase in renal dysfunction and systemic inflammation in vivo. Systemic overexpression of HSP27 appears to counteract the direct proximal tubule-protective effects of HSP27 by exacerbating NK1.1 inflammatory cytotoxicity and increasing the release of proinflammatory keratinocyte-derived cytokine (KC) in vivo (4).

In this study, we questioned whether selective renal expression of HSP27 in mice through lentiviral gene delivery (as opposed to global overexpression) would provide renal protection against IR injury. We tested the hypothesis that selective expression of HSP27 in the kidneys of mice would improve renal function and reduce necrosis, inflammation, and apoptosis after IR injury. We used intrarenal gene delivery with a lentiviral vector that expresses either enhanced green fluorescent protein (EGFP) or human HSP27 coexpressing EGFP (EGFP-huHSP27). We then probed for expression of the transgene in the injected kidney and other tissues. Following injection with a lentivirus encoding EGFP or EGFP-huHSP27, we subjected the mice to renal IR injury. We show that mice renally injected with the EGFP-huHSP27 lentivirus had significant renal protection against IR injury with reduced necrosis, inflammation, and apoptosis compared with mice injected with the EGFP lentivirus.

MATERIALS AND METHODS

Intrarenal lentivirus delivery in C57BL/6 mice. C57BL/6 mice were obtained from Harlan (Indianapolis, IN). A lentivirus encoding EGFP-huHSP27 was generated by subcloning huHSP27 cDNA into a modified shuttle vector, CMV-pLL3.7, where the insert expression is driven by a CMV promoter followed by an IRES-EGFP sequence for simultaneous coexpression of the EGFP reporter gene and hemagglutinin (HA) tag. When expressed, this vector yields two independent proteins, EGFP and the huHSP27 protein fused with the HA tag. The lentivirus was produced by a triple transfection of CMV-huHSP27-pLL3.7 or CMV-pLL3.7 (10 μg), pSVG (vesicular stomatitis virus G; 7 μg; Invitrogen, Carlsbad, CA), and pΔ8.9 (5 μg; from Dr. Van Parjs, MIT, Cambridge, MA) as previously described (25, 26). In vivo virus transduction in mice was performed as described by Nakamura et al. (35) with modifications. In anesthetized mice, after temporary occlusion of the left renal pedicle, a 31G needle attached to a 1-ml syringe was inserted at the lower pole of the left kidney parallel to the long axis under the capsule and was carefully pushed toward the upper pole. As the needle was slowly removed, 100 μl of a filter-purified lentivirus cocktail (EGFP or EGFP-huHSP27, ∼5 × 104 IU/μl) or saline was injected within the capsule. Mice were subjected to renal IR 48 h after saline or virus injection as described below. Preliminary studies showed that lentivirus-mediated EGFP or EGFP-huHSP27...
protein as well as mRNA expression in kidney parenchyma were robust after 48 h.

**Determination of transgene expression with intrarenal lentivirus injection.** We detected the expression of EGFP or EGFP-huHSP27 48 h after intrarenal injection of a lentivirus in C57BL/6 mice using fluorescence microscopy and immunohistochemistry, as described previously (20). In addition, total RNA was extracted from renal cortices and RT-PCR assays were performed for EGFP and mouse GAPDH as described previously (26, 27) (Table 1).

**Renal IR injury.** Renal IR injury procedures were performed 48 h after intrarenal injection of saline, EGFP, or an EGFP-huHSP27 lentivirus in C57BL/6 mice as described (20). In addition, renal IR injury procedures were also performed using mice overexpressing (OE) human HSP27 or wild-type (WT; same genetic background as OE mice but lacking transgene encoding huHSP27) mice. This strain has been described previously (36) and was backcrossed against C57BL/6 mice for at least four generations. After Columbia University Institutional Animal Care and Use Committee approval, adult (25–30 g) male mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg or to effect; Henry Schein Veterinary, Indianapolis, IN) and placed supine on a heating pad under a warming light to maintain body temperature at 37°C. Additional pentobarbital sodium was given as needed on the basis of the mouse’s response to a tail pinch. Bilateral flank incisions were made, and following right nephrectomy, a microaneurysm clip occluded the left renal pedicle (artery and vein) for 30 min. This duration of ischemia was chosen to maximize the reproducibility of renal injury and to minimize mortality rates for these mice. After 30 min, the microaneurysm clip was removed, renal reperfusion was confirmed (color changes), 0.5 ml of saline was given intraperitoneally, the microaneurysm clip was removed, renal reperfusion was confirmed (color changes), 0.5 ml of saline was given intraperitoneally, the wounds were closed in two layers, and the animals were allowed to awaken from anesthesia. The sham mice were subjected to anesthesia and right nephrectomy only.

**Plasma creatinine level.** Plasma creatinine was measured by an enzymatic creatinine reagent kit according to the manufacturer’s instructions (Thermo Fisher Scientific, Waltham, MA). This method of creatinine measurement largely eliminates the interferences from serum plasma chromagens well known to the Jaffé method (12).

**Assessment of renal inflammation.** Renal inflammation after IR injury was determined 1) with detection of neutrophil infiltration with immunohistochemistry 24 h after renal IR and 2) by measuring mRNA encoding markers of inflammation, including keratinocyte-derived cytokine (KC), intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), and tumor necrosis factor-α (TNF-α). Immunohistochemistry for neutrophils was performed as described previously (24, 28) with a monoclonal antibody against PMN (clone 7/4). A primary antibody that recognized IgG2a (MA121, Serotec, Raleigh, NC) was used as a negative isotype control in all experiments. Semiquantitative RT-PCR assays for proinflammatory mRNAs were performed as described previously (26, 27) (Table 1).

**F-actin staining after renal IR injury.** As breakdown of filamentous (F)-actin occurs early after renal IR, we visualized the F-actin cytoskeleton 24 h after renal IR by staining with phalloidin (red) as an early index of renal injury (32, 33) as described previously (20). The mean fluorescent intensity after background correction was calculated from five random blinded sections with identical surface areas per slide to quantify F-actin degradation after renal IR. To minimize the variations in fluorescent intensity, slides from sham-operated animals and animals subjected to renal IR injury were processed together.

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**Table 1. Primers used to amplify mRNAs encoding human HSP27, mouse HSP27, GAPDH, and proinflammatory cytokines based on published GenBank sequences for mice**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Accession Number</th>
<th>Sequence (Sense/Antisense)</th>
<th>Product Size, bp</th>
<th>Cycle Number</th>
<th>Annealing Temperature, °C</th>
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<tr>
<td>EGFP</td>
<td>NC_013179</td>
<td>5'-GACATGAGGAGGAGGACCTTT-3'</td>
<td>379</td>
<td>40</td>
<td>58</td>
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<tr>
<td></td>
<td></td>
<td>5'-TGCTCAGGTTGAGGTTGGG-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse KC</td>
<td>J04596</td>
<td>5'-GGATGAGGCTGCGCTGAGG-3'</td>
<td>203</td>
<td>26</td>
<td>60</td>
</tr>
<tr>
<td>Mouse MIP-2</td>
<td>X53798</td>
<td>5'-CTCCGCTCGGCAACCTATG-3'</td>
<td>282</td>
<td>22</td>
<td>60</td>
</tr>
<tr>
<td>Mouse ICAM-1</td>
<td>X52264</td>
<td>5'-GTCCTCTTCTCTCCCTG-3'</td>
<td>409</td>
<td>21</td>
<td>60</td>
</tr>
<tr>
<td>Mouse TNF-α</td>
<td>X02611</td>
<td>5'-TACTGAACTTCGGGGTGATTGG-3'</td>
<td>290</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>Mouse MCP-1</td>
<td>NM_011333</td>
<td>5'-ACACGACGCTCGACGACGACG-3'</td>
<td>312</td>
<td>22</td>
<td>60</td>
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<tr>
<td>Human HSP27</td>
<td>NM_001540</td>
<td>5'-TTAATGTGCTCTGTCGCGG-3'</td>
<td>259</td>
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<td>5'-CACCAAGGCTGCGCTGAGG-3'</td>
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<tr>
<td>Mouse GAPDH</td>
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<td>5'-GGCGACCGCTGCTGGTGAACG-3'</td>
<td>450</td>
<td>15</td>
<td>65</td>
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HSP27, heat shock protein 27; EGFP, enhanced green fluorescent protein; ICAM-1, intercellular adhesion molecule-1; KC, keratinocyte-derived chemokine; MCP-1, monocyte chemoattractant protein 1; MIP-2, macrophage inflammatory protein 2; TNF-α, tumor necrosis factor-α. Respective anticipated RT-PCR product size, PCR cycle number for linear amplification, and annealing temperatures used for each primer are also provided.
Immunohistochemistry for human HSP27 and assessment of colocalization with F-actin. Twenty-four hours after renal IR, kidneys were embedded in Tissue-Tek Optimal Cutting Temperature Compound (Sakura Finetek, Torrance, CA) and cut into 7-μm sections. The sections were air-dried overnight and fixed in 3.7% paraformaldehyde in PBS and briefly washed in PBS. To reduce background staining, the sections were incubated in 1% bovine serum albumin dissolved in PBS for 10 min at room temperature. The sections were...

![Fig. 1](image1.jpg)

Fig. 1. Expression and colocalization of human heat shock protein 27 (huHSP27) and enhanced green fluorescent protein (EGFP) in the kidney after lentiviral gene delivery. A: representation of 4 immunohistochemistry fluorescence photomicrographs for (top) EGFP expression (green) and (middle) huHSP27 (red) in C57BL/6 mice renally injected with either (left) an EGFP or (right) EGFP-huHSP27 lentivirus 48 h earlier. With 0.5-μm thickness z-sections, we were able to show that huHSP27 (red) and EGFP (green) colocalize together (yellow; bottom). B: representative gel images of RT-PCR results from 4 experiments for GAPDH and EGFP from mice renal cortices. Mouse kidneys were injected with the EGFP-huHSP27-encoding lentivirus 48 h before RT-PCR. Representative results from injected and contralateral (noninjected) kidneys are shown.
incubated with anti-human HSP27 antibody (Abcam, Cambridge, MA) antibody overnight at 4°C, then stained with Alexa Fluor 350 (blue)-labeled phalloidin (Invitrogen) for 30 min at 37°C in a humidified chamber in the dark. Excess phalloidin was removed by washing twice in PBS. After washing with PBS, the sections were incubated with a goat anti-rabbit Alexa Fluor 594-conjugated secondary antibody (red) for 1 h at room temperature in the dark and mounted with Vectashield (Vector Laboratories, Burlingame, CA). Colocalization (magenta) of F-actin (blue) with huHSP27 (red) was determined with z-sections taken with a step size of 0.5 μm (for total z-distance of 5 μm) with an Olympus Spinning Disk Confocal System microscope (Olympus America, Center Valley, PA) and analyzed with Slidebook (Intelligent Imaging Innovations, Philadelphia, PA) software using Pearson’s correlation as described (20).

Protein determination. Protein content was determined with the Thermo Scientific bicinchoninic acid protein assay reagent with bovine serum albumin as a standard.

Reagents. Unless otherwise specified, all chemicals were obtained from Sigma (St. Louis, MO).

Statistical analysis. The data were analyzed with one-way ANOVA plus Tukey’s post hoc multiple comparison test to compare mean values across multiple treatment groups. The ordinal values of the Jablonski scale were analyzed by the Kruskal-Wallis nonparametric test with Dunn’s posttest comparison among groups. In all cases, P < 0.05 was taken to indicate significance. All data are expressed as means ± SE.

RESULTS

In vivo renal expression of EGFP or EGFP-huHSP27 after intrarenal lentiviral gene delivery in C57BL/6 mice. Intrarenal lentivirus injection had no adverse effects on C57BL/6 mice. Figure 1A (∼400, top, representative of 4 photomicrographs) demonstrates that intrarenal delivery of a lentivirus encoding EGFP or EGFP-huHSP27 resulted in robust expression of EGFP in the corticomedullary junction (primarily S3 segments of proximal tubule as the major tubular segment represented) 48 h after injection. There was minimal EGFP expression (similar to baseline green autofluorescence) in the contralateral kidney (data not shown). We confirmed the expression of huHSP27 in EGFP-huHSP27 renally-injected mice using immunohistochemistry. Figure 1A shows that mice injected with the EGFP-huHSP27 lentivirus demonstrate robust expression of huHSP27 (red), whereas the mice injected with the EGFP lentivirus failed to show expression (middle). Furthermore, with 0.5-μm z-sections, we were able to show that huHSP27 (red) and EGFP (green) colocalize together (yellow, bottom).

Figure 1B (representative of 4 experiments) shows the expression of EGFP mRNA (detected with RT-PCR, 40-cycle amplification) in the renal cortices of C57BL/6 mice renally injected with the EGFP-huHSP27 lentivirus. We failed to detect EGFP mRNA in the contralateral kidneys of EGFP-huHSP27 lentivirus-injected mice.

C57BL/6 mice renally expressing EGFP-huHSP27 are protected against renal IR injury. After 30 min of renal ischemia and 24 h of reperfusion, plasma creatinine (mg/dl) increased significantly in both mice renally injected with saline or a lentivirus encoding EGFP compared with sham-operated mice injected with saline or EGFP (Fig. 2). In contrast, mice renally injected with the lentivirus encoding EGFP-huHSP27 showed significant renal protection after IR injury (Fig. 2).

C57BL/6 mice renally expressing EGFP-huHSP27 show less renal tubular necrosis after renal IR. Thirty minutes of renal ischemia followed by 24 h of reperfusion resulted in significant renal injury in both mice renally injected with saline or a lentivirus encoding EGFP (Fig. 3, representative of 6 experiments) as evidenced by severe tubular necrosis, corticomedullary congestion and hemorrhage, and development of proteinaceous casts in all mouse kidney sections. However, mice renally injected with the lentivirus encoding EGFP-huHSP27 had significantly less renal tubular damage and necrosis after renal IR. The Jablonski scale renal injury score histology grading is used to grade renal tubular necrosis after murine renal IR injury (16). Thirty minutes of renal ischemia and 24 h of reperfusion resulted in severe acute tubular necrosis in both mice renally injected with saline (renal injury score = 3.3 ± 0.3, n = 5) or the lentivirus encoding EGFP (renal injury score = 3.1 ± 0.2, n = 8), whereas mice renally injected with the lentivirus encoding EGFP-huHSP27 had a significantly lower renal injury score (1.8 ± 0.3, n = 5, P < 0.001 vs. mice injected with saline or EGFP).
Blood KC mRNA are increased in global huHSP27 OE mice but not in C57BL/6 mice renally injected with the EGFP-huHSP27 lentivirus. We have previously shown that huHSP27 OE mice had increased renal injury with elevated creatinine levels following renal IR compared with WT mice (4), and we confirm these findings in the current study (data not shown). Whole-blood KC mRNA was upregulated in huHSP27 OE mice compared with huHSP27 WT mice (Fig. 5A). In contrast, C57BL/6 mice renally injected with the EGFP-huHSP27 lentivirus did not demonstrate increased whole-blood KC mRNA compared with C57BL/6 mice renally injected with the EGFP lentivirus (Fig. 5B).

C57BL/6 mice renally expressing EGFP-huHSP27 show reduced renal neutrophil infiltration after renal IR. Immunohistochemical assays showed an increase in neutrophil infiltration (Fig. 6, representative of 4 experiments) in mice renally injected with saline or with the lentivirus encoding EGFP at 24 h after renal IR injury. The mice renally injected with the lentivirus encoding EGFP-huHSP27 had reduced neutrophil infiltration after renal IR.

C57BL/6 mice renally expressing EGFP-huHSP27 show reduced renal apoptosis after IR. We failed to detect significant TUNEL-positive cells in kidney sections from sham-operated mice renally injected with saline, the lentivirus encoding EGFP, or EGFP-huHSP27 (data not shown). Mice renally injected with saline or with the EGFP lentivirus 48 h before 30 min of renal ischemia and 24 h of reperfusion showed many TUNEL-positive cells in the corticomedullary junction (Fig. 7, representative of 4 experiments). Mice renally injected with lentivirus encoding EGFP-huHSP27 had reduced TUNEL-positive renal tubule cells.

C57BL/6 mice expressing EGFP-huHSP27 show reduced disruption of renal proximal tubule F-actin architecture. As expected, renal IR injury resulted in severe disruption of renal proximal tubule F-actin compared with sham-operated mice. In Fig. 9A (representative of 5 experiments), postischemic disruption of the F-actin cytoskeleton in renal proximal tubular epithelial cells is shown. Mice subjected to sham surgery showed intense staining in the tubular epithelial cell basal plasma membrane. In contrast, kidneys from mice renally injected with the EGFP-encoding lentivirus and subjected to renal IR showed significant loss of F-actin staining in the tubular epithelial cells. Mice injected...
with the EGFP-huHSP27-encoding lentivirus show reduced disruption or increased polymerized form of renal proximal tubule F-actin architecture after IR. Mean fluorescent F-actin intensity also showed reduced renal proximal tubule F-actin degradation in mice undergoing renal IR injury after renal injection with the EGFP-huHSP27-encoding lentivirus compared with mice injected with the EGFP-encoding lentivirus (Fig. 9B, n = 5).

C57BL/6 mice renally expressing EGFP-huHSP27 show enhanced colocalization of F-actin with huHSP27. Figure 10 demonstrates partial colocalization of F-actin with huHSP27 (representative of 4 experiments). Figure 10B shows robust expression of huHSP27 (red fluorescence) in the kidney 48 h after renal injection with the EGFP-huHSP27 lentivirus compared with renal injection with the EGFP lentivirus (Fig. 10A). Figure 10, C and D, demonstrates F-actin expression (blue fluorescence) in the kidney following lentivirus injection. When the panels are merged (Fig. 10, E and F), enhanced colocalization of huHSP27 and F-actin can be observed (magenta) in kidneys injected with the EGFP-huHSP27 lentivirus (Fig. 10F).

**Discussion**

The major finding of this study is that selective renal overexpression of huHSP27 in the kidney protected mice against renal IR injury in vivo. We showed that intraparenchymal...
Mal injection of a lentivirus into the kidney resulted in preferential expression of the transgene product in the injected kidney. Mice injected with a lentivirus encoding EGFP-huHSP27 were protected against renal tubular necrosis, apoptosis, and inflammation after renal IR injury compared with mice injected with an EGFP-encoding lentivirus. In addition, huHSP27 expression colocalized with F-actin in mice injected with the EGFP-huHSP27 lentivirus. Therefore, intrarenal lentiviral delivery of HSP27 may be a therapeutic option to protect against renal IR injury.

In previous studies, we demonstrated that activation of renal A1ARs resulted in renal protection both in vitro and in vivo (21, 24, 26, 28) that was at least partially mediated by phosphorylation and upregulation of HSP27 protein expression (18, 26). In addition, we recently demonstrated that selective renal expression of A1ARs in A1AR knockout mice via a lentiviral vector protected mice against renal IR injury and led to upregulation of HSP27 (20).

HSPs are a group of molecular chaperones that are upregulated after various cellular stresses, including heat shock, hypoxia, ischemia, and toxin exposures (1, 3). Increased HSP27 expression protects a cell from injury or death by acting as a chaperone to facilitate proper polypeptide folding, assembly of oligomers, transport, and conformational changes. HSP27 can also inhibit apoptosis and stabilize the actin cytoskeleton, leading to increased resistance against cell death.
HSP27 overexpression has been shown to be protective in the brain, liver, lung, and heart (5, 29, 38, 41). HSP27 can be phosphorylated or upregulated to protect the cell against stress or injury (6) and has also been shown to translocate to the nucleus to interact with transcription factors and stimulate gene transcription (9).

HSP27 is involved in the response of the kidney to ischemic injury. Ischemia caused an accumulation of HSP25 (also known as HSP27) in the cortex and outer medulla in rats (40) and resulted in an increased association of HSP25 with the cytoskeletal components of proximal convoluted tubule cells (37). In addition, overexpression of HSP27 reduced sublethal renal epithelial cell injury at cell contact sites via a c-Src-dependent mechanism (11). These suggest a role for HSP27 in maintaining actin and cytoskeletal integrity in the kidney after ischemia. Another role may involve membrane channel proteins as the interactions of various HSPs induced after ischemia, including HSP25, were involved in stabilizing Na-K-ATPase in in vitro assays of rat renal cortex after in vivo ischemia (2).

We previously demonstrated that kidney proximal tubule cells cultured from HSP27 OE mice showed increased resistance against H2O2-induced necrosis in vitro compared with cells cultured from HSP27 WT mice (4). In addition, HSP27 OE mice were protected against hepatic IR injury in vivo compared with HSP27 WT mice (5). However, we were surprised to find that HSP27 OE mice had increased inflammation and renal injury after renal IR in vivo compared with HSP27 WT mice (4). Global overexpression of HSP27 led to increased neutrophil, T lymphocyte, and NK1.1+ cell infiltration after renal IR compared with WT mice and was associated with increased blood and kidney KC levels. In this study, we sought to determine whether selective renal expression of HSP27, as opposed to global overexpression, would lead to improved renal function after IR in vivo.

Using a lentiviral vector, we were able to selectively transduce both EGFP and EGFP-huHSP27 in the renal cortex and corticomedullary junction of the kidney. The corticomedullary junction is the main site of injury in our model of warm renal IR and is composed mainly of S3 segments of the proximal tubule (8, 14). We confirmed that there was selective protein expression by demonstrating robust EGFP expression in injected kidneys compared with noninjected kidneys (Fig. 1) and that mice injected with the EGFP-huHSP27 lentivirus expressed huHSP27 protein (immunocytochemistry, Fig. 1A; RT-PCR, Fig. 4A). Therefore, using our method of lentiviral

Fig. 9. Reduction in F-actin disruption with lentiviral gene huHSP27 delivery after renal IR injury. A: representative (of 5 experiments) fluorescent photomicrographs of phalloidin labeling (red) to visualize F-actin in renal proximal tubules from C57BL/6 mouse kidneys. Sham kidneys demonstrate intact F-actin architecture. Mouse kidneys were renally injected with either an EGFP- or EGFP-huHSP27-encoding lentivirus 48 h earlier and subjected to 30-min renal ischemia and 24-h reperfusion. #, Proximal tubules with disrupted F-actin staining; *, intact F-actin cytoskeleton. We show that proximal tubules from kidneys injected with the EGFP-huHSP27 lentivirus show better preserved F-actin (red) than the proximal tubules from kidneys injected with the EGFP-encoding lentivirus. B: quantification of mean fluorescent proximal tubule F-actin intensity as a measure of F-actin preservation in kidney sections (n = 5). Values are means ± SE and analyzed with 1-way ANOVA plus Tukey’s post hoc multiple comparison test. #P < 0.001 vs. sham group. *P < 0.001 vs. EGFP IR group.
injection, we were able to directly test the hypothesis that selective expression of huHSP27 in the kidney protects mice against renal IR injury.

Selective expression of huHSP27 in the kidneys of mice protected against renal IR injury as evidenced by a reduction in plasma creatinine levels. This was associated with a reduction in the renal expression of the proinflammatory cytokines TNF-α and KC, in contrast to our previous results in HSP27 OE mice showing elevated ICAM-1, MCP-1, TNF-α, and KC after renal IR compared with WT mice (4). In addition, we demonstrated that with selective renal expression of huHSP27, whole-blood KC mRNA levels were not upregulated as they were in huHSP27 OE mice (Fig. 5). TNF-α has been shown to rise early and to mediate neutrophil infiltration after renal IR (7, 30) as well as cardiac IR (15). The deleterious effects of TNF-α were reduced with administration of TNF-α binding protein or anti-TNF-α antibody. KC has also been shown to rise after renal IR (34) and to mediate neutrophil infiltration.

Fig. 10. Lentivirus-mediated expression of hu-HSP27 promotes colocalization of F-actin and huHSP27. Representative fluorescent photomicrographs of phalloidin labeling (blue) to visualize F-actin (C and D) and huHSP27 (A and B) protein immunocytochemistry (red) in renal proximal tubules from C57BL/6 mouse kidneys. Mouse kidneys were renally injected with an EGFP-encoding lentivirus (A, C, and E) or EGFP-huHSP27-encoding lentivirus (B, D, and F). HSP27 and F-actin colocalize together (E and F; magenta), shown with 0.5-μm-thick z-sections. Images are representative of 4 independent experiments.
after renal IR injury (31). With EGFP-huHSP27 lentivirus injection into the kidney, there was reduced neutrophil infiltration as well as significantly less renal tubular necrosis of the “outer stripe” of the outer medulla and reduced renal tubular apoptosis (as shown by TUNEL staining, DNA laddering). Last, we demonstrated significant loss of the renal tubular F-actin cytoskeleton structure after renal IR in EGFP-injected mice, whereas EGFP-huHSP27-injected mice had better preservation of the F-actin cytoskeleton. Stress or injury has been shown to promote association or colocalization of HSP27 and F-actin, leading to cytoprotection in vitro (39, 42) and in vivo (20), and we confirm these findings in our study. Therefore, we have shown in multiple ways that selective renal expression of huHSP27 protects mice against renal IR injury.

These findings of selective renal expression of huHSP27 are in contrast to our previous study demonstrating that global overexpression of huHSP27 in mice worsened renal IR injury with increased inflammation, neutrophil infiltration, and worsened renal function. Chronic compensatory changes are always of concern when transgenic mice are studied. It is possible that the huHSP27 OE transgenic mice used in our previous study had long-term compensatory (extrarenal) systemic changes from chronic, global overexpression of huHSP27 that caused the increased renal IR injury. Selective renal expression of huHSP27 in our current study, on the other hand, was acute (~48 h), and the mice may not have had adequate time to develop these compensatory changes. It is possible that chronic, global overexpression of huHSP27 caused activation of inflammatory mediators, including leukocytes and NK1.1+ cells, leading to increased renal IR injury. This is supported by the findings that after splenectomy or NK1.1+ cell depletion, huHSP27 OE mice had decreased renal IR injury compared with huHSP27 WT mice (4). In addition, transfer of splenocytes or NK1.1+ cells from huHSP27 OE mice to huHSP27 WT mice led to increased renal IR injury.

As discussed previously (20), considering the advantages and disadvantages of various viral vectors, including an adenovirus, adeno-associated virus, retrovirus, and lentivirus, we chose to use a lentivirus to introduce the transgene into the kidney to selectively express huHSP27 in vivo. The advantages of lentiviral gene delivery include stable and long-lasting gene transduction and its ability to infect nondividing postmitotic cells (10). As in our previous study (20), we demonstrated robust expression of the transgene in the cortex, outer medulla, and corticomedullary junction, which are the areas most susceptible to injury after IR due to the delicate balance between oxygen delivery and consumption because of the high ATP demand and relatively low blood flow found here.

In summary, we show in this study that selective renal expression of the EGFP-huHSP27 transgene via a lentiviral vector is possible in the mouse kidney and that this selective renal overexpression protects mice subjected to renal IR injury. These findings may have important clinical implications, as they imply that kidney-specific expression of HSP27 through lentiviral delivery is a viable therapeutic option in attenuating the effects of renal IR.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**REFERENCES**


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