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Activation of hepatocyte growth factor receptor, c-met, in renal tubules is required for renoprotection after acute kidney injury

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Abstract

Hepatocyte growth factor is a pleiotropic protein that promotes injury repair and regeneration in multiple organs. Here, we show that after acute kidney injury (AKI), the HGF receptor, c-met, was induced predominantly in renal tubular epithelium. To investigate the role of tubule-specific induction of c-met in AKI, we generated conditional knockout mice, in which the c-met gene was specifically disrupted in renal tubules. These Ksp-met^{-/-} mice were phenotypically normal and had no appreciable defect in kidney morphology and function. However, in AKI induced by cisplatin or ischemia-reperfusion injury, the loss of tubular c-met substantially aggravated renal injury. Compared with controls, Ksp-met^{-/-} mice displayed higher serum creatinine, more severe morphologic lesions, and increased apoptosis, which was accompanied by an increased expression of Bax and Fas ligand and decreased phosphorylation-activation of Akt. In addition, ablation of c-met in renal tubules promoted chemokine expression and renal inflammation after AKI. Consistently, ectopic expression of hepatocyte growth factor in vivo protected the kidneys against AKI in control mice, but not in Ksp-met^{-/-} counterparts. Thus, our results suggest that tubule-specific c-met signaling is crucial in conferring renal protection after AKI, primarily by its anti-apoptotic and anti-inflammatory mechanisms.

Keywords

HGF; c-met; acute kidney injury; apoptosis; inflammation

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DISCLOSURES

None

INTRODUCTION

Acute kidney injury (AKI) is a devastating clinical condition with high rate of morbidity and mortality.^{1, 2} Despite decades of research, there is no effective therapy for patients with AKI in the clinical setting. Extensive studies have suggested that the pathogenesis of AKI involves exaggerated inflammation, tubular cell apoptosis and/or necrosis.³⁻⁵ After ischemic, toxic or septic insults, pro-inflammatory signal cascade is activated, which leads to chemokine expression and release, as well as renal infiltration of T cells and macrophages. Injured tubular cells also undergo apoptosis and/or necrosis, resulting in tubular cell depletion and cast formation inside the lumen of renal tubules.^{4, 6} In this context, identifying an effective strategy to both inhibit renal inflammation and promote cell survival is essential for minimizing kidney damage and accelerating repair and recovery after AKI.

Hepatocyte growth factor (HGF) is a pleiotropic factor that plays a wide variety of roles in diverse biologic processes such as organ development, tissue homeostasis and injury repair.⁷⁻⁹ All the biologic actions of HGF are mediated by a single transmembrane receptor, c-met, a protein tyrosine kinase.¹⁰ HGF expression is restricted to cells of mesenchymal origin, whereas c-met receptor is more ubiquitously expressed, predominantly in epithelial cells.¹¹ Upon binding of its ligand, c-met receptor is activated, leading to its auto-phosphorylation on tyrosine residues. This triggers cascades of signal transduction and ultimately controls the activities of its target cells. Of interest, emerging evidence indicates that HGF possesses potent anti-apoptosis and anti-inflammation capacity, making it especially well suited to promote injury repair and regeneration after tissue damage.¹²⁻¹⁵

HGF and its c-met receptor are known to play a role in both kidney development and in the maintenance of kidney homeostasis.¹⁶⁻¹⁸ We have previously demonstrated that loss of c-met receptor in a podocyte-specific manner renders mice more susceptible to injury in adriamycin nephropathy, resulting in more severe podocyte apoptosis and albuminuria.¹⁹ Likewise, disruption of HGF signaling aggravates renal interstitial fibrosis after obstructive injury.²⁰ Both HGF and c-met receptor are up-regulated in the kidneys after AKI, and administration of exogenous HGF promotes tubular repair and recovery.^{17, 18} However, because HGF can target almost all types of cells, including intrinsic kidney cells and infiltrated cells, it remains elusive whether HGF signaling in a particular cell type is critical for mediating its renoprotective actions.

In this study, we generated conditional knockout mice in which c-met receptor is specifically disrupted in tubular epithelia of the kidneys. By using two models of AKI induced by cisplatin and ischemia-reperfusion injury (IRI), respectively, we show that mice with tubule-specific ablation of c-met receptor are more susceptible to injury. Our results establish that tubule-specific HGF-c-met signaling is pivotal for renal protection against AKI.

RESULTS

Induction of c-met in renal tubules after AKI

To study the role of endogenous HGF/c-met signaling in AKI, we first examined the expression of c-met in mouse kidneys after cisplatin injection, a toxic AKI model characterized by acute tubular injury, apoptosis and necrosis, and renal inflammation.^{1, 3, 21} Western blot analyses of whole kidney lysates revealed a greater than 4-fold increase of renal c-met protein in mice treated with cisplatin, compared with vehicle controls (Figure 1, a and b). Immunohistochemical staining also showed an increased c-met protein expression predominantly in renal tubules at 3 days after cisplatin injection (Figure 1d, arrows). To define the tubular segment-specificity of c-met induction in this model, we utilized double immunostaining for c-met (red) and various tubular markers (green) in the kidney after cisplatin injection. As shown in Figure 1e, c-met protein was induced in virtually all major tubular segments of the kidneys, including proximal tubules, distal tubules and collecting collect, after AKI induced by cisplatin. Together with earlier reports in other AKI models,^{17, 18} these results indicate that an increased c-met expression is a common response of renal tubular epithelium after acute injury.

Generation of mutant mice with tubule-specific ablation of c-met

To investigate the potential role of tubular c-met induction, we generated conditional knockout mice in which c-met gene is selectively disrupted in renal tubules by utilizing the Cre-LoxP system.²² Homozygous c-met floxed mice were mated with Ksp-Cre transgenic mice expressing Cre recombinase under the control of the tubule-specific Ksp-cadherin promoter (Figure 2a). Mice with tubule-specific ablation of c-met, designated as Ksp-met^{-/-} (genotype: c-met^{fl-fl}, Cre), were generated (Figure 2b). Homozygous c-met floxed mice (genotype: c-met^{fl-fl}) were used as controls throughout the experiments. As shown in Figure 2, c and d, c-met protein levels were significantly reduced in the kidneys lysates of Ksp-met^{-/-} mice, compared with controls. Notably, renal c-met expression was not completely abolished because c-met is ubiquitously expressed in all kidney cells.¹¹ Immunohistochemical staining also revealed a significant reduction of c-met protein in renal tubular epithelium of the Ksp-met^{-/-} mice under cisplatin-stimulated conditions, compared to the controls (Figure 2, e and f). Of note, Ksp-met^{-/-} mice were phenotypically normal under basal conditions and displayed no appreciable abnormality in kidney structure and function (Figure 2, g through j).

Tubule-specific ablation of c-met aggravates AKI

We next examined the effects of c-met ablation in AKI induced by cisplatin. While two out of nine Ksp-met^{-/-} mice died (22.2% mortality rate) within 3 days after cisplatin injection, all of seven control mice survived in the same period under the identical conditions, suggesting a protective effect of tubular c-met. In the surviving mice, serum creatinine levels at 3 days after cisplatin were significantly higher in Ksp-met^{-/-} group than in the controls (Figure 3a). Of interest, despite this difference in renal function, both control and Ksp-met^{-/-} mice underwent similar weight loss (~2 g per day), as shown in Figure 3b. Consistent with renal function data, Ksp-met^{-/-} kidneys exhibited more severe morphological injury, characterized by loss of tubule brush borders, tubular cell death and detachment, as well as

cast formation in the lumen of tubules (Figure 3e, enlarged area), comparing to the controls (Figure 3d). Together, these data demonstrate that loss of c-met receptor in a tubule-specific fashion aggravates cisplatin-induced AKI.

Ablation of c-met exacerbates tubular cell apoptosis after AKI

To explore the mechanisms underlying the protective role of c-met, we examined apoptotic cell death in the kidneys of control and Ksp-met^{-/-} mice after AKI. As shown in Figure 4a, TUNEL staining revealed considerable apoptosis in the kidneys of control mice at 3 days after cisplatin injection. However, the number of apoptotic cells in Ksp-met^{-/-} kidneys was significantly higher than that in the controls (Figure 4, a and b), indicating that c-met exerts its cytoprotective action by inhibiting tubular cell apoptosis. To determine which tubular segments are susceptible to apoptosis in this model, we used double immunostaining for cleaved caspase-3 (red) and various tubular segment-specific markers (green). As shown in Figure 4, c through f, apoptosis, as determined by cleaved caspase-3 positivity, occurred predominantly in the proximal tubule and collect duct epithelia. Not surprisingly, more cleaved caspase-3-positive cells were detected in these tubular segments after cisplatin injection in Ksp-met^{-/-} mice, compared with the controls (Figure 4, c through f).

We further examined renal expression and activation of Akt-protein kinase B (PKB), a central player in regulating cell survival. As shown in Figure 4, g and h, tubule-specific loss of c-met receptor markedly inhibited Akt phosphorylation in the kidneys after AKI. As Akt phosphorylates and negatively regulates Bax,²³ a pro-apoptotic member of the Bcl-2 family that plays a crucial role in mediating mitochondrial dysfunction and cell apoptosis,^{24, 25} we next examined Bax protein expression. As shown in Figure 4, g and i, Bax protein was increased in the kidneys of Ksp-met^{-/-} mice after AKI induced by cisplatin, comparing to the controls.

Besides the intrinsic apoptosis pathway involving mitochondria and Bax, c-met may also affect the apoptotic signaling mediated by the death receptor, Fas. As shown in Figure 4, g and j, we found that FasL expression was significantly induced in the injured kidneys of Ksp-met^{-/-} mice after AKI, comparing to control mice. Collectively, these results suggest that tubule-specific loss of c-met exacerbates kidney injury by promoting apoptosis through multiple pathways.

Ablation of c-met augments renal inflammation after AKI

To assess the role of HGF/c-met signaling on renal inflammation in AKI, we next examined pro-inflammatory cytokine expression and the infiltration of inflammatory cells in the kidneys of control and Ksp-met^{-/-} mice after cisplatin injection. TNF- α is a critical pro-inflammatory cytokine thought to contribute to cisplatin-induced AKI and is produced by T-cells and activated macrophages in renal injury.³ As shown in Figure 5a, qRT-PCR demonstrated a significant increase in TNF- α mRNA expression in Ksp-met^{-/-} mice after cisplatin injection, compared with the controls. Similarly, Western blotting showed a ~2-3 fold increase of renal TNF- α protein in the Ksp-met^{-/-} mice after cisplatin injection (Figure 5, b and c). As shown in Figure 5, d and e, tubule-specific ablation of c-met caused significantly more CD3⁺ T cell infiltration into the kidneys at 3 days after cisplatin

injection. More CD45-positive cells were also observed in the kidneys of *Ksp-met*^{-/-} mice after cisplatin injection, comparing to controls (Figure 5, d and f). Therefore, it is clear that tubule-specific ablation of *c-met* augments renal inflammation after AKI.

Loss of tubular HGF signaling also worsens AKI induced by ischemia-reperfusion injury

To confirm the broad implications of HGF signaling in conferring renal protection after AKI, we sought to investigate whether HGF also plays a role in ameliorating AKI induced by ischemia-reperfusion injury (IRI).²⁶ As shown in Figure 6a, serum creatinine levels at 1 day after IRI were significantly higher in *Ksp-met*^{-/-} mice compared to controls. *Ksp-met*^{-/-} kidneys also showed more severe morphological injury, characterized by tubular cell dropout and loss of brush borders (Figure 6, b and c). Apoptosis was also more prevalent in the *Ksp-met*^{-/-} mice compared to the controls, as demonstrated by TUNEL staining (Figure 6d). Furthermore, expression of Bax and FasL proteins was markedly increased in the kidneys of *Ksp-met*^{-/-} mice, while phospho-Akt levels were decreased (Figure 6, e-h). In short, these results indicate that loss of *c-met* signaling exacerbates ischemic AKI as well.

We further examined the effect of *c-met* deficiency on renal inflammation after ischemic AKI. As shown in Figure 7, a and b, qRT-PCR demonstrated an increased mRNA expression of pro-inflammatory TNF- α and monocyte chemoattractant protein-1 (MCP-1) in the kidneys of *Ksp-met*^{-/-} mice at 1 day after IRI, compared to the controls. Immunofluorescence staining also showed greater numbers of CD3⁺ T cells and CD45⁺ leukocytes in *Ksp-met*^{-/-} kidneys than that in the controls (Figure 7, c through f), suggesting that loss of tubular *c-met* signaling exacerbates renal inflammation after ischemic AKI.

Ectopic expression of HGF protects against inflammation and tubular apoptosis

To ascertain the protective role of HGF/*c-met* signaling in preserving renal tubular epithelium, we sought to evaluate the effect of exogenous HGF on kidney structure and function after IRI. To this end, mice were intravenously injected with HGF expression plasmid (pCMV-HGF) or empty vector (pcDNA3) by using a hydrodynamic-based gene delivery approach. Previous studies have shown that this method leads to substantial increases in HGF in both kidney tissue and the circulation.^{27, 28} Consistently, HGF plasmid injection markedly induced HGF mRNA expression in the kidneys, as shown in Figure 8a. We then induced IRI at 12 h after HGF plasmid injection. As shown in Figure 8, b through d, ectopic expression of exogenous HGF significantly reduced serum creatinine at 24 h after IRI and led to marked improvement in renal pathology. Furthermore, HGF expression inhibited both TNF- α mRNA (Figure 8e) and protein expression (Figure 8f). Exogenous HGF also repressed renal Bax expression after IRI (Figure 8f). These data clearly demonstrate a direct and dominant role for HGF/*c-met* signaling in conferring renoprotection after AKI.

Activation of tubular *c-met* signaling is essential for renal protection after AKI

To further determine whether tubular *c-met* receptor is required for mediating renal protection by exogenous HGF after AKI, we administered the HGF expression plasmid (pCMV-HGF) or empty vector (pcDNA3) into control and *Ksp-met*^{-/-} mice, respectively. At 16 h after plasmid injection, mice were injected with cisplatin to induce AKI and

sacrificed at 3 days. While all of 7 control mice survived after injection with pCMV-HGF and cisplatin, 3 out of 6 *Ksp-met*^{-/-} mice died after the same treatments. The death rate of *Ksp-met*^{-/-} mice after cisplatin injection was similar in the groups pre-administered with or without pCMV-HGF, suggesting that renoprotection elicited by exogenous HGF is dependent on an intact c-met receptor in renal tubules. Similar data were obtained when serum creatinine level was measured in the survived mice and estimated in dead mice (approximately 2.5 mg-dl), which was based on the average value of serum creatinine level in AKI mice prior to death. As presented in Figure 9a, while expression of pCMV-HGF reduced serum creatinine in control mice, it did not significantly affect kidney dysfunction in *Ksp-met*^{-/-} mice after cisplatin injection. Regardless of pCMV-HGF expression, *Ksp-met*^{-/-} kidneys also exhibited more severe morphological injury, compared with the controls (Figure 9, b and c).

We further examined apoptotic cell death in the kidneys of control and *Ksp-met*^{-/-} mice after various treatments. As shown in Figure 9, c and d, co-immunofluorescence staining for cleaved caspase-3 (red) and tubular basement membrane marker laminin (green) revealed a decrease of apoptotic cells in the tubular compartment of the kidneys in control mice injected with pCMV-HGF. However, in *Ksp-met*^{-/-} mice, expression of exogenous HGF appeared to have little effect on tubular cell apoptosis after cisplatin injection (Figure 9, c and d). Similarly, renal expression of Bax proteins was reduced in the control mice injected with pCMV-HGF, but not in *Ksp-met*^{-/-} mice (Figure 9e). Together, these data demonstrate that activation of tubular c-met signaling is essential for renal protection elicited by exogenous HGF after AKI.

DISCUSSION

As the sole receptor for HGF, the abundance of c-met receptor not only dictates the magnitude of its signaling but also determines the site-specificity of its actions.¹⁸ It is well recognized that HGF and its c-met receptor constitutes a signal circuit that plays a crucial role in promoting tissue repair and regeneration after injury in multiple organs.^{8, 29} However, because c-met receptor is ubiquitously expressed in almost all cell types, it remains elusive whether HGF/c-met signaling in a particular cell type is especially important for its protective actions. In this study, we demonstrate that tubule-specific ablation of c-met receptor significantly aggravates tubular cell apoptosis, augments renal inflammation and worsens kidney dysfunction in mouse models of AKI. It should be noted that the difference in renal injury between control and *Ksp-met*^{-/-} groups is most likely underestimated, because a few mice with the most severe AKI died selectively in the *Ksp-met*^{-/-} group. The effect of c-met ablation on kidney injury is clearly independent of the type of injurious stimuli, because both cisplatin (toxic) and IRI (ischemic) cause the similar deterioration of AKI in *Ksp-met*^{-/-} mice, compared to the controls. These results indicate that loss of c-met receptor in a tubule-specific fashion renders the mice vulnerable to both toxic and ischemic injury, and suggest that tubular c-met signaling is essential for conferring renal protection after AKI.

HGF receptor is constitutively expressed, at low level, in the tubular epithelium of adult kidneys.^{11, 18} However, mice with tubule-specific ablation of c-met display normal

phenotype, indicating that HGF/c-met signaling in renal tubules is dispensable under normal physiologic conditions. This result is not without precedent, as several previous studies demonstrate that knockout of c-met in different cell types such as glomerular podocytes, renal collecting duct epithelial cells and hepatocytes does not result in structural and functional abnormality in mice as well.^{19, 20, 30} Therefore, as one of many growth factors present in a given tissue, HGF/c-met function in a particular cell type under normal circumstances is likely redundant. However, possibility also exists that because of the incomplete ablation of c-met in Ksp-met^{-/-}mice, the residual receptor in renal tubular epithelia may be sufficient for supporting tubular physiology in normal conditions.

After AKI, c-met receptor is up-regulated in renal tubules (Figure 1).^{17, 18} Using co-staining for c-met and various tubular segment-specific markers, it is found that c-met is induced in all major tubular segments of the kidneys after AKI, although its induction occurs predominantly in AQP1-positive proximal tubule and AQP3-positive collecting duct epithelia (Figure 1). Of interest, these nephron segments are also most susceptible to injury in AKI, as illustrated by the induction and activation of caspase-3 in these sites (Figure 4). These results are also in line with a recent report on nephron specific uptake mechanism of cisplatin in the kidneys.³¹ Such a close, segment-specific association between c-met induction and injury in AKI suggests that up-regulation of c-met receptor could be a defensive response of the kidneys in an attempt to protect against the catastrophic damage to tubular epithelial cells. Consistent with this view, tubule-specific depletion of c-met aggravates kidney injury after AKI induced by both toxic and ischemic insults. Furthermore, expression of exogenous HGF gene is able to protect kidneys against AKI in control mice, but not in Ksp-met^{-/-}counterparts (Figure 9). It should be noted that a significant proportion of proximal tubules (about 50%) does not express Cre in Ksp-Cre mice,³² and therefore c-met receptor is not knocked out in all proximal tubules in this model. In this regard, the importance of tubular c-met receptor in conferring renal protection is even underestimated in this study.

The mechanism underlying the renoprotective actions of c-met signaling in AKI are likely attributable to its ability to inhibit apoptosis and inflammation. Of many cellular events that may contribute to the pathogenesis of AKI, excessive apoptosis and inflammation are two most studied mechanisms that lead to tubular collapse and kidney failure.^{4, 33} Tubular cell apoptosis is tightly controlled by the delicate balance between pro- and anti-apoptotic forces, in which Akt-protein kinase B and Bcl-2 family of proteins are key players.³⁴ Using cultured proximal tubular epithelial cells in vitro, we previously have shown that HGF promotes tubular cell survival by inhibiting apoptosis via activating Akt phosphorylation and modulating Bcl-2 family protein expression.^{15 35} Indeed, knockout of tubular c-met receptor results in a decreased Akt phosphorylation and increased Bax expression. Of particularly interest, loss of c-met in a tubule-specific manner also induces renal expression of FasL, a protein that triggers apoptosis via extrinsic death receptor-dependent pathway.^{36, 37} Collectively, it becomes clear that c-met signaling promotes tubular cell survival in vivo through mechanisms involving both intrinsic, mitochondria-dependent and extrinsic, death receptor-dependent pathways.

Acute kidney injury also provokes inflammation, as manifested by renal infiltration of inflammatory cells such as T cells and monocytes-macrophages. Interestingly, tubule-specific ablation of c-met receptor leads to an enhanced peritubular inflammatory infiltration, suggesting a critical role for tubular HGF signaling in controlling renal inflammation. This is not completely surprising, as tubular epithelial cells make up the bulk of renal parenchyma and are the primary target of ischemic or toxic insults. In response to cell stress or injuries after AKI, tubular epithelium instigates its production and release of inflammatory cytokines, creating pro-inflammatory niches, in which the gradients of chemotactic cytokines provide a directional signal for guiding the infiltration of inflammatory cells to the injured sites.^{5, 33} Consistent with this notion, mice with tubule-specific ablation of c-met receptor have an increased level of MCP-1 and TNF- α expression, and augmented infiltration of inflammatory cells after AKI. Conversely, ectopic expression of exogenous HGF inhibits cytokine expression and ameliorates kidney injury (Figure 8). The ability of c-met signaling to inhibit renal inflammation after AKI is in line with earlier in vitro observations, in which HGF represses NF- κ B activation and inhibits pro-inflammatory cytokine expression in tubular epithelial cells, as well as in endothelial cells and macrophages.^{13, 14, 38} In short, anti-inflammation appears to be one of the key mechanisms underlying the renoprotection of HGF after AKI in vivo.

In summary, we here demonstrate that tubule-specific, endogenous c-met signaling is essential for renal protection against AKI induced by either toxic (cisplatin) or ischemic (IRI) insults, as genetic ablation of c-met receptor selectively in renal tubules aggravates kidney dysfunction. Such beneficial effects of HGF/c-met are apparently mediated by its ability to inhibit apoptosis and inflammation. Although we cannot completely rule out the possibility that other cell types may contribute to the HGF/c-met-mediated renal protection via different mechanisms as well, our studies for the first time illustrate that tubule-specific activation of c-met signaling is required and could have profound impact on renal protection after AKI.

MATERIALS AND METHODS

Mice and genotyping

c-met floxed mice were created by homologous recombination using a c-met gene fragment with loxP sites flanking exon 16, as described previously,^{19, 30} and kindly provided by Dr. Snorri S. Thorgeirsson (National Cancer Institute, NIH, Bethesda, MD, USA). Transgenic mice that expressed Cre recombinase under the control of kidney-specific Ksp-cadherin promoter (ksp-Cre) were generated as reported elsewhere.³² By mating c-met floxed mice with Ksp-Cre transgenic mice, conditional knockout mice (Ksp-met^{-/-}) in which c-met gene was specifically disrupted in renal tubular epithelia (genotype c-met^{fl-fl}, Cre^{+/-}) were created. These mice were cross-bred with homozygous c-met floxed mice (genotype c-met^{fl-fl}) to generate offspring with 50% Ksp-met^{-/-} mice and 50% control mice (c-met floxed mice) within the same litters. A routine PCR protocol was used for genotyping of tail DNA samples with the following primer pairs: Cre transgene, 5'-AGG-TGT-AGA-GAA-GGC-ACT-TAGC-3' and 5'-CTA-ATC-GCC-ATC-TTC-CAG-CAG-G-3', which generated a 411-bp fragment; and c-met genotyping, 5'-TTA-GGC-AAT-GAG-GTG-TCC-CAC-3'

and 5'-CCA-GGT-GGC-TTC-AAA-TTC-TAA-3', which yielded 380-bp band for the floxed alleles. All animals were born normally at the expected Mendelian frequencies. At baseline they were normal in size and did not display any gross physical or behavioral abnormalities. Animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Mouse models of acute kidney injury

Acute kidney injury (AKI) in mice was induced by a single intraperitoneal injection of cisplatin (Sigma, St. Louis, MO). Cisplatin was freshly prepared at 1 mg/ml in saline and administered by the single intraperitoneal injection at a dose of 30 mg/kg as described elsewhere.³⁹ At 3 days after injection, mice were sacrificed, and serum and kidney samples were collected for various analyses. We also utilized another AKI model induced by renal ischemia-reperfusion injury (IRI).^{6, 26} Briefly, after mice were anesthetized, a midline abdominal incision was made and bilateral renal pedicles were clipped for 35 minutes using microaneurysm clamps. After removal of the clamps, reperfusion of the kidneys was visually confirmed. The incision was then closed and the animal was allowed to recover. During the ischemic period, body temperature was maintained between 35~37.5°C using a temperature-controlled heating system. Blood and tissue samples were obtained at sacrifice at 24 hours post-IRI.

Plasmid injection

The HGF expression plasmid (pCMV-HGF) driven under a human cytomegalovirus promoter which contains full-length human HGF cDNA was cloned as described elsewhere.⁴⁰ The empty expression plasmid vector pcDNA3 was purchased from Invitrogen (San Diego, CA). Plasmid DNA was administered into mice by a hydrodynamic-based gene transfer technique via rapid injection of a large volume of DNA solution through the tail vein, as described elsewhere.⁴⁰ Briefly, 20 µg of plasmid DNA was diluted in 1.8 ml of saline and injected via the tail vein into the circulation within 5 to 10 seconds. Mice from control group were injected with 20 µg of empty plasmid vector pcDNA3 in 1.8 ml of saline in an identical fashion.⁴⁰ Twelve hours after plasmid injection, renal ischemia-reperfusion injury surgery was carried out in mice. Blood and tissue samples were obtained at 24 hours post-IRI.

Determination of serum creatinine

Serum creatinine level was determined by use of the QuantiChrom creatinine assay kit, according to the protocols specified by the manufacturer (BioAssay Systems, Hayward, CA). The level of serum creatinine was expressed as mg per deciliter (dl).

Histology and immunohistochemical staining

Paraffin-embedded mouse kidney sections (3-µm thickness) were prepared and stained with hematoxylin-eosin (HE) or periodic acid-Schiff (PAS) reagent by standard protocol. Immunohistochemical staining for c-met receptor was performed using a specific rabbit polyclonal antibody (sc-161; Santa Cruz Biotechnology, Santa Cruz, CA), according to the established protocol as described previously.⁴¹

Immunofluorescent staining

Kidney cryosections were fixed with 3.7% paraformalin for 15 min at room temperature and immersed in 0.2% Triton X/100 for 10 min. After blocking with 10% donkey serum in PBS for 1 hour, slides were immunostained with primary antibodies against CD3 (sc-20047) and CD45 (sc-1178; Santa Cruz Biotechnology). Primary antibodies in PBS containing 10% normal donkey serum were applied to cells overnight at 4°C, and, after washing, slides were incubated for 1 hour with CY3-conjugated, affinity-purified secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). Stained cells were mounted with Vectashield anti-fade mounting media using DAPI (4', 6-diamidino-2-phenylindole, HCl) to visualize the nuclei (Vector Laboratories, Burlingame, CA). The CD3 and CD45-positive cells were defined as positive cytoplasmic membrane staining (red) with a DAPI-positive nuclear staining (blue), and expressed as the numbers of positive cells per high power field (hpf). For double immunofluorescence staining, two sets of slides were immunostained with anti-c-met or anti-cleaved caspase-3 (#9664, Cell Signaling Technology, Danvers, MA) and one of the following antibodies: anti-aquaporin 1 (AQP1; AB2219), anti-Thiazide-sensitive NaCl Cotransporter (NCC; AB3553) or anti-aquaporin 3 (AQP3; AB3067; Millipore, Billerica, MA). Double immunostaining for cleaved caspase-3 and laminin (L-8271, Sigma, St. Louis, MO) was also performed. Slides were viewed using an Eclipse E600epi fluorescence microscope equipped with a digital camera (Nikon, Melville, NY)

Detection of apoptotic cells

Apoptotic cell death was determined by using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining with the DeadEnd Colorimetric Apoptosis Detection System (Promega, Madison, WI), as described previously.²⁶

Real-time RT-PCR

Total RNA isolation and real-time RT-PCR were carried out by the procedures described previously.⁴² Briefly, first strand cDNA synthesis was carried out by using a reverse transcription system kit according to the instructions of the manufacturer (Promega). Quantitative, real-time RT-PCR (qRT-PCR) was performed on ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) as described previously.⁴² The sequences of the primer pairs were as follows: mouse TNF- α , 5-cgtagcaaaccaccaagtg-3 (sense) and 5-ccttgaagagaacctgggag-3 (anti-sense); mouse MCP-1, 5-cccactcacctgctgctac-3 (sense) and 5-ttcttggggtcagcacaga-3. PCR was run under standard conditions. The mRNA levels of various genes were calculated after normalizing to β -actin.

Western blot analysis

Kidney tissues were lysed with radioimmune precipitation assay (RIPA) buffer containing 1% NP-40, 0.1% SDS, 100 μ g/ml PMSF, 1% protease inhibitor cocktail, and 1% phosphatase I and II inhibitor cocktail (Sigma) on ice. The supernatants were collected after centrifugation at 13,000 \times g at 4°C for 15 min. Protein expression was analyzed by Western blot analysis as described previously.²⁶ The primary antibodies used were as follows: anti-c-met (sc-8057; Santa Cruz Biotechnology), anti-phospho-Akt (Ser473) (#4060) (Cell

Signaling Technology), anti-Bax (sc-493), anti-FasL (sc-6237), anti-actin (sc-1616; Santa Cruz Biotechnology) and anti-GAPDH (AM4300; Ambion, Austin, TX).

Statistical analyses

All data were expressed as mean \pm SEM. Statistical analysis of the data was performed using SigmaStat software (Jandel Scientific Software, San Rafael, CA). Comparison between groups was made using one-way ANOVA, followed by the Student-Newman-Keuls test. $P < 0.05$ was considered significant.

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REFERENCES

1. Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney Int.* 2008; 73:994–1007. [PubMed: 18272962]
2. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol.* 2003; 14:2199–2210. [PubMed: 12874476]
3. Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest.* 2002; 110:835–842. [PubMed: 12235115]
4. Havasi A, Borkan SC. Apoptosis and acute kidney injury. *Kidney Int.* 2011; 80:29–40. [PubMed: 21562469]
5. Sharfuddin AA, Molitoris BA. Pathophysiology of ischemic acute kidney injury. *Nat Rev Nephrol.* 2011; 7:189–200. [PubMed: 21364518]
6. Linkermann A, Brasen JH, Himmerkus N, et al. Rip1 (receptor-interacting protein kinase 1) mediates necroptosis and contributes to renal ischemia-reperfusion injury. *Kidney Int.* 2012; 81:751–761. [PubMed: 22237751]
7. Liu Y. Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action. *Am J Physiol Renal Physiol.* 2004; 287:F7–F16. [PubMed: 15180923]
8. Nakamura T, Sakai K, Matsumoto K. Hepatocyte growth factor twenty years on: Much more than a growth factor. *J Gastroenterol Hepatol.* 2011; 26(Suppl 1):188–202. [PubMed: 21199531]
9. Liu ZX, Nickel CH, Cantley LG. HGF promotes adhesion of ATP-depleted renal tubular epithelial cells in a MAPK-dependent manner. *Am J Physiol Renal Physiol.* 2001; 281:F62–70. [PubMed: 11399647]
10. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol.* 2010; 11:834–848. [PubMed: 21102609]
11. Zhang X, Li Y, Dai C, et al. Sp1 and Sp3 transcription factors synergistically regulate HGF receptor gene expression in kidney. *Am J Physiol Renal Physiol.* 2003; 284:F82–F94. [PubMed: 12473536]
12. Herrero/Fresneda I, Torras J, Franquesa M, et al. HGF gene therapy attenuates renal allograft scarring by preventing the profibrotic inflammatory-induced mechanisms. *Kidney Int.* 2006; 70:265–274. [PubMed: 16710352]
13. Gong R, Rifai A, Dworkin LD. Hepatocyte growth factor suppresses acute renal inflammation by inhibition of endothelial E-selectin. *Kidney Int.* 2006; 69:1166–1174. [PubMed: 16501492]
14. Giannopoulou M, Dai C, Tan X, et al. Hepatocyte growth factor exerts its anti-inflammatory action by disrupting nuclear factor- κ B signaling. *Am J Pathol.* 2008; 173:30–41. [PubMed: 18502824]
15. Zhang J, Yang J, Liu Y. Role of Bcl-xL induction in HGF-mediated renal epithelial cell survival after oxidant stress. *Int J Clin Exp Pathol.* 2008; 1:242–253. [PubMed: 18784816]

16. Liu Y. Hepatocyte growth factor and the kidney. *Curr Opin Nephrol Hypertens.* 2002; 11:23–30. [PubMed: 11753083]
17. Rabkin R, Fervenza F, Tsao T, et al. Hepatocyte growth factor receptor in acute tubular necrosis. *J Am Soc Nephrol.* 2001; 12:531–540. [PubMed: 11181801]
18. Liu Y, Tolbert EM, Lin L, et al. Up-regulation of hepatocyte growth factor receptor: an amplification and targeting mechanism for hepatocyte growth factor action in acute renal failure. *Kidney Int.* 1999; 55:442–453. [PubMed: 9987069]
19. Dai C, Saleem MA, Holzman LB, et al. Hepatocyte growth factor signaling ameliorates podocyte injury and proteinuria. *Kidney Int.* 2010; 77:962–973. [PubMed: 20375988]
20. Ma H, Saenko M, Opuko A, et al. Deletion of the Met receptor in the collecting duct decreases renal repair after ureteral obstruction. *Kidney Int.* 2009; 76:868–876. [PubMed: 19675527]
21. Sheikh-Hamad D, Timmins K, Jalali Z. Cisplatin-induced renal toxicity: possible reversal by N-acetylcysteine treatment. *J Am Soc Nephrol.* 1997; 8:1640–1644. [PubMed: 9335396]
22. Dai C, Huh CG, Thorgeirsson SS, et al. β -cell-specific ablation of the hepatocyte growth factor receptor results in reduced islet size, impaired insulin secretion, and glucose intolerance. *Am J Pathol.* 2005; 167:429–436. [PubMed: 16049329]
23. Gardai SJ, Hildeman DA, Frankel SK, et al. Phosphorylation of Bax Ser184 by Akt regulates its activity and apoptosis in neutrophils. *J Biol Chem.* 2004; 279:21085–21095. [PubMed: 14766748]
24. Ruiz-Vela A, Opferman JT, Cheng EH, et al. Proapoptotic BAX and BAK control multiple initiator caspases. *EMBO Rep.* 2005; 6:379–385. [PubMed: 15776018]
25. Wei MC, Zong WX, Cheng EH, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science.* 2001; 292:727–730. [PubMed: 11326099]
26. Zhou D, Li Y, Lin L, et al. Tubule-specific ablation of endogenous β /catenin aggravates acute kidney injury. *Kidney Int.* 2012; 82:537–547. [PubMed: 22622501]
27. Dai C, Yang J, Liu Y. Single injection of naked plasmid encoding hepatocyte growth factor prevents cell death and ameliorates acute renal failure in mice. *J Am Soc Nephrol.* 2002; 13:411–422. [PubMed: 11805170]
28. Yang J, Chen S, Huang L, et al. Sustained expression of naked plasmid DNA encoding hepatocyte growth factor in mice promotes liver and overall body growth. *Hepatology.* 2001; 33:848–859. [PubMed: 11283849]
29. Mizuno S, Matsumoto K, Nakamura T. HGF as a renotropic and anti-fibrotic regulator in chronic renal disease. *Front Biosci.* 2008; 13:7072–7086. [PubMed: 18508717]
30. Huh CG, Factor VM, Sanchez A, et al. Hepatocyte growth factor-c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci U S A.* 2004; 101:4477–4482. [PubMed: 15070743]
31. Ciaramboli G, Deuster D, Knief A, et al. Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions. *Am J Pathol.* 2010; 176:1169–1180. [PubMed: 20110413]
32. Shao X, Somlo S, Igarashi P. Epithelial-specific Cre-lox recombination in the developing kidney and genitourinary tract. *J Am Soc Nephrol.* 2002; 13:1837–1846. [PubMed: 12089379]
33. Kinsey GR, Li L, Okusa MD. Inflammation in acute kidney injury. *Nephron Exp Nephrol.* 2008; 109:e102–107. [PubMed: 18802372]
34. Vasudevan KM, Garraway LA. AKT signaling in physiology and disease. *Curr Top Microbiol Immunol.* 2010; 347:105–133. [PubMed: 20549472]
35. Liu Y. Hepatocyte growth factor promotes renal epithelial cell survival by dual mechanisms. *Am J Physiol Renal Physiol.* 1999; 277:F624–F633.
36. Villa/Morales M, Fernandez/Piqueras J. Targeting the Fas-FasL signaling pathway in cancer therapy. *Expert Opin Ther Targets.* 2012; 16:85–101. [PubMed: 22239437]
37. Lettau M, Paulsen M, Schmidt H, et al. Insights into the molecular regulation of FasL (CD178) biology. *Eur J Cell Biol.* 2011; 90:456–466. [PubMed: 21126798]
38. Coudriet GM, He J, Trucco M, et al. Hepatocyte growth factor modulates interleukin-6 production in bone marrow derived macrophages: implications for inflammatory mediated diseases. *PLoS ONE.* 2011; 5:e15384. [PubMed: 21072211]

39. Wei Q, Dong G, Franklin J, et al. The pathological role of Bax in cisplatin nephrotoxicity. *Kidney Int.* 2007; 72:53–62. [PubMed: 17410096]
40. Dai C, Yang J, Bastacky S, et al. Intravenous administration of hepatocyte growth factor gene ameliorates diabetic nephropathy in mice. *J Am Soc Nephrol.* 2004; 15:2637–2647. [PubMed: 15466268]
41. Tan X, He W, Liu Y. Combination therapy with paricalcitol and trandolapril reduces renal fibrosis in obstructive nephropathy. *Kidney Int.* 2009; 76:1248–1257. [PubMed: 19759524]
42. Ding H, Zhou D, Hao S, et al. Sonic hedgehog signaling mediates epithelial-mesenchymal communication and promotes renal fibrosis. *J Am Soc Nephrol.* 2012; 23:801–813. [PubMed: 22302193]

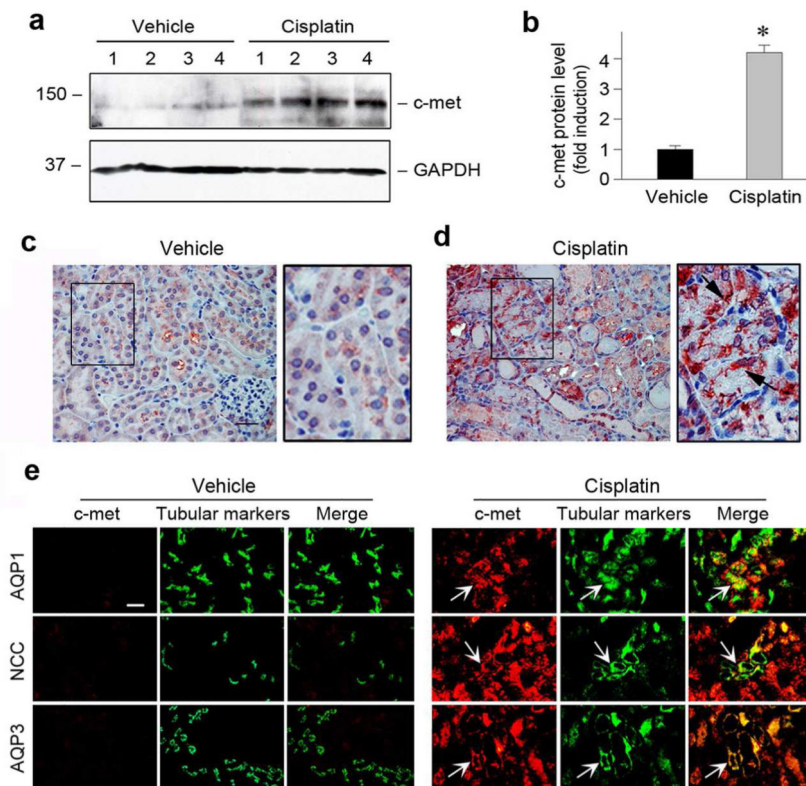


Figure 1. Induction of c-met expression in the kidneys after acute kidney injury
(a) Western blots demonstrate renal c-met protein levels after AKI induced by cisplatin. Mouse kidneys were collected at 3 days after injection of either vehicle or cisplatin. Numbers (1 through 4) indicate each individual animal in a given group. **(b)** Quantitative determination of renal c-met protein levels in vehicle- and cisplatin-treated groups. * $P < 0.05$ versus vehicle controls ($n = 4$). **(c, d)** Representative micrographs show renal c-met staining in mice treated with vehicle **(c)** or cisplatin **(d)**. Boxed area is enlarged. Arrows indicate positive staining. Scale bar, 50 μm . **(e)** Co-staining for c-met and tubular segment-specific markers in the kidneys after cisplatin injection. Immunofluorescence staining demonstrated the co-staining of c-met (red) and various tubular markers (green) in the kidneys at 3 days after cisplatin injection. Segment-specific tubular markers used are as follows: proximal tubule, aquaporin-1 (AQP1); distal tubule, thiazide-sensitive NaCl cotransporter (TSC)-NCC; and collecting duct, aquaporin-3 (AQP3). Arrowheads indicate c-met-positive tubules. Scale bar, 50 μm .

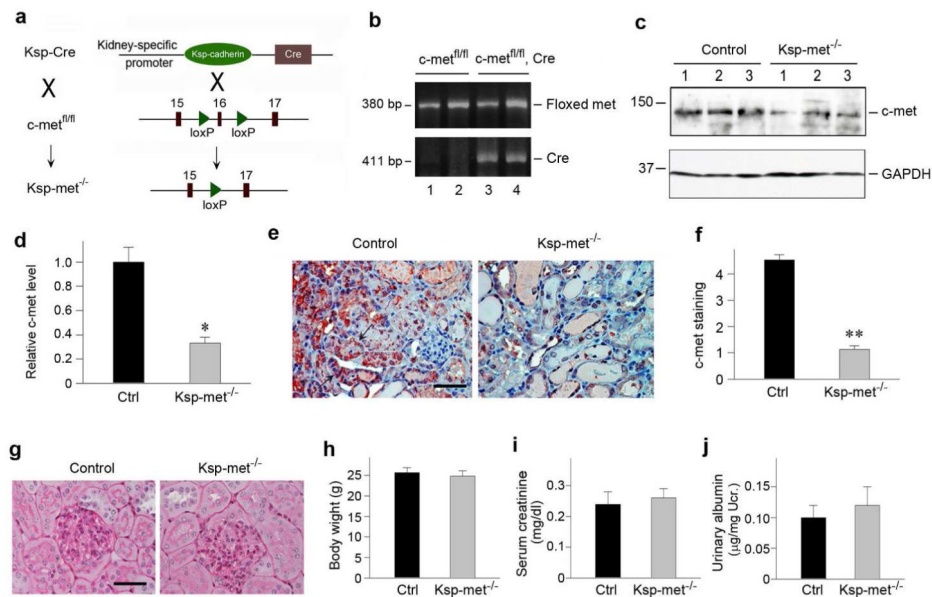


Figure 2. Generation of the tubule-specific c-met knockout mice

(a) Diagram shows the strategy of cross-breeding of the c-met floxed mice (c-met^{fl/fl}) with Cre transgenic mice under the control of Ksp-cadherin promoter (Ksp-Cre). Exons 15 through 17 of c-met gene were indicated. LoxP sites were also denoted. (b) Genotyping of the mice by PCR analysis of genomic DNA. Lanes 1 and 2 show the genotyping of the control mice used in this study (genotype: c-met^{fl/fl}), whereas lane 3 and 4 demonstrate the genotyping of the tubule-specific c-met knockout mice (genotype: c-met^{fl/fl}, Cre), designated as Ksp-met^{-/-}. (c, d) Western blot analyses demonstrated a substantial reduction of renal c-met protein in Ksp-met^{-/-} mice. Representative Western blot (c) and quantitative data (d) are presented. Kidney lysates were made from control and Ksp-met^{-/-} mice at 3 days after cisplatin injection. Numbers (1, 2 and 3) indicate each individual animal in a given group. **P* < 0.05 versus controls (n = 4). (e) Representative micrographs show renal c-met staining in the control and Ksp-met^{-/-} mice at 3 days after cisplatin injection. Arrows indicate positive renal tubules. Scale bar, 50 μm. (f) Semi-quantitative analysis show a substantial reduction of c-met staining in Ksp-met^{-/-} mice after AKI. ***P* < 0.01 versus controls. (g-j) Mice with tubule-specific ablation of c-met receptor are phenotypically normal. (g) Representative micrographs show the morphology of control and Ksp-met^{-/-} kidneys. Scale bar, 30 μm. There was no differences in body weight (h), serum creatinine (i), and urinary albumin level (j) between control and Ksp-met^{-/-} mice in normal physiological conditions (n=4).

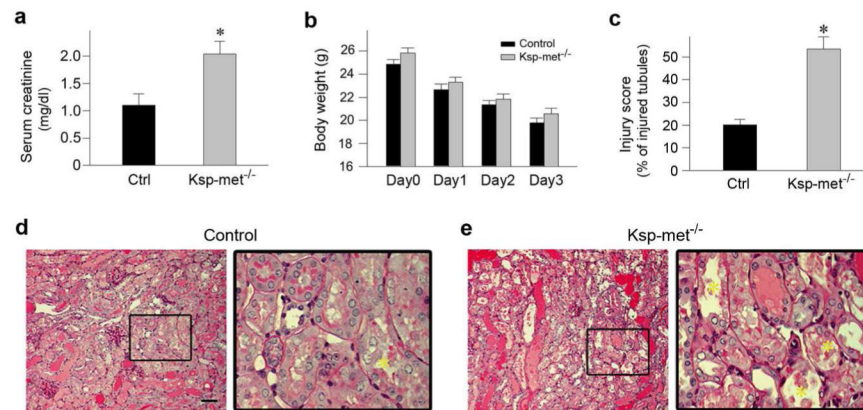


Figure 3. Tubule-specific ablation of c-met aggravates kidney dysfunction and histologic lesions after AKI

(a) Serum creatinine level in control and Ksp-met^{-/-} mice at 3 days after cisplatin injection. * $P < 0.05$ ($n = 7$). (b) Change of body weight in mice following the treatment of cisplatin. (c) Morphological injury assessed in the PAS-stained kidney sections in control and Ksp-met^{-/-} mice. ** $P < 0.01$ ($n = 7$). (d, e) Representative micrographs of the kidneys at 3 days after cisplatin injection in control (d) and Ksp-met^{-/-} mice (e). Boxed areas were enlarged and presented. Yellow asterisks in the enlarged boxed areas indicate the injured tubules. Scale bar, 50 μm .

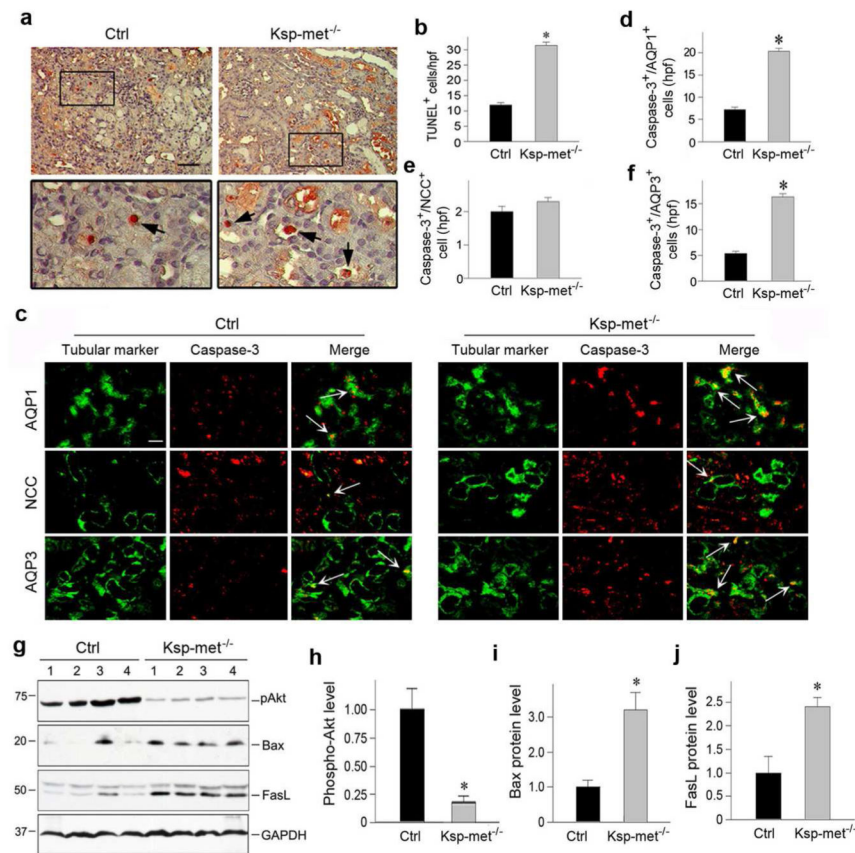


Figure 4. Tubule-specific ablation of c-met promotes apoptosis after AKI induced by cisplatin (a) Representative micrographs show apoptotic cell death detected by TUNEL staining after AKI in the control and Ksp-met^{-/-} mice. Arrows in the enlarged boxed areas indicate apoptotic cells. Scale bar, 50 μ m. (b) Quantitative determination of apoptotic cells in renal cortical and medullar regions at 3 days after cisplatin injection. Data are presented as numbers of apoptotic cells per high power field (hpf). ** $P < 0.05$ (n = 6). (c-f) Loss of tubular c-met signaling promoted renal tubule cells apoptosis. Co-immunofluorescence staining demonstrated the co-staining of cleaved-caspase-3 (red) and various tubular markers (green) in the kidneys at 3 days after cisplatin injection. Segment-specific tubular markers used are as follows: proximal tubule, aquaporin-1 (AQP1); distal tubule, thiazide-sensitive NaCl cotransporter (TSC)-NCC; and collecting duct, aquaporin-3 (AQP3). Arrowheads indicate cleaved-caspase-3-positive cells. Scale bar, 50 μ m. Quantitative data on double staining for the cleaved caspase-3 and tubular segment-specific markers are presented in Panel (d), (e) and (f), respectively. * $P < 0.05$ (n = 4). (g-j) Tubular ablation of c-met suppressed renal Akt phosphorylation and promoted Bax, FasL protein expression. Representative Western blot (g) and quantitative data on phosphor-Akt (h), Bax (i) FasL (j) and are presented. Numbers (1, 2, 3 and 4) indicate each individual animal in a given group. * $P < 0.05$ (n = 4).

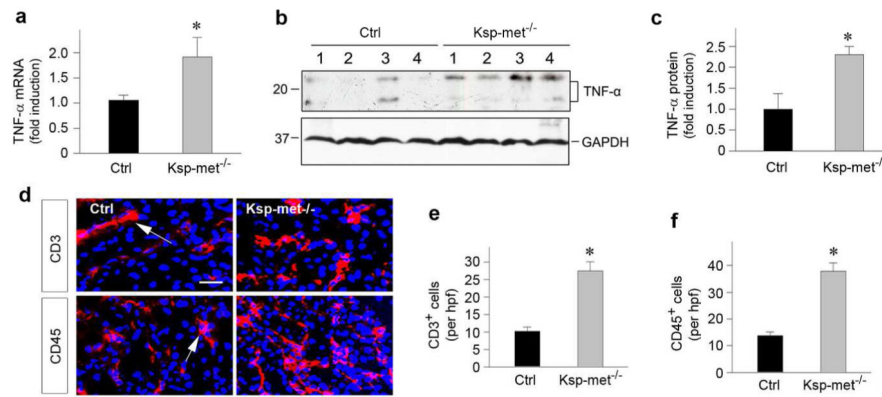


Figure 5. Endogenous c-met signaling inhibits renal inflammation after AKI induced by cisplatin (a) Quantitative, real-time RT-PCR (qRT-PCR) demonstrate an increased expression of pro-inflammatory cytokine TNF- α mRNA in Ksp-met $^{-/-}$ kidneys, comparing to controls. * $P < 0.05$ ($n = 6$). (b, c) Western blots demonstrate renal TNF/ α protein levels in the control and Ksp-met $^{-/-}$ mice at 3 days after cisplatin injection. Representative Western blot (b) and quantitative data (c) are presented. (d) Immunofluorescence staining revealed an increased infiltration of CD3 $^{+}$ T cells and CD45 $^{+}$ leukocytes in the kidneys at 3 days after cisplatin injection. Arrows indicate positive staining. Scale bar, 30 μ m. (e, f) Quantitative data are presented as numbers of CD3 $^{+}$ (e) or CD45 $^{+}$ cells (f) per high power field (hpf). * $P < 0.05$ ($n = 5$).

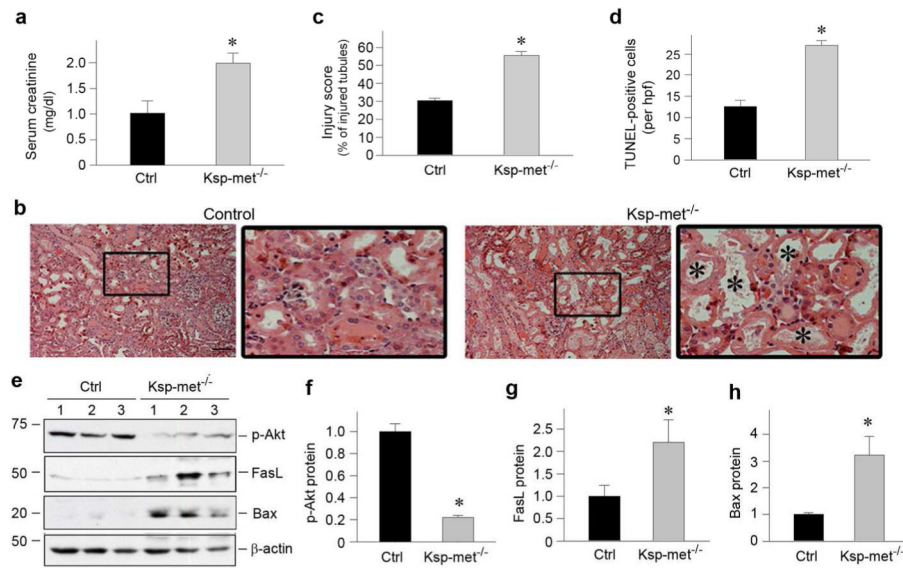


Figure 6. Loss of tubular c-met aggravates AKI induced by ischemia-reperfusion injury (IRI) (a) Serum creatinine level in the control and Ksp-met^{-/-} mice at 1 day after renal IRI. * $P < 0.05$ (n=3-4). (b) Representative micrographs of the kidneys in control and Ksp-met^{-/-} mice at 1 day post-IRI. Asterisks in the enlarged boxed areas indicate injured tubules. Scale bar, 50 μ m. (c) Quantitative assessment of renal injury. Injury score (% of injured tubules) are presented. * $P < 0.05$ (n=3). (d) Quantitative determination of apoptotic cells in the kidneys of control and Ksp-met^{-/-} mice at 1 day after IRI. Data are presented as numbers of apoptotic cells per high power field (hpf). * $P < 0.05$ (n = 3). (e-h) Tubular loss of c-met suppressed renal Akt phosphorylation and promoted FasL and Bax protein expression in ischemic AKI. Western blot (e) and quantitative data on phosphor-Akt (f), FasL (g) and Bax (h) are presented. Numbers (1, 2 and 3) indicate each individual animal in a given group. * $P < 0.05$ (n = 3).

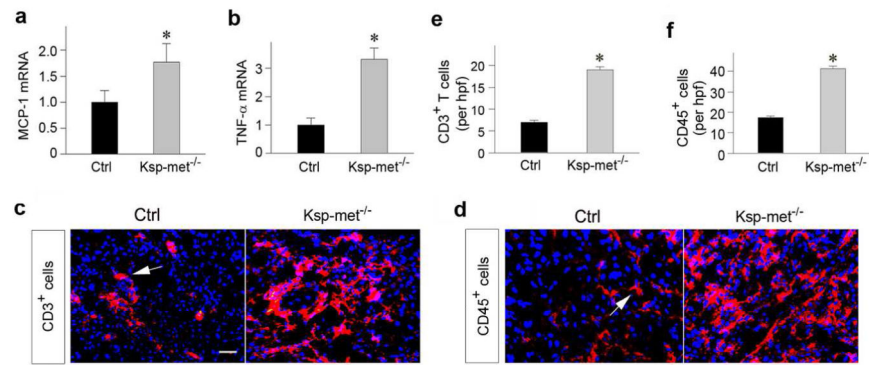


Figure 7. Tubular loss of c-met aggravates renal inflammation in ischemic AKI
(a, b) qRT-PCR demonstrate an increased expression of pro-inflammatory cytokines TNF- α and MCP-1 in Ksp-met^{-/-} kidneys, comparing to controls. * $P < 0.05$ (n = 3-4). **(c, d)** Immunostaining shows an increased renal infiltration of CD3⁺ T cells and CD45⁺ leukocytes in the kidneys of Ksp-met^{-/-} mice at 1 day after IRI. Arrows indicate positive staining. Scale bar, 50 μ m. **(e, f)** Quantitative data are presented as numbers of positive cells per high power field (hpf). * $P < 0.05$ (n = 5).

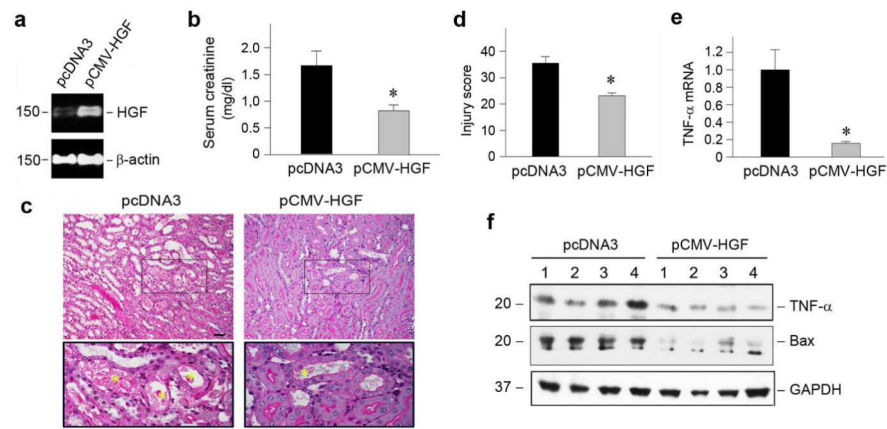


Figure 8. Expression of exogenous HGF by single plasmid injection *in vivo* protects against ischemic AKI in mice

(a) RT/PCR show exogenous human HGF mRNA expression in the kidneys at 1 day after single plasmid injection. (b) Serum creatinine level in mice injected with either empty vector (pcDNA3) or human HGF expression vector (pCMV-HGF). Serum was collected at 1 day after IRI. $*P < 0.05$ (n=4). (c) Representative micrographs of the kidneys in mice injected with pcDNA3 or pCMV-HGF plasmid at 1 day after IRI. Yellow asterisks in the enlarged boxed areas indicate the injured tubules. Scale bar, 50 μ m. (d) Quantitative assessment of renal injury. Injury score (% of injured tubules) are presented. $*P < 0.05$ (n=4). (e) qRT-PCR demonstrates an decreased expression of TNF- α mRNA in the kidneys of mice injected with pCMV-HGF, comparing to pcDNA3 controls. $*P < 0.05$ (n = 4). (f) Ectopic expression of HGF by single plasmid injection also suppresses renal expression of TNF- α and Bax proteins. Numbers (1, 2, 3 and 4) indicate each individual animal in a given group.

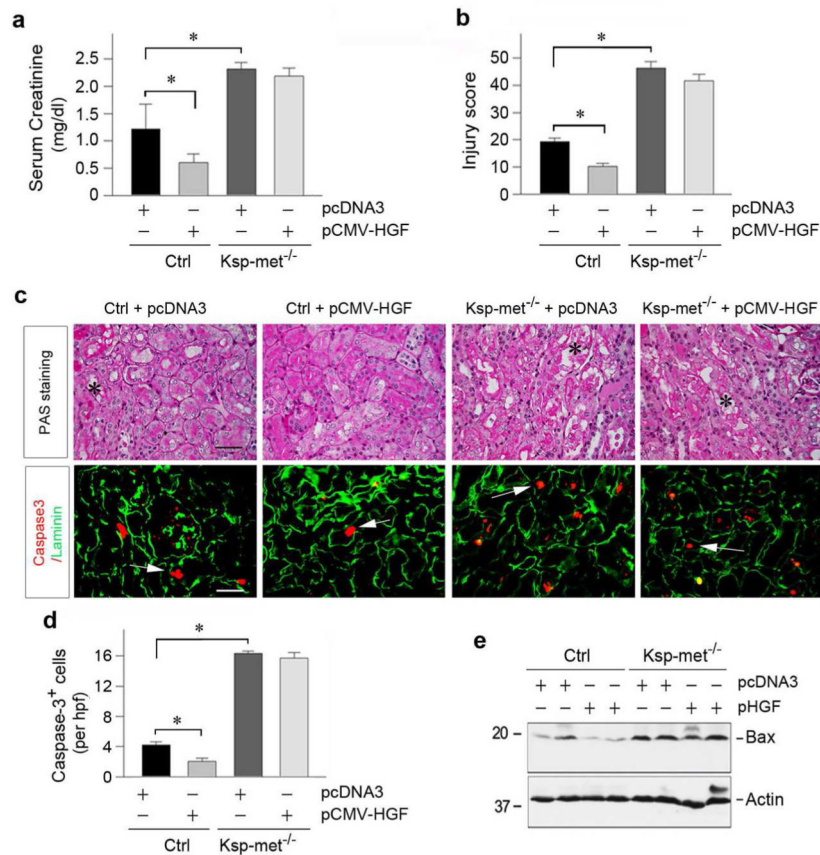


Figure 9. Activation of tubular c-met signaling is essential for renal protection after AKI

(a) Serum creatinine level in control and Ksp-met^{-/-} mice administrated with either empty vector (pcDNA3) or HGF expression vector (pCMV-HGF) at 3 days after cisplatin injection. **P* < 0.05 (n = 4-7). (b, c) Morphological injury assessed in the PAS-stained kidney sections in control and Ksp-met^{-/-} mice administrated with either pcDNA3 or pCMV-HGF at 3 days after cisplatin injection. Representative micrographs of the kidneys (c) and quantitative assessment of injury (b) are presented. **P* < 0.05 versus control mice injected with pcDNA3 (n = 3). Scale bar, 50 μm. (c, d) Co-immunofluorescence staining for cleaved-caspase-3 (red) and laminin (green) demonstrated that apoptosis largely occurred in the tubular compartment of kidneys (c) at 3 days after cisplatin injection. Scale bar, 50 μm. Quantitative data are presented in Panel (d). **P* < 0.05 versus control mice injected with pcDNA3 (n = 3). (e) Western blots demonstrate renal Bax protein levels in the control and Ksp-met^{-/-} mice administrated with either pcDNA3 or pCMV-HGF at 3 days after cisplatin injection.