Hepatocyte Growth Factor Receptor in Acute Tubular Necrosis

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Abstract. In acute tubular necrosis, there are early transient increases in circulating and local bioactive hepatocyte growth factor (HGF) levels and renal HGF receptor (c-MET) gene expression. It has therefore been suggested that endogenous HGF may play a role in initiating renal repair. To test this hypothesis, changes in the levels, activity, and anatomic distribution of c-MET protein were characterized in relation to the onset and localization of DNA synthesis in kidneys of rats with ischemia-induced acute tubular necrosis. Whole-kidney c-MET protein levels were significantly increased in the injured kidneys 12 h after injury and rose to a maximum after 1 d, exceeding the control values by sevenfold. Eight days after injury, c-MET levels, although decreasing, were still elevated above control values. An increase in the levels of activated c-MET, i.e., tyrosine-phosphorylated c-MET, was also evident as early as 12 h after injury. Histologic analyses demonstrated that the increase in c-MET immunoreactivity was most marked in the most severely damaged nephron segments in the outer medulla. In injured proximal tubules, the receptor was redistributed from an apical location to an intracellular location. DNA synthesis was increased in the injured kidneys, especially in the outer medulla, where the increase in c-MET protein levels was most prominent. The increase in DNA synthesis was first detected 12 h after the initial increase in activated c-MET levels. It is concluded that the early increases in the levels of c-MET protein and activated receptor support the hypothesis that HGF participates in the initiation of renal regeneration. In addition, the persistent elevation of c-Met protein levels suggests that prolonged and even late treatment with HGF may be of therapeutic value.

Recovery of normal renal function after acute tubular necrosis (ATN) requires regeneration of damaged tubular epithelium, a process in which growth factors play an important role. Several studies have examined the ability of selected growth factors to modulate the course of experimental ATN in rats. Earlier studies indicated that epidermal growth factor attenuates the severity and duration of acute renal failure (1), and we and other investigators recently demonstrated that insulin-like growth factor I treatment is also effective (2,3). Hepatocyte growth factor (HGF) is another growth factor that may play a role in renal regeneration after injury, and its administration does have a salutary effect on the course of experimental acute renal failure, stimulating DNA synthesis and accelerating recovery (4–6). HGF is a potent mitogen not only for hepatocytes but also for endothelial cells and several types of epithelial cells, including renal tubular cells (7,8). This growth factor is produced throughout the body in cells of mesenchymal origin and is secreted in an immature inactive form that is released into the circulation or locally stored bound to the extracellular matrix (7). In the rat kidney, HGF is produced in the peritubular interstitium, most likely by endothelial cells and macrophages (9). In addition to stimulating cell growth and migration in cell culture, it stimulates the formation of tubular structures and cellular differentiation (7,8). HGF is secreted as a biologically inactive precursor that is activated by serine protease cleavage. These proteases are induced in injured tissues and serve to increase the formation of the mature active form (10). Active HGF acts on target cells through a cell membrane tyrosine kinase receptor known as c-MET. Binding of HGF to c-MET causes receptor homodimerization, auto-phosphorylation, and activation of tyrosine kinase activity, which in turn activates complex downstream signaling cascades, including the phosphatidylinositol-3-kinase and mitogen-activated protein kinase pathways (7,8).

Support for a role for endogenous HGF in regeneration after ATN comes from studies that demonstrated early transient increases in HGF levels in injured kidneys. HGF gene expression is simultaneously increased in uninjured tissues such as liver, lung, and spleen (4,11,12), and these tissues then release the growth factor into the circulation. HGF bioactivity in kidney and plasma is increased, reaching a peak 12 h after injury and returning to baseline levels after 2 to 3 d, indicating that HGF functions in both an endocrine manner and a local paracrine/autocrine manner (4,13). Kidney c-MET mRNA lev-

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els are also transiently increased after ischemia- and folic acid-induced injuries (13–15), and whole-kidney c-MET protein levels are elevated 12 and 24 h after folic acid administration (13). Taken together, the early increases in circulating HGF levels and kidney c-MET expression suggest that this ligand-receptor pair may play a role in promoting regeneration of injured kidneys. To more firmly establish whether HGF and c-MET do indeed participate in the regenerative process, we have examined the response of the receptor in greater detail. We report that, in rats with ischemic ATN, both the level and state of activation of the c-MET receptor protein increase early after injury, before the onset of DNA synthesis. Furthermore, the increase in c-MET levels is most prominent in the more severely injured nephron segments, where maximal DNA synthesis is observed. Therefore, our data, although not providing direct evidence for a role for HGF in renal regeneration, do support the hypothesis that HGF participates in the early regenerative process. Finally, we also report that c-MET protein levels remain elevated at a time when the elevated circulating HGF levels are known to have returned to baseline values (4,13), suggesting that prolonged and even delayed treatment with HGF may be of therapeutic value in acute renal failure.

### Materials and Methods

#### Animals and Tissue Collection

Male Sprague-Dawley rats, weighing approximately 225 g and allowed free access to food and water, were anesthetized with intraperitoneally administered xylazine (10 mg/kg) and ketamine (75 mg/kg). Body temperature was maintained at 37°C. The kidneys were exposed via a flank incision (2). In one-half of the rats, the renal pedicle was clamped for 35 min (ATN group); the other rats served as sham-operated control animals. After recovery from the procedure, the ATN rats were allowed ad libitum dietary intake and the control animals were pair-fed. Twelve hours or 1, 2, or 8 d after surgery, the rats were anesthetized, blood was collected, and the kidneys were rapidly excised and frozen in liquid nitrogen. Other rats were given 100 mg/kg bromodeoxyuridine (BrdU) 2 h before euthanasia, at which time the kidneys were perfusion-fixed, via the abdominal aorta, with cold 4% paraformaldehyde for 10 min. Kidney slices and small bowel samples (positive control samples) were then stored overnight in cold 4% paraformaldehyde and embedded in paraffin blocks, from which 5-μm sections were cut and processed for immunohistochemical analysis.

#### Solution Hybridization/RNase Protection Assays

Total RNA was extracted according to the method of Chomczynski, and mRNA amounts were quantified by means of a solution hybridization/ribonuclease protection assay, as described previously (16). In brief, total RNA (20 μg) was hybridized overnight at 42°C with approximately 200,000 cpm of [α-32P]CTP-labeled antisense c-MET RNA probe in 75% formamide/400 mM NaCl. After hybridization, the mixture was added to RNase digestion buffer containing RNase A (100 mg/ml) and RNase T1 (2000 U/ml) and was incubated at 30°C for 30 min. Proteinase K (200 mg/ml) was then added, and incubation was continued at 37°C for 30 min. Protected hybrids were then precipitated with ethanol and size-separated on a 5% polyacrylamide/8 M urea denaturing gel. Autoradiographs were obtained, and protected bands were measured by densitometry. Sample loading was controlled by measuring the level of ethidium bromide-stained 18S rRNA in samples of total RNA subjected to electrophoresis through a denaturing agarose gel containing 1.7% formaldehyde. Protection assays were repeated at least once.

#### Preparation of Riboprobes

The c-MET template was kindly provided by Dr. Paul Godowski (Genentech Inc.). This cDNA fragment was prepared by first performing reverse transcription of total rat liver mRNA using two primers with homology to both the human and mouse c-MET mRNA sequences, i.e., 5′-CGTAGGATCCATATGGCTGGGACTT-3′ and 5′-GCGATGATCCACTTAACTGCTTGG-3′ (17,18). After PCR amplification, the resulting 221-bp cDNA fragment was inserted into a pBluescript II SK(+) plasmid. The plasmid was linearized with SrfI, and the T7 promoter was used for transcription of the riboprobe.

#### Preparation of Crude Kidney Membranes

For measurement of c-MET levels in kidney plasma membranes, crude membranes were isolated, as described previously, from control and ATN kidneys 2 and 8 d after surgery (16). In brief, kidney homogenates were prepared in 8% (wt/wt) sucrose and subjected to differential centrifugation. Crude membranes were collected after centrifugation at 47,000 × g, washed, resuspended in calcium-free Krebs-Ringer Hepes buffer (pH 7.4), and stored at −80°C.

#### Western Immunoblotting

c-MET and phosphorylated c-MET were detected according to previously described methods. In brief, frozen kidney was homogenized under liquid nitrogen and homogenized in 5 volumes of ice-cold RIPA lysis buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 μg/ml pepstatin, 1 mM sodium vanadate, and 1 mM NaF. The homogenate was incubated at 4°C with shaking for 1 h and then clarified by centrifugation at 40,000 × g for 30 min. Plasma membranes were solubilized in the same buffer. For detection of c-MET, the supernate was added to sodium dodecyl sulfate (SDS) sample buffer with 2-mercaptoethanol, and the solution was heated for 5 min at 95°C. For detection of c-MET phosphorylation, 1 mg of tissue extract was incubated overnight at 4°C with anti-murine c-MET antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and precipitated with protein A-agarose. The immunoprecipitates were washed several times and resuspended in SDS sample buffer with 2-mercaptoethanol and were then heated at 95°C for 5 min. The solubilized antibodies (50-μg aliquots) were separated by SDS-polyacrylamide gel electrophoresis (7.5% running gel) and transferred to nitrocellulose membranes, which were blocked with 5% nonfat dry milk for detection of c-MET or 1% bovine serum albumin in Tris-buffered saline with 0.05% Tween for detection of tyrosine phosphorylation. The membranes were then incubated overnight at 4°C with 1 μg/ml anti-murine c-MET or anti-phosphotyrosine antibody (Santa Cruz Biotechnology), washed, and incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody; proteins were detected using the enhanced chemiluminescence method (Amersham, Arlington Heights, IL) and were quantified by densitometry.

#### Immunohistochemical Analyses

c-MET and BrdU were detected with appropriate antisera by means of the avidin-biotin immunoperoxidase procedure. For localization of c-MET, two different, affinity-purified, rabbit IgG polyclonal antibodies were used at dilutions of 1:400. One antibody was directed against the last 21 amino acids of the carboxyl terminus of the mouse
c-MET protein (Santa Cruz Biotechnology). The other antibody was directed against the extracellular domain of human c-MET and was prepared by stimulating antibodies against a recombinant c-MET-IgG fusion protein (19). For detection of c-Met, deparaffinized kidney sections were incubated first with 3% hydrogen peroxide for 30 min (to block endogenous peroxidase) and then with 10% nonimmune goat serum for 1 h at room temperature (to prevent nonspecific antibody binding), followed by overnight incubation at 4°C with the murine or human anti-c-MET antibody. After a wash with phosphate-buffered saline, sections were incubated with biotinylated goat anti-rabbit IgG (dilution, 1:400; Vector Laboratories) for 30 min, followed by incubation with horseradish peroxidase-conjugated streptavidin for 30 min at room temperature. The secondary antibody was observed after the addition of 3,3’-diaminobenzidine in 0.02% urea/hydrogen peroxide. Kidneys from at least three control rats and three ATN rats were examined at each time point of the study. Nonspecific signals were excluded by incubating sections with nonimmune rabbit serum or with anti-c-MET antibody that had been preabsorbed with the c-MET-IgG fusion protein or with a tumor necrosis factor receptor-IgG fusion protein (20). Sections were counterstained with Gill’s hematoxylin. For detection of BrdU, the sections were incubated for 25 min at 37°C in phosphate-buffered saline with 0.1% trypsin and 0.1% CaCl₂ and were then incubated for 45 min at 70°C with 95% formamide in 0.15 trisodium citrate. After the sections were rinsed, they were incubated for 1 h at room temperature with mouse monoclonal anti-BrdU antibody diluted 1:50 in 1% fetal bovine serum (Dako Products, Carpenteria, CA). The secondary antibody was biotinylated goat anti-mouse IgG (Vector) and was detected using the peroxidase reaction, as described above.

**DNA Synthesis**

DNA synthesis was determined from the incorporation of BrdU into DNA, as measured by immunohistochemical analyses, and was quantitated by counting the number of labeled tubular cell nuclei in six randomly selected, high-power (×400) fields in each kidney cortex.

**Biochemical Measurements**

Serum creatinine levels were determined in a Beckman creatinine analyzer (Beckman Instruments, Fullerton, CA). Tissue protein concentrations were determined using the Bio-Rad method (Bio-Rad Laboratories, Richmond, CA).

**Statistical Analyses**

For comparison between groups, data were analyzed with the unpaired t test. P values of <0.05 were considered significant. Results are expressed as mean ± SEM.

### Table 1. Serum Creatinine Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Creatinine Levels (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.5 d</td>
</tr>
<tr>
<td>Sham</td>
<td>0.6 ± 0.03</td>
</tr>
<tr>
<td>ATN</td>
<td>2.0 ± 0.19ᵇ</td>
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ᵃ n = 3 to 8 rats/group per time point. ATN, acute tubular necrosis.
ᵇ P < 0.01.
ᶜ P < 0.05.

### Results

**Serum Creatinine Levels**

In rats subjected to bilateral renovascular occlusion, serum creatinine levels were elevated at 12 h and returned to control values by day 8 (Table 1).

**Kidney c-MET mRNA Levels Are Increased after Ischemic Injury**

As shown in Figure 1, there were significant increases in c-MET mRNA levels 6 and 24 h after injury. This finding is consistent with earlier reports (4,13,15).

**Kidney c-MET Protein Levels Are Increased after Injury**

To determine whether the increase in c-MET gene expression is followed by an increase in cellular receptor protein levels, we measured whole-kidney c-MET protein levels using Western immunoblotting. The results are presented in Figures 2 and 3A. As early as 12 h after injury, there was a significant increase in c-MET protein levels, which reached a maximum at 24 h. At that time, the c-MET levels in the ATN rat kidneys were 7 times greater than those in the sham-operated control kidneys. The c-MET levels then decreased but were still significantly higher than control values after 8 d. Because kidney c-MET levels reflect protein levels in whole cells and because the immunohistochemical studies described below demonstrated redistribution of the receptors after injury, we also measured plasma membrane c-MET levels on days 2 and 8. Like the cell levels, plasma membrane c-MET levels increased after injury (Figure 4).

**Onset of Increased DNA Synthesis Occurs after the Increase in c-MET Protein Levels**

In contrast to the early increase in c-Met protein levels, DNA synthesis in the ATN kidneys at 12 h after injury did not differ from that observed in the control kidneys (Figure 3B). By 24 h, there was a marked (18-fold) increase in DNA synthesis, which was sustained at 48 h and returned to baseline values by day 8.

**Phosphorylated c-MET Levels Are Increased after Renal Injury**

As depicted in Figure 5, there were distinct increases in phosphorylated c-Met levels at 12 and 24 h after surgery in the
injured kidneys, compared with control values. Phosphorylated receptors were barely detectable in the control animals and were thus not quantifiable by densitometry. Because the receptor protein levels were greater in the ATN samples (Figure 2), the increase in phosphorylation in this group could simply reflect an increase in the number of receptors in the basal state of autophosphorylation. Alternatively, the increased phosphorylation could reflect a true increase in the state of receptor tyrosine phosphorylation, or a combination of both of these possibilities. Whichever is the case, it is likely that the increased levels of phosphorylated c-MET promote the action of HGF. Because this increase in the levels of phosphorylated receptors preceded the onset of DNA synthesis in the injured kidneys (Figure 3B), this finding lends support to the hypothesis that endogenous HGF participates in initiating the regeneration of injured kidneys. Because the earliest time point examined was 12 h after the insult, it is possible that an increase in phosphorylated receptor levels may have occurred even earlier.

**c-MET Protein Levels Are Increased in the Injured Regions of the Kidney, and c-MET Protein Is Redistributed within Damaged Proximal Tubule Cells**

Low-power views of control kidney sections demonstrated low-level c-MET immunoreactivity in all regions of the kidney, but especially evident in the outer medulla (Figure 6A). The ATN kidneys exhibited a general increase in c-MET immunostaining, which was strongest in the outer medulla (the region of the kidney sustaining the most severe injury) (Figure
day, control and ATN. We also observed that two tumor necrosis factor receptor-IgG fusion protein failed to (Figure 6C). In contrast, preincubation of antibody with a c-MET-IgG fusion protein largely blocked the c-MET signal preabsorbed antibody was tested (20). Preabsorption with a c-MET-IgG fusion protein might be caused by nonspecific binding of the anti-c-MET antibody to damaged cells, the effects of using antigen-preabsorbed antibody or omitting this antibody altogether were.

Figure 5. Western immunoblot analysis of kidney plasma membrane c-Met protein. Plasma membranes were prepared from rats 2 and 8 d after ischemic injury or a sham operation and were processed as described for Figure 2 (n = 3 rats/group per time point).

6B). It is noteworthy that BrdU immunoreactivity (a measure of DNA synthesis) was maximally increased in this same region of the injured kidneys (Figure 6E). These findings provide further support for a role for HGF in renal regeneration. To exclude the possibility that this increase in c-MET immunostaining might reflect nonspecific binding of the anti-c-MET antibody to damaged cells, the effect of using antigen-preabsorbed antibody was tested (20). Preabsorption with a c-MET-IgG fusion protein largely blocked the c-MET signal (Figure 6C). In contrast, preincubation of antibody with a tumor necrosis factor receptor-IgG fusion protein failed to block the signal (data not shown). We also observed that two different c-MET-specific antibodies, i.e., anti-murine and anti-human c-MET antibodies, yielded essentially identical results and no signal was obtained with nonimmune rabbit serum (data not shown).

At higher magnification, an interesting pattern of c-MET distribution in the cortex was apparent. In control kidneys, the c-MET signal was strongest in the distal tubules and collecting ducts. In those nephron segments, staining was localized mostly in the apical and basolateral membranes (Figure 7A). The proximal tubular cells also stained for c-MET. There the signal was clearly present in the brush border membrane. In occasional proximal tubules, there were infrequent areas of cytoplasmic staining. Focal staining of glomerular epithelial cells was also evident. In the injured cortex, a range of patchy changes was evident, reflecting the variable severity of the ischemic damage. These findings are presented in Figure 7B for kidneys obtained 24 h after injury. Similar patterns were observed after 12 and 48 h. Figure 7B demonstrates that, in the injured cortex, some proximal tubules exhibited the same apical polar c-MET distribution as observed in control kidneys. In other tubules, the c-MET signal was present in a punctate pattern in the cell cytosol. This appearance is consistent with the presence of receptors in endocytotic vesicles and may reflect endocytosis of the receptor after it is activated by HGF. The more severely injured cells were diffusely stained, and the detached cells were intensely stained. These latter changes likely reflect the loss of polarity that follows cellular injury (22). Despite this receptor redistribution, it is important to note that the immunoblot analysis demonstrated overall increases in whole-cell and plasma membrane receptor protein levels (Figure 4).

Examination of the outer stripe of the outer zone of the medulla of control kidneys revealed diffuse c-MET staining in all nephron segments (Figure 7C). Interestingly, in contrast to the cortical proximal tubules, outer medullary proximal tubular cells lacked brush border c-MET immunostaining and c-MET appeared to be localized intracellularly. This variance in immunostaining likely reflects differences in the structure and function of the different segments of the proximal tubules. The ATN kidneys demonstrated an increase in immunoreactivity in the outer stripe of the medulla, and this was most intense in the injured nephrons (presumably, the straight portion of the proximal tubules) (Figure 7D). Both detached and attached cells were heavily stained. We suggest that the increase in c-MET staining in the detached cells reflects an unsuccessful protective response to injury that occurs before detachment and cellular necrosis. In the inner stripe of the outer zone of the medulla, c-MET expression was evident in all nephron segments (Figure 8A). In the injured kidneys, the tubular cells appeared swollen and were diffusely immunostained (Figure 8B). The papillae of the control kidneys demonstrated c-MET staining distributed throughout the collecting ducts (Figure 8C). The localization of the signal was poorly defined, but staining appeared more prominent in the apical membrane. Staining was also evident in interstitial cells and thin-walled structures consistent in appearance with the thin limbs of Henle and the vasa recta. There did not seem to be any major difference in c-MET immunostaining between control and ATN kidneys (Figure 8D). Eight days after the original ischemic insult, the intensity of immunostaining in the ATN kidneys decreased (data not shown), which is consistent with the Western immunoblots presented earlier. Many but not all of the proximal tubules had regained the apical localization of c-MET staining at that time. Light staining of the cellular interstitial infiltrate was present at that time. To rule out the possibility that the altered c-MET immunostaining in the ATN kidneys might be caused by nonspecific binding of the anti-c-MET antibody to damaged cells, the effects of using antigen-preabsorbed antibody or omitting this antibody altogether were tested (20). Preabsorption with a c-MET-IgG fusion protein largely but not completely blocked the c-MET signal (Figure 9A), whereas in the absence of the antibody there was no
immunostaining at all (Figure 9B). Therefore, there seems to be a low level of nonspecific immunostaining, which cannot account for the changes in c-MET expression observed in the injured kidneys. It is also possible that the low level of immunostaining observed reflects incomplete preabsorption of the anti-c-MET antibody.

**Discussion**

Administration of recombinant HGF to rodents with acute renal failure accelerates recovery, and it has been suggested that endogenous HGF may play a role in the natural spontaneous recovery process. The aim of these experiments was to test this hypothesis. To this end, we examined the changes in the expression and activity of the HGF receptor, c-MET, in the kidneys of rats with ischemia-induced ATN. We observed that, as early as 12 h after the ischemic insult, there was a fourfold increase in c-MET protein levels. This was preceded by an increase in c-MET gene expression that was evident at 6 h after ischemia. c-MET protein levels increased to a maximum after 24 h, at which time the levels in the ATN kidneys exceeded the values for the sham-operated control kidneys by sevenfold. Levels then decreased, but even after 8 d the levels were still threefold higher than those in control animals. This increase in whole-kidney c-MET protein levels was accompanied by a similar increase in kidney plasma membrane c-MET levels, which would favor an increase in tissue sensitivity to HGF. It is important to note that the increase in kidney c-MET protein levels preceded the increase in DNA synthesis, which was first evident 24 h after injury. Furthermore, the greatest increases in c-MET and DNA synthesis occurred in the same region of the kidney, namely the outer medulla, where injury was most marked. Because circulating and local kidney HGF levels are also elevated as early as 2 to 6 h after renal injury (4,13), this sequence of events supports the hypothesis that HGF plays a role in initiating renal regeneration, and it is consistent with the recent findings of Liu et al. (13) in folic acid-induced acute renal failure. Additional support is provided by our finding that c-MET tyrosine phosphorylation, a process that reflects receptor activation, is increased within 12 h after injury. Whether the increased tyrosine phosphorylation reflects an increase in the number of receptors in the basal state of phosphorylation and/or an absolute increase in the state of receptor phosphorylation is unclear, because the number of receptors was increased in the injured kidneys. In any case, because tyrosine phosphorylation is a...
phosphorylation of c-MET serves to activate signal transduction, the increase in phosphorylation supports a role for HGF in renal regeneration.

c-MET immunostaining in normal rat kidneys was strongest in the cortical distal tubules and collecting ducts and medullary collecting ducts. In these nephron segments, c-MET appeared to be localized to the apical and basolateral membranes. Immunostaining in the proximal tubular cells was weak and localized mainly to the brush border membrane. Liu et al. (23) also described prominent staining of distal tubular cells, with weak staining of proximal tubules, but did not comment on the subcellular receptor distribution. Because the kidneys were not perfusion-fixed in that study, the apical location of c-MET may have been obscured. Those authors did note that early diabetes is associated with an increase in c-MET protein levels, and they proposed that the HGF/c-MET system may play a role in diabetic renal hypertrophy, as has been suggested for compensatory renal growth (9). Crepaldi et al. (24) observed that c-MET is localized exclusively to the basolateral pole of cultured MDCK cells (a dog kidney cell line with distal nephron features). This finding may well reflect a cell line phenomenon. In the same study, those authors localized c-MET to the basolateral pole of the epithelium lining the human colon. Similarly, Nusrat et al. (25) localized c-MET to the basolateral membrane of native crypt epithelial cells of human intestine. In contrast, Tsarfaty et al. (26) reported that c-MET is localized to the apical pole of cells lining human breast tissue mammary ducts. These varying descriptions of the polar distribution of c-MET may reflect tissue-specific cellular differences in the localization of the receptor, as well as perhaps technical differences.

We observed striking changes in the cellular distribution and intensity of c-MET immunostaining in acutely injured rat kidneys, which was not readily evident when the antibody was preabsorbed with the antigen. In the outer medulla, which was the most prominent site of ischemia-induced ATN and subsequent DNA synthesis, there was a marked increase in c-MET levels, especially in the more severely damaged cells. Both detached and attached cells were heavily immunostained. Because increases in c-MET mRNA and protein levels were detected as early as 6 and 12 h after injury, respectively, and because cellular necrosis and detachment evolve during a 12- to 24-h period (27), we suggest that immunostaining of the detached cells reflects a failed protective response that occurred before detachment. However, it might be argued that the increase in c-MET levels in the injured cells might in some way contribute to their injury. Arguing against this are reports that early treatment with HGF ameliorates the severity of injuries (4–6).

In the cortex, there were patchy changes in the proximal reference.
tubules. Some tubules maintained the same apical polar distribution of c-MET as observed in control kidneys, whereas other tubules exhibited a punctate pattern of staining in the cell cytosol. In more severely injured proximal tubular cells, there was a loss of the apical distribution of c-MET, with staining in the cytosol; cells that had detached from the basement membrane were intensely and diffusely stained. These latter changes likely reflect the general loss of polarity that follows ischemic tubular cell injury (22). The situation in the modestly injured cells is less clear. In these cells, the punctate intracellular distribution of the receptor may reflect increased receptor-mediated endocytosis in response to elevated HGF levels (21). Indeed, in human patients with ATN, there is a marked increase in urinary HGF levels above the low levels that are normally observed, and it is conceivable that elevated tubular fluid HGF levels could serve to activate the apical receptors, causing receptor-mediated endocytosis (28). The activated receptors could then activate other signaling proteins and so induce a regenerative response. Also relevant is the report by Tajima et al. (21), who noted a decrease in plasma membrane HGF receptor binding in the remaining kidney after uninephrectomy and in regenerating liver. They attributed this decrease in specific binding to either increased receptor occupancy or increased internalization in response to elevated circulating HGF levels. Taking that report together with all of our findings, we propose that tubular cells respond to injury by increasing c-MET expression, a response that is followed by increased receptor activation, receptor internalization, signal

Figure 8. Immunohistochemical localization of c-MET in the inner stripe of the outer zone of the medulla and papilla of sham-operated control and ATN rats 24 h after an ischemic episode. Magnification, ×220. Inner stripe of the outer zone of the medulla of control (A) and ATN (B) kidneys. The tubular cells of the injured kidney appear swollen and are diffusely stained. Papillae of control (C) and ATN (D) kidneys.

Figure 9. (A) Photomicrograph of the cortex of 24-h post-ATN kidneys probed with anti-c-Met antibody that had been preabsorbed with the receptor protein used to stimulate antibody formation. (B) Photomicrograph of the outer stripe of the outer zone of the medulla probed with secondary antibody only; the anti-c-MET antibody was omitted. The photomicrographs correspond to the regions shown in Figure 7, B and D, respectively. Magnification, ×220.
transduction, and subsequent promotion of a regenerative response. Furthermore, when the injury is severe and cell polarity is lost, it seems that the receptors become distributed throughout the cell. Examination of the kidneys 8 d after injury revealed that many but not all of the proximal tubules demonstrated normalization of receptor distribution.

In conclusion, we have demonstrated that, after an ischemic renal insult, there are early increases in c-MET protein levels and activity that precede the onset of DNA synthesis. Furthermore, the greatest increases in receptor protein expression and DNA synthesis both occur in the most severely injured nephron segments in the outer medulla. Taken together, this sequence of events and their anatomic colocalization support the hypothesis that HGF and c-MET participate in promoting recovery from injury. Finally, it is important to note that the c-MET levels remain elevated as much as 8 d after the ischemic insult. This finding, together with the report that serum HGF levels are elevated only transiently after acute experimental renal injury, suggests that prolonged and even late treatment with HGF may be of therapeutic benefit.

Acknowledgments

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