Hepatocyte growth factor/*c-met* signaling pathway is required for efficient liver regeneration and repair

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Hepatocyte growth factor/scatter factor *c-met* signaling pathway is of central importance during development as well as in tumorigenesis. Because homozygous null mice for either *hgf/sf* or *c-met* die *in utero*, we used Cre/*lox*P-mediated gene targeting to investigate the function of *c-met* specifically in the adult liver. Loss of *c-met* appeared not to be detrimental to hepatocyte function under physiological conditions. Nonetheless, the adaptive responses of the liver to injury were dramatically affected. Mice lacking *c-met* gene in hepatocytes were hypersensitive to Fas-induced apoptosis. When injected with a low dose of anti-Fas antibody, the majority of these mice died from massive apoptosis and hemorrhagic necrosis, whereas all wild-type mice survived with signs of minor injury. After a challenge with a single necrogenic dose of CCl_4, *c-met* conditional knockout mice exhibited impaired recovery from centrolobular lesions rather than a deficit in hepatocyte proliferation. The delayed healing was associated with a persistent inflammatory reaction, overproduction of osteopontin, early and prominent dystrophic calcification, and impaired hepatocyte scattering/migration into diseased areas. These studies provide direct genetic evidence in support of the critical role of *c-met* in efficient liver regeneration and suggest that disruption of *c-met* affects primarily hepatocyte survival and tissue remodeling.

Hepatocyte growth factor/scatter factor *c-met* signaling pathway (HGF/SF-Met) supports diverse biological processes including development, proliferation, scattering, and branching morphogenesis (1–4). HGF/SF-Met signaling is also of critical importance in tumorigenesis, particularly in the invasive and metastatic stages (5, 6). The receptor encoded by the *c-met* gene is a disulfide linked α/β heterodimer that is generated by proteolytic processing of a single polypeptide precursor (7). It has been demonstrated that the activation of the multiple signal transduction pathways downstream of *c-met* takes place via the multidocking site, a short sequence motif near the C terminus of the β chain (8). Furthermore, the two tyrosine residues (Tyr-1349 and Tyr-1356) located within the multidocking site have been shown to be of crucial importance in recruiting several signal transducer and adaptor molecules that act downstream of *c-met* (8–10). The *c-met* belongs to a small receptor family that includes *Ron* and *sea* (9, 11, 12). All three receptors share the heterodimeric structural motif of an extracellular α-unit and a transmembrane β-unit harboring the intrinsic tyrosine kinase activity, and belong to the phylogenetic tree of plexin and semaphorin receptors (13). The ligands for both *c-met* and *Ron*, HGF/SF and macrophage stimulating protein (MSP), respectively, belong to the plasminogen family of proteins, but are unique in that neither possesses proteolytic activity (13).

The importance of *c-met* during liver development has been demonstrated previously. Knockout mice for both *c-met* (14) and HGF (15, 16) fail to complete the developmental process and die *in utero* between embryonic days 13.5 and 16.5 with multiple abnormalities, including signs of underdeveloped liver. The importance of the intact *c-met* receptor for hepatocyte survival during development has been elegantly demonstrated by the use of mutant mice with modified Met receptors displaying enhanced recruitment of specific effectors (17). For example, neither Met^{2P} (optimal motif for phosphatidylinositol 3-kinase recruitment) nor Met^{2S} (optimal motif for src recruitment) were sufficient for HGF-mediated survival of hepatocytes in the developing liver, though partial rescue of myoblast migration was achieved.

In adult livers, HGF/*c-met* signaling induces diverse biological activities including mitogenesis, antifibrosis, and cyto-protection (2). Administration of an agonistic anti-Fas antibody to mice rapidly leads to massive liver apoptosis and liver failure with similar pathology and pathogenesis seen in human fulminant hepatic failure (18). Administration of human recombinant HGF (rhHGF) before anti-Fas antibody injection demonstrated a potent dose-dependent inhibition of liver apoptosis and lethal hepatic failure exemplifying the powerful survival function of the HGF/SF-Met signaling pathway in liver physiology (19). The antifibrotic effects of HGF/*c-met* have been demonstrated in a rat model of lethal liver cirrhosis in which a HGF-based gene therapy suppressed an increase in transforming growth factor (TGF)-β1-inhibited fibrogenesis and hepatocyte apoptosis, and produced a complete resolution of fibrosis in the cirrhotic liver (20).

To directly address the role of *c-met* in adult liver physiology, as well as its impact on regeneration, survival, and response to injury, a conditional knockout mouse line was generated by using Cre-*lox*P-mediated gene targeting (21–23). Inactivation of the mouse *c-met* gene was accomplished by a conditional deletion of exon 16 containing a critical ATP-binding site in the intracellular tyrosine kinase (TK) domain, essential for the activation of *c-met* signaling (14). Here we report that, although selective ablation of the *c-met* gene in adult mouse hepatocytes appears not to be detrimental, the reparative responses of the liver to acute injury are dramatically affected.

Methods

Generation of the *c-met*$^{fl/fl}$ Mouse. Exon 16 of the mouse *c-met* gene was modified by conditional gene targeting in HM-1 embryonic stem cells. The targeting construct (Fig. 1*A*) introduced an upstream *lox*P site in the intron preceding exon 16 and a floxed neomycin resistance cassette in the intron immediately downstream of exon 16. Three independent clones gave germline transmission of the targeted allele. The *c-met*$^{+/fl}$ mice were crossed with EIIaCre transgenic mice (24) to generate both

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Abbreviations: HGF/SF-Met, hepatocyte growth factor/scatter factor *c-met* signaling pathway; rhHGF, recombinant human HGF; AlbCre, albumin-Cre; PB, phenobarbital; PH, partial hepatectomy; uPA, urokinase-type plasminogen activator.

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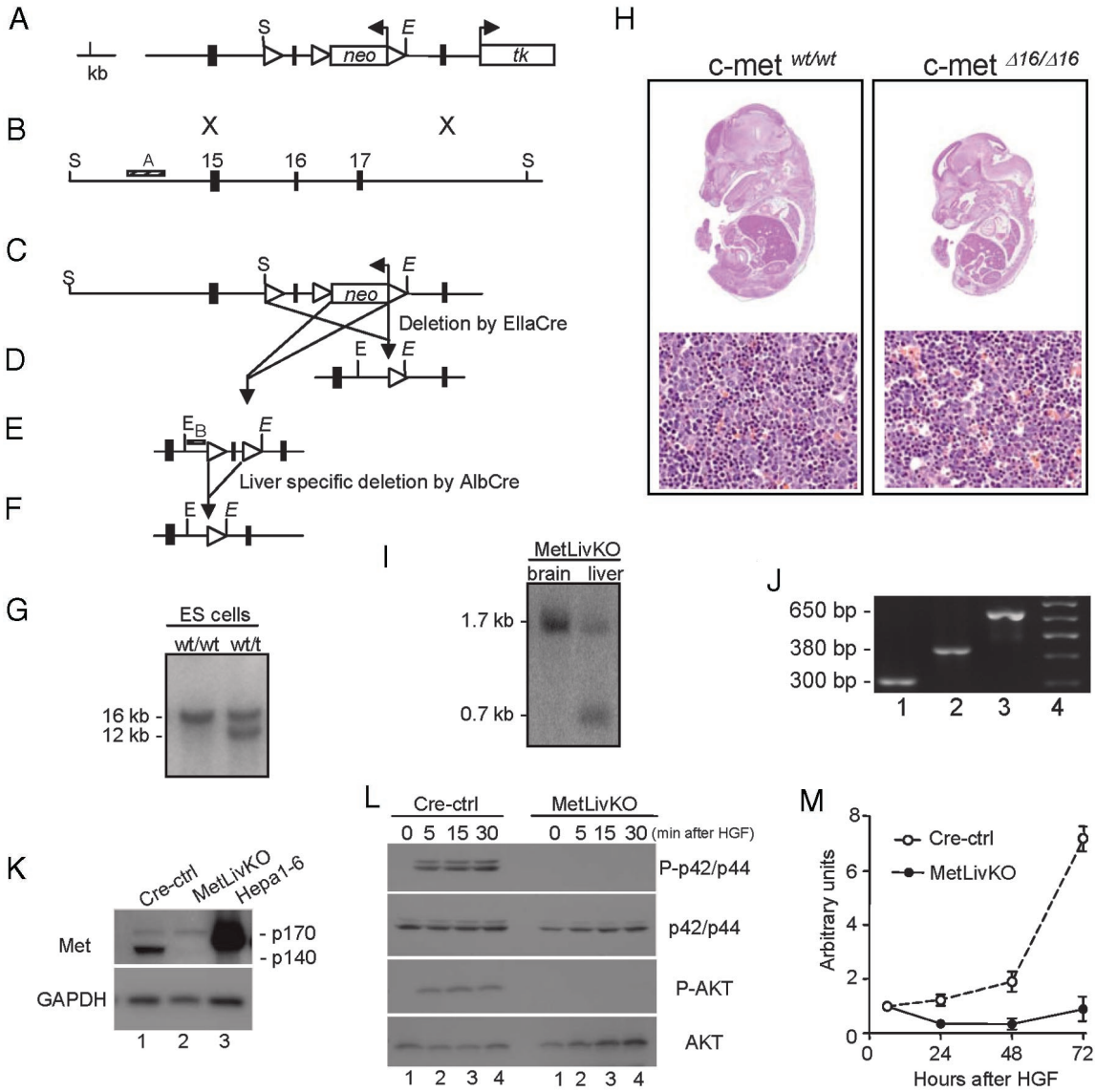

Fig. 1. Conditional deletion of exon 16 of mouse *c-met* gene. (*A*) *c-met* targeting vector. *loxP* sites are indicated by open triangles. *neo*, neomycin resistance gene; *tk*, herpes simplex thymidine kinase gene. (*B*) *c-met* wild-type allele. Filled boxes indicate c-*met* exons 15, 16, and 17. Hatched box indicates position of the probe A used for selection of targeted embryonic stem clones. (*C*) *c-met* targeted allele. S, *Spe*I; E, *Eco*RI. (*D*) Deleted allele ($c\text{-}met^{\Delta 16}$) produced by crossing mice heterozygous for targeted allele ($c\text{-}met^{+/fl}$) to EllaCre transgenic mice. (*E*) Floxed allele ($c\text{-}met^{fl}$). B, internal probe. (*F*) Liver-specific deletion of exon 16 by crossing $c\text{-}met^{fl/fl}$ mice to AlbCre transgenic mice. (*G*) Southern analysis of wild-type and targeted alleles. *Spe*I fragment specific to wild-type (16 kb) and targeted (12 kb) allele are shown. (*H*) $c\text{-}met^{+/\Delta 16}$ and $c\text{-}met^{\Delta 16/\Delta 16}$ embryos at embryonic day 14.5 and corresponding liver sections stained with hematoxylin/eosin. (Original magnification, ×25 and ×100, respectively.) (*I*) Southern analysis of tissue-specific deletion of floxed allele. The 1.7-kb floxed and 0.7-kb deleted alleles are shown. DNA extracted from 3-week-old MetLivKO mouse. (*J*) PCR analysis of genomic DNA from isolated hepatocytes. Lane 1, $c\text{-}met^{+/+}$; lane 2, $c\text{-}met^{fl/fl}$; lane 3, $c\text{-}met^{fl/fl}$;AlbCre. PCR fragments corresponding to wild-type (300 bp), floxed (380 bp), and deletion-specific (650 bp) fragments are shown. (*K*) Western blot of cell lysates from cultured hepatocytes with indicated genotypes using anti-c-Met antibody. Hepa1–6, positive control. (*L*) Western blots showing phosphorylation status of p42/p44 MAPK and AKT upon HGF stimulation in cultured hepatocytes. (*M*) Response to rhHGF in primary hepatocyte cultures. Proliferation was measured in 96-well plates (1×10^4 cells per well) by colorimetric assay (Roche Diagnostics).

$c\text{-}met^{+/\Delta 16}$ and $c\text{-}met^{+/fl}$ mice (Fig. 1 *E* and *F*) that were used to generate $c\text{-}met^{fl/fl}$ and $c\text{-}met^{\Delta 16/\Delta 16}$ mice. The $c\text{-}met^{fl/fl}$ mice were then crossed with mice hemizygous for the albumin-Cre (AlbCre) transgene (The Jackson Laboratory) expressing Cre exclusively in the postnatal liver. The ($c\text{-}met^{fl/+}$;AlbCre)F_1 mice so generated were interbred with $c\text{-}met^{fl/+}$ littermates lacking the AlbCre transgene. This breeding scheme yielded ($c\text{-}met^{fl/fl}$;$\text{AlbCre}^{+/-}$)F_2 mice designated as MetLivKO and littermate control mice, $c\text{-}met^{+/+}/\text{AlbCre}^{+/-}$ (Cre control). The established mouse lines were maintained in a 129SV/C57BL/6 background through brother/sister mating. Genotypes of all mice were assessed either by PCR or Southern analysis of tail DNA. (Details are available upon request to S.S.T.)

Animal Studies. Eight- to 12-week-old mice were used unless otherwise indicated. By this age, the efficiency of DNA excision by the AlbCre transgene exceeded 90% (data not shown and ref. 25). Partial hepatectomy was performed under metofane anesthesia as described (26). For acute CCl_4 studies, 0.4 μg/g weight of CCl_4 in mineral oil (Sigma) was injected i.p. either into untreated mice or after three daily doses of 100 μg/kg of phenobarbital (PB) (Sigma). One hour before death, animals

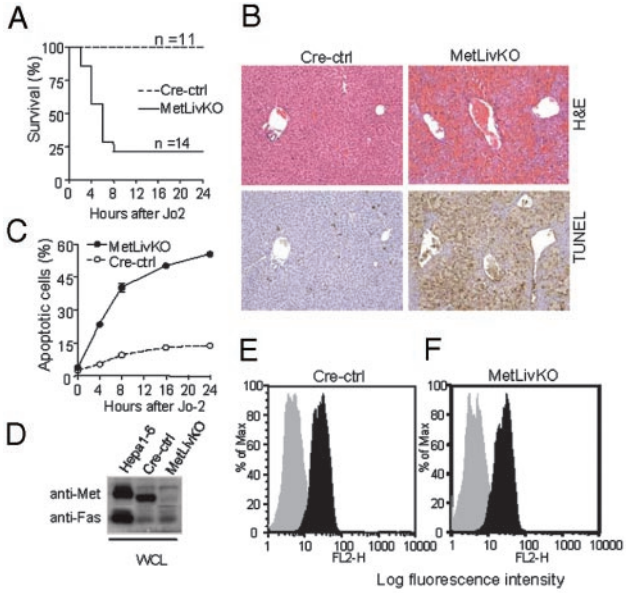

Fig. 2. The MetLivKo mice are more sensitive to Fas-mediated apoptosis. (*A*) Reduced survival in MetLivKO mice. (*B*) Hematoxylin and eosin and terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining 6 h after Jo-2 injection. (Original magnification, ×200.) (*C*) Assessment of apoptosis *in vitro*. Data represent cumulative average number of apoptotic cells ± SEM. At least 500 nuclei were counted from duplicate slides. (*D*) Western blot analysis of Met and Fas in whole cell lysates (WCL) from cultured hepatocytes. Hepa1–6, positive control. (*E* and *F*) Surface expression of Fas on freshly isolated hepatocytes determined by FACS (black histograms). Gray histograms, unstained cells.

received i.p. injections of BrdUrd (150 mg/kg) (Boehringer Mannheim). For Fas studies, mice were injected i.p. with 0.165 μg/g weight of Jo-2 mAb (Pharmingen). All studies were done according to National Institutes of Health guidelines for animal care.

Hepatocyte Isolation and Culture. Hepatocytes were isolated by two-step collagenase perfusion of liver followed by isodensity purification in Percoll (27). Cells were plated at a density of 2.5×10^4 per cm^2 in two-well Lab-Tek chamber slides and 10-cm dishes (Nalge Nunc) or fibrinogen-coated (1 μg/cm^2, Upstate Biotechnology) glass-bottom culture dishes (Mat Tek) and grown in Ham's F-12/DMEM at 37°C with 5% CO_2 as described (27). After overnight incubation, cells were synchronized for 2 h by serum deprivation. For HGF stimulation, cells were treated with 50 ng/ml of rhHGF (R & D Systems) for 5–30 min and whole cell lysates collected. For Fas apoptosis studies, slides were treated with 0.5 μg/ml of Jo-2 mAb in the presence of rhHGF for the indicated time, fixed in methanol/acetic acid (3:1), and stained with propidium iodine (PI). For the phagocytosis assay, FITC-labeled *Escherichia coli* bioparticles (100 per cell) were mixed with purified rabbit polyclonal IgG specific for *E. coli* as directed by Molecular Probes. Phagocytosis was allowed to proceed for 2–8 h in the presence of rhHGF. Fifteen minutes before observation with a Zeiss LSM510 confocal microscope, cells were washed two times with serum-free medium and loaded with 0.2 μg/ml of LysoTracker Red DND-99 (Molecular Probes). All experiments were performed in duplicate and repeated by using independent cultures from at least two to four animals.

Immunostaining and Immunoblotting. Apoptosis was assayed on 5 μM paraffin liver sections fixed in 10% formalin by using the terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL)/peroxidase kit (Intergen). BrdUrd incorporation was detected by using anti-BrdUrd (1:100, Becton Dickinson). At least 1,000 cells were counted per animal to determine the percentage of BrdUrd-positive cells (labeling index). Immunostaining for F4/80 macrophage surface antigen (1:100, Serotec) and osteopontin (1 μg/ml, R & D Systems) was performed

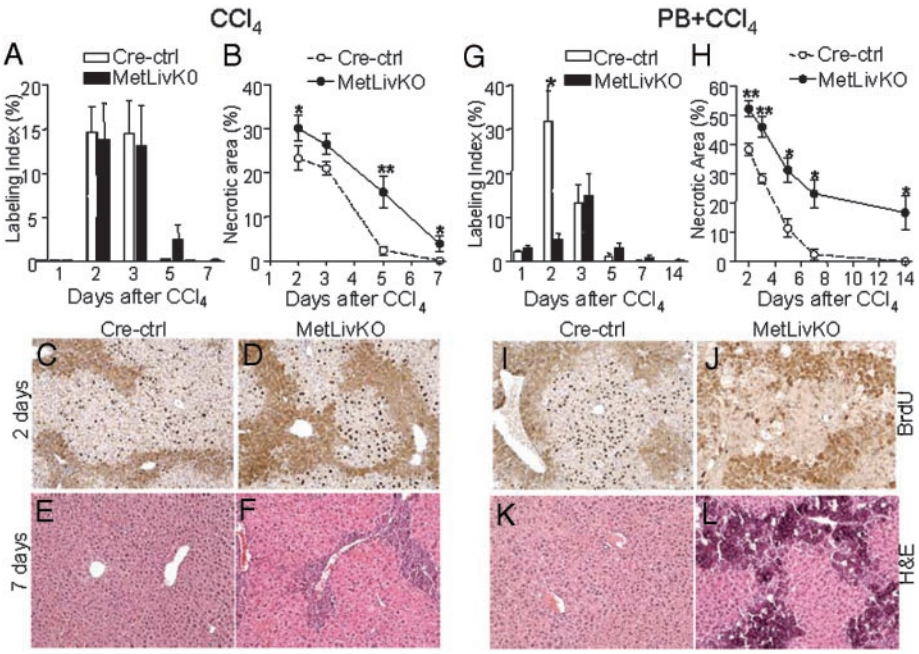

Fig. 3. *c-met* is required for liver healing but not hepatocyte proliferation. (*A* and *G*) Kinetics of BrdUrd incorporation. (*B* and *H*) Computer based morphometry of necrotic areas on hematoxylin/eosin-stained sections (NIH IMAGE). For each liver, necrotic areas within a 21-mm^2 randomly selected field were measured. All data are means ± SEM; $n = 5$: *, $P < 0.05$; **, $P < 0.001$ as determined by Student's *t* test. (*C*, *D*, *I*, and *J*) BrdUrd immunostaining counterstained with heamotoxylin. (*E*, *F*, *K*, and *L*) Hematoxylin/eosin staining. (Original magnification, ×200.)

following recommendations of the manufacturers. For Fas staining, the suspension of freshly isolated hepatocytes was incubated for 20 min at 4°C with phycoerythrin (PE)-conjugated Fas (1:100, Pharmingen), washed twice with PBS, and analyzed on a FACSCalibur (BD Bioscience) flow cytometer. Immunoblotting was performed according to the recommendations of suppliers. Anti-Met and anti-Fas were purchased from Santa Cruz Biotechnology and used at 2 μg/ml. Anti-p42/44 mitogen-activated protein kinase, antiphospho-p42/44, anti-AKT, and antiphosporylated AKT were all used at a 1:1,000 dilution (Cell Signaling).

Results and Discussion

Generation and Characterization of *c-met*$^{fl/fl}$ Mouse. After standard electroporation of the targeting vector and culture of embryonic stem (ES) cells, five correctly targeted clones containing all three loxP sites were identified (Fig. 1 *A–G*). Three of the independent homologous recombinant ES clones produced germ-line transmission of the targeted allele (*t*). When heterozygous *c-met*$^{+/t}$ mice were intercrossed, no homozygous *c-met*$^{t/t}$ pups were born, suggesting that the neo cassette was interfering with the transcription of the *c-met* gene. Therefore, the *neo* cassette was deleted from the targeted allele by crossing the *c-met*$^{+/t}$ mice with EIIa-Cre mice (24) (Fig. 1 *D* and *E*), and mice containing the floxed allele (*c-met*$^{+/fl}$) or the deleted allele (*c-met*$^{+/\Delta 16}$) were generated. We confirmed that the *c-met*$^{\Delta 16/\Delta 16}$ phenotype (Fig. 1*H*) characterized by embryonic lethality and abnormal liver development was similar to that reported for the HGF and *c-met* null mice (14–16).

The *c-met*$^{fl/fl}$ mice were viable, grew at a normal rate, were fertile, and had no apparent histological or physiological abnormalities observed for >1 year. To accomplish a selective inactivation of the *c-met* gene in hepatocytes, the *c-met*$^{fl/fl}$ mice were crossed with AlbCre transgenic mice (25) to generate MetLivKO and Cre control mice. There was no detectable level of *c-met* deletion in the nonhepatic tissues, confirming the tissue specificity of AlbCre-mediated *c-met* disruption (Fig. 1*I*). PCR analysis of genomic DNA isolated from the MetLivKO hepatocytes showed the presence of only the 650-bp fragment specific for the deleted allele (Δ16) (Fig. 1*J*). Western blot analysis revealed the p170 precursor with only trace amounts of the mature p140 form of c-Met (Fig. 1*K*). In addition, both *c-met*-dependent signaling via p42/p44 and AKT and HGF growth stimulation were completely abolished in the MetLivKO hepatocytes (Fig. 1 *L* and *M*). These biochemical data combined with the *in vivo* phenotype of *c-met*$^{\Delta 16/\Delta 16}$ embryos indicate that conditional deletion of exon 16 effectively inactivated the *c-met* gene.

Increased Sensitivity to Fas-Induced Apoptosis. Because mutated *c-met* in the adult quiescent liver does not appear to be detrimental (>1 year observation), we sought to address the functional importance of *c-met* in diverse adaptive responses of the liver. To test vulnerability to injury, we first evaluated the response of MetLivKO mice to apoptotic stimuli induced by Fas antibody. This was of considerable interest because not only does pretreatment with rhHGF before anti-Fas antibody injection demonstrate a potent dose-dependent inhibition of liver apoptosis and lethal hepatic failure (19), but it was recently shown that direct binding between c-Met and Fas receptor inhibited Fas activation and thus prevented apoptosis (28). Massive hepatocyte death and hemorrhagic liver destruction in MetLivKO mice began within the first 2–4 h after injection of the Jo-2 antibody; and by 8 h, 80% of the animals died of acute liver failure. In contrast, all Cre control mice survived and had only minor liver injury after being exposed to the same dose of the Jo-2 antibody (Fig. 2 *A* and *B*). Fas stimulation *in vitro* also triggered a significantly higher apoptotic response in primary cultures of *c-met* deleted hepatocytes than Cre control hepatocytes (Fig. 2*C*). No significant changes were found in the levels of Fas protein or the surface density of the Fas receptor in mice of both genotypes (Fig. 2 *D–F*), implying that Fas expression is not affected by the lack of c-Met under physiological conditions. However, the observed sensitivity of the MetLivKO mice to Fas-mediated apoptosis may be due to the reduced amount of c-Met protein and thus attenuated Met-Fas sequestration (28). Alternatively, the loss of antiapoptotic signaling by HGF/*c-met* in MetLivKO mice may potentiate the death-inducing function of the Fas ligand (29). These data indicate that intact *c-met* signaling is critical for protecting the liver against Fas-induced apoptosis.

Delayed Healing After Liver Injury in MetLivKO Mice. We next determined whether HGF/*c-met* signaling is required for liver regeneration induced by a standard two-thirds partial hepatectomy (PH) (26, 30). MetLivKO mice were more vulnerable to surgery, and most of the animals were either moribund or died by 48 h after PH (8 of 10). Morphologically, *c-met* ablated regenerating livers displayed numerous areas of necrosis, signs of jaundice, and diffused macro- or microvesicular steatosis (data not shown). Therefore, to further address regeneration and injury in MetLivKO mice and to avoid the mortality associated with PH, we used a model of toxic liver injury induced by a single injection of CCl_4 (31). After a single CCl_4 exposure, all MetLivKO mice survived and showed the same kinetics and magnitude of proliferation as AlbCre controls (Fig. 3 *A*, *C*, and *D*), suggesting that HGF is not the sole factor controlling hepatocyte proliferation and that other growth factor signaling pathways may compensate for the loss of HGF/*c-met*. However, amplification of CCl_4-mediated injury by pretreatment with phenobarbital (PB), known to increase CCl_4 metabolism and

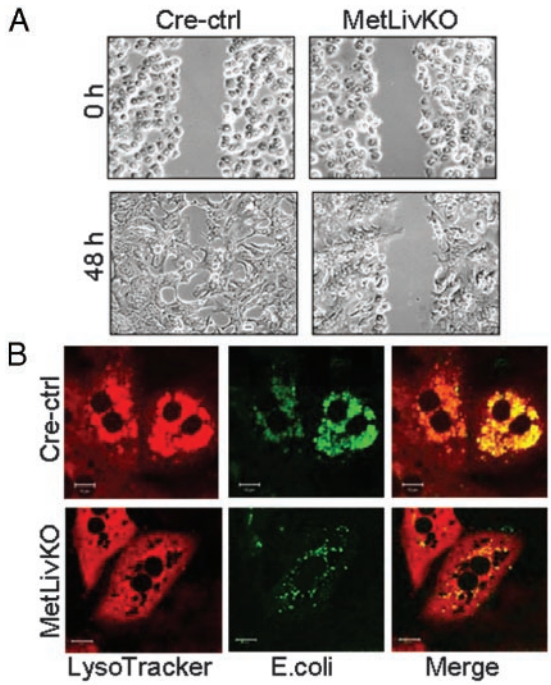

Fig. 4. Lack of *c-met* signaling results in impairment of motility and phagocytosis. (*A*) Linear scrape wounds made in subconfluent monolayers of primary hepatocytes 4 h after plating were allowed to heal in the serum-free medium in the presence of 50 ng/ml rhHGF. (Original magnification, ×100.) (*B*) Representative confocal microscopy images of hepatocytes exposed to *E. coli* (green, *Center*) and lysosomal-specific fluorochrome (red, *Left*). Colocalization of green and red pixels appears as yellow (merge, *Right*). (Scale bar, 10 μm.)

toxicity (31), resulted in a significant inhibition of hepatocyte proliferation in MetLivKO mice (Fig. 3 *G*, *I*, and *J*). Thus, the lack of HGF/*c-met* signaling becomes detrimental in the context of more extensive liver injury. In striking contrast, the recovery from liver necrosis was impaired in the MetLivKO mice (Fig. 3 *B*, *E*, and *F*), even when the proliferative response was comparable to Cre controls (Fig. 3 *A* and *B*) and was further aggravated by pretreatment with PB (Fig. 3*H*).

Although the attenuated proliferative response may contribute to the diminished recovery, together the data suggest that the primary deficiencies in MetLivKO livers involve tissue remodeling and survival after injury rather than proliferation. The initial CCl_4-mediated liver damage was larger in MetLivKO mice, but the difference in the areas of necrosis between knockout and control mice was relatively small. Nevertheless, the *c-met*-deleted livers remained diseased and showed persistent centrolobular lesions for at least 14 days after PB+CCl_4 treatment compared to a rapid restoration of normal liver architecture in similarly treated Cre control mice (Fig. 3 *H*, *K*, and *L*). The repair impediment could not be accounted for by continuous apoptosis/necrosis because no significant differences either in the number of terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL)-positive hepatocytes or serum levels of aspartate aminotransferase between MetLivKO and wild-type livers were observed (not shown and Fig. 6, which is published as supporting information on the PNAS web site). High protease activities known to degrade extracellular matrix proteins (MMP-2 and MMP-9) found in MetLivKO livers also made it unlikely that the delayed resolution of the necrotic lesions was caused by attenuated proteolysis (Fig. 6). Therefore, the functional competence of *c-met*-deleted hepatocytes was examined to determine which of the lost *c-met* function(s) may contribute to the disruption of tissue remodeling and thus may be responsible for converting a normally acute injury into a chronic lesion. Given the well documented role of *c-met* in regulating cell motility (5, 6), we hypothesized that loss of *c-met* might reduce the motogenic/scattering function and thus attenuate or prevent the hepatocyte migration into the necrotic area associated with the healing process. We observed that primary hepatocytes from MetLivKO mice failed to migrate in a standard wound healing assay (Fig. 4*A*). Furthermore, the reorganization of the actin cytoskeleton and extensive ruffling seen in cultured hepatocytes from Cre control mice after HGF stimulation were absent in MetLivKO hepatocytes (Fig. 7, which is published as supporting information on the PNAS web site). Similarly, during compensatory liver regeneration after CCl_4 exposure, *c-met*-deleted hepatocytes maintained structural integrity through E-cadherin mediated cell–cell adhesion, whereas Cre control hepatocytes acquired a motile phenotype and, as part of the tissue remodeling process, actively invaded the necrotic borders (Fig. 7).

We next asked whether the loss of motogenic capacity would affect the proficiency of hepatocytes to phagocytose. Although hepatocytes are not considered "professional phagocytes," they can phagocytose both bacteria and apoptotic bodies (32). To examine this function, primary hepatocyte cultures were incubated with opsonized *E. coli* (Fig. 4*B*). Bacterial uptake was significantly reduced in *c-met* null cells, suggesting that loss of phagocytic activity might be a contributing factor in generating persistent liver injury (33).

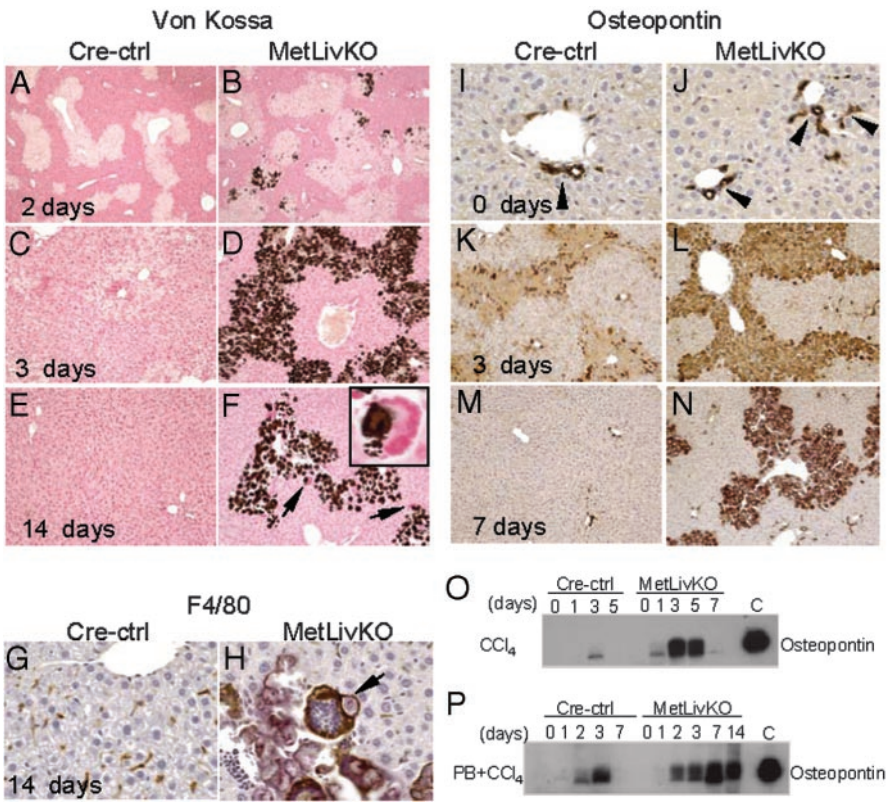

Fig. 5. Abnormalities of liver remodeling after injury with CCl_4+PB. (*A–F*) Calcium deposition was visualized by Von Kossa stain. (*F Inset*) Accumulation of calcium inside giant multinucleated cells shown by arrows. (*G* and *H*) F4/80 staining of Kupffer cells. Arrow points to a giant multinucleated Kupffer cell. (*I–N*) Expression of osteopontin. (*I* and *J*) Osteopontin staining in biliary epithelial cells (arrowheads). Slides were counterstained with hematoxylin. (Original magnification, ×50 in *A* and *B*, ×100 in *C–F* and *K–N*, and ×400 in *G–J* and *Inset* in *F*). (*O* and *P*) Immunoblots with antiosteopontin. (*P*) Samples at time 0 were obtained after the last phenobarbital injection. *C*, recombinant mouse osteopontin (R & D Systems).

Conversion of Acute to Chronic Injury in MetLivKO Livers. The most prominent demonstration of the rapid conversion from acute to chronic injury in MetLivKO livers was the persistent elevation of inflammatory cytokines (Fig. 6) as well as the early and extensive deposition of calcium within the CCl_4-induced necrotic areas (Fig. 5 *A–F*). Accordingly, multinucleated giant cells expressing the F4/80 surface antigen of Kupffer cells were broadly distributed throughout the calcified hepatic lesions, reflecting impediment in phagocyte-mediated clearance of necrotic cells (Fig. 5 *G* and *H*). Furthermore, osteopontin, a multifunctional molecule associated with inflammation, dystrophic calcification, and tissue remodelling (34), was highly up-regulated in the diseased MetLivKO livers (Fig. 5 *I–P*). This is of interest because the migration response to osteopontin is executed by means of cell surface integrins and activation of the *c-met* signaling pathway (35, 36). Osteopontin is also known to have prosurvival activity in a variety of cell types by binding to $\alpha v\beta 3$ integrin and CD44, both of which also employ, at least to some extent, the HGF/*c-met* signaling pathway to elicit their biological effects (37, 38).

The phenotype observed in the MetLivKO mice after CCl_4 exposure is strikingly similar to that seen in plasminogen- and urokinase-type plasminogen activator (uPA)-deficient mice after CCl_4 treatment (39, 40). Although neither the initial development of liver injury after CCl_4 exposure nor the proliferative response were affected in the plasminogen- and uPA-deficient mice, the capacity of mutated livers to clear necrotic tissue and to repopulate the affected area by regenerating hepatocytes was dramatically impaired, similar to the findings in MetLivKO mice. Also, dystrophic calcification of the injured areas was observed in the plasminogen-deficient mice.

In the absence of *c-met* signaling, there was a clear relationship between the extent of the CCl_4-induced injury (i.e., loss of mass) and the attenuation of DNA synthesis, suggesting that the capacity of *c-met*-deleted hepatocytes to proliferate may depend on the extent of tissue loss. Similarly, retarded liver regeneration after standard PH was observed in the uPA-deficient mouse, possibly because of the attenuated proleolytic maturation of pro-HGF (41). Because uPA converts plasminogen into plasmin, which activates matrix metaloproteinases and thereby promotes the release of pro-HGF from the matrix (1), it seems likely that the increase in tissue loss (e.g., after two-third PH) would result in delayed liver regeneration in the plasminogen-deficient mice.

Both the level and processing of plasminogen are intact (unpublished data) in the MetLivKO mouse. Because *c-met* is only deleted in the hepatocytes, we hypothesize that the "common deficiency" in tissue remodeling after injury shared with the plasminogen- and uPA-deficient mice involves the HGF/SF-Met signaling pathway. The inefficient activation of the pro-HGF in the plasminogen- and uPA-deficient mice may significantly contribute to the delayed repair after acute liver injury observed in these models. Furthermore, these data suggest that the intact HGF/SF-Met signaling pathway and plasminogen activation are both required for efficient repair and remodeling after tissue injury.

Taken together these studies provide direct genetic evidence in support of the central role of *c-met* signaling in liver regeneration and repair. The *c-met*$^{fl/fl}$ mouse should provide a useful model for identifying functions and molecular mechanisms associated with HGF/*c-met* signaling in the adult organism.

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