Increased Carbon Tetrachloride-Induced Liver Injury and Fibrosis in FGFR4-Deficient Mice

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Carbon tetrachloride (CCl₄) intoxicification in rodents is a commonly used model of both acute and chronic liver injury. Recently, we showed that mice in which FGFR4 was ablated from the germline exhibited elevated cholesterol metabolism and bile acid synthesis coincident with unrepressed levels of cytochrome P450 7A (CYP7A), the rate-limiting enzyme in cholesterol disposal. Of the four fibroblast growth factor (FGF) receptor genes expressed in adult liver, FGFR4 is expressed specifically in mature hepatocytes. To determine whether FGFR4 plays a broader role in liver-specific metabolic functions, we examined the impact of both acute and chronic exposure to CCl₄ in FGFR4-deficient mice. Following acute CCl₄ exposure, the FGFR4-deficient mice exhibited accelerated liver injury, a significant increase in liver mass and delayed hepatolobular repair. Chronic CCl₄ exposure resulted in severe fibrosis in livers of FGFR4-deficient mice compared to normal mice. Analysis at both mRNA and protein levels indicated an 8-hour delay in FGFR4-deficient mice in the down-regulation of cytochrome P450 2E1 (CYP2E1) protein, the major enzyme whose products underlie CCl₄-induced injury. These results show that hepatocyte FGFR4 protects against acute and chronic insult to the liver and prevents accompanying fibrosis. The results show that FGFR4 acts by promotion of processes that restore hepatolobular architecture rather than cellularity while limiting damage due to prolonged CYP2E1 activity. (*Am J Pathol* 2002, 161:2003–2010)

Metabolism in the liver protects tissues in higher organisms from potentially harmful blood-borne environmental chemicals. Ironically, the metabolic products of detoxification reactions that protect other tissues from effects of the primary toxicant can be destructive to the liver when in excess or chronically present. Administration of carbon

tetrachloride (CCl₄) to rodents is a widely used model to study mechanisms of hepatic injury. CCl₄ causes hepatocyte injury that is characterized by centrilobular necrosis that is followed by hepatic fibrosis. Systemic administration of growth factors and cytokines, epidermal growth factor (EGF),¹ hepatocyte growth factor (HGF),² and interleukin-6 (IL-6) in IL-6-deficient mice³ reduces CCl₄-induced hepatic injury. It is unclear whether the systemic application is through direct activation of receptor signaling systems in hepatocytes or mediated indirectly by other liver cell types or organ sites. Moreover, EGF, HGF, and IL-6 play positive roles in the regenerative response of the liver to cellular loss from partial hepatectomy or damage. Thus, it is difficult to dissect a direct role of the receptor signaling systems in modulation of hepatocyte-specific, metabolic-induced hepatic damage from enhanced restoration of liver cellularity through delayed apoptosis or enhanced cell division. The pericellular matrix-linked heparan sulfate-fibroblast growth factor (FGF) receptor complex is an intrinsic sensor of perturbation and remodeling in the extracellular environment.⁴ One or more members of the family of polypeptide ligands, transmembrane tyrosine kinase, and heparan sulfate chains appear present in all tissues. The system plays key roles in sensing and signaling changes during embryonic development and maintenance of homeostasis among cellular compartments in the adult.^{4,5} Of the four FGFR isotypes and their numerous splice variants, only FGFR4 is expressed in mature hepatocytes.⁶ The deletion of *FGFR4* from the genome causes no obvious developmental abnormalities in mice, including the liver.^{7,8} Moreover, the livers of FGFR4-deficient mice regenerate on schedule after partial hepatectomy that suggests that either FGFR4 is compensated for or plays no key role in the regenerative response.⁸ Despite the lack of effect on development and regeneration, FGFR4-deficient mice exhibit a chronically depleted gall bladder and an elevated pool and fecal excretion of bile acids.⁸ The elevated cholesterol metabolism and bile acid synthesis was coincident with unrepressed levels of CYP7A, the rate-limiting enzyme in cholesterol disposal. This indicated

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that FGFR4 signaling interfaces with metabolite-controlled transcription networks in hepatocytes, independent of liver cell proliferation. Here we show that the absence of FGFR4 resulted in accelerated and increased liver injury and fibrosis after CCl₄ administration. The degradation of cytochrome P450 2E1 (CYP2E1), the major enzyme involved in metabolism of CCl₄ whose products in excess result in liver injury, was delayed in the FGFR4-deficient mice after CCl₄ treatment. These data are a second example of how pericellular matrix-linked FGFR4 communicates the status of the extracellular environment to hepatocyte metabolic networks. They suggest an important role of hepatocyte FGFR4 in limitation of the extent of toxin product-induced liver injury and fibrosis and restoration of the hepatolobular architecture rather than cellularity.

Materials and Methods

Animals and Administration of CCl₄

Disruption of the mouse *fgfr4* locus was carried out in 129 Sv strain-derived ES cells as described.⁷ Mice used in the study were limited to 7-to-8-week-old females. Mice were maintained in 12-hour light/12-hour dark cycles with free access to food and water. Three to five mice were used for each experimental group as described in the text. For acute CCl₄-induced liver damage study, a single dose of 2.0 ml/kg of body weight (2:5 v/v in mineral oil) was administered by intraperitoneal (IP) injection. For chronic CCl₄-induced liver damage study, a dose of 2.0 ml/kg of body weight of CCl₄ was administered IP twice per week. Livers were excised for analysis after the mice were weighed, anesthetized, and exsanguinated. Neither FGFR4 (+/+) nor FGFR4 (-/-) mice exhibited overt symptoms or mortality from the single acute dose of CCl₄. The chronic damage protocol resulted in a 10% mortality rate in both wild-type and mutant animals. All procedures were performed in accordance with the Institutional Animal Care and Use Committee at the Institute of Biosciences and Technology, Texas A&M University System Health Science Center.

Enzyme Analyses

Blood plasma levels of alanine transaminase (amino-transferase) (ALT) activity were measured using the GP-Transaminase kit (No. 505-P, Sigma, St. Louis, MO). Plasminogen activator activity was assessed by zymography. One hundred μ g of total liver protein was subjected to 10% SDS-PAGE under non-reducing conditions. The acrylamide gel was washed for 30 minutes in 2.5% Triton X-100/PBS and for 30 minutes in distilled water and then placed on a casein gel containing 2% (w/v) nonfat dry milk, 1% (w/v) agarose, and 15 μ g/ml plasminogen (Roche, Indianapolis, IN) in PBS, and incubated in a humid chamber at 37°C until caseinolytic bands were visible and then photographed. Protein kinase A (PKA) activity was measured using the SignalTECT cAMP-dependent protein kinase (PKA) assay system (No. V7480, Promega, Madison, WI).

Hepatocyte DNA Synthesis

Two hours before sacrifice of the animals for analysis, 50 μ g per g body weight of bromodeoxyuridine (BrdU) was administered intraperitoneally. The livers were removed and weighed at the times indicated in the text. BrdU incorporation in fixed liver sections was visualized with an anti-BrdU monoclonal antibody (No. 2531, Sigma) and an alkaline phosphatase-conjugated second antibody. Positive hepatocytes were counted, and BrdU incorporation was expressed as the percentage of the number of labeled hepatocytes in four or five visual fields.

Histological Analysis and Measurement of Collagen

Liver tissues were fixed overnight in Histochoice Tissue Fixative MB (No. H120-4L, Amresco, Solon, OH), dehydrated through a series of ethanol treatments, and embedded in paraffin according to standard procedure. Sections were prepared and stained with hematoxylin and eosin and for collagen using Sirius Red (0.02%).

Quantitative analysis of collagen in Sirius Red-stained liver sections was performed by morphometric analysis. Briefly, liver sections were stained with Sirius Red, and slides were computer analyzed to calculate the percentage of collagen in total liver tissue area using Scion Image Beta 4.0.2 (Scion Corporation, downloaded from www.scioncorp.com).

mRNA Analysis by Northern Hybridization and RNase Protection

Total RNA was isolated from livers with the Ultraspec RNA Isolation system (No. BL-10200, Biotecx Laboratories, Houston, TX), and specific mRNAs were measured by Northern blot hybridization. Briefly, about 20 μ g of RNA was separated electrophoretically on 1% agarose gel containing 2.2 mol/L formaldehyde and transferred to BrightStar-Plus positively charged nylon membranes (No. 10104, Ambion, Austin, TX). The mouse *cyp2e1* and β -actin cDNAs were labeled with P³² by random primer labeling method. The membrane was first hybridized with the *cyp2e1* probe in ULTRAhyb ultrasensitive hybridization buffer (No. 8670, Ambion) overnight at 42°C, followed by washing and autoradiography. The amount of radiographical product was quantitated using a phosphorimager (Molecular Dynamics, Sunnyvale, CA). The same membrane was stripped of probe and then hybridized with the β -actin cDNA, followed by washing, autoradiography, and quantitation by the phosphorimager. Densitometric units between samples were normalized for RNA load by division of the density by the density of the internal β -actin in each sample. Experimental values were expressed in units relative to the level of expression in wild-type mice that was assigned a value of one as described in the text.

For RNase protection, 50 μ g of total liver RNA was hybridized with 1×10^5 cpm of [P³²]-labeled specific mouse plasminogen or urokinase-type plasminogen ac-

Figure 1. Injury in FGFR4 (-/-) livers after acute CCl₄ treatment. **A:** ALT levels after acute CCl₄ treatment. Values are the mean $\pm$ SD ($n = 4$ mice). Significance of differences between FGFR4 (+/+) and (-/-) mice at 12, 18, 24, 30, 38, and 48 hours was $P < 0.05$. **B:** Accelerated hepatocyte DNA synthesis in FGFR4 (-/-) livers after acute CCl₄ treatment. Values are the mean $\pm$ SD ($n = 4$ mice). **C:** Increased liver mass in FGFR4 (-/-) livers after acute CCl₄ treatment. Values are the mean $\pm$ SD ($n = 4$ mice). Values of FGFR4 (-/-) livers were significantly higher than that of FGFR4 (+/+) livers at all time points except day 0 ($P < 0.05$).

tivator (uPA) antisense riboprobes with β -actin riboprobes in the same reaction mixture. After treatment with ribonuclease, protected products were analyzed on 5% polyacrylamide sequencing gels, followed by autoradiography. Size of protection products was determined from the product of a DNA sequencing reaction parallel to the protection assays. The amount of each radiographical product was quantitated using a phosphor-imager (Molecular Dynamics).

Immunochemical Analysis by Western Blot

For immunochemical analysis, livers were homogenized in PBS containing 0.5% sodium deoxycholate and 0.1% SDS and centrifuged. The protein concentration was determined using the BCA Protein Assay reagent (No. 23225X, Pierce, Rockford, IL). A total of 25 μ g of protein was subjected to 12% SDS-PAGE, transferred to Hybond-P membrane (Amersham, Piscataway, NJ) which was incubated with 1:2000 dilution of rabbit anti-human CYP2E1 antiserum (a gift from Dr. Jerome M. Lasker, Mount Sinai School of Medicine), washed, and then incubated with 1:20,000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA). Bands were visualized by development with the Amersham ECL-Plus detection reagents (Amersham) and quantitated using an Alphamager (Alpha Innotech, San Leandro, CA). Experimental values were expressed in units relative to the level of expression in wild-type mice that was assigned a value of one as described in the text.

Statistical Analyses

Values were expressed as the mean $\pm$ SD from the number of replications described in the text. The statistical significance of differences between mean values ($P < 0.05$) was evaluated using the two-tailed Student's *t*-test.

Results

Liver Injury after Acute CCl₄ Administration

Twelve hours after a single injection of CCl₄, lytic liver damage monitored by blood plasma ALT levels in FGFR4 (-/-) mice was four times that of wild-type FGFR4 (+/+) mice (Figure 1A). Both the rise and fall of plasma ALT in response to CCl₄ preceded that of wild type, and, at the peak, was elevated by 1.5 times in the FGFR4-deficient mice. Analysis of cell proliferation by incorporation of BrdU revealed that peak CCl₄-induced DNA synthesis in hepatocytes in the FGFR4 (-/-) mice preceded that of wild-type FGFR4 (+/+) mice by 10 to 12 hours. At the 48-hour peak of DNA synthesis when 75% of hepatocytes were labeled in normal mice, labeling in the mutant mice had already dropped to 30% in hepatocytes. Hepatocyte DNA synthesis in the livers of both types of mice was equal at 72 hours and returned to baseline by 168 hours post-CCl₄ treatment (Figure 1B). Along with the early release of ALT, the accelerated DNA synthesis in FGFR4 (-/-) livers is consistent with increased sensitivity to injury caused by the CCl₄, causing earlier onset of the regenerative response. A quantitative analysis of areas under both the curves for ALT release and BrdU incorporation suggested that despite early onset in the FGFR4-deficient mice, the overall extent of ALT release and cell proliferation induced by the single acute dose of CCl₄ was similar between the FGFR4 (+/+) and FGFR4 (-/-) mice. However, the single acute dose of CCl₄ caused a significant increase in wet weight liver mass specifically in the FGFR4 (-/-) mice. At 24 hours post-CCl₄ administration, wet weight increased in FGFR4-deficient mice by 1.4-fold. Livers in the deficient mice were 1.8 times larger ($P < 0.05$) at the plateau at 72 hours (Figure 1C). The notable increase in liver mass persisted after 168 hours post-CCl₄ administration when livers were quiescent in respect to DNA synthesis.

Figure 2. Delayed repair in livers of FGFR4 (-/-) mice after acute CCl₄ treatment. **A:** Appearance of whole livers. The exaggerated white punctate lacy appearance of livers from FGFR4 (-/-) mice 168 hours post-CCl₄ treatment is indicated by the arrow. **B:** Abnormal repair of zone 3 injury. Necrotic areas are indicated by arrows. Paraffin-embedded sections were stained with hematoxylin and eosin. Magnification, $\times 150$.

Visual inspection of the livers revealed non-transparent white punctate foci indicative of focal damage in FGFR4 (-/-) livers at 168 hours post-CCl₄ treatment, while the appearance of untreated null mice and the treated livers in FGFR4 (+/+) mice were near normal (Figure 2A). Histological examination confirmed that the livers of FGFR4 (-/-) mice suffered hepatocellular damage more rapidly due to the single acute dose of CCl₄ than wild-type controls (Figure 2B). Significant injury was detectable in FGFR4-deficient mice relative to wild-type in histological zone 3⁹ at 12 hours after administration of CCl₄ (not shown). Although total liver mass was larger and surviving hepatocytes were 30% larger than wild type in FGFR4 (-/-) livers 96 hours after CCl₄ administration, the general pathology was similar in both wild-type and mutant livers with cell debris and inflammatory cells apparent in areas of necrosis (Figure 2B). At 168 hours when all DNA synthesis had ceased, large damaged areas in which necrotic and inflammatory cells were abundant were notable in the livers of the FGFR4 (-/-) mice while the livers of FGFR4 (+/+) mice were essen-

tially normal (Figure 2B). Taken together, these results suggest that significant toxic injury caused by the single acute dose of CCl₄ appeared earlier in the FGFR4 (-/-) livers, causing an accelerated schedule of hepatocyte DNA synthesis. Moreover, restoration of the normal hepatolobular structure was delayed and the results of the cellular damage persistent, giving rise to an increase in total liver mass in the FGFR4-deficient animals. This was despite the fact that DNA synthesis was accelerated in the mutant mice, and the total magnitude of cellular damage assessed by ALT and cellular regeneration by DNA synthesis was similar to wild-type mice.

Abnormal Activation of Urokinase Type Plasminogen Activator in FGFR4-Deficient Mice after Acute CCl₄ Administration

Liver repair after a toxic injury requires restoration of cellularity coordinated with the timely proteolytic clearance of matrix components and necrotic cells. Mice deficient in plasminogen and uPA exhibit impaired hepatolobular restoration, and, thus, it has been proposed that both play key roles in the proteolytic clearance of matrix components and necrotic cells after acute CCl₄-induced liver injury.^{10,11} We determined whether the expression of either plasminogen or uPA was impaired in the FGFR4-deficient livers. Analysis of mRNA levels by ribonuclease protection (RPA) revealed that there was no difference in plasminogen mRNA expression after acute CCl₄ administration (Figure 3A). Surprisingly, a significant increase of uPA expression over livers of wild-type animals was apparent at day 3 that was sustained through day 7 in FGFR4 (-/-) mice (Figure 3B). In FGFR4 (+/+) livers, uPA mRNA expression was elevated at days 4 and 5 post-CCl₄ treatment and declined to baseline at days 6 and 7 coincident with restoration of the damaged liver to normal. The activity profile of uPA reflected the mRNA expression profile (Figure 3C). These results suggest that a deficiency of plasminogen or uPA does not underlie the delay in hepatolobular restoration in FGFR4-deficient liver. On the contrary, FGFR4 signaling may be a factor that limits net uPA expression and activity during the remodeling process after cellularity is restored.

Liver Injury and Fibrosis after Chronic CCl₄ Administration

Both normal and FGFR4-deficient mice were administered 2.0 ml/kg of body weight of CCl₄ twice per week. Examination of paraffin-embedded sections of livers after 3 weeks revealed extensive zone 3 injury which bridged from vein to vein in livers of the FGFR4 (-/-) mice. In contrast, livers from FGFR4 (+/+) mice exhibited mild injury (Figure 4A). Sirius Red stain revealed an extensive collagenous network characteristic of fibrosis in the FGFR4-deficient mouse livers (Figure 4B). Fibrotic networks radiated out from the bridges of fibril-forming collagen in zone 3, which were much less acute in the FGFR4 (+/+) animals (Figure 4B). Quantitative image

Figure 3. Chronic elevation of uPA in FGFR4-deficient mice after acute CCl₄ administration. **A** and **B**: Expression of plasminogen (plg) and uPA mRNA in FGFR4 (+/+) and FGFR4 (-/-) livers after acute CCl₄ administration. mRNA was determined by RNase protection as described in Materials and Methods. Total RNA was isolated from three mouse livers in each group at the indicated times after acute CCl₄ administration and pooled before analysis. P, probes. **C**: Zymography of uPA activity in FGFR4 (+/+) and FGFR4 (-/-) liver extracts after acute CCl₄ administration. Bands indicate zones of casein lysis due to plasmin from plasminogen activated by uPA. Numbers at the bottom of each panel indicate the density of each band at the indicated times divided by that in untreated livers.

analysis (see Materials and Methods) indicated that collagen staining was elevated over 40 times in the FGFR4-deficient mice compared to only five times in wild-type mice (Figure 4C). These results indicate that the absence of hepatocyte FGFR4 resulted in pronounced long-term damage and fibrosis due to the chronic treatment with CCl₄.

Absence of FGFR4 Delays Down-Regulation of Liver CYP2E1 Induced by CCl₄

Since FGFR4 down-regulates cytochrome P450 enzyme, CYP7A, at the level of transcription to modulate liver bile acid synthesis from cholesterol, we investigated the impact of deletion of FGFR4 on CYP2E1. CYP2E1 is thought to be the key enzyme involved in metabolism of CCl₄ whose bioactive products result in CCl₄-induced liver injury.^{12,13} Expression level and stability determine the extent of CCl₄-induced liver injury. Ethanol potentiates CCl₄-induced liver injury¹⁴⁻¹⁶ by increasing the synthesis¹⁷ and stabilization¹⁸ of CYP2E1. α -hederin prevents

Figure 4. Increased injury and fibrosis in FGFR4 (-/-) livers due to chronic CCl₄ administration. **A**: Increased zone 3 injury in FGFR4 (-/-) livers. Mice were subjected to chronic CCl₄ treatment for 21 days; livers were excised, tissue sections prepared and stained with hematoxylin and eosin as described in Materials and Methods. Magnification, $\times 60$. **B**: Increased hepatofibrosis in FGFR4 (-/-) livers. Liver tissue sections were stained with Sirius Red. Magnification, $\times 60$. **C**: Quantitative analysis of collagen in Sirius Red-stained liver sections. Percentage of liver collagen in WT or FGFR4 (-/-) mice after chronic CCl₄ treatment for 21 days. Liver sections were stained with Sirius Red and slides were analyzed by morphometric image analysis, digitized, and quantitated as described in Materials and Methods to calculate the percentage of collagen in total liver tissue area. Values are the mean $\pm$ SD ($n = 4$ mice). Values of FGFR4 (-/-) livers were significantly higher than that of FGFR4 (+/+) livers after chronic CCl₄ treatment for 21 days ($P < 0.05$).

CCl₄-induced hepatotoxicity by decreasing the expression and activity of the CYP2E1 enzyme.¹⁹ Analysis of expression of the *cyp2e1* gene at both the mRNA (Figure 5A) and protein (Figure 5B) levels indicated that expression was equal in normal untreated livers of both FGFR4 (+/+) and FGFR4 (-/-) mice. However, following administration of a single dose of CCl₄ that causes acute damage and the regenerative response, the level of *cyp2e1* mRNA exhibited a drop to 50% of the level of untreated animals in both FGFR4 (+/+) and FGFR4 (-/-) mice at 4 and 8 hours post-treatment (Figure 5C). A further 30% de-

Figure 5. Delayed down-regulation of CYP2E1 in livers of FGFR4 ($-/-$) mice. **A** and **B**: Expression of *cyp2e1* mRNA and protein in untreated mice. In **A**, mRNA was determined by Northern blot hybridization as described in Materials and Methods using 25 μ g total RNA isolated from three livers from three wild-type ($+/+$) or FGFR4-deficient ($-/-$) mice. β -actin was probed as a loading control. In **B**, CYP2E1 was determined by immunoblot using 25 μ g protein from the three livers. **C**: Effect of acute CCl_4 treatment on expression of *cyp2e1* mRNA. Total RNA was isolated from three mouse livers in each group at the indicated times after acute CCl_4 administration and pooled before Northern blotting. Quantitation of bands relative to β -actin controls and the calculated estimate of the change relative to 0 time controls indicated in **C** and **D** was described in Materials and Methods. **D**: Delay in down-regulation of CYP2E1 in livers from FGFR4-deficient mice. Liver protein was isolated from three mice in each group at the indicated times after acute CCl_4 administration and pooled before Western blotting.

crease at 12 hours to 20% of untreated controls was apparent specifically in the FGFR4 ($-/-$) livers, which may be due to the accelerated degradation of *cyp2e1* mRNA in more severe areas of necrosis in the FGFR4 ($-/-$) livers. However, a much more significant difference between the wild-type and mutant mouse livers was in the rate of down-regulation of CYP2E1 protein levels

Figure 6. Accelerated decrease of PKA activity in FGFR4-deficient livers after acute CCl_4 administration. Values are the mean $\pm$ SD ($n = 3$ mice). Significance of differences between FGFR4 ($+/+$) and ($-/-$) livers at 4 hours was $P < 0.01$.

which has been shown to be due to CCl_4 -induced post-translational degradation in normal mouse livers.²⁰ Four and 8 hours after administration of CCl_4 , CYP2E1 dropped to 40% and 2% of the levels apparent in untreated livers, respectively (Figure 5D). Although the drop in CYP2E1 at 4 hours post-treatment was similar to wild type in livers of the FGFR4 ($-/-$) mice, CYP2E1 levels at 8 hours in the FGFR4 ($-/-$) mice were still that at 4 hours in wild-type mice. This additional 4 hours of sustained CYP2E1 activity may contribute to the accelerated time frame of the damage and response in FGFR4-deficient mice.

Absence of FGFR4 Accelerated the Decrease in Protein Kinase A Activity in Liver after an Acute Dose of CCl_4

Rapid degradation of CYP2E1 has been suggested to involve hormone-activation of cAMP-dependent protein kinase A, phosphorylation of CYP2E1, and phosphorylation- and ubiquitin-dependent degradation.²¹⁻²³ The FGF signaling system has been shown to activate PKA in other systems such as SK-N-MC cells²⁴ and NIH 3T3 cells.²⁵ We tested whether PKA activity was different between CCl_4 -treated wild-type and mutant mouse livers before the 4 to 8 hour window in which CYP2E1 was persistent in FGFR4-deficient mice. PKA activity increased slightly at 2 hours post- CCl_4 treatment in the livers from both types of animals and peaked at 4 hours in the wild-type mice (Figure 6). In contrast, PKA activity in FGFR4 ($-/-$) livers dropped to 60% that of wild type at 4 hours ($P < 0.01$). At 8 hours post- CCl_4 treatment, PKA activity was again equal in both types. These results suggest a window between 2 to 8 hours post-treatment where PKA activity may be deficient coincident with liver FGFR4 deficiency which may contribute to delay in degradation of CYP2E1.

Discussion

Here we show that hepatocyte FGFR4 plays an important role in the orderly restoration of liver mass and morphology independent of hepatocyte proliferation after both acute and chronic toxic insult to the liver. In the latter case, FGFR4 contributes to the limitation of resultant liver fibrosis. Mice devoid of FGFR4 display a delay in repair of zone 3 injury due to a one-time toxic dose of CCl₄ and severe fibrotic damage caused by repeated exposure to the same agent. Metabolism of xenobiotics and other potentially insulting agents, such as CCl₄, occurs predominantly in the pericentral zone (zone 3) of the liver where its products cause hepatocyte injury which can be of both short- and long-term consequences to the liver.^{26–28} Energy metabolism is partitioned between the periportal zone (zone 1), where oxidative energy metabolism, glucose release, and amino acid utilization occur, and the pericentral zone (zone 3), where glucose uptake, ketogenesis, and diverse biotransformations, such as cholesterol metabolism and bile acid synthesis, occur in addition to xenobiotic and toxin metabolism. Previously, we showed that FGFR4-deficient mice exhibited elevated cholesterol metabolism and bile acid synthesis suggesting that FGFR4 signaling plays a role in the process predominantly through a dampening effect.⁸ The effect of the FGFR4 deficiency was traced to elevated expression of the rate-limiting enzyme in the conversion, CYP7A, at the mRNA level that was not repressed by dietary cholesterol. Here we show that the absence of FGFR4 provides a 4 or more hour delay in degradation of CYP2E1, the rate-limiting step in the detoxification of CCl₄ that results in hepatocyte-damaging by-products as a consequence of the conversion. Thus, the control of damage by FGFR4 signaling may be due, in part, to its delay to the timetable for degradation of CYP2E1. In contrast to depression of CYP7A in cholesterol metabolism and bile acid synthesis at the mRNA level, FGFR4 appears to exert its effect on CYP2E1 at the protein level. Our results suggest that FGFR4 may sustain PKA activity sufficiently to prevent prolonged stability and activity of CYP2E1 in damaged livers. Further work is needed to link FGFR4 signaling to phosphorylation of CYP2E1 by PKA. The fact that both bile acid synthesis and CCl₄ transformation occur in zone 3 hepatocytes and both are modulated by FGFR4 suggests that FGFR4 may play an important role in signaling external perturbation to potentially damaging metabolic networks, particularly in zone 3 hepatocytes.

FGFR4 (–/–) hepatocytes may be more sensitive to damage that causes accelerated DNA synthesis and proliferation of hepatocytes; however, the FGFR4 (–/–) livers exhibited areas filled with debris and inflammatory cells at 168 hours post-CCl₄ well after cellularity was restored. The latter phenotype was similar to mice devoid of plasminogen and plasminogen activators that had no evidence of increased sensitivity to the same CCl₄ insult.^{10,11,29,30} Similar to FGFR4 (–/–) mice,⁸ hepatocellular proliferation was not impaired and liver size increased out of proportion to body weight independent of an increase in cell number.¹¹ This indicated a role of plasminogen and uPA-mediated processes similar to he-

patocyte FGFR4 in hepatolobular restoration independent of cell proliferation. FGF signaling impacts plasminogen activator activity in a positive mode coincident with cell proliferation or tissue remodeling in a number of different experimental systems. These include remodeling of the vasculature in angiogenesis,³¹ germinal vesicle breakdown in ovarian follicles,³² and myoblast migration and fusion.³³ In the latter, FGF2 down-regulates uPA expression coincident with delay of myoblast fusion in differentiating myoblasts. Surprisingly, the FGFR4 (–/–) mice exhibited a constitutively elevated level of uPA beginning at 72 hours post-CCl₄ insult suggesting that FGFR4 signaling may normally down-regulate uPA in damaged livers. These results clearly show that the FGFR4 (–/–) phenotype is not a consequence of deficient plasminogen/plasminogen activator activity. Whether FGFR4 signaling is downstream of and up-regulated by uPA, and down-regulates uPA activity in feedback mode, eg, deficient FGFR4 signaling mediates the phenotype caused by deficient plasminogen/plasminogen activator, is a subject of future study. Likewise, it is of interest to know whether chronic uPA activity eventually contributes to the delay of normal hepatolobular restoration.

It has become increasingly clear that the ubiquitous and extremely diverse members of the FGF family of activating FGF polypeptides, FGFR transmembrane kinases, and heparan sulfate oligosaccharide chains combine in a tissue-specific mode to sense perturbation and maintain homeostasis, both in diverse developing and adult tissues.^{4,6,34} The FGFR4 complex, the specific iso-type of the four FGFR kinases present in mature hepatocytes,^{4,6} is in a strategic position to monitor homeostasis and perturbation of the perilobular liver environment through its matrix-associated heparan sulfate subunit and matrix-controlled activating FGF ligands.⁴ Our results suggest a dual role of hepatocyte FGFR4, both in limitation of the product-induced damage during biochemical detoxification and the reconstitution of normal architecture occurring as a consequence of toxic insult. Modulation of hepatocyte FGFR4 signaling may be a useful target for therapeutic modulation of toxin-induced liver injury and fibrosis and the FGFR4-deficient mice a useful model for study of both high-level acute and low-level chronic liver insult, including cirrhosis.

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