Role of Fn14 in acute alcoholic steatohepatitis in mice

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Karaca G, Xie G, Moylan C, Swiderska-Syn M, Guy CD, Krüger L, Machado MV, Choi SS, Michelotti GA, Burkly LC, Diehl AM. Role of Fn14 in acute alcoholic steatohepatitis in mice. Am J Physiol Gastrointest Liver Physiol 308: G325–G334, 2015. First published December 18, 2014; doi:10.1152/ajpgi.00429.2013.—TNF-like weak inducer of apoptosis (TWEAK) is a growth factor for bipotent liver progenitors that express its receptor, fibroblast growth factor-inducible 14 (Fn14), a TNF receptor superfamily member. Accumulation of Fn14+ progenitors occurs in severe acute alcoholic steatohepatitis (ASH) and correlates with acute mortality. In patients with severe ASH, inhibition of TNF-α increases acute mortality. The aim of this study was to determine whether deletion of Fn14 improves the outcome of liver injury in alcohol-consuming mice. Wild-type (WT) and Fn14 knockout (KO) mice were fed control high-fat Lieber deCarli diet or high-fat Lieber deCarli diet with 2% alcohol (ETOH) and injected intraperitoneally with CCl4 for 2 wk to induce liver injury. Mice were euthanized 3 or 10 days after CCl4 treatment. Survival was assessed. Liver tissues were analyzed for cell death, inflammation, proliferation, progenitor accumulation, and fibrosis by quantitative RT-PCR, immunoblot, hydroxyproline content, and quantitative immunohistochemistry. During liver injury, Fn14 expression, apoptosis, inflammation, hepatocyte replication, progenitor and myofibroblast accumulation, and fibrosis increased in WT mice fed either diet. Mice fed either diet expressed similar TWEAK/Fn14 levels, but ETOH-fed mice had higher TNF-α expression. The ETOH-fed group developed more apoptosis, inflammation, fibrosis, and regenerative responses. Fn14 deletion did not reduce hepatic TNF-α expression but improved all injury parameters in mice fed the control diet. In ETOH-fed mice, Fn14 deletion inhibited TNF-α induction and increased acute mortality, despite improvement in liver injury. Fn14 mediates wound-healing responses that are necessary to survive acute liver injury during alcohol exposure.

alcohol; liver injury; liver fibrosis; liver progenitors

FIBROBLAST GROWTH FACTOR -inducible 14 (Fn14) is a tumor necrosis factor (TNF) superfamily receptor for TNF-like weak inducer of apoptosis (TWEAK) (12). TWEAK is expressed by macrophages (3) and acts as mitogen for Fn14+ liver progenitor cells (3, 12, 30). Although TWEAK-Fn14 interaction promotes expansion of progenitor populations that are involved in liver regeneration (13), it also induces fibrosis in many organs, including liver, kidney, and heart (15, 22, 21). Moreover, TWEAK-Fn14 pathway activation stimulates proinflam-
were euthanized 72 h after the last CCl₄ injection to determine acute effects of ETOH on liver injury. Seventeen additional WT and Fn14 KO mice were fed chow diets for 10 days after completion of 2 wk of ETOH + CCl₄ treatment and then euthanized to assess how deletion of Fn14 impacted recovery from ASH. To evaluate the effects of treatment on proliferative activity, nine WT and nine Fn14 KO mice were routinely injected with bromodeoxyuridine (BrdU, 50 μg/g body wt ip) 2 h before they were euthanized. A more detailed list of the experimental groups is provided in Table 1.

Immunochemistry. Formalin-fixed, paraffin-embedded liver tissues were cut into 5-μm-thick sections and mounted on glass slides. Sections were deparaffinized with xylene, dehydrated with ethanol, and then incubated for 10 min in 3% hydrogen peroxide to block endogenous peroxidase. For antigen retrieval, the sections were heated in 10 mM sodium citrate buffer (pH 6.0) for 10 min or incubated with pepsin (catalog no. 00-3009, Invitrogen) for 5 min. Nonspecific binding was inhibited by 15 min of incubation in protein blocking solution. Sections were incubated with primary antibodies. 3,3'-Diaminobenzidine reagent (catalog no. K3466, Dako) was applied in the presence of hydrogen peroxide to visualize target proteins. 3′-Diaminobenzidine reagent (catalog no. K3466, Dako) was applied in the presence of hydrogen peroxide to visualize target proteins. Immunohistochemistry on livers from mice that were treated with CCl₄ was performed following the manufacturer’s instructions.

RESULTS

Subacute liver injury triggers hepatic accumulation of Fn14⁺ cells. To determine if alcohol ingestion influenced changes in Fn14 expression during liver injury, we performed quantitative RT-PCR. Western blot analysis, and immunohistochemistry on livers from mice that were treated with CCl₄ while being fed the control or the ETOH diet. Results were compared with those from mice fed the same diets but treated with vehicle. Expression of Fn14 mRNA and protein was barely detectable in vehicle-treated mice fed either diet. Regardless of alcohol consumption, CCl₄ strongly induced Fn14 expression. Moreover, when CCl₄ was withdrawn and chow diets were resumed, Fn14 expression fell back to baseline assessed by morphometric analysis using MetaView software (Universal Imaging, Downington, PA) in 10 randomly chosen ×20 fields per section per mouse, as described elsewhere (19). TUNEL, Ki67, PCNA, BrdU, and Sox9 staining was quantified by counting the numbers of hepatocytes with stained nuclei in 10 randomly chosen ×10 fields per section per mouse.

mRNA quantification by real-time RT-PCR. Total RNA was isolated using commercial reagents (TRIZol, Invitrogen). RNA concentration and purity were determined with a spectrophotometer (model ND-1000, NanoDrop Technologies, Palo Alto, CA), and samples with a 260 nm-to-280 nm absorbance ratio >1.8 were used in subsequent analyses. RNA was quantified by RT-PCR as described elsewhere (34). All samples were analyzed in duplicate. Gene expression levels were normalized to the reference gene S9, and fold change was calculated by the comparative threshold (2⁻△△Ct) method. Sequences of primers are listed in Table 2.

Western blot analysis. Protein extracts were prepared by homogenization of liver tissue in RIPA buffer (catalog no. R0278, Sigma) and quantified (Pierce). Proteins were visualized by Western blot analysis using the primary antibodies Fn14 (Abcam) and β-actin (Santa Cruz Biotechnology).

Hydroxyproline assay. Liver hydroxyproline content was quantified in flash-frozen liver samples, as described elsewhere (27). Concentrations were calculated from a standard curve prepared with high-purity hydroxyproline (Sigma-Aldrich) and expressed as milligrams of hydroxyproline per gram of liver.

Assessment of hepatic injury. Serum alanine aminotransferase levels were measured using commercially available kits (Biotron Diagnostics) according to the manufacturer’s instructions.

Statistical analysis. Values are means ± SE from at least three animals per group (see Table 1 for details). Student’s t-test was used for comparisons between two groups, and one- or two-way ANOVA followed by Tukey’s multiple-comparison post hoc test was used for comparisons among more than two groups. Survival was calculated using log-rank analysis. P < 0.05 was considered to be statistically significant.

Table 1. Detailed usage of mice in experiments

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Before Treatment</th>
<th>After Treatment (Analyzed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Fn14 KO</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>ETOH</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>C + CCl₄</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>ETOH + CCl₄ + 10 days off</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Total number</td>
<td>37</td>
<td>32</td>
</tr>
</tbody>
</table>

WT, wild-type; Fn14 KO, fibroblast growth factor-inducible 14 knockout; C, control (high-fat Lieber DiCarli diet); ETOH, high-fat Lieber DiCarli diet with 2% alcohol. In the ETOH + CCl₄ treatment group, WT and 9 Fn14 KO mice received bromodeoxyuridine. *All mice died within 2 wk. No mice died during the 10-day recovery period.

Table 2. Sequence of mouse primers

<table>
<thead>
<tr>
<th>Product</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>Collagen 1α1</td>
<td>GAGCGGAGATCGTCTGACGTC</td>
</tr>
<tr>
<td>Desmin</td>
<td>TGACGTCCTGGCAGTACGGAGC</td>
</tr>
<tr>
<td>Fn14</td>
<td>TACGTCCTGGCAGTACGGAGC</td>
</tr>
<tr>
<td>IL-6</td>
<td>AAGCCCAAGGCTTCTTGAAGAGCAGATAGCAG</td>
</tr>
<tr>
<td>S9</td>
<td>GACTCCGGAGACCTCGAGGATCAG</td>
</tr>
<tr>
<td>αSMA</td>
<td>GATGGAGACCGACAGAAGAGAAGAGAAGAGAAGAG</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TCCGTAGAAACCAGCAGCAGAGAGAAGAGAAGAG</td>
</tr>
<tr>
<td>LGR5</td>
<td>GATTTGAGACGGAGACAGAAGAGAAGAGAAGAG</td>
</tr>
</tbody>
</table>

αSMA, α-smooth muscle actin.
levels (Fig. 1, A, B, and D). Thus, subacute liver injury provided a strong stimulus for Fn14 induction, and, at these levels of ingestion, alcohol did not appreciably alter injury-related increases in hepatic Fn14 expression.

Immunostaining with anti-Fn14 antibody or control IgG demonstrated that a subpopulation of nonnecrotic hepatocytes in CCl4-treated WT mice specifically expressed Fn14 (Fig. 1, C–E), whereas no Fn14 mRNA or protein was detected in Fn14-deficient (Fn14 KO) CCl4-treated mice. Since ETOH feeding alone was insufficient to induce significant Fn14 expression, we chose to study the effects of Fn14 deletion on the liver injury induced by exposure to the control diet/H11001 CCl4 or the ETOH diet/H11001 CCl4.

Deletion of Fn14 increases mortality, despite improving liver injury. In WT mice, CCl4 induced necrosis in the liver. Consistent with findings reported by others (24), a greater degree of acute injury was noted when alcohol was present in the diet of animals treated with CCl4 (Fig. 2A). The histopathological findings demonstrating more extensive hepatocellular injury in the ETOH + CCl4 group correlated with elevations in serum alanine aminotransferase (ALT; Fig. 2B). Similarly treated Fn14 KO mice developed less histological necrosis and inflammation than WT mice (Fig. 2A). Consistent with decreased injury on hematoxylin-eosin staining, TUNEL staining demonstrated that ETOH + CCl4 induced greater hepatocyte apoptosis than CCl4 alone, while Fn14 deletion significantly reduced the number of apoptotic hepatocytes relative to WT animals (Fig. 2C). Despite the decrease in overall injury, approximately one-quarter of the Fn14 KO mice that were treated with ETOH/H11001 CCl4 died during the treatment period, while all the other Fn14 KO mice survived, as did all the WT mice, including the ETOH/H11001 CCl4 group (Fig. 2D). Thus, Fn14 deletion selectively increased liver injury-related mortality in ETOH-consuming mice. However, Fn14 KO and WT mice that survived acute liver injury and were examined 10 days after discontinuation of ETOH/H11001 CCl4 were similar with regard to hepatic hematoxylin-eosin staining, serum ALT, and hepatocyte apoptosis markers (Fig. 2, A–C). Furthermore, no surviving mice in either group died during the recovery period.

Deletion of Fn14 reduces proinflammatory cytokine expression. Severe ASH in humans is associated with dramatic induction of proinflammatory cytokines, such as TNF-α (10).
TNF-α plays a key role in the pathogenesis of ETOH-mediated liver injury in rodent models (35). However, neutralization of TNF-α increased acute mortality in patients with severe acute ASH (21). To determine if Fn14 influenced inflammatory responses to liver injury in our models, we evaluated whole liver expression of two key inflammatory cytokines, TNF-α and interleukin-6 (IL-6), and one macrophage-specific marker, F4/80.

CCl4 treatment increased mRNA levels of both cytokines in WT mice. ETOH-consuming mice demonstrated severalfold greater induction of TNF-α and IL-6 than mice fed the control diet. Fn14 deletion did not prevent TNF-α mRNA upregulation in mice fed the control diet and treated with CCl4 but abrogated the additive effects of ETOH on TNF-α induction during liver injury (Fig. 3, A and B). Fn14 is a member of the TNF receptor superfamily, and its natural ligand is TWEAK. Unlike TNF-α, CCl4 treatment increased mRNA levels of both cytokines in WT mice. ETOH-consuming mice demonstrated severalfold greater induction of TNF-α and IL-6 than mice fed the control diet. Fn14 deletion did not prevent TNF-α mRNA upregulation in mice fed the control diet and treated with CCl4 but abrogated the additive effects of ETOH on TNF-α induction during liver injury (Fig. 3, A and B). Fn14 is a member of the TNF receptor superfamily, and its natural ligand is TWEAK. Unlike TNF-α,
hepatic expression of TWEAK did not increase during CCl4 treatment in any of the WT mice. Indeed, Fn14 deletion increased (rather than decreased) TWEAK mRNA levels in all groups (Fig. 4). Fn14 deletion inhibited induction of IL-6 in mice fed the ETOH or the control diet, further suggesting that the inhibitory effects of Fn14 deletion on TNF-α expression in ETOH-fed mice were specific (Figs. 3, A and B). Also, although ETOH diet-fed mice tended to accumulate more F4/80+/H11001 cells than control diet-fed mice during liver injury, Fn14 deletion inhibited CCl4-related accumulation of macrophages similarly in both groups of mice (Fig. 3C). The aggregate data suggest that induction of TNF-α during ETOH-associated liver injury occurs via Fn14-dependent and -independent mechanisms and that the former are particularly important for increasing hepatic TNF-α production. Withdrawal of the injury-inducing factors resulted in a decline of TNF-α and IL-6 in WT mice, leading to similar expression of these cytokines in WT and Fn14 KO livers at the end of the 10-day recovery period.

Deletion of Fn14 inhibits liver progenitor accumulation and impairs hepatocyte proliferation. Fn14 is expressed by progenitor cells, which can differentiate into hepatocytes (1, 12, 13). The effects of subacute injury on expression of other progenitor markers and hepatocyte proliferation and the impact of Fn14 deletion on these parameters were evaluated by quantitative immunohistochemistry and quantitative RT-PCR analysis (Figs. 5–7). Rates of hepatocyte turnover are known to be low in healthy adult mice. Consistent with this, very few hepatocytes in the healthy livers of control diet-fed WT mice were labeled with the proliferation markers Ki67 and PCNA (25, 37; data not shown). These mice also demonstrated very few cells that were positive for pan-CK, a liver progenitor marker that is also expressed by Fn14+ cells (30; data not shown). Staining for LGR5 [an endodermal progenitor marker (11)], αFP [a marker of hepatocytic progenitors (29)], and Sox9 [a bipotent liver progenitor marker (9)] was observed rarely and localized primarily around periportal areas (data not shown).

CCl4 injections increased numbers of Ki67- and PCNA-labeled hepatocytes in WT mice (Fig. 5, A and B). Injury-related accumulation of proliferating hepatocytes was greater in ETOH diet-fed mice (Fig. 5, A and B), which also demonstrated more liver injury than control diet-fed controls (Fig. 2). CCl4 injections also increased progenitors in both groups of WT mice and promoted greater expansion of progenitor cells in ETOH-fed mice (Figs. 6 and 7). This was accompanied by increased whole liver mRNA expression of LGR5, αFP, and Sox9 in the ETOH + CCl4 group (Fig. 6B and Fig. 7, B and C). Furthermore, careful examination of serially stained sections demonstrated that Fn14+ cells were closely localized with LGR5+ cells and αFP+ cells but distinct from cells that expressed pan-CK or Sox9 (Figs. 7A and 8A), consistent with our previous findings after partial hepatectomy (13).
Baseline levels of hepatocyte proliferation and progenitor accumulation were similar in Fn14 KO and WT mice fed control diets. After CCl4 treatment, however, Fn14 KO mice accumulated fewer pan-CK-, LGR5-, aFP-, and Sox9-expressing cells, expressed less keratin 7 [a biliary/progenitor cell marker (7)] mRNA, and showed decreased induction of Ki67 and PCNA labeling in hepatocytes. The reduced proliferative and progenitor responses occurred in control- and ETOH diet-fed Fn14 KO mice (Fig. 5, A and B, Fig. 6, and Fig. 7, B–D). BrdU incorporation confirmed significantly decreased hepatocyte proliferation during liver injury in Fn14 KO mice (Fig. 5C). Hence, Fn14 promotes liver progenitor accumulation

![Image](https://example.com/file.png)

**Fig. 7.** Deletion of Fn14 impairs liver progenitor expansion. A: representative immunohistochemistry of Fn14, LGR5, a-fetoprotein (aFP), pan-CK, and Sox9 in liver sections from WT mice fed ETOH diet and treated with CCl4 for 2 wk. Original magnification \(\times 10\). CV, central vein; PV, portal vein. B–D: morphometry data and mRNA expression for aFP (B) and Sox9 (C) and mRNA expression for keratin 7 (Krt7; D) for livers from WT and Fn14 KO mice. Results are expressed as fold change compared with control. Results were normalized to the housekeeping gene S9. All comparisons were calculated by 2-way ANOVA followed by Tukey’s multiple-comparison post hoc test: \(*P < 0.05, ***P < 0.001.\)
and hepatocyte proliferative activity during subacute liver injury. After discontinuation of ETOH and CCl₄, hepatic pan-CK, LGR5, and Sox9 protein and/or mRNA expression fell to similar levels in surviving WT and Fn14 KO mice, while the number of Ki67- and PCNA-labeled hepatocytes declined by ≥50% in both groups (Fig. 5, A and B). Declines in regenerative responses (Figs. 5–7) paralleled the resolution of liver injury (Fig. 2) and accompanying fall in Fn14 expression (Fig. 1).

**Deletion of Fn14 reduces liver fibrosis.** Fibrosis occurs during severe ASH (10). CCl₄ injections increased hepatic fibrosis in WT mice. As with liver injury (Fig. 2) and injury-related regenerative responses (Figs. 5–7), consumption of ETOH diets exacerbated liver fibrosis, as evidenced by increased Sirius red staining (Fig. 9, A and B), collagen 1α1 mRNA (Fig. 9C), and liver hydroxyproline content (Fig. 9D). Because myofibroblasts derived from activated hepatic stellate cells (HSC) are major sources of collagen-producing cells in injured livers (14), we evaluated several HSC activation markers, including αSMA and desmin, by quantitative immunohistochemistry and quantitative RT-PCR (Fig. 10). Both markers increased at the protein and mRNA levels during injury and were greater in ETOH- than control diet-fed mice during CCl₄ treatment.

Fn14 KO mice demonstrated significantly less liver fibrosis than WT mice after CCl₄ injections, regardless of diet. Fibrosis...
as assessed by morphometry) and collagen gene expression (as assessed by quantitative RT-PCR) in Fn14 KO mice were about half the levels observed in WT livers (Fig. 9, B and C). The hepatic hydroxyproline content was also lower in CCl4-treated Fn14 KO than CCl4-treated WT mice (Fig. 9C). Consistent with the decrease in liver fibrosis, Fn14 KO livers also accumulated only about half as many myofibroblastic cells as WT livers (Fig. 10). Deletion of Fn14 did not retard regression of fibrosis when ETOH and CCl4 were withdrawn in surviving animals (Figs. 9 and 10).

DISCUSSION

In this study we examined, for the first time, the effect of Fn14 deletion in an animal model that mimics features of human ASH. Similar to patients with severe ASH, ETOH-consuming mice with subacute liver injury demonstrated striking increases in Fn14 mRNA and protein expression without a concomitant increase in TWEAK expression (Fig. 4) (1). In mice, as in humans (10, 20), severe ASH is also associated with increased production of inflammatory cytokines and macrophage accumulation, liver cell death, a progenitor response, and fibrosis. In the current animal model, the intensity of each of these processes generally paralleled the level of Fn14 expression, being significantly weaker in Fn14 KO mice than WT controls. These data suggest that targeting Fn14 might improve the outcome of ASH. Therefore, it is particularly noteworthy that we observed the opposite effect when Fn14 was deleted. Namely, acute mortality was higher in animals deficient in Fn14. Interestingly, all deaths occurred during the 2nd wk of ETOH feeding and were restricted to the subgroup of Fn14 KO mice that received CCl4 injections to induce subacute liver injury. When ETOH and CCl4 were withdrawn, no further mortality occurred.

The reduced short-term survival in the Fn14 KO group is not easily explained by differences in liver injury, because serum aminotransferases and hepatocyte apoptosis were similar in WT and Fn14 KO mice that survived ETOH + CCl4 treatment. However, surviving ETOH diet-fed Fn14-deficient mice demonstrated significantly decreased progenitor accumulation and fibrosis compared with ETOH diet-fed WT mice immediately upon termination of treatment. The latter findings raise the possibility that the increase in ETOH-related mortality might have resulted from defective liver wound healing during subacute liver injury, a concept supported by recent evidence for impaired liver regeneration and increased mortality in Fn14 KO mice after partial hepatectomy (13). In this regard, it is noteworthy that hepatic expression of TNF-α was significantly higher in ETOH- than control diet-fed mice during liver injury and that Fn14 deletion selectively suppressed the ETOH-related “super”-induction of TNF-α. TNF-α has important proregenerative actions (5). In humans with severe ASH, treatments that inhibited TNF-α increased short-term mortality (21), and this negative outcome was attributed to loss of the proregenerative effects of TNF-α (18). Thus it is possible that inhibition of TNF-α induction by Fn14 deletion is responsible for the high mortality in Fn14 KO mice. Definitive proof would require supplementation of TNF-α in the Fn14 KO mice either pharmacologically or genetically. Since the Fn14 KO was global, it is also conceivable that the increased mortality might have resulted from loss of Fn14 function in extrahepatic tissue(s). Further research is needed to clarify this, because necropsies were not performed in the current study. While it will be interesting to determine whether liver-specific deletion of Fn14 impacts subacute liver injury-related mortality, the clinical relevance of such studies will be doubtful, because it is not feasible to achieve liver-specific inhibition of Fn14 in
patients with ASH by a genetic or a pharmacological approach. Moreover, the present work in WT mice demonstrates that hepatic Fn14 expression declined rapidly once ASH-inducing insults were stopped. Indeed, levels of Fn14 mRNA and protein were found to be similar in WT and Fn14 KO mice within 10 days. At that time point, both groups also had comparable levels of liver function, progenitors, fibrosis, and inflammatory cytokine levels. Similar findings were observed when these studies were repeated in mice that are genetically deficient in TWEAK (data not shown), although the present studies and at least one other study (1) demonstrated that TWEAK itself was not elevated when ETOH injury was augmented with injections of CCl4. These findings might be consistent with those seen in human ASH and support evidence that Fn14 is able to signal in a ligand-independent manner (33). Lack of TWEAK mRNA induction also does not preclude the possibility that TWEAK mRNA induction might have occurred at some other time point(s), that local levels of TWEAK protein increased in our system, or that the injured livers might have been exposed to increased levels of soluble TWEAK that were generated by some extrahepatic source. The aggregate data, nevertheless, indicate that inhibition of Fn14 activity does not facilitate the resolution of ASH-related injury, casting doubt about the utility of treating ASH patients with Fn14 or TWEAK inhibitors. It is possible that pharmacological targeting of Fn14 or TWEAK may not have the same effect as genetic deletion of Fn14. Although we did not examine the effects of anti-TWEAK antibodies in the current studies, we recently published a very comprehensive comparison of Fn14 deletion, TWEAK deletion, and anti-TWEAK antibody treatment in several hundred mice at various time points after partial hepatectomy. In those experiments, all the approaches to abrogate TWEAK-Fn14 signaling had the same effect: each profoundly inhibited liver regeneration (both accumulation of liver progenitors and replication of mature-appearing liver cells) (13). Thus it is unlikely that dramatic differences will occur between pharmacological inhibition of Fn14 or TWEAK and genetic deletion of Fn14 in the current model.

Our findings do, however, support the concept that recovery from ASH (and other injuries that kill hepatocytes) requires hepatocyte regeneration. The present study is important, because it provides novel evidence that Fn14 regulates regenerative responses to ASH, a clinically important form of human liver injury. Because Fn14 promotes progenitor-mediated regeneration (12, 30) and both progenitor accumulation and hepatocyte proliferative activity were impaired when Fn14 was absent, our findings suggest that progenitors mediate the outcomes of ASH. Although the role of liver progenitor cells during liver injury is still poorly defined (28), we and others have reported that expansion of progenitor cells occurs during many types of hepatic injury in humans and mice and proposed that progenitor compartments contribute considerably to the proper restoration of functional hepatic parenchyma (4, 11, 26, 36). Thus, inhibition of Fn14 might negatively affect liver regeneration by disrupting processes that normally modulate progenitor fate during and after ASH-related liver injury. Somewhat surprisingly, we also observed that Fn14 influences liver repair during ASH by supporting HSC differentiation into myofibroblasts. In the present study, stellate cell transformation into myofibroblasts was disrupted during ASH in mice lacking Fn14, and this accompanied reduced accumulation of liver progenitors and impaired wound-healing responses during acute ASH, including decreased hepatocyte proliferative activity and decreased survival. On the basis of these results (worse acute mortality during ASH and no obvious long-term benefits after ASH), we conclude that it would be premature to treat patients with severe acute ASH with Fn14/TWEAK signaling inhibitors.

REFERENCES


