HMGB1 recruits hepatic stellate cells and liver endothelial cells to sites of ethanol-induced parenchymal cell injury

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First published October 3, 2013; doi:10.1152/ajpgi.00151.2013.—Alcoholic liver disease is one of the most common causes of liver cirrhosis and portal hypertension worldwide; however, the mechanisms by which ethanol leads to liver injury and fibrosis are not fully understood. The process by which injured cells release molecules that recruit inflammatory cells in absence of infection has been referred to as “sterile inflammation.” Although prior studies have characterized this process for recruitment of inflammatory cells, the mechanisms by which LEC and HSC participate are less understood.

Owing to its unique anatomic location downstream from the gut along with its fenestrated and permeable sinusoidal characteristics, the liver is continuously exposed to pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides (LPS) or endogenous danger-associated molecular patterns (DAMPs). In this study, we test the concept that HMGB1 may be released from ethanol-treated liver parenchymal cells and contribute to HSC and LEC recruitment. Ethanol stimulation of rat hepatocytes and HepG2 cells resulted in translocation of HMGB1 from the nucleus as assessed by Western blot. HMGB1 protein levels were increased in the supernatant of ethanol-treated hepatocytes compared with vehicle-treated cells. Migration of both HSC and LEC was increased in response to conditioned medium for ethanol-treated hepatocytes (CMEtOH) compared with vehicle-treated hepatocytes (CMVEH) (P < 0.05). Recombinant HMGB1 (100 ng/ml) also stimulated migration of HSC and LEC compared with vehicle stimulation (P < 0.05 for both HSC and LEC). HMGB1 stimulation of HSC increased the phosphorylation of Src and Erk and HMGB1-induced HSC migration was blocked by the Src inhibitor PP2 and the Erk inhibitor U0126. Hepatocytes release HMGB1 in response to ethanol with subsequent recruitment of HSC and LEC. This pathway has implications for HSC and LEC recruitment to sites of ethanol-induced liver injury.

HMGB1; ethanol; hepatocyte; hepatic stellate cells (HSC); liver endothelial cells (LEC)

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penicillin/streptomycin (ScienCell) under standard tissue culture conditions (a humidified 5% carbon dioxide incubator at 37°C). LECs were cultured in medium containing 5% fetal bovine serum, 2% endothelial cell growth supplement, and 1% penicillin-streptomycin.

Table 1. Primer sequence

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HMGB1 was more visible in the cytoplasm of ethanol-treated HepG2 cells compared with control (G). HMGB1, green; blue, nucleus; red, cytoplasm (original magnification ×65). Results were obtained from at least 3 independent experiments. Data in bar graphs represent means ± SE. *P < 0.05; NS, not significant.

Fig. 1. Ethanol induces high-mobility group box 1 (HMGB1) cytosolic translocation and release from hepatocytes. Rat hepatocytes were treated with ethanol (0–100 mM) for 24 h and supernatants were collected for Western blot assay and HMGB1 ELISA. Western blot assay shows significant increase of HMGB1 protein levels in the supernatants of ethanol-treated cells (A). HMGB1 concentration was increased in the supernatants of ethanol-treated hepatocytes by HMGB1 ELISA (B). Rat hepatocytes were treated with ethanol (0–100 mM) for 24 h and mRNa was prepared for real-time PCR. There was no significant change in HMGB1 mRNA levels after ethanol treatment (C). Ethanol- or vehicle-treated hepatocytes were collected and stained with Trypan blue. There was no significant difference in the proportion of viable cells between vehicle-treated cells and ethanol-treated cells. In MTS and caspase 3/7 assay performed with HepG2 cells, neither necrosis nor apoptosis was observed in response to ethanol (D). Rat hepatocytes (E) and HepG2 cells (F) were treated with ethanol (0–100 mM) for 24 h and nuclear and cytoplasmic fractions were isolated and prepared for SDS-PAGE. HMGB1 expression was significantly decreased in nuclear fractions and increased in cytoplasmic fraction after ethanol treatment by Western blot analysis. In immunocytochemistry study, HMGB1 was more visible in the cytoplasm of ethanol-treated HepG2 cells compared with control (G). HMGB1, green; blue, nucleus; red, cytoplasm (original magnification ×65). Results were obtained from at least 3 independent experiments. Data in bar graphs represent means ± SE. *P < 0.05; NS, not significant.
tion time. The resultant supernatants were collected to use as EtOH CM for conditioned medium from ethanol-stimulated HepG2 cells and EtOH DMEM for basal DMEM containing ethanol.

Isolation of nuclear and cytoplasmic proteins and Western blotting. Cells were washed twice with ice-cold PBS and homogenized in a cell lysis buffer at 4°C for 20 min. After centrifugation, the protein concentration in the lysates was measured by a Bradford assay. In some experiments nuclear and cytoplasmic cell lysates from HepG2 cells and rat hepatocytes were collected for Western blot analysis by using previously validated protocols (3). Lysates containing 30–50 μg of proteins were heated for 3 min at 100°C. Protein lysates were separated on a 12 or 15% acrylamide gel and transferred to polyvinylidene difluoride membranes (GE Healthcare, Buckinghamshire, UK). After 60-min incubation with 5% nonfat dry milk (Bio-Rad) or 5% albumin from bovine serum (Sigma-Aldrich) at room temperature to block the nonspecific binding, membranes were incubated at 4°C overnight with specific primary antibodies and then, for 2 h with secondary antibodies conjugated to horseradish peroxidase at 4°C. Membranes were washed and protein bands were detected with an enhanced chemiluminescence detection system (ECL Plus, Santa Cruz Biotechnology) according to the manufacturer’s instructions. When necessary, membranes were stripped and reprobed with an anti-GAPDH antibody (1:107). Digitalization of films was performed with a scanner (Epson V750, Nagano, Japan). Quantification of band density was performed by use of Image J 1.40G (NIH, Bethesda).

HMGB1 ELISA. HMGB1 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (IBL, Tokyo, Ontario, Canada) that detects rat and mouse HMGB1 according to the manufacturer’s instructions.

Real-time PCR. Total RNA was extracted using TRIzol reagent according to the manufacturer’s instructions (Invitrogen Life Technologies). The reverse-transcription reaction was performed by using 1 μg total RNA that was reverse-transcribed into the first-strand cDNA by Superscript II reverse transcriptase with random primers (Invitrogen Life Technologies). PCR mixture was prepared with SYBR Green PCR Master Mix (PE Applied Biosystems, Foster City, CA) by using the primers as shown in Table 1. Thermal cycling conditions were 10 min at 95°C followed by 40 cycles of 95°C for 15 s and 60°C for 1 min on an ABI PRISM 7000 Sequence Detection

Fig. 2. HMGB1 induces migration of human (h) hepatic stellate cells (HSC) and liver endothelial cells (LEC). HSC and LEC (3,000 cells/well) were loaded into the upper wells of Boyden assay chamber and recombinant HMGB1 (0–100 ng/ml) or conditioned medium from ethanol-stimulated hepatocytes (CMEtOH) compared with vehicle-stimulated hepatocytes (CMEtOH) was collected from the media of rat hepatocytes (CMEtOH) treated with ethanol (10, 50, and 100 mM) for 24 h. Migration of HSC (A) and LEC (B) was significantly increased in response to recombinant HMGB1. Similar findings were noted in response to CMEtOH (C with HSC and D with LEC). In separate experiment evaluating the effect of ethanol on cell migration, migration of HSC (E) and LEC (F) was significantly increased in EtOH CM compared with EtOH DMEM. (*P < 0.05). CMe100, conditioned medium from 100 mM ethanol-stimulated hepatocytes; CMe50, conditioned medium from 50 mM ethanol-stimulated hepatocytes; EtOH CM, conditioned medium from ethanol-stimulated HepG2 cells; EtOH DMEM, basal DMEM containing ethanol. Results depicted are compiled from at least 3 experiments. Data represents means ± SE. *P < 0.05.
System (PE Applied Biosystems). Gene expression was normalized with rat 14S mRNA or mouse β-actin mRNA content.

Migration assay. Migration of HSC and LEC was evaluated by Boyden chamber assay in response to conditioned medium or recombinant HMGB1. Boyden assay was performed as previously described (7). In brief, modified Boyden chambers (Becton Dickinson, Heidelberg, Germany) were used with filters (8 μm pores, Neuro Probe, Gaithersburg, MD) coated with collagen type I (50 μg/ml). HMGB1 (100 ng/ml) or conditioned medium was added to the lower chamber and 3,000 cells in 50 μl of serum-free DMEM were added to the upper chamber. In some experiments, HMGB1-neutralizing antibody was added in lower wells of Boyden chambers. For inhibition of RAGE or TLR4 receptors, HSC and LEC were preincubated for 30 min at 4°C with 40 μg/ml anti RAGE (R&D Systems) or 5 μg/ml anti-TLR4 (Biolegend) or murine control IgG (Biolegend) (5). After 4 h incubation at 37°C, cells remaining on the upper surface of filters were scraped off with a cotton swab and cells on the lower surface were fixed with ethanol and stained with DAPI. Cells were counted by use of the ImagePro program. Results are the means ± SE of the number of cells counted in ×4 low-power fields per filter.

Cell viability and caspase 3/7 assay. To evaluate the cytotoxicity of ethanol toward hepatocytes, cell viability assay was performed. In brief, after incubation of hepatocytes with ethanol (0, 10, 50, and 100 mM) for 24 h, the cells were exposed to a 0.4% Trypan blue solution for 3 min and viewed under a light microscope. Cell viability was defined as the ratio of unstained cells to the total number of cells.

Fig. 3. HMGB1 is essential for CMe50-induced migration of HSC and LEC. In Boyden chamber assay, CMe50-induced cell migration was blocked by HMGB1-neutralizing antibody (1 μg/ml) in HSC (A) and LEC (B). HepG2 cells were transfected with HMGB1-siRNA. HMGB1 protein expression was markedly decreased by HMGB1-siRNA transfection, and nuclear (N) levels were reduced in response to ethanol based on Western blot assay. GAPDH blot is shown from nuclear and cytosolic (C) preparations (C). CMe50 from HMGB1-siRNA-transfected cells did not induce migration of HSC (D) or LEC (E), whereas the number of migrated cells was significantly increased by CMe50 from control-siRNA-transfected cells. Results depicted are compiled from at least 3 experiments. Data represents means ± SE. *P < 0.05.
HepG2 cells (1 × 10⁴/well) were used for MTS (CellTiter 96 Aqueous Non-Radioactive Cell Proliferation assay, Promega) and caspase 3/7 assay (Apo-One Homogeneous Caspase-3/7 Assay, Promega) with incubation time 4 h or 30 min, respectively. Camptothecin (10 and 20 μM) was used as a positive control for apoptosis.

**Immunocytochemistry.** HepG2 cells were treated with basal DMEM containing 100 mM ethanol for 24 h. The cells were fixed with 2.5% paraformaldehyde for 5 min and washed three times with PBS. For permeability, the cells were exposed to 0.1% Triton X-100 in PBS for 5 min. After blocking with 10% FBS in PBS, the cells were incubated with the HMGB1 antibody overnight at +4°C, and then secondary antibody (Alexa Fluor 488 goat anti-rabbit IgG) was applied for 1 h at room temperature. Phalloidin and ToTo3 were used to stain cytoplasm and nucleus for 20 min, respectively.

**Immunohistochemistry.** Paraffin-embedded sections of mouse liver were deparaffinized and rehydrated. Heat-induced antigen retrieval was performed in sodium citrate buffer pH 6. After blocking endogenous peroxidase for 15 min, the sections were blocked with 10% FBS in PBS for 1 h at room temperature. The sections were incubated with the HMGB1 antibody overnight at +4°C, and then biotinylated secondary antibody (Vector Laboratories) was applied for 1 h at room temperature. ABC reagent (Vector Laboratories) was used to develop the stain, and brown staining in cytoplasm was quantitated in randomly chosen sections.

**In vivo ethanol feeding studies in mice.** C57B6 mice were fed with Lieber-DeCarli liquid diet (32% calories from ethanol, 5% volume) or nonalcohol isocaloric control liquid diet for 4 wk as described (24). All animal work was performed under Mayo IACUC oversight.

**Statistical analysis.** Data expressed were as means ± SE of data obtained from at least three independent experiments and compared by Mann-Whitney U-test or ANOVA for multiple comparison parametric data. A P value less than 0.05 was considered statistically significant.

**RESULTS**

**Ethanol induces HMGB1 release from hepatocytes.** To examine whether ethanol stimulates HMGB1 release from hepatocytes, rat hepatocytes were treated with 0, 10, 50, and 100 mM of ethanol for 24 h and supernatants were collected and assayed for HMGB1 release by Western blot analysis and ELISA. HMGB1 expression by Western blot analysis (Fig. 1A) and HMGB1 concentration by HMGB1 ELISA (Fig. 1B) were significantly increased in the supernatant of cells incubated with ethanol in a concentration-dependent manner. There was no significant change in HMGB1 mRNA expression after ethanol treatment (Fig. 1C), suggesting that the increased release of HMGB1 does not involve increased transcription. Because HMGB1 can be passively released from necrotic cells, viability of hepatocytes after ethanol treatment was also evaluated. Cell viability did not differ between hepatocytes treated with ethanol and vehicle for 24 h (Fig. 1D). This result was supported by data using HepG2 cells, in which ethanol dosing showed neither necrosis nor apoptosis (Fig. 1D). Since cytoplasmic HMGB1 translocation correlates with eventual cellular release (12), Western blot analysis was performed with nuclear and cytoplasmic protein lysates of ethanol-treated rat hepatocytes to determine whether ethanol treatment induces the translocation of HMGB1 from nucleus to cytoplasm in hepatocytes. Nuclear HMGB1 protein levels decreased while cytoplasmic levels increased in response to ethanol treatment, with levels in the total lysates remaining unchanged (Fig. 1E; top and bottom). Similar results were observed in ethanol-treated...
HepG2 cells (Fig. 1F). This was further corroborated by immunocytochemistry studies showing cytoplasmic translocation of HMGB1 in HepG2 cells treated with ethanol (Fig. 1G). Collectively, these data indicate that ethanol actively stimulates HMGB1 release from hepatocytes.

Recombinant and ethanol-stimulated hepatocyte HMGB1 induce migration of hepatic stellate cells and liver sinusoidal endothelial cells. The effect of HMGB1 on migration of HSC and LEC was evaluated by modified Boyden chamber assay. Recombinant human HMGB1 induced migration of human HSCs (Fig. 2A) and human LECs (Fig. 2B) in a concentration-dependent manner. Similarly, significantly greater numbers of HSC (Fig. 2C) and LEC (Fig. 2D) migrated in the Boyden assay in response to CME100 compared with CMVEH. Interestingly, the migration of cells decreased in CMe100 (conditioned medium from 100 mM ethanol-stimulated hepatocytes) compared with CMe50 (conditioned medium from 50 mM ethanol-stimulated hepatocytes). This is consistent with prior studies in which increasing HMGB1 levels were less effective in stimulating migration of endothelial progenitor cells, possibly due to changes in protein conformation or receptor-ligand binding kinetics (5). To exclude an effect of residual ethanol from CME100 on HSC migration, EtOH CM and EtOH DMEM were subjected to Boyden chamber assay and their effect on cell migration were compared in both HSC and LEC. The results showed significantly higher migration in EtOH CM compared with EtOH DMEM, excluding the possibility that increased migration in response to EtOH CM is due to residual EtOH in the conditioned medium (Fig. 2, E and F). Next, to confirm that HMGB1 was causative in CME100-induced migration, experiments were performed with HMGB1-neutralizing antibody. The migratory effect of CME100 was blocked by HMGB1-neutralizing antibody in both HSC (Fig. 3A) and LEC (Fig. 3B). To further evaluate the effect of HMGB1 on the cell migration, CMVEH and CMVEH were prepared from HepG2 cells after HMGB1-siRNA transfection. HepG2 cells were used in this assay because they are more amenable to transfection than primary hepatocytes. HMGB1 protein levels were significantly decreased in HMGB1-siRNA-transfected cells compared with control-siRNA-transfected cells and nuclear levels were reduced in response to ethanol (Fig. 3C). More cells migrated in response to CME100 of control-siRNA-transfected cells, compared with CME100 of HMGB1-siRNA-transfected cells for both HSCs (Fig. 3D) and LECs (Fig. 3E). In total, these data indicate that HMGB1 stimulates migration of both HSC and LEC.

HMGB1 requires Src/Erk pathway for HSC migration but not for LEC migration. We next sought to identify signaling mechanisms that mediate HSC and LEC migration in response to HMGB1. Since Src and Erk are implicated in liver nonparenchymal cell migration (3, 48), we evaluated the changes of the activation of Src and Erk in response to recombinant HMGB1. After treatment with HMGB1 (100 ng/ml) for varying durations (0–90 min), cells were collected for Western blot assay. Both Src and Erk were activated after 15-min stimulation with recombinant HMGB1 in HSC (Fig. 4A). This response was not observed in LEC, in which only small and nonsignificant changes were observed in p-Src after extended duration of incubation (Fig. 4B).

To further explore the role of Src in HMGB1-induced migration, Boyden chamber assay was performed after blocking Src by two different approaches. First, HSCs and LECs were pretreated with 10 μM of the Src inhibitor PP2 for 30 min and Boyden chamber assay was performed. PP2 attenuates...
HMGB1-induced migration of HSC (Fig. 5A). However, PP2 did not affect HMGB1-induced migration of LEC (Fig. 5B). Next, cells were transfected with a Src dominant-negative mutant retroviral construct (Src-Y419F) that attenuates Src function or a Src-wild-type (Src-WT) retroviral construct (48).

In Boyden chamber assay, cell migration was increased in response to recombinant HMGB1 protein in Src-WT HSCs, but not Src-Y419F mutant-transfected HSCs (Fig. 5C). However, transfection of Src-Y419F mutant construction did not affect HMGB1-induced migration of LEC (Fig. 5D). These results suggested that Src is required for the migratory effect of HMGB1 on HSC but not LEC.

In Western blot assay, PP2 pretreatment also blocked HMGB1-induced Erk phosphorylation in HSC (Fig. 6A), whereas this response was not significant in LEC (Fig. 6B). These results suggest that Erk may be downstream of Src in HMGB1 migration signaling in HSC. Therefore, we further explored role of Erk in HMGB1-induced migration. Boyden chamber assay was performed after pretreatment of cells with the Erk inhibitor U0126. HMGB1-induced migration of HSC was blocked by 30-min pretreatment with U0126 (5 μM) (Fig. 6C), whereas U0126 did not affect HMGB1-induced migration of LEC (Fig. 6D). Thus the Src/Erk pathway plays a pivotal role in HMGB1-induced HSC migration but not for HMGB1-induced LEC migration. In LEC, HMGB1 (100 ng/ml) increased Akt activation by Western blot assay (Fig. 7A). Additionally, HMGB1-induced migration of LEC was blocked by 30-min pretreatment with Akt inhibitor (Fig. 7B). These data suggest that Akt may be involved in LEC migration in response to HMGB1.

**HMGB1 induced cell migration via TLR4 and RAGE in HSC.** We performed experiments with inhibitory antibodies against two major HMGB1 receptors, TLR4 and RAGE, to evaluate their role in HMGB1-induced cell migration in HSC and LEC. Neutralizing anti-TLR4 and anti-RAGE antibodies significantly blocked the HMGB1-induced cell migration in HSC (Fig. 8A). However, neither anti-TLR4 nor anti-RAGE antibodies blocked the HMGB1-induced migration in LEC (Fig. 8B). These data indicate that HMGB1 induces cell migration through TLR4 and RAGE in HSC.

**HMGB1 in alcohol-induced liver injury in vivo.** To evaluate the role of HMGB1 in alcohol-induced liver injury in vivo, mice were fed alcohol (24). Steatosis was documented in liver tissue of alcohol-fed mice as previously described (Fig. 9A). HMGB1 mRNA levels from total liver were significantly increased in alcohol-fed mice (Fig. 9B). Increased HMGB1 mRNA tissue levels in vivo are likely a result of infiltrating inflammatory cells that also generate HMGB1 since HMGB1...
mRNA levels from hepatocytes in vitro were not elevated in response to ethanol (Fig. 1C). HMGB1 cytoplasmic translocation was also detected in hepatocytes in alcohol-fed mice as assessed by immunohistochemistry (Fig. 9C). Although serum HMGB1 levels were increased by 30%, this change did not reach statistical significance (Fig. 9D). These data suggest that HMGB1 translocation occurs in alcohol-induced liver injury in vivo. In vivo studies with more inflammatory and/or fibrotic alcohol feeding models will be useful to explore this further.

**DISCUSSION**

In this study, we examined the role of HMGB1 in the hepatocyte response to ethanol. Our results indicate that ethanol induces HMGB1 secretion by hepatocytes. In addition, secreted HMGB1 induces migration of HSC and LEC. We also uncover specific signaling mechanisms by which this is achieved. For example, migration of HSC requires activation of the nonreceptor tyrosine kinase Src as well as activation of the kinase Erk. In total, the work identifies a pathway that may contribute to alcohol injury responses in liver.

Previous studies have demonstrated that intranuclear HMGB1 can be actively secreted or passively released into extracellular space in response to pathogens or in response to necrotic cell injury (1, 2, 25, 32, 34, 41, 43). Extracellular HMGB1 in turn acts as a recruitment signal to induce an inflammatory reaction (41, 43). This process has been best characterized in liver in context of ischemia-reperfusion injury (9). Our results indicate that ethanol also activates the HMGB1 injury pathway. In our experiments, HMGB1 release was likely active since cell viability was intact in response to ethanol and thus release was not a result of the passive release of intracellular contents by necrotic cells. The steps by which HMGB1 is actively released from cells remain under active investigation, although our initial data show that active release via exosomes seems to be less likely than a traditional secretory pathway (data not shown).

![Fig. 7. Akt may contribute to HMGB1-induced LEC migration. Western blotting and Boyden chamber assay were performed on LEC treated with recombinant HMGB1 (100 ng/ml). Serum-starved LEC was treated with recombinant HMGB1 for different time course (0–90 min). Total cell lysates were used for Western blotting. Akt phosphorylation was increased in time 60 and 90 min (A). HMGB1-induced cell migration was attenuated by 30-min pretreatment with Akt inhibitor (B). Results were obtained from at least 3 independent experiments. Data in bar graphs represent means ± SE and are expressed as number of cells in the filter. *P < 0.05.](image-url)

![Fig. 8. Akt may contribute to HMGB1-induced LEC migration. Western blotting and Boyden chamber assay were performed on LEC treated with recombinant HMGB1 (100 ng/ml). Serum-starved LEC was treated with recombinant HMGB1 for different time course (0–90 min). Total cell lysates were used for Western blotting. Akt phosphorylation was increased in time 60 and 90 min (A). HMGB1-induced cell migration was attenuated by 30-min pretreatment with Akt inhibitor (B). Results were obtained from at least 3 independent experiments. Data in bar graphs represent means ± SE and are expressed as number of cells in the filter. *P < 0.05.](image-url)
Prior studies have shown that HMGB1 acts as a chemokine for various cell types (10, 33, 45). However, the molecular steps by which HMGB1 stimulates cell migration are not fully understood especially in HSC and LEC (8, 13, 42). In the present studies we found that HMGB1 induces migration of both HSC and LEC; however, the mechanisms of cell motility were different in the two cell types. We used a number of complementary approaches to explore the role of the Src and Erk intracellular mediators in cell migration. These studies revealed that although HMGB1 utilizes both Src and Erk for HSC migration, these mediators are not required for HMGB1-induced migration of LEC. Further studies will be required to elucidate how HMGB1 induces LEC migration; however, Akt may be a likely mediator based on the present studies and prior literature (48).

LPS is increasingly recognized as an instigator of diverse liver injury processes. In alcohol-induced injury, there is an increase in gut permeability that increases LPS delivery to the liver through the portal vein. LPS acts on a multitude of liver cells that express TLR4 to induce responses that include cell activation and migration (9, 17, 36). Furthermore, recent studies indicate that sterile inflammation, that is, injury in the absence of infection, can induce similar responses (28). These nonmicrobial endogenous danger signals are termed DAMPs and include HMGB1, heat-shock protein, uric acid, and double-stranded genomic DNA (20, 28, 34). Interestingly, HMGB1 has been implicated not only as a potential TLR4 agonist but also as a molecule that is produced and released in response to TLR4 activation (16). Thus HMGB1 could contribute to sterile inflammation through both of these mechanisms. These responses are important for wound healing reactions to tissue injury (20), and our work implicates HMGB1 in these processes in HSC.

Prior studies have shown that HMGB1 can induce cell migration (10, 33) and proinflammatory cytokine production (34, 43). Our study supports a potential role for this molecule in ethanol-induced liver injury. Ethanol induces translocation and secretion of HMGB1 from the hepatocytes. HMGB1 in turn induces migration of HSCs and LECs to the injured sites via Src/Erk and Akt pathways, respectively. The results support the concept that ethanol-induced HMGB1 release may play an important role in the pathogenesis of chronic inflammation and recruitment of cells contributing to the wound response in alcoholic liver disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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