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Cylindromatosis Gene CYLD Regulates Hepatocyte Growth Factor Expression in Hepatic Stellate Cells Through Interaction With Histone Deacetylase 7

Rajeswara R. Pannem,[1*] Christoph Dorn,[2*] Claus Hellerbrand,[2**] and Ramin Massoumi[1**]

Hepatic fibrosis is considered as a physiological wound-healing response to liver injury. The process involves several factors, such as hepatocyte growth factor (HGF), which restrains hepatic injury and facilitates reversibility of fibrotic reaction in response to an acute insult. Chronic liver injury and sustained inflammation cause progressive fibrosis and, ultimately, organ dysfunction. The mechanisms tipping the balance from restoration to progressive liver tissue scarring are not well understood. In the present study, we identify a mechanism in which the tumor-suppressor gene, *cylindromatosis* (CYLD), confers protection from hepatocellular injury and fibrosis. Mice lacking CYLD ($CYLD^{-/-}$) were highly susceptible to hepatocellular damage, inflammation, and fibrosis and revealed significantly lower hepatic HGF levels, compared to wild-type (WT) animals. Exogenous application of HGF rescued the liver injury phenotype of $CYLD^{-/-}$ mice. In the absence of CYLD, gene transcription of HGF in hepatic stellate cells was repressed through binding of histone deacetylase 7 (HDAC7) to the promoter of HGF. In WT cells, CYLD removed HDAC7 from the HGF promoter and induced HGF expression. Of note, this interaction occurred independently of the deubiquitinating activity of CYLD. *Conclusions:* Our findings highlight a novel link between CYLD and HDAC7, offering mechanistic insight into the contribution of these proteins to progression of liver disease. Thus, through regulation of HGF level, CYLD ameliorates hepatocellular damage and liver fibrogenesis. (HEPATOLOGY 2014;60:1066-1081)

Hepatic fibrosis can be considered as a physiological wound-healing response to liver injury. The process also engages factors, including hepatocyte growth factor (HGF), restraining hepatic injury and facilitating reversibility of the fibrotic reaction in response to an acute insult. However, chronic liver injury (CLI) and sustained inflammation cause progressive fibrosis and, ultimately, organ dysfunction. Activation of hepatic stellate cells (HSCs) is a key event of hepatic fibrogenesis. Upon activation, HSCs transdifferentiate into myofibroblasts and produce the major fraction of extracellular matrix (ECM) proteins during hepatic fibrogenesis.[1-3] The most well-

Abbreviations: Ab, antibody; ALI, acute liver injury; Akt, protein kinase B; BDL, bile duct ligation; b.w., body weight; CaMK, Ca^{2+}/calmodulin-dependent kinase; CLD, chronic liver disease; CLI, chronic liver injury; CM, conditioned medium; CYLD, cylindromatosis; DEN, diethylnitrosamine; ECM, extracellular matrix; FACS, fluorescence-activated cell sorting; GalN, galactosamine hydrochloride; HAC, histone acetylation; HATs, histone acetyltransferases; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HDACi, HDAC inhibitors; HGF, hepatocyte growth factor; HSCs, hepatic stellate cells; ICAM-1, intracellular adhesion molecule 1; IF, immunofluorescent; IHC, immunohistochemistry; IL, interleukin; IP, intraperitoneally; JNK, Jun N-terminal kinase; KCs, Kupffer cells; KO, knockout; MCD, methionine and choline deficient; MCP-1, monocyte chemoattractant protein 1; mRNA, messenger RNA; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor kappa B; PBS, phosphate-buffered saline; siRNA, small interfering RNA; α-SMA, alpha-smooth muscle actin; TAK1, TGF-β1-activated kinase 1; TGF-β, transforming growth factor beta; TNF, tumor necrosis factor; TRAF2, TNF receptor-associated factor 2; TPX, trapoxin; TSA, trichostatin A; WT, wild type.

From the [1]Department of Laboratory Medicine, Medicon Village, Lund University, Lund, Sweden; and [2]Department of Internal Medicine I, University Hospital Regensburg, Regensburg, Germany.

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**These authors contributed equally to this work.*

***These authors share senior authorship.*

known profibrogenic mediators expressed by activated HSCs include transforming growth factor beta (TGF-β) and platelet-derived growth factor, whereas HGF functions as an antifibrogenic mediator.[2,3] Although the effects of HGF in reducing accumulation of ECM and development of hepatic fibrosis have been well studied, the exact mechanism regulating HGF expression in HSCs in response to liver injury and inflammation remains unknown.

CYLD is a deubiquitination enzyme acting as a tumor suppressor in different types of cancer.[4] Deubiquitin activity of CYLD interferes with components of distinct signaling pathways, thereby regulating different cellular processes, such as proliferation and survival.[5-7] CYLD is down-regulated in hepatocellular carcinoma (HCC), and forced expression of CYLD in HCC cell lines triggers a significant decrease in nuclear factor kappa B (NF-κB) activity.[8,9] A recent study demonstrated that deletion of CYLD exon 9 generates a deubiquitinase-deficient form of CYLD, which causes hepatic inflammation, fibrosis, and cancer.[10] The phenotype of these mice reflects elevated TGF-β1-activated kinase 1 (TAK1) ubiquitination and Jun N-terminal kinase (JNK) activation caused by lack of CYLD-mediated cleavage of polyubiquitin chains from TAK1.[10] Another recent study showed that deletion of CYLD exon 7/8, a short splice variant of CYLD, leads to biliary fibrosis and increased susceptibility for chemically induced HCC.[11] Despite these important findings, the current knowledge about the CYLD-mediated signaling pathway that ameliorates hepatocellular damage and liver fibrogenesis is limited.

Histone acetylation (HAC) is associated with active gene transcription and histone deacetylation with transcriptional repression. Acetylation status of histones is controlled through opposing actions of two classes of enzymes: histone acetyltransferases (HATs), which transfer acetyl groups to lysine residues within the N-terminal tails of core histones, and histone deacetylases (HDACs), which remove the acetyl groups.[12,13] Generally, HAC loosens the structure of chromatin, facilitates access of the transcriptional machinery to DNA, and initiates gene transcription.[12] In contrast, HDACs remove acetyl groups from histone proteins and reverse the effects of HATs through alterations in transcription.[13] To date, 18 human HDACs have been identified and grouped into four classes. HDAC7 is a member of a class of HDACs that shuttle between the cytoplasm and nuclei. HDAC7 suppresses expression of the angiogenesis suppressor gene, A kinase anchor protein 12,[14] Nuclear factor of activated T cells, cytoplasmic 1,[15] c-jun,[16] and the cell-cycle inhibitor gene, Reprimo.[17] More recently, it was shown that forced accumulation of HDAC7 in the nucleus of cytotoxic T cells suppresses expression of genes encoding cytokines, cytokine receptors, and adhesion molecules.[18] The function of HDACs, including HDAC7, is blocked using HDAC inhibitors (HDACi). These compounds interact with the catalytic domain of HDACs and alter gene expression. HDACi reduce expression of inflammatory and fibrotic genes, thereby exhibiting strong antifibrotic characteristics in the liver. In these studies, treatment with HDACi inhibited HSC activation and reduced liver fibrosis.[19-21] However, the HDAC target of HDACi in reducing HSC activation has not been identified.

In the present study, we observed that, upon hepatic injury, cylindromatosis (CYLD) regulates expression of hepatocyte growth factor (HGF) through direct interactions that prevent HDAC7 recruitment to the promoter of HGF. Elevated levels of HGF in the presence of CYLD reduced hepatocellular death and hepatic fibrosis.

Materials and Methods

Cells and Cell Culture. Primary murine hepatocytes and primary murine and human HSCs were isolated and cultured as previously described.[22-24] *In vitro* HSC activation was achieved through cell culture on uncoated tissue-culture dishes as previously described.[23,24]

Animals and Experimental Murine Models of Hepatic Injury. Generation of CYLD KO (knockout; $CYLD^{-/-}$) mice has previously been described.[25] Mice were maintained in specific pathogen-free housing at the Clinical Research Center (Malmö, Sweden) and at the University Hospital Regensburg (Regensburg, Germany), and animal experiments were performed according to national and international guidelines of the European Union (EU). At the age of 10-12 weeks, male C57BL/6 WT (wild-type; $CYLD^{+/+}$) and $CYLD^{-/-}$ mice underwent different models of acute liver injury (ALI) and CLI (5-6 animals per group). For short-term studies of hepatocellular injury and inflammation, diethylnitrosamine

Address reprint requests to: Claus Hellerbrand, M.D., Department of Internal Medicine I, University Hospital Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany. E-mail: claus.hellerbrand@ukr.de; fax: +49 941 9447154.

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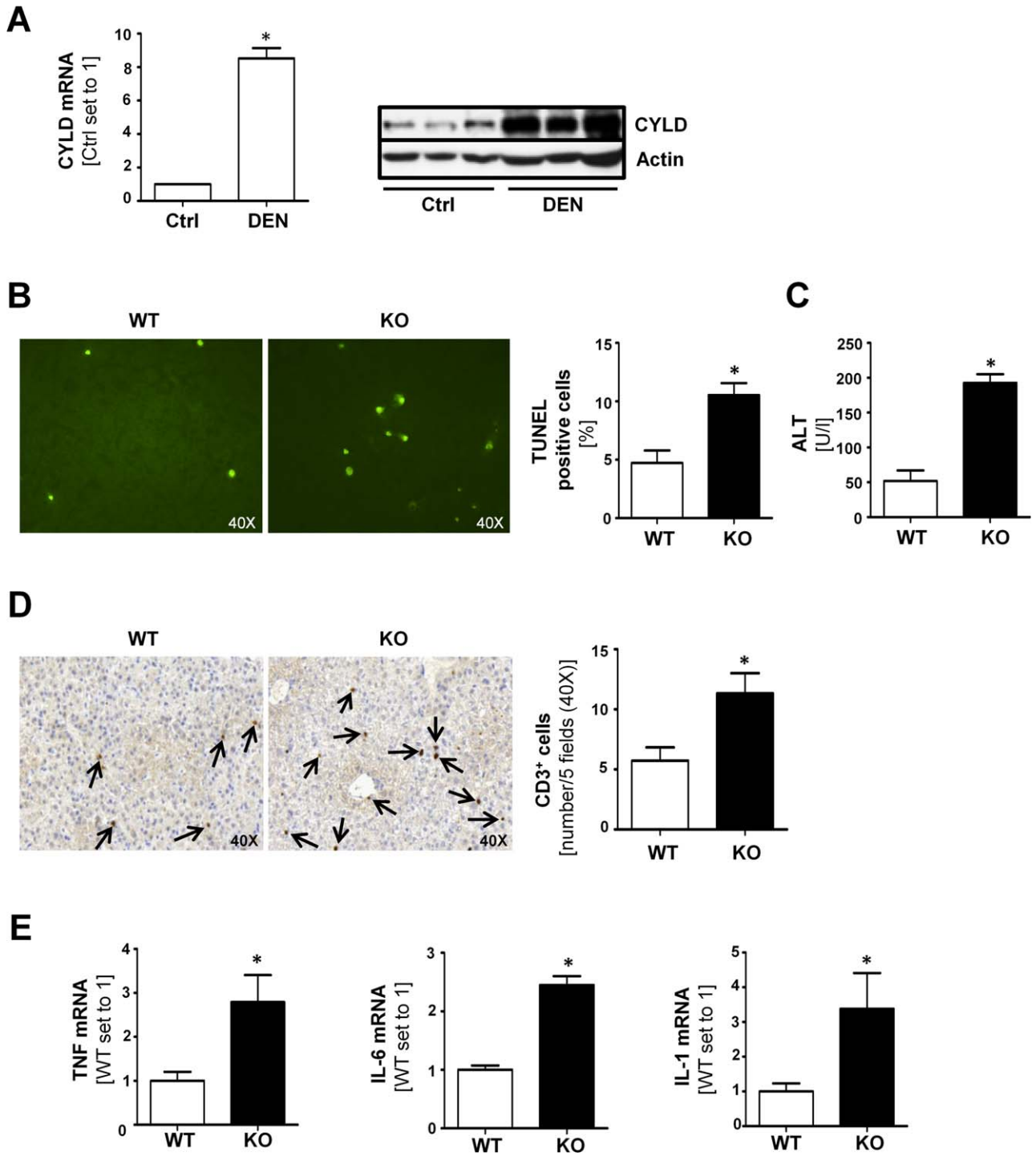

Fig. 1. *CYLD*$^{-/-}$ mice are more susceptible to ALI than WT mice. (A) Analysis of hepatic CYLD mRNA and protein expression levels in mice after DEN treatment for 24 hours (n = 5/group), compared to control mice. (B) Evaluation of apoptotic cells in liver using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay in 15-day-old DEN-treated WT (n = 5) and CYLD-deficient (KO; n = 5) animals after 24 hours. (C) Quantification of serum levels of alanine aminotransferase (ALT) in DEN-treated WT and KO mice. (D) The number of CD3-positive inflammatory cells in liver of WT and KO mice after DEN treatment for 24 hours. (F) Hepatic mRNA expression of TNF, IL-6, and IL-1 in WT and KO mice after DEN treatment for 6 hours. *$P < 0.05$.

(DEN; Sigma-Aldrich, St. Louis, MO) was intraperitoneally (IP) injected (100 μg/g body weight [b.w.]), and animals were sacrificed at the indicated time points. CCl_4-induced ALI was analyzed 24 hours after IP injection of 1 μL of CCl_4/g b.w. (diluted with corn oil). Galactosamine hydrochloride (GalN) plus tumor necrosis factor (TNF)-induced ALI were analyzed 6 hours after IP injection of 1 mg of GalN/g b.w. and

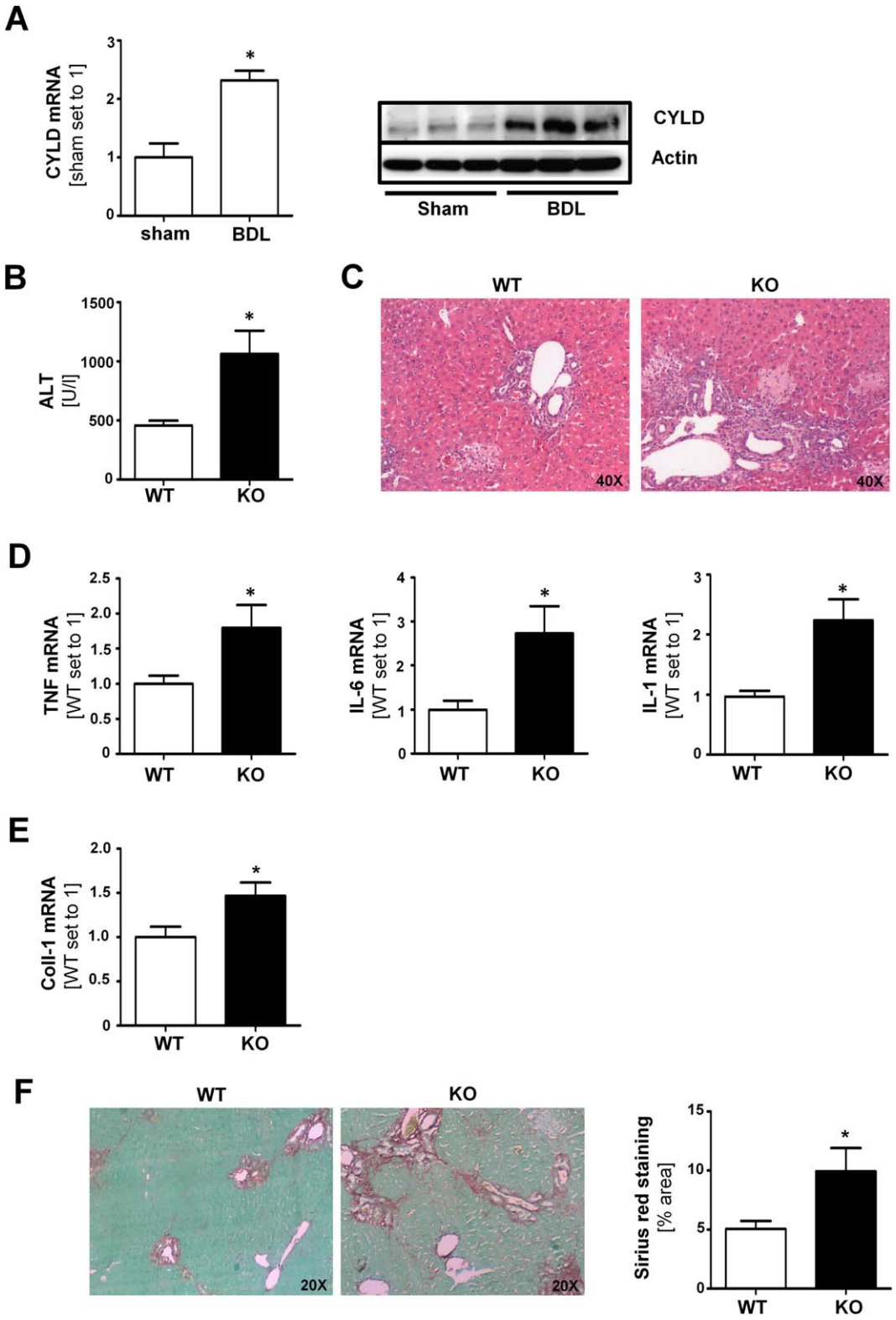

Fig. 2. *CYLD*$^{-/-}$ mice are more susceptible to CLI than WT mice. (A) Analysis of hepatic CYLD mRNA and protein expression in BDL and sham-operated (sham) WT mice (n = 6/group). (B) Alanine aminotransferase (ALT) serum levels in BDL WT and KO mice. (C) Hematoxylin and eosin staining of liver tissue sections from BDL WT and KO mice, showing marked bile duct proliferation and necrotic areas. (D) Analysis of proinflammatory (TNF, IL-6, and IL-1) and (E) profibrogenic (collagen type I; Coll-1) hepatic gene expression in BDL WT and KO mice. (F) Sirius Red/fast green staining of hepatic tissue from BDL WT and KO mice and quantification of stained areas. *$P < 0.05$.

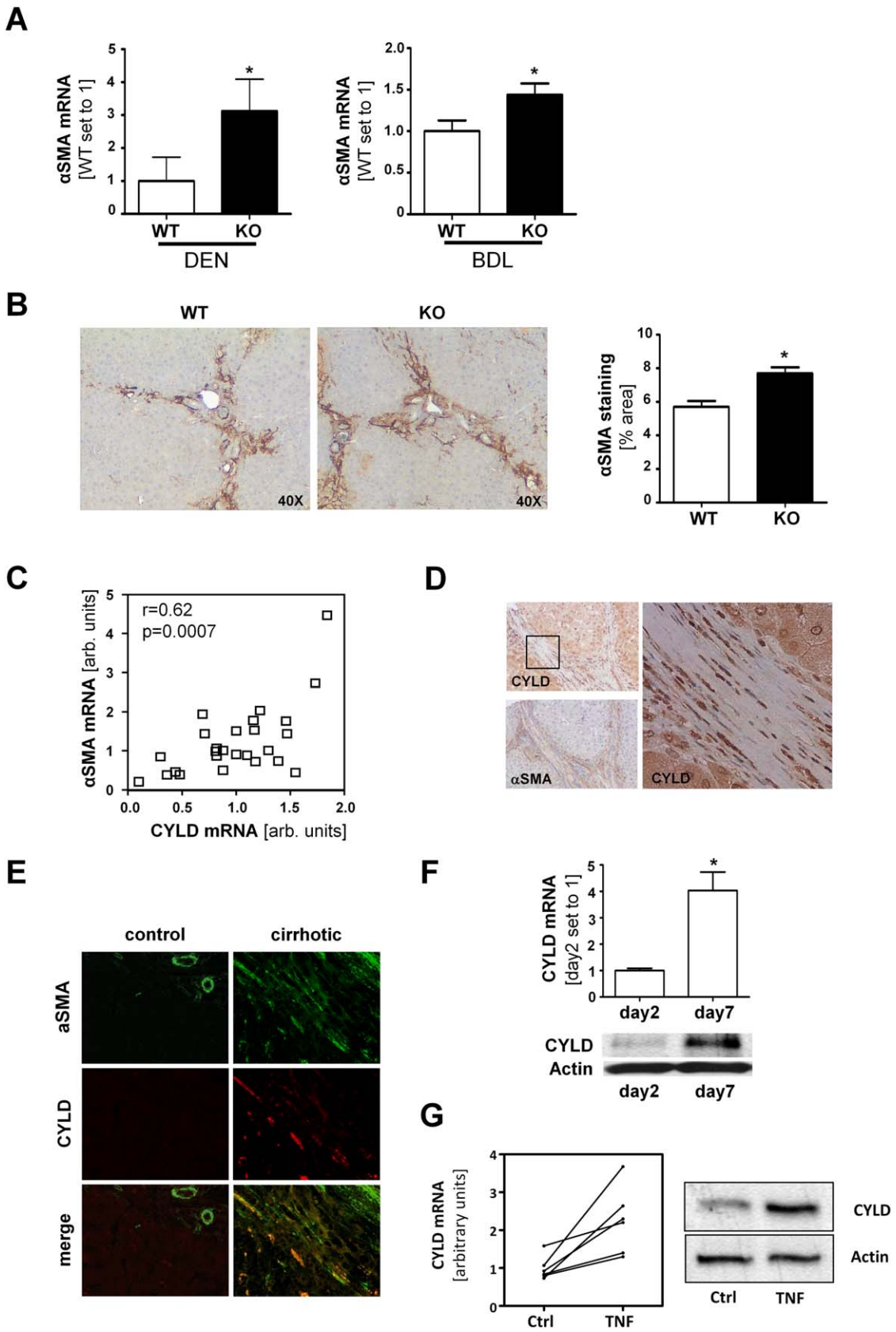
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Fig. 3.

0.02 μg of TNF/g b.w. (diluted with phosphate-buffered saline [PBS]). In addition, bile duct ligation (BDL) or sham operations were performed as previously described,[26] and animals were sacrificed at 3 weeks after the operation. A nonalcoholic steatohepatitis (NASH)-inducing methionine- and choline-deficient diet (MCD diet) or control diet was fed for 6 weeks. The MCD diet was purchased from MP Biomedicals (Solon, OH); all other chows were purchased from Ssniff (Soest, Germany). To assess the effect of recombinant HGF on DEN-mediated liver injury in $CYLD^{-/-}$ mice, a single IP injection of recombinant murine HGF (1.68 μg/kg; R&D Systems, Abingdon, UK) or vehicle (PBS) was administered to $CYLD^{-/-}$ mice. Twenty-four hours later, an IP injection with DEN (100 mg/kg b.w.) was administered together with an intravenous HGF or vehicle (PBS) application. All mice were sacrificed through heart puncture under deep ketamine/xylazine (2:1) anesthesia, and liver tissue and blood samples were collected for further analyses.

Human Tissue Specimens. Formalin-fixed, paraffin-embedded human liver tissues for immunohistochemical (IHC) analysis were retrieved from the surgical pathology files of the Institute of Pathology of the University of Regensburg. Human liver tissue for cell isolation and expression analyses was obtained according to the guidelines of the charitable state-controlled foundation, Human Tissue and Cell Research (Regensburg, Germany). Informed consent was obtained from all patients, and the study was approved through the local ethics committee of the University of Regensburg.

Statistical Analyses. Statistical analyses were performed using GraphPad Prism5 software (GraphPad Software Inc., San Diego, CA). Results are expressed as the means ± standard error (range) or percent. Two-tailed P values were calculated using Mann-Whitney's U test. A correlation analysis was performed using Pearson's product-moment correlation coefficient. A P value <0.05 was considered statistically significant.

Additional Methods. Detailed methodology is described in the Methods section of the Supporting Information.

Results

CYLD Deficiency Aggravates Hepatocellular Damage, Inflammation, and Fibrosis in Response to ALI and CLI. CYLD is ubiquitously expressed in human and murine livers (Supporting Fig. 1A), but mice lacking the CYLD gene[25] exhibited regular liver architecture and hepatocellular integrity under physiological conditions (Supporting Fig. 1B-E). To understand the role of CYLD under pathophysiological conditions, we exposed WT ($CYLD^{+/+}$) and CYLD-deficient ($CYLD^{-/-}$) mice to models of ALI and CLI. The acute toxicity of DEN or CCl_4 in liver caused a marked up-regulation of hepatic CYLD expression in WT mice (Fig. 1A and Supporting Fig. 2). $CYLD^{-/-}$ mice displayed a significantly increased number of apoptotic cells (Fig. 1B), higher levels of serum transaminases (Fig. 1C), and an increased number of recruited inflammatory cells (Fig. 1D and Supporting Fig. 3A), compared to $CYLD^{+/+}$ mice, in response to acute hepatic injury through DEN. These results were consistent with the enhanced levels of the proinflammatory genes (Fig. 1E and Supporting Fig. 3B,C) and with multifocal areas of necrosis (Supporting Fig. 4) in livers of DEN-treated $CYLD^{-/-}$ mice. In contrast, $CYLD^{-/-}$ control mice untreated or injected with vehicle (PBS or mineral oil) did not show any significant differences in infiltration of inflammatory or apoptotic cells, compared to WT liver tissue. In addition, levels of serum transaminases and hepatic proinflammatory gene expression were similar between $CYLD^{-/-}$ and $CYLD^{+/+}$ mice (Supporting Fig. 5).

To simulate chronic hepatic injury, we used 3-week BDL, an established model of extrahepatic cholestasis in rodents.[26] Similar to the DEN and CCl_4 models, we observed a marked up-regulation of hepatic CYLD expression in control mice (Fig. 2A). In the BDL model, $CYLD^{-/-}$ mice showed more hepatocellular

Fig. 3. CYLD expression is increased in activated HSCs, and CYLD deficiency leads to enhanced HSC activation in response to hepatic injury. (A) Analysis of hepatic α-SMA mRNA expression in DEN-treated (left panel; n = 5/group) and BDL (right panel; n = 6/group) WT and CYLD-deficient (KO) mice. (B) α-SMA IHC staining of hepatic tissue from BDL WT and KO mice and quantification of stained areas (*$P < 0.05$, compared with WT). (C) Correlation analysis of CYLD and α-SMA mRNA expression levels in liver tissue from 27 patients with CLD using quantitative polymerase chain reaction. (D) IHC staining of CYLD and α-SMA revealing strong CYLD immunosignals in α-SMA-positive cells in fibrotic septa of cirrhotic human livers (magnification right panel: 100×). (E) IF staining of CYLD and α-SMA in hepatic tissue from patients with CLD, revealing a strong CYLD immunosignal in α-SMA-positive cells in fibrotic septa of cirrhotic human liver. (F) Comparison of CYLD mRNA and protein expression in quiescent (day 2) and activated (day 7) human HSCs using quantitative polymerase chain reaction and western blotting analyses (*$P < 0.05$, compared with day 2). (G) Expression of CYLD mRNA (from 6 different donors) and protein in untreated (control) and TNF (10 ng/mL for 24 hours)-stimulated primary human HSCs.

damage (Fig. 2B,C), increased levels of proinflammatory genes, and inflammatory cell markers (Fig. 2D and Supporting Fig. 6), compared to $CYLD^{+/+}$ mice. Furthermore, we applied a second model of CLI, in which we fed mice an MCD diet, causing NASH. In this model, CYLD deficiency did not affect triglyceride

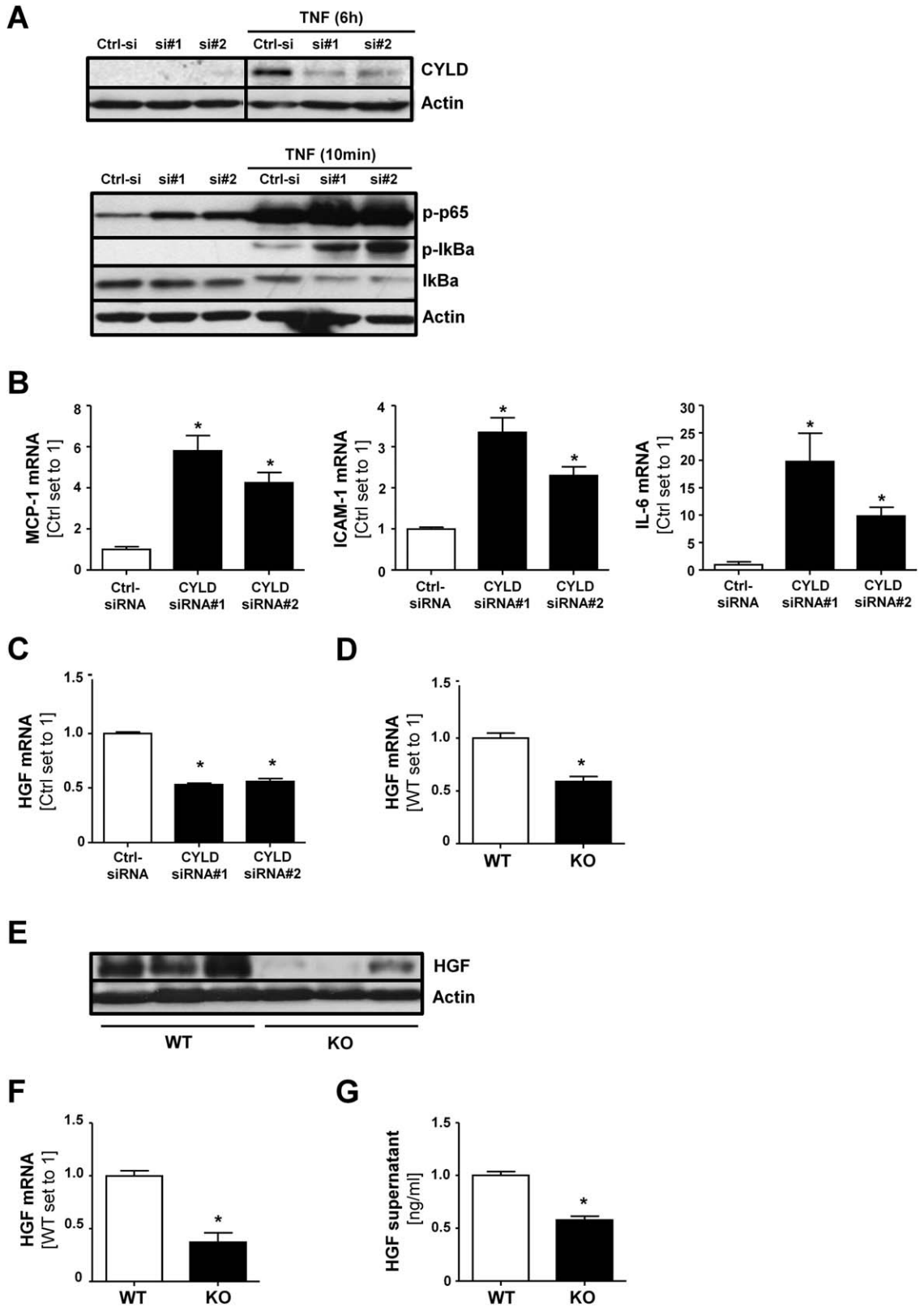


Fig. 4.

levels in liver and serum (Supporting Fig. 7), but exacerbated hepatocellular damage and inflammation (Supporting Fig. 8). Moreover, CLI induced higher hepatic expression levels of profibrogenic genes (Fig. 2E and Supporting Fig. 8), and Sirius Red staining revealed enhanced hepatic collagen deposition in $CYLD^{-/-}$, compared to $CYLD^{+/+}$, mice (Fig. 2F). In both models, $CYLD^{-/-}$ and $CYLD^{+/+}$ control mice (fed with control diet or sham operated) showed similar levels of proinflammatory and -fibrogenic gene expression, and neither $CYLD^{-/-}$ nor $CYLD^{+/+}$ mice revealed significant infiltration with inflammatory or apoptotic cells in liver (Supporting Figs. 8 and 9).

Next, we compared JNK and TAK activation in $CYLD^{-/-}$ and $CYLD^{+/+}$ mice using CCl_4 and GalN/TNF treatment. We did not observe any significant differences in JNK and TAK activation comparing nontreated hepatocytes isolated from $CYLD^{-/-}$ and $CYLD^{+/+}$ mice (Supporting Fig. 10A). In GalN/TNF-treated liver and hepatocytes, we detected elevated JNK activation in $CYLD^{-/-}$, compared to $CYLD^{+/+}$, mice (Supporting Fig. 10B,C). Furthermore, CCl_4 treatment promoted activation of TAK1 and ubiquitination of TNF receptor-associated factor 2 (TRAF2) in $CYLD^{-/-}$, compared to $CYLD^{+/+}$, livers and hepatocytes (Supporting Fig. 10B-E). Both GalN/TNF and CCl_4 treatment increased cell death in $CYLD^{-/-}$, compared to $CYLD^{+/+}$, hepatocytes (Supporting Fig. 10B).

CYLD Expression Is Increased in Activated HSCs. Hepatic injury results in activation of the HSCs, which is characterized by *de novo* expression of alpha-smooth muscle actin (α-SMA).[2,3] In ALI and CLI models, enhanced hepatic α-SMA expression in $CYLD^{-/-}$ mice, compared to $CYLD^{+/+}$ mice, was reflecting more advanced HSC activation (Fig. 3A) and IHC confirmed an increased number of α-SMA-positive cells in chronically injured $CYLD^{-/-}$, compared to $CYLD^{+/+}$, liver tissue (Fig. 3B). In contrast, neither $CYLD^{-/-}$ nor $CYLD^{+/+}$ mice showed significant HSC activation under nontreated conditions (Supporting Fig. 11). Notably, a striking correlation between CYLD and α-SMA expression was observed in hepatic tissue from patients with chronic liver disease (CLD; Fig. 3C). IHC analysis revealed similar CYLD-staining intensity of hepatocytes in control and cirrhotic human liver tissues (Supporting Fig. 12), but a strong CYLD immunosignal in α-SMA-positive cells in fibrotic septa of cirrhotic human livers (Fig. 3D). Double immunofluorescence (IF) analysis confirmed colocalization of CYLD and α-SMA in cirrhotic liver tissue (Fig. 3E). Moreover, in an *in vitro* model of HSC activation, we observed that activated HSCs (day 7) expressed higher levels of CYLD than quiescent HSCs (day 2; Fig. 3F), and CYLD expression in activated HSCs was further induced in response to TNF stimulation (Fig. 3G). Together, these findings indicated that CYLD expression is inducible during HSC activation and in response to proinflammatory cytokine stimulation.

The Functional Role of CYLD in Activated HSCs. To obtain further insight into the role of CYLD in HSCs, we suppressed CYLD expression in activated human HSCs and observed that expression of CYLD interfered with activation of the classic NF-κB pathway (Fig. 4A) and expression of NF-κB target genes, such as monocyte chemoattractant protein 1 (MCP-1), intracellular adhesion molecule 1 (ICAM-1), and interleukin (IL)-6 (Fig. 4B), which are up-regulated during HSC activation.[2] In contrast, and unexpectedly, CYLD suppression in activated HSCs reduced expression of HGF (Fig. 4C), but not expression of other growth factors in human HSCs (Supporting Fig. 13A). To validate these findings, we isolated HSCs from $CYLD^{+/+}$ and $CYLD^{-/-}$ mice. Activation of murine $CYLD^{-/-}$ HSCs revealed enhanced expression of NF-κB-regulated pro-inflammatory genes, compared to WT cells (Supporting Fig. 13B) and electrophoresis mobility shift assay analysis confirmed enhanced NF-κB binding activity in TNF-stimulated $CYLD^{-/-}$ HSCs, compared to $CYLD^{+/+}$ HSCs (Supporting Fig. 14A). In contrast, we found no differences in activation of TAK1 and JNK comparing $CYLD^{-/-}$ and $CYLD^{+/+}$ HSCs (Supporting Fig. 14B). Liver tissues or quiescent HSCs isolated from $CYLD^{+/+}$ and $CYLD^{-/-}$ mice revealed no significant differences in HGF expression under noninduced conditions (Supporting Fig. 15A,B). In contrast, hepatic HGF expression was significantly lower in $CYLD^{-/-}$ than in

Fig. 4. Reduced HGF expression in CYLD-deficient HSCs and $CYLD^{-/-}$ mice in models of hepatic injury. (A) Western blotting analysis of CYLD protein expression in human HSCs treated by TNF (10 ng/mL for 6 hours) and its suppression by transfection with CYLD-siRNA (si#1 and si#2; 4 days posttransfection). Control cells were transfected with negative control siRNA. Western blotting analyses disclosed the effects of siRNA-mediated CYLD suppression on p65 and IκBa phosphorylation (basal or upon TNF stimulation; 10 ng/mL for 10 minutes). (B) Quantification of mRNA expression of NF-κB target genes MCP-1, ICAM-1, and IL-6 and (C) HGF in human HSCs after transfection with CYLD-siRNA (4 days posttransfection); negative control siRNA served as the control (*$P < 0.05$, compared to control-siRNA). (D) Expression levels of HGF at the mRNA and (E) protein level in livers from WT and KO mice after treatment with DEN for 6 hours (n = 6/group). (F) Quantification of HGF mRNA in activated HSCs (left panel) and (G) HGF protein in the supernatant of activated HSCs (right panel) isolated from WT and KO mice. *$P < 0.05$, compared to WT.

CYLD$^{+/+}$ mice in models of DEN- or CCl_4-induced liver injury (Fig. 4D,E and Supporting Fig. 15C), similar to the HGF expression in activated HSCs isolated from *CYLD*$^{-/-}$ and *CYLD*$^{+/+}$ mice (Fig. 4F,G). In contrast, expression of other growth factors was either similar or enhanced (Supporting Fig. 13C,D). These data indicate CYLD as a critical regulator of HGF in activated HSCs.

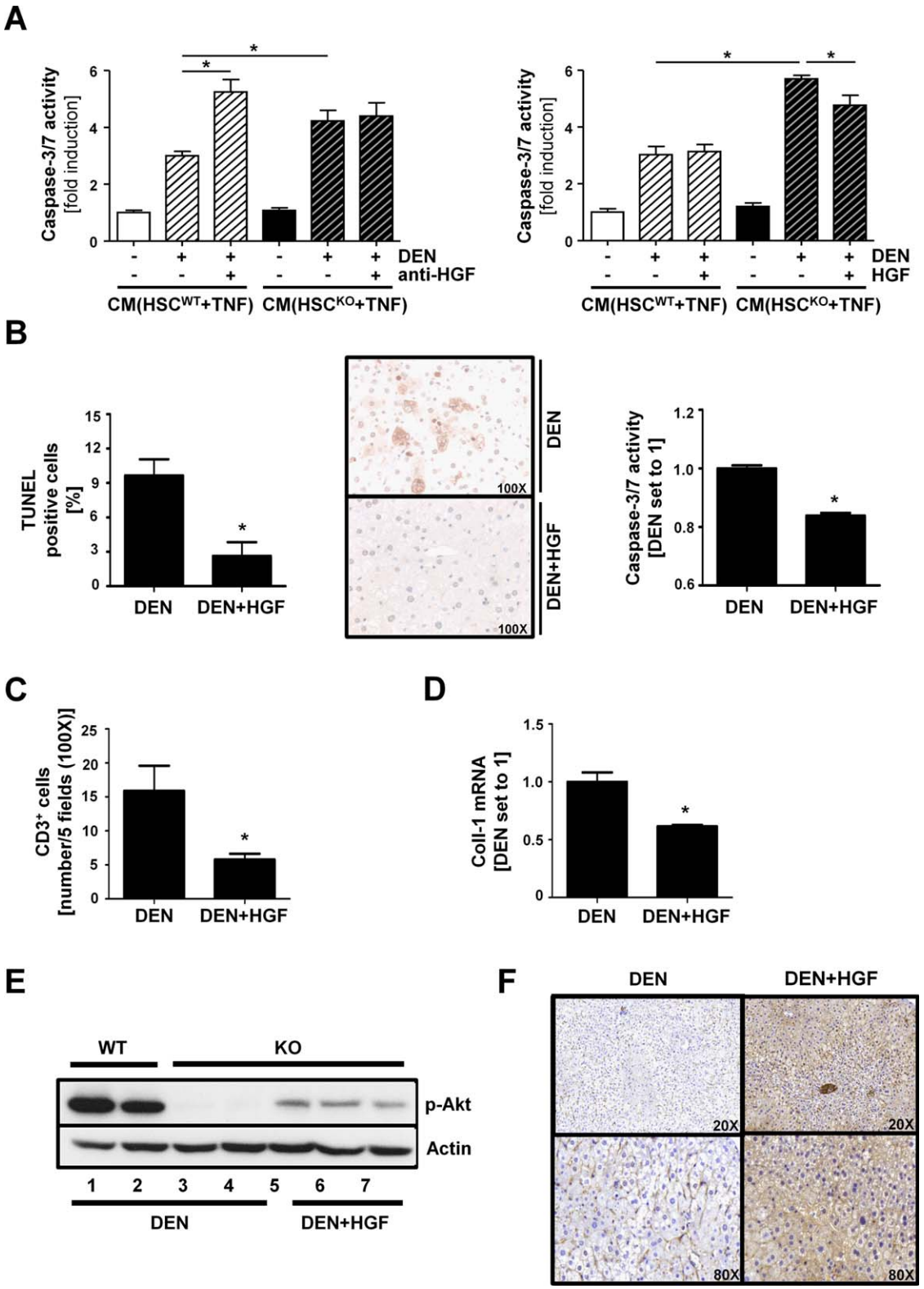
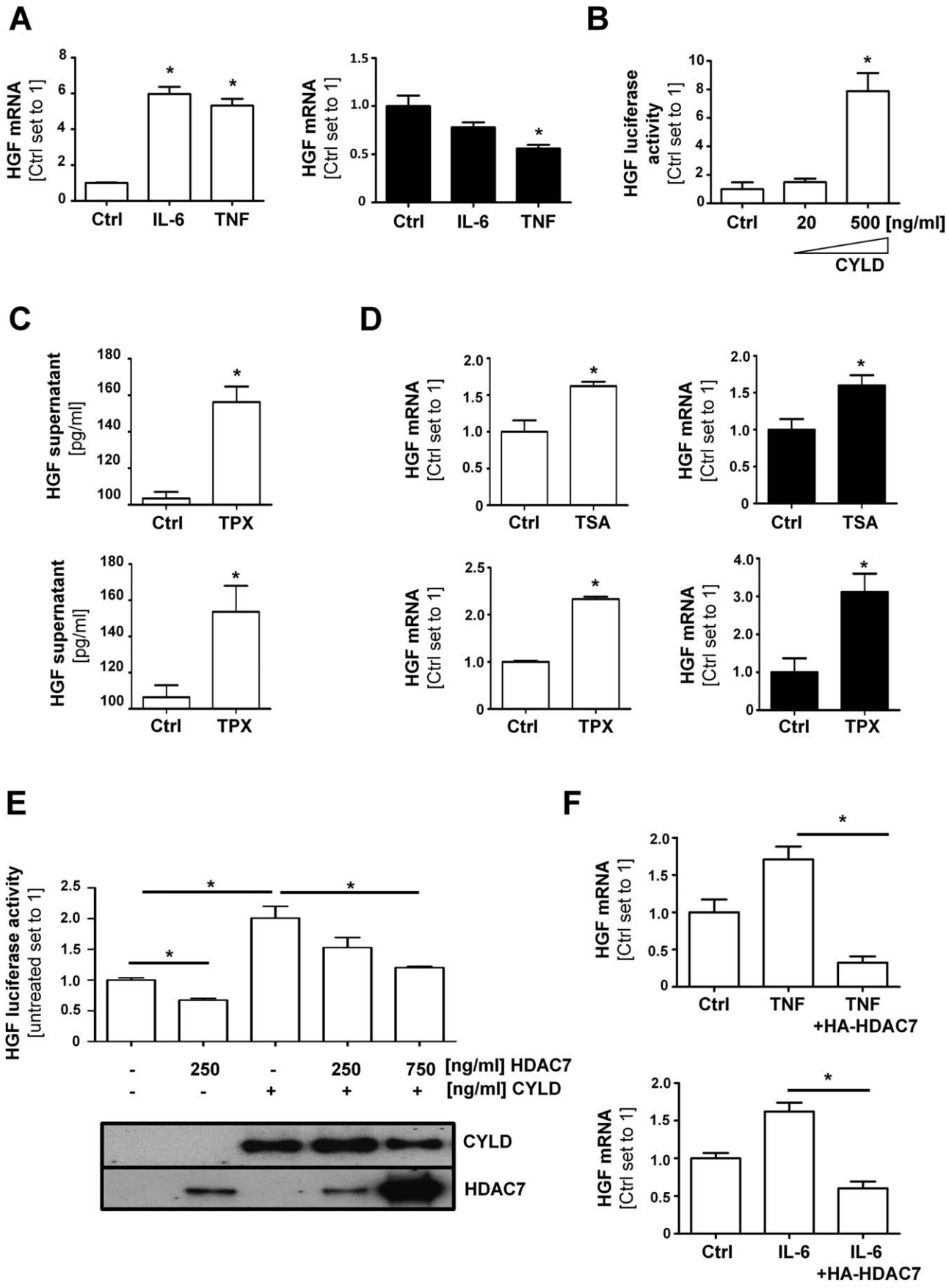


Fig. 5.

Effect of CYLD-Mediated HGF Regulation in Activated HSCs. Activated HSCs are the major source of hepatic HGF, which plays a critical role in tissue protection and regeneration and provides less susceptibility to chronic inflammation and fibrosis.[27-30] To characterize the effect of reduced HGF expression on hepatocellular damage in CYLD-deficient HSCs, we incubated primary hepatocytes, isolated from $CYLD^{+/+}$ mice, with conditioned medium (CM) from activated HSCs derived from $CYLD^{+/+}$ or $CYLD^{-/-}$ mice, before treatment with DEN. This procedure promoted caspase-3/7 activity, which was significantly higher in hepatocytes stimulated with CM from $CYLD^{-/-}$, compared with those treated with CM from $CYLD^{+/+}$ HSCs (Fig. 5A). Inhibition of HGF downstream signaling, after addition of neutralizing anti-HGF antibody (Ab), enhanced DEN-induced caspase-3/7 activity in hepatocytes treated with CM from $CYLD^{+/+}$ HSCs, but not in hepatocytes incubated with CM from $CYLD^{-/-}$ HSCs (Fig. 5A, left panel). Conversely, supplementation with recombinant HGF did not affect caspase-3/7 activity in hepatocytes incubated with CM from $CYLD^{+/+}$ HSCs, but alleviated DEN-induced caspase-3/7 activity in hepatocytes treated with CM from $CYLD^{-/-}$ HSCs (Fig. 5A, right panel). Next, we assessed whether exogenous HGF ameliorates hepatocellular injury in CYLD-deficient mice. Injection of recombinant HGF into $CYLD^{-/-}$ mice before acute DEN-induced hepatic injury significantly reduced hepatic cell death (Fig. 5B), proinflammatory gene expression (Supplorting Fig. F16A), inflammatory cell recruitment (Fig. 5C), and profibrogenic gene expression (Fig. 5D; Supporting Fig. 16B). The potent activity of HGF in preventing cell death in distinct types of cells, including hepatocytes, is typically mediated through activation of protein kinase B (Akt).[31-33] DEN treatment markedly increased activation of Akt in $CYLD^{+/+}$, but not in $CYLD^{-/-}$, hepatic tissue (Fig. 5E). Furthermore, in CYLD-deficient mice, injection of HGF before DEN treatment elevated activation of Akt (Fig. 5E,F). In $CYLD^{+/+}$ mice, HGF application did not significantly affect apoptosis, proinflammatory and -fibrogenic gene expression, or Akt activation (Supporting Fig. 17). Taken together, these results suggest that lower HGF levels in CYLD-deficient mice result in pronounced hepatic injury, inflammation, and profibrogenic gene expression, associated with the development and progression of hepatic injury.

CYLD Regulates HGF Levels in HSCs Through Interaction With HDAC7. Next, we identified the mechanisms through which CYLD induces HGF expression in HSCs. The cytokines, IL-6 and TNF, primarily secreted from activated Kupffer cells (KCs) in response to hepatic injury, stimulate HGF production in HSCs.[34,35] Both cytokines promoted HGF expression and secretion in HSCs isolated from $CYLD^{+/+}$ mice, but not in CYLD-deficient HSCs (Fig. 6A). Furthermore, luciferase assays demonstrated substantially increased HGF promoter activity after CYLD transfection (Fig. 6B), indicating that CYLD regulates expression of HGF at the transcriptional level. HDACs balance the acetylation activities of HACs during chromatin remodeling. HDACs are essential in regulating gene transcription, and inhibition of these enzymes suppresses HGF expression in tumor stroma.[36] To determine whether the repression of HGF in the absence of CYLD is dependent on histone acetylation, HSCs were treated with the potent HDACi, trichostatin A (TSA) or trapoxin (TPX). This treatment generated an increase of HGF levels in both WT and CYLD-deficient HSCs (Fig. 6C,D). HDAC7 is a member of the HDAC class IIa enzymes, possessing nucleocytoplasmic shuttling properties.[37] It was recently shown that TNF derepresses expression of HDAC7 target genes.[38] To determine whether CYLD regulates HDAC7 in HSCs, thereby affecting HGF production, we overexpressed CYLD and observed that CYLD overrode HDAC7-mediated inhibition of HGF promoter activity (Fig. 6E) and overexpression of HDAC7 inhibited TNF- or IL6-induced HGF expression (Fig. 6F).

Fig. 5. HGF ameliorates DEN-induced liver injury and fibrosis. (A) Caspase-3/7 activity in primary hepatocytes from WT mice, quantified using the Apo-One Homogeneous Caspase-3/7 assay (Promega, Madison, WI). One day after isolation, hepatocytes were preincubated for 16 hours with CM derived from TNF-stimulated (10 ng/mL for 48 hours) activated HSCs (30,000 cells/cm^2 in 150 μL/cm^2 serum-free Dulbecco's modified Eagle's medium, high glucose, for 48 hours), isolated from WT or CYLD-deficient (KO) mice, as described in the Materials and Methods section. In addition, cells were incubated with (left panel) a neutralizing anti-mHGF Ab (AF2207; 1 μg/mL; anti-goat immunoglobulin G served as a control; R&D Systems, Minneapolis, MN) or (right panel) with recombinant murine HGF (from PeproTech, Hamburg, Germany), as previously described. Subsequently, apoptosis was induced through stimulation with DEN (10 mM for 16 hours). (B) The number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells, and caspase-3/7 activity in livers from WT and KO mice treated with DEN (n = 3), or HGF and DEN (n = 3). (C) The level of inflammatory cells (CD3-positive cells) in livers of KO mice treated with DEN, or HGF and DEN. (D) Hepatic mRNA expression of collagen type-I (Coll-1) in KO mice treated with DEN, or HGF and DEN (n = 3-6). (E) Expression of activated Akt in livers from DEN-treated (24 hours) WT and KO mice or HGF- and DEN-treated (48 hours) KO mice. (F) IHC analysis of phospho-Akt in livers of DEN, or HGF- and DEN-treated KO mice. *$P < 0.05$.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IL-6 +HA-HDAC7

Fig. 6.

Furthermore, IL-6 and TNF stimulation reduced nuclear translocation of HDAC7 (Fig. 7A) and its recruitment to the promoter of HGF (Fig. 7B) in WT, but not in CYLD-deficient, HSCs (Fig. 7A,B). DNA immunoprecipitation assay confirmed direct binding of HDAC7 to the HGF promoter (Supporting Fig. 18). Most important, knocking down HDAC7 expression using small interfering RNA (siRNA) or short hairpin RNA resulted in elevated levels of HGF messenger RNA (mRNA) in both $CYLD^{+/+}$ and $CYLD^{-/-}$ HSCs (Fig. 7C). In addition, overexpression of the shuttling-deficient HDAC7 mutant (HDAC7-mNES) prevented induction of HGF in $CYLD^{+/+}$ HSCs (Fig. 7D). Thus, interaction of CYLD with HDAC7 in the cytoplasm in response to IL-6 or TNF stimulation might reflect reduced nuclear translocation and recruitment to the HGF promoter. Endogenous immunoprecipitation and colocalization studies showed that, in nonstimulated $CYLD^{+/+}$ HSCs, HDAC7 was primarily localized in nuclei, whereas stimulation with TNF promoted translocation of HDAC7 from the nucleus to the cytoplasm (Fig. 7E, upper panels). In contrast, HDAC7 was constitutively localized in the nucleus of $CYLD^{-/-}$ HSCs in the absence or presence of TNF (Fig. 7E, lower panels). Furthermore, intense cytoplasmic colocalization (Fig. 7E) and an association (Fig. 7F) between HDAC7 and CYLD were detected in stimulated cells. However, total levels of HDAC7 did not differ between nonstimulated and stimulated cells (Fig. 7F).

Overexpression of a catalytic mutant version of CYLD (CYLD-C/S) blocked HDAC7 nuclear localization, suggesting that deubiquitinating activity of CYLD is not responsible for blocking the nuclear localization of HDAC7 after IL-6 or TNF stimulation (Fig. 8A). Furthermore, FLAG pull-down assays, using a series of CYLD deletion mutants, showed that a construct containing the third CAP-Gly domain and the proline-rich region of CYLD (CYLD 222-956) interacted with purified HDAC7 (Fig.8 B,C). These results suggest that IL-6 and TNF promote the binding of HDAC7 to CYLD while blocking nuclear translocation and recruitment of HDAC7 to the HGF promoter. However, in the absence of CYLD, HDAC7 mediates the down-regulation of HGF expression, which promotes hepatocellular damage, inflammation, and fibrosis in response to hepatic injury.

Discussion

Deubiquitination is a highly regulated process implicated in different signaling pathways, and mutations in several deubiquitination enzymes have been linked to acute and chronic inflammatory diseases. CYLD is a deubiquitination enzyme acting as a tumor suppressor in different types of human cancer, including HCC.[8,39] CYLD has previously been shown to affect mechanisms associated with acute and chronic hepatic injury.[10,11] In these studies, mice with a hepatocyte-specific expression of a deubiquitinase-deficient form of CYLD or mice with deletion of CYLD's TRAF2-binding site (exon 7/8 deleted) suffered from hepatic inflammation, fibrosis, and cancer through uncontrolled TAK1 and JNK activation.[10,11] In contrast to these findings, complete deletion of CYLD in the present study did not affect the number of apoptotic cells, liver damage, or inflammation under noninducing conditions. These phenotypic differences may be explained by CYLD deletion being complete in our study while only partial in previous studies.[10,11] Here, we have uncovered a novel mechanism by which CYLD expression in HSCs protects from hepatic injury independently of its deubiquitin activity.

An underlying cause of the hepatoprotective and antifibrogenic effects of CYLD is the regulatory function of this protein on HGF, a pleiotropic signaling molecule expressed and secreted through activated HSCs. Especially in injured liver tissue, activation of HGF is promoted by factors secreted from hepatocytes, which further protects hepatocytes from cellular damage and inhibits hepatic fibrosis.[40-42] Hepatic HGF expression was significantly lower in $CYLD^{-/-}$ than in $CYLD^{+/+}$ mice upon hepatic injury, and exogenous application of HGF rescued the liver injury

Fig. 6. CYLD regulates HGF levels in HSCs through interaction with HDAC7. (A) Expression of HGF mRNA in untreated (Ctrl), IL-6- (25 ng/mL for 12 hours), and TNF (10 ng/mL for 12 hours)-treated primary HSCs isolated from WT (white bars) and $CYLD^{-/-}$ (black bars) mice. (B) HGF luciferase promoter activity in human HSCs transfected full-length CYLD. HSCs were transfected with pGL-2-HGF-1029 luciferase promoter construct (200 ng/well) alone, or pGL-2-HGF-1029 (200 ng/well) and FLAG-CYLD (20 or 500 ng/well) for 24 hours. (C) Levels of HGF in supernatants from human HSCs, before and after treatment with TSA (5 ng/mL) or TPX (2 ng/ml) for 24 hours. (D) HGF mRNA levels in $CYLD^{+/+}$ HSCs (white bars) and $CYLD^{-/-}$ HSCs (black bars) before and after treatment with TSA or TPX for 24 hours. (E) HGF promoter activity in human HSCs, transfected with HDAC7 (250 or 750 ng/well) and full-length CYLD (500 ng/well) after 24 hours (upper panel). Lower panel shows levels of CYLD and HDAC7 protein in transfected cells. (F) HGF expression at the mRNA level in mock- or HA-HDAC7-transfected (9 μg expression plasmid) WT HSCs in the absence (Ctrl) or presence of TNF (10 ng/mL for 48 hours, upper panel) or IL-6 (25 ng/mL for 48 hours, lower panel) $^*P < 0.05$, compared to control.

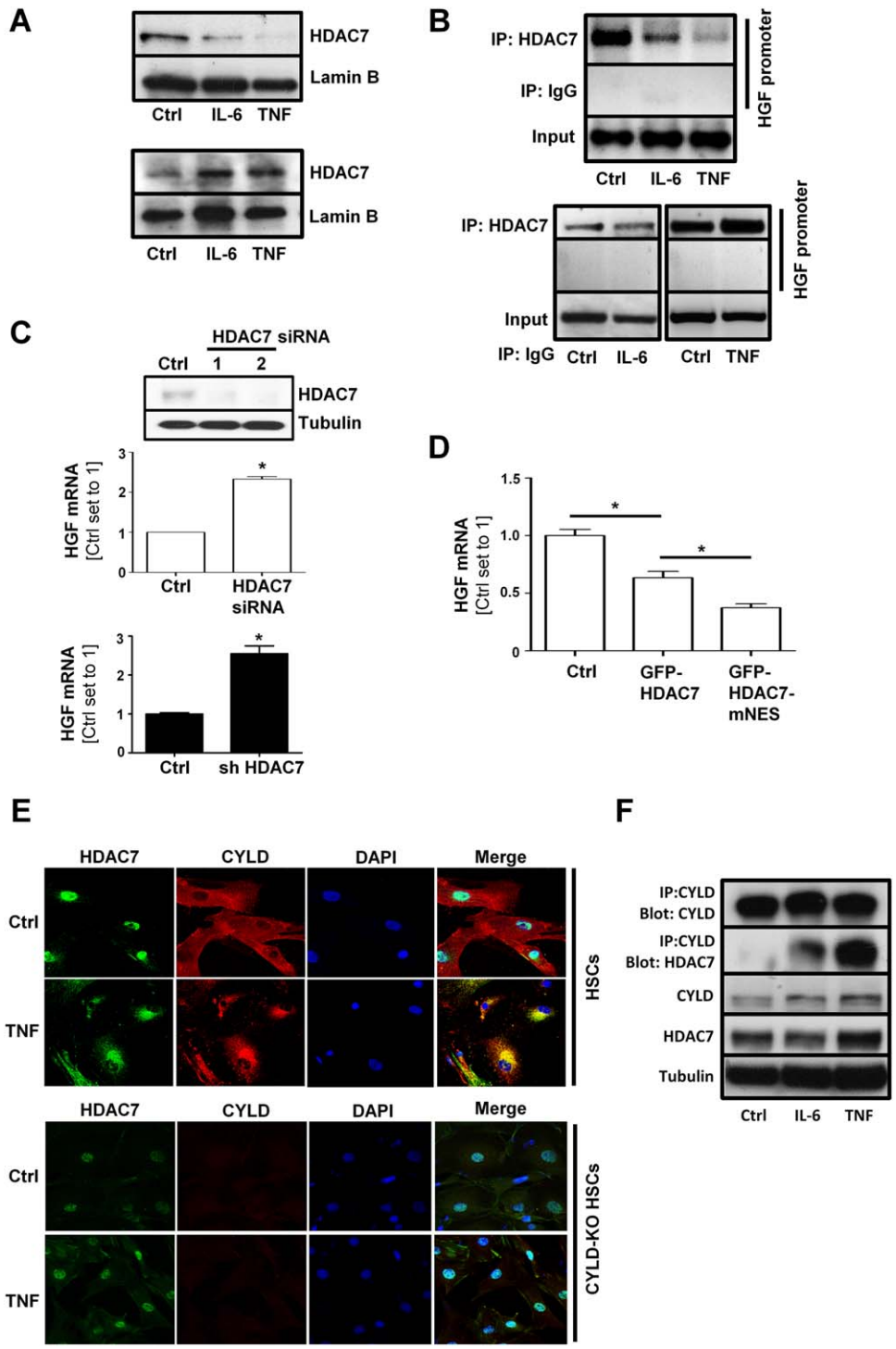

Fig. 7. Proinflammatory mediators promote interaction of CYLD with HDAC7. (A) The level of HDAC7 in the nucleus of untreated (control), IL-6- (25 ng/mL for 24 hours), and TNF (10 ng/mL for 24 hours)-treated WT HSCs (upper panel) and $CYLD^{-/-}$ HSCs (lower panel). (B) Chromatin immunoprecipitation (IP) of HGF promoter in control, IL-6- (25 ng/mL for 24 hours), and TNF (10 ng/mL for 24 hours)-stimulated WT HSCs (upper panel) and $CYLD^{-/-}$ HSCs (lower panel). (C) Upper panel: Levels of HDAC7 protein in $CYLD^{+/+}$ HSCs, treated with HDAC7-specific siRNA for 48 hours using two different siRNA-oligos, and in control HSCs (untreated). Lower panel: Expression of HGF mRNA in $CYLD^{-/-}$ HSCs transfected with control or shHDAC7 (100 ng/mL) plasmid for 24 hours ($n = 3$). (D) Expression of HGF mRNA in WT HSCs transfected with 9 μg of green fluorescent protein (GFP)-HDAC7 or mutant GFP-HDAC7-mNES for 48 hours ($n = 3$). (E) Localization of HDAC7 (green) and CYLD (red) in $CYLD^{+/+}$ HSCs (upper panel) and $CYLD^{-/-}$ HSCs (lower panel), in the absence or presence of 10 ng/mL of TNF for 24 hours, using confocal IF microscopy. (F) Endogenous immunoprecipitation (IP), using CYLD Ab in IL-6- (25 ng/mL) or TNF (10 ng/mL)-treated (24 hours), and control (untreated) $CYLD^{+/+}$ HSCs. $^*P < 0.05$. IgG, immunoglobulin G; DAPI, 4′,6-diamidino-2-phenylindole.

phenotype observed in CYLD-deficient mice. This treatment resulted in reduced hepatic cell death and elevated activation and phosphorylation of Akt. In this pathway, cell survival by HGF is mediated through signaling from its receptor, c-Met, to phosphatidylinositol 3-kinase and the phosphorylation of Akt.[43] Akt, in turn, exerts antiapoptotic effects through phosphorylation of B-cell lymphoma 2–associated death promoter and procaspase-9, which promotes cell survival.[31-33] Taken together, these results suggest that CYLD-induced HGF production inhibits hepatic injury and cell death associated with development and progression of hepatic fibrosis.

As a mechanism through which CYLD induces HGF expression in HSCs, we identified the interaction of HGF with HDAC7. HDAC7 belongs to the

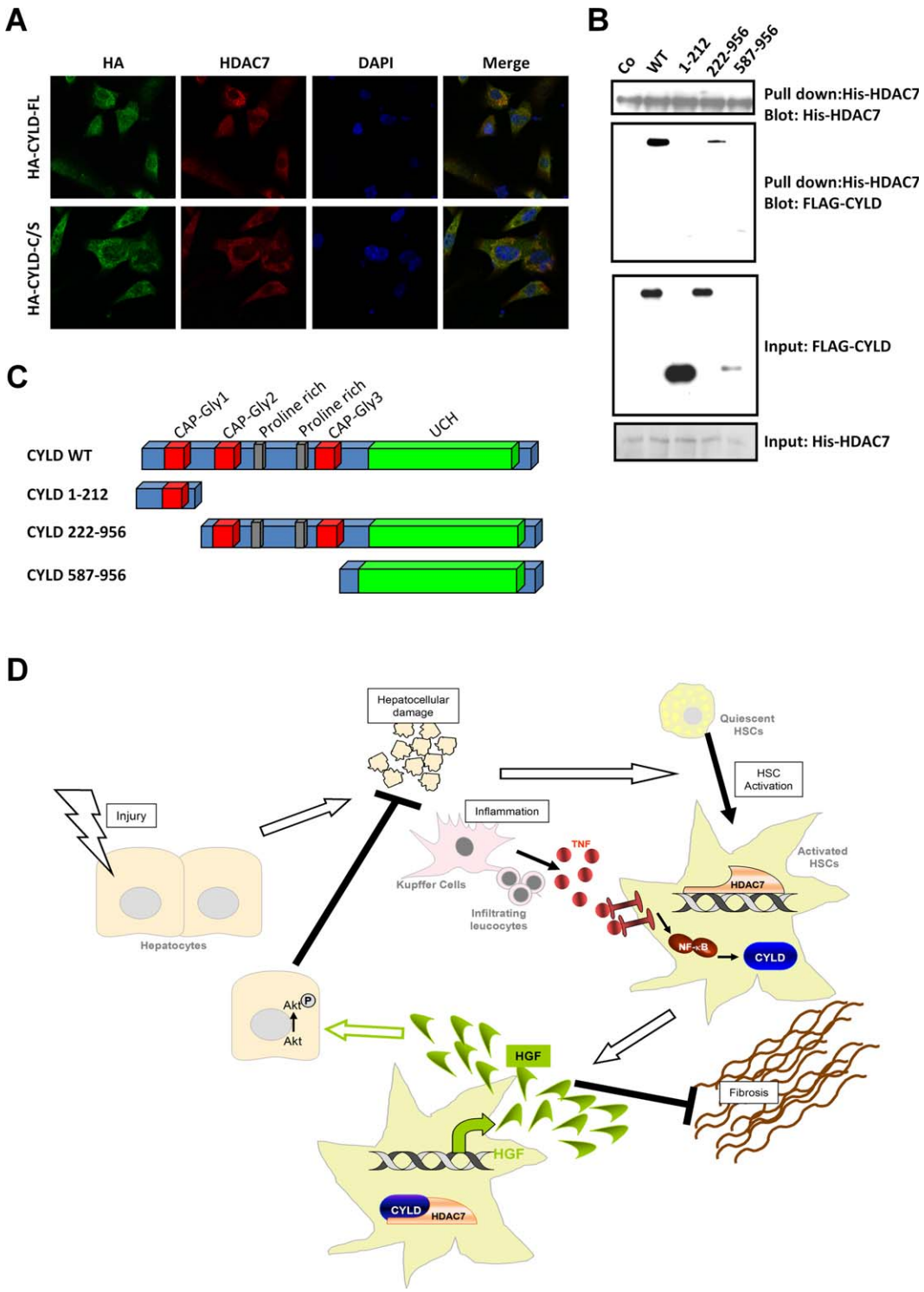

Fig. 8. CYLD deubiquitinase activity is not responsible for blocking the nuclear localization of HDAC7. (A) Analysis of subcellular colocalization of endogenous HDAC7 in HA-CYLD-FL (HA-tagged full-length WT CYLD) or HA-CYLD-C/S (HA-tagged catalytically mutant CYLD) transfected primary CYLD$^{-/-}$ HSCs. (B) *In vitro* pull-down assay, using His-HDAC7 and either full-length CYLD (CYLD-WT) or deletion of mutants of CYLD (CYLD 1-212, CYLD 222-956, or CYLD 587-956). (C) Schematic presentation of WT CYLD and truncation mutants of CYLD. The three CAP-Gly domains are depicted in red, proline-rich domains in gray, and the UCH domain in green. (D) Model of the up-regulation of HGF in HSCs by inactivation of HDAC7 through CYLD, which further protects hepatocytes against injury and inhibits hepatic fibrosis. DAPI, 4',6-diamidino-2-phenylindole.

HDAC class IIa enzymes, possessing nucleocytoplasmic shuttling properties.[37] It was shown recently that 14-3-3 and Ca^{2+}/calmodulin-dependent kinase (CaMK) I control the subcellular localization of HDAC-7. CaMK I–mediated phosphorylation of HDAC7 stimulates HDAC7 export from the nucleus, and in the cytoplasm, HDAC7 directly interacts with 14-3-3 proteins, leading to the derepression of HDAC7 target genes.[37] Here, we showed that deletion of the HDAC7 gene in HSCs increased levels of HGF. In contrast to WT HSCs, knockdown of CYLD did not change HGF levels in HDAC7-deficient HSCs, indicating the importance of CYLD-HDAC7 interaction for regulation of HGF expression in HSCs. We observed that HDAC7 was primarily localized in nuclei of HSCs, whereas stimulation with TNF or IL-6 promoted translocation of HDAC7 from the nucleus to the cytoplasm and binding to CYLD. This interaction further prevented the nuclear translocation of HDAC7 and recruitment to the HGF promoter. Consistent with this finding, it has recently been shown that TNF derepressed expression of HDAC7 target genes.[38] However, it has yet to be determined whether HDAC7 phosphorylation and binding to 14-3-3 are necessary for CYLD interaction. However, we observed a direct interaction between purified CYLD and HDAC7 protein *in vitro*, suggesting an alternative mechanism of HDAC7 inactivation in the cytoplasm induced through CYLD, independent of HDAC7 phosphorylation and 14-3-3 interaction.

In summary, our findings highlight a novel link between CYLD and HDAC7, offering mechanistic insight into the contribution of these proteins to the progression of liver disease. Notably, interaction between CYLD and HDAC7 occurs independently of the deubiquitin activity of CYLD and functions in the presence of proinflammatory cytokines secreted from infiltrating inflammatory cells, resident macrophages, and HSCs, activated in response to liver injury. We propose a model in which CYLD-regulated hepatic HGF expression plays a crucial role in the interaction of hepatocytes with nonparenchymal liver cells and (infiltrating) inflammatory cells, providing protection against hepatocellular injury and inflammation and, consequently, the development and progression of hepatic fibrosis (Fig. 8D). We propose that this CYLD-regulated pathway works together with other important signaling pathways in HSCs and other cell types, including hepatocytes, KCs, and recruited inflammatory cells for restraining hepatic injury and fibrosis.

In addition, the novel CYLD-HDAC7 connection might be critical for other biological processes, considering the pleiotropic function of this HDAC under physiological and pathological conditions. Thus, these findings provide an avenue for potential strategies to selectively affect HDAC7 under inflammatory conditions, in CLDs, and, potentially, in conditions beyond hepatic disorders.

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References

1. Gieling RG, Burt AD, Mann DA. Fibrosis and cirrhosis reversibility - molecular mechanisms. Clin Liver Dis 2008;12:915-937, xi.
2. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134:1655-1669.
3. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-218.
4. Massoumi R. CYLD: a deubiquitination enzyme with multiple roles in cancer. Future Oncol 2011;7:285-297.
5. Courtois G. Tumor suppressor CYLD: negative regulation of NF-kappaB signaling and more. Cell Mol Life Sci 2008;65:1123-1132.
6. Sun SC. CYLD: a tumor suppressor deubiquitinase regulating NF-kappaB activation and diverse biological processes. Cell Death Differ 2010;17:25-34.
7. Massoumi R. Ubiquitin chain cleavage: CYLD at work. Trends Biochem Sci 2010;35:392-399.
8. Hellerbrand C, Bumes E, Bataille F, Dietmaier W, Massoumi R, Bosserhoff AK. Reduced expression of CYLD in human colon and hepatocellular carcinomas. Carcinogenesis 2007;28:21-27.
9. Chu L, Gu J, He Z, Xiao T, Liu X. Adenoviral vector expressing CYLD augments antitumor activity of TRAIL by suppression of NF-kappaB survival signaling in hepatocellular carcinoma. Cancer Biol Ther 2006;5:615-622.
10. Nikolaou K, Tsagaratou A, Eftychi C, Kollias G, Mosialos G, Talianidis I. Inactivation of the deubiquitinase CYLD in hepatocytes causes apoptosis, inflammation, fibrosis, and cancer. Cancer Cell 2012; 21:738-750.
11. Urbanik T, Boger RJ, Longerich T, Becker K, Ehrenberg KR, Hovelmeyer N, et al. Liver specific deletion of CYLDexon7/8 induces severe biliary damage, fibrosis and increases hepatocarcinogenesis in mice. J Hepatol 2012;57:995-1003.
12. Lee KK, Workman JL. Histone acetyltransferase complexes: one size doesn't fit all. Nat Rev Mol Cell Biol 2007;8:284-295.
13. Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 2006; 6:38-51.
14. Turtoi A, Mottet D, Matheus N, Dumont B, Peixoto P, Hennequiere V, et al. The angiogenesis suppressor gene AKAP12 is under the epigenetic control of HDAC7 in endothelial cells. Angiogenesis 2012;15:543-554.
15. Jin Z, Wei W, Dechow PC, Wan Y. HDAC7 inhibits osteoclastogenesis by reversing RANKL-triggered beta-catenin switch. Mol Endocrinol 2013;27:325-335.
16. Ma C, D'Mello SR. Neuroprotection by histone deacetylase-7 (HDAC7) occurs by inhibition of c-jun expression through a deacetylase-independent mechanism. J Biol Chem 2011;286:4819-4828.

17. Malik S, Jiang S, Garee JP, Verdin E, Lee AV, O'Malley BW, et al. Histone deacetylase 7 and FoxA1 in estrogen-mediated repression of RPRM. Mol Cell Biol 2010;30:399-412.
18. Navarro MN, Goebel J, Feijoo-Carnero C, Morrice N, Cantrell DA. Phosphoproteomic analysis reveals an intrinsic pathway for the regulation of histone deacetylase 7 that controls the function of cytotoxic T lymphocytes. Nat Immunol 2011;12:352-361.
19. Rombouts K, Niki T, Wielant A, Hellemans K, Geerts A. Trichostatin A, lead compound for development of antifibrogenic drugs. Acta Gastroenterol Belg 2001;64:239-246.
20. Niki T, Rombouts K, De Bleser P, De Smet K, Rogiers V, Schuppan D, et al. A histone deacetylase inhibitor, trichostatin A, suppresses myofibroblastic differentiation of rat hepatic stellate cells in primary culture. HEPATOLOGY 1999;29:858-867.
21. Rombouts K, Knittel T, Machesky L, Braet F, Wielant A, Hellemans K, et al. Actin filament formation, reorganization and migration are impaired in hepatic stellate cells under influence of trichostatin A, a histone deacetylase inhibitor. J Hepatol 2002;37:788-796.
22. Bosserhoff AK, Moser M, Scholmerich J, Buettner R, Hellerbrand C. Specific expression and regulation of the new melanoma inhibitory activity-related gene MIA2 in hepatocytes. J Biol Chem 2003;278: 15225-15231.
23. Hellerbrand C, Jobin C, Licato LL, Sartor RB, Brenner DA. Cytokines induce NF-kappaB in activated but not in quiescent rat hepatic stellate cells. Am J Physiol 1998;275:G269-G278.
24. Muhlbauer M, Weiss TS, Thasler WE, Gelbmann CM, Schnabl B, Scholmerich J, Hellerbrand C. LPS-mediated NFkappaB activation varies between activated human hepatic stellate cells from different donors. Biochem Biophys Res Commun 2004;325:191-197.
25. Massoumi R, Chmielarska K, Hennecke K, Pfeifer A, Fassler R. Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. Cell 2006;125:665-677.
26. Gabele E, Froh M, Arteel GE, Uesugi T, Hellerbrand C, Scholmerich J, et al. TNFalpha is required for cholestasis-induced liver fibrosis in the mouse. Biochem Biophys Res Commun 2009;378:348-353.
27. Nakamura T, Sakai K, Nakamura T, Matsumoto K. Hepatocyte growth factor twenty years on: Much more than a growth factor. J Gastroenterol Hepatol 2011;26(Suppl 1):188-202.
28. Inagaki Y, Higashi K, Kushida M, Hong YY, Nakao S, Higashiyama R, et al. Hepatocyte growth factor suppresses profibrogenic signal transduction via nuclear export of Smad3 with galectin-7. Gastroenterology 2008;134:1180-1190.
29. Suzumura K, Hirano T, Son G, Iimuro Y, Mizukami H, Ozawa K, Fujimoto J. Adeno-associated virus vector-mediated production of hepatocyte growth factor attenuates liver fibrosis in mice. Hepatol Int 2008;2:80-88.
30. Lin Y, Xie WF, Chen YX, Zhang X, Zeng X, Qiang H, et al. Treatment of experimental hepatic fibrosis by combinational delivery of urokinase-type plasminogen activator and hepatocyte growth factor genes. Liver Int 2005;25:796-807.
31. Schulze-Bergkamen H, Brenner D, Krueger A, Suess D, Fas SC, Frey CR, et al. Hepatocyte growth factor induces Mcl-1 in primary human hepatocytes and inhibits CD95-mediated apoptosis via Akt. HEPATOLOGY 2004;39:645-654.
32. Moumen A, Patane S, Porras A, Dono R, Maina F. Met acts on Mdm2 via mTOR to signal cell survival during development. Development 2007;134:1443-1451.
33. Moumen A, Ieraci A, Patane S, Sole C, Comella JX, Dono R, Maina F. Met signals hepatocyte survival by preventing Fas-triggered FLIP degradation in a PI3k-Akt-dependent manner. HEPATOLOGY 2007;45: 1210-1217.
34. Fujiyoshi M, Ozaki M. Molecular mechanisms of liver regeneration and protection for treatment of liver dysfunction and diseases. J Hepatobiliary Pancreat Sci 2011;18:13-22.
35. Kariv R, Enden A, Zvibel I, Rosner G, Brill S, Shafritz DA, et al. Triiodothyronine and interleukin-6 (IL-6) induce expression of HGF in an immortalized rat hepatic stellate cell line. Liver Int 2003;23:187-193.
36. Matsumoto Y, Motoki T, Kubota S, Takigawa M, Tsubouchi H, Gohda E. Inhibition of tumor-stromal interaction through HGF/Met signaling by valproic acid. Biochem Biophys Res Commun 2008;366: 110-116.
37. Kao HY, Verdel A, Tsai CC, Simon C, Juguilon H, Khochbin S. Mechanism for nucleocytoplasmic shuttling of histone deacetylase 7. J Biol Chem 2001;276:47496-47507.
38. Gao C, Cheng X, Lam M, Liu Y, Liu Q, Chang KS, Kao HY. Signal-dependent regulation of transcription by histone deacetylase 7 involves recruitment to promyelocytic leukemia protein nuclear bodies. Mol Biol Cell 2008;19:3020-3027.
39. Pannem RR, Dorn C, Ahlqvist K, Bosserhoff AK, Hellerbrand C, Massoumi R. CYLD controls c-MYC expression through the JNK-dependent signaling pathway in hepatocellular carcinoma. Carcinogenesis 2014;35:461-468.
40. Matsuda Y, Matsumoto K, Ichida T, Nakamura T. Hepatocyte growth factor suppresses the onset of liver cirrhosis and abrogates lethal hepatic dysfunction in rats. J Biochem 1995;118:643-649.
41. Matsuda Y, Matsumoto K, Yamada A, Ichida T, Asakura H, Komoriya Y, et al. Preventive and therapeutic effects in rats of hepatocyte growth factor infusion on liver fibrosis/cirrhosis. HEPATOLOGY 1997;26:81-89.
42. Ueki T, Kaneda Y, Tsutsui H, Nakanishi K, Sawa Y, Morishita R, et al. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. Nat Med 1999;5:226-230.
43. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 2010;11:834-848.

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