DEP Domain–Containing mTOR–Interacting Protein Suppresses Lipogenesis and Ameliorates Hepatic Steatosis and Acute-on-Chronic Liver Injury in Alcoholic Liver Disease

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Alcoholic liver disease (ALD) is characterized by lipid accumulation and liver injury. However, how chronic alcohol consumption causes hepatic lipid accumulation remains elusive. The present study demonstrates that activation of the mechanistic target of rapamycin complex 1 (mTORC1) plays a causal role in alcoholic steatosis, inflammation, and liver injury. Chronic-plus-binge ethanol feeding led to hyperactivation of mTORC1, as evidenced by increased phosphorylation of mTOR and its downstream kinase S6 kinase 1 (S6K1) in hepatocytes. Aberrant activation of mTORC1 was likely attributed to the defects of the DEP domain-containing mTOR-interacting protein (DEPTOR) and the nicotinamide adenine dinucleotide–dependent deacetylase sirtuin 1 (SIRT1) in the liver of chronic-plus-binge ethanol-fed mice and in the liver of patients with ALD. Conversely, adenoviral overexpression of hepatic DEPTOR suppressed mTORC1 signaling and ameliorated alcoholic hepatosteatosis, inflammation, and acute-on-chronic liver injury. Mechanistically, the lipid-lowering effect of hepatic DEPTOR was attributable to decreased proteolytic processing, nuclear translocation, and transcriptional activity of the lipogenic transcription factor sterol regulatory element-binding protein-1 (SREBP-1). DEPTOR-dependent inhibition of mTORC1 also attenuated alcohol-induced cytoplasmic accumulation of the lipogenic regulator lipin 1 and prevented alcohol-mediated inhibition of fatty acid oxidation. Pharmacological intervention with rapamycin alleviated the ability of alcohol to up-regulate lipogenesis, to down-regulate fatty acid oxidation, and to induce steatogenic phenotypes. Chronic-plus-binge ethanol feeding led to activation of SREBP-1 and lipin 1 through S6K1-dependent and independent mechanisms. Furthermore, hepatocyte–specific deletion of SIRT1 disrupted DEPTOR function, enhanced mTORC1 activity, and exacerbated alcoholic fatty liver, inflammation, and liver injury in mice. Conclusion: The dysregulation of SIRT1–DEPTOR–mTORC1 signaling is a critical determinant of ALD pathology; targeting SIRT1 and DEPTOR and selectively inhibiting mTORC1–S6K1 signaling may have therapeutic potential for treating ALD in humans. (HEPATOLOGY 2018; 68:496–514).
need to develop new pathophysiology-oriented therapies in human ALD.

The mechanistic target of rapamycin (mTOR) is a conserved protein kinase that forms two functional complexes, termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 regulates cellular processes including cell growth, proliferation, and metabolism. While mTORC1 inhibition prevents apoptosis in cancer cells, the potential effect of mTORC1 on alcoholic liver injury has not been investigated. Because DEP domain-containing mTOR-interacting protein (DEPTOR) has recently emerged as an mTOR binding protein that inhibits the mTOR kinase, the role of DEPTOR in affecting the pathogenesis of ALD needs more investigation. Because overexpression of the nicotinamide adenine dinucleotide–dependent deacetylase sirtuin 1 (SIRT1) suppresses mTORC1 activity and attenuates fatty liver in genetically obese ob/ob mice, we hypothesized that abnormal regulation of mTORC1 is functionally linked to the development of ALD. To test this hypothesis, we used a recently developed mouse model of chronic-plus-binge ethanol feeding, which is characterized by hepatic steatosis and acute-on-chronic liver injury and closely resembles the pathogenesis and drinking pattern of ALD in humans.

Here, we provide clinical evidence that mTORC1 functions as a central regulator of the ethanol-sensing machinery that links lipid metabolic dysregulation to hepatocellular apoptosis in mice and in patients with ALD. Our data illustrate that (1) DEPTOR, a critical negative regulator of mTORC1, protects mice from alcohol-induced fatty liver, inflammation, and liver damage; (2) mTORC1 is necessary for alcohol to activate hepatic lipogenesis and to inhibit fatty acid oxidation in mice; (3) overexpression of the inactive S6 kinase 1 (S6K1), the downstream kinase of mTORC1, ameliorates alcoholic hepatic steatosis largely through reduced sterol regulatory element-binding protein-1 (SREBP-1)–dependent de novo lipogenesis; and (4) defective SIRT1 is coupled to the down-regulation of DEPTOR and up-regulation of mTORC1 and lipogenesis in patients with ALD. Therefore, targeting the SIRT1–DEPTOR–mTORC1 axis may have therapeutic potential in human ALD.

Materials and Methods

ANIMAL MODELS

The Bin-binge mouse model of early-stage ALD was recently developed. Liver (hepatocyte)–specific SIRT1 knockout (SIRT1 LKO) mice were achieved by crossing albumin-Cre recombinase...
transgenic mice with floxed SIRT1Δex4 mice containing the deleted SIRT1 allele with floxed exon 4. (11)

All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio.

HUMAN LIVER TISSUE SAMPLES

Normal human liver samples and ALD tissues were obtained from donor livers or recipient livers during liver transplantation from the Liver Tissue Procurement and Distribution System at the University of Minnesota as described. (10,12)

STATISTICAL ANALYSIS

Data are presented as means ± standard error of the mean (SEM). Using GraphPad Prism 5.0 software, results were analyzed by one-way analysis of variance between multiple groups, when appropriate, and by a two-tailed Student t test between two groups. P < 0.05 was considered statistically significant.

Results

mTORC1 ACTIVATION IS EVIDENT IN THE MOUSE MODEL OF CHRONIC-PLUS-BINGE ETHANOL FEEDING

Because many patients with ALD have a history of chronic drinking superimposed by excessive binge drinking, (1) a mouse model of chronic-plus-binge ethanol feeding was used, as described. (9,10) Hematoxylin and eosin staining showed that chronic-binge ethanol administration resulted in steatosis containing small and large lipid droplet vacuoles within hepatocytes compared with pair-fed mice (Fig. 1A). Liver and plasma triglyceride levels were significantly higher in mice after chronic-binge ethanol feeding than those in pair-fed mice. Hepatic and plasma cholesterol levels, body weight, and food intake were comparable between two groups of the mice. Importantly, plasma alanine aminotransferase (ALT) levels were increased more than 3-fold in chronic-binge ethanol-fed mice compared with pair-fed mice (Fig. 1B). The Thr389 phosphorylation of S6K1, which is modulated by mTOR, (13,14) was increased 3.3-fold. The phosphorylation of the S40 ribosomal protein S6 at Ser235/Ser236, the best-characterized substrate of S6K1, was increased ~5-fold. The Thr37/Thr46 phosphorylation of 4E-BP1, another substrate of mTORC1, was also enhanced 3-fold, further validating the inappropriate mTORC1 signaling in chronic-binge ethanol-fed mice. S6K1 activity was positively correlated with hepatic triglyceride content in mice (Fig. 1B). Furthermore, immunohistochemical analysis revealed that elevated positive signals of S6 phosphorylation were colocalized primarily in the cytoplasm of lipid droplet-rich hepatocytes of chronic-binge ethanol-fed mice (Fig. 1C).

To determine whether mTORC1 is involved in ALD pathology, multiple markers of mTORC1 signaling were determined. Autophosphorylation of mTOR at Ser2481 was increased ~2-fold in chronic-binge ethanol-fed mice compared with pair-fed mice (Fig. 1B). The Thr389 phosphorylation of S6K1, which is modulated by mTOR, (13,14) was increased 3.3-fold. The phosphorylation of the S40 ribosomal protein S6 at Ser235/Ser236, the best-characterized substrate of S6K1, was increased ~5-fold. The Thr37/Thr46 phosphorylation of 4E-BP1, another substrate of mTORC1, was also enhanced 3-fold, further validating the inappropriate mTORC1 signaling in chronic-binge ethanol-fed mice. S6K1 activity was positively correlated with hepatic triglyceride content in mice (Fig. 1B). Furthermore, immunohistochemical analysis revealed that elevated positive signals of S6 phosphorylation were colocalized primarily in the cytoplasm of lipid droplet-rich hepatocytes of chronic-binge ethanol-fed mice (Fig. 1C).

We further observed that expression of Rictor, a major component of mTORC2, was unaffected. The Ser473 phosphorylation at the Akt hydrophobic site, which can be catalyzed by mTORC2, (3) was slightly decreased by ethanol feeding (Fig. 1D; Supporting Fig. S1C). It is possible that activation of mTORC1 by ethanol may trigger an S6K1-dependent negative feedback loop toward insulin receptor substrate 1–phosphoinositide 3-kinase signaling, leading to dampened activation of Akt. (3) Taken together, aberrant activation of mTORC1, but not mTORC2, is associated with alcoholic hepatic steatosis.

HEPATIC DEPTOR IS SUPPRESSED BY CHRONIC-PLUS-BINGE ETHANOL FEEDING

To elucidate the mechanism underlying the up-regulation of mTORC1 by ethanol, we sought which upstream regulator of mTORC1 is involved in ALD. Expression of regulatory-associated protein of mTOR (Raptor), a critical scaffold and activator that stimulates mTORC1 activity, (3) was not significantly affected by ethanol feeding (Fig. 1D). Endogenous levels of tuberous sclerosis complex 2 (TSC2), a negative regulator of mTORC1, (3) and the activity of extracellular signal–regulated kinase 1/2, the upstream kinases of the TSC complex, were comparable between the two groups (Fig. 1D; Supporting Fig. S1D,E). DEPTOR has been recently identified as an endogenous inhibitor of the mTOR kinase. (5) While DEPTOR activity is largely modulated through the control of DEPTOR protein levels in cultured cells, (5) we explored the function of DEPTOR on lipid metabolism. DEPTOR was highly expressed in the cytoplasm
FIG. 1. Chronic-plus-binge ethanol feeding leads to inhibition of DEPTOR and activation of mTORC1 signaling and promotes the development of fatty liver in mice. (A) Effect of chronic-binge ethanol feeding on metabolic parameters and plasma ALT levels. Representative hematoxylin and eosin staining of hepatic steatosis in a mouse model of chronic-plus-binge ethanol feeding. Original magnification: ×10 or ×20. (B) Representative immunoblots for phosphorylation of mTOR, S6K1, S6, and 4E-BP1 in livers from four mice in each group. (C) Positive immunostaining for phosphorylated S6 (brown color) was predominantly located in lipid-rich hepatocytes (yellow arrows) in ethanol-fed mice. (D) Effect of chronic-binge ethanol feeding on key regulators of mTORC1 and mTORC2. (E) Chronic-binge ethanol feeding leads to an impairment of hepatic DEPTOR. Notably, positive staining for DEPTOR is visualized mainly in the cytoplasm of hepatocytes in pair-fed mice, and this effect is reduced in ethanol-fed mice. Original magnification: ×20 or ×40. Linear regression between hepatic DEPTOR levels and p-S6 phosphorylation in normal and ethanol-fed mice. Data are presented as mean ± SEM, n = 6-8 in each group. *P < 0.05 versus pair-fed mice. (F) Effect of ethanol on DEPTOR and mTORC1 signaling in AML12 mouse hepatocytes. Abbreviations: EtOH, ethanol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; H&E, hematoxylin and eosin; p, phosphorylation.
FIG. 2. Adenoviral overexpression of hepatic DEPTOR inhibits mTORC1 and improves hepatic steatosis in chronic-binge ethanol-fed mice. (A) Schematic representation of the interaction regions between mTOR and DEPTOR. DEPTOR inhibits mTOR activity by binding the PDZ domain of DEPTOR to the FAT domain of the mTOR kinase. Immunoblotting analyses confirmed adenovirus-mediated overexpression of DEPTOR in human HepG2 cells. (B) Hepatic overexpression of either DEPTOR protein (~46 kDa) or GFP (~27 kDa) in mice is confirmed. (C) Overexpression of DEPTOR improves hepatic steatosis and lowers triglyceride accumulation in ethanol-fed mice. (D) Overexpression of DEPTOR represses the induction of mTORC1 toward the downstream signaling in ethanol-fed mice. (E) Positive staining for phosphorylated S6 in hepatocytes (yellow arrows) in ethanol-fed mice is reduced by overexpressing DEPTOR. (F) Increased DEPTOR levels correlate with decreased S6 phosphorylation and lowered hepatic triglyceride content in mice. Data are presented as mean ± SEM, n = 6–8. *P < 0.05 versus ethanol-fed mice with Ad-GFP injection. Abbreviations: EtOH, ethanol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; H&E, hematoxylin and eosin; p, phosphorylation.
FIG. 3. Hepatic overexpression of DEPTOR ameliorates alcohol-mediated dysregulation of lipid metabolism in chronic-binge ethanol-fed mice. (A) The active, nuclear form of SREBP-1 is increased in ethanol-fed mice and decreased by DEPTOR overexpression. (B,C) The nuclear translocation of SREBP-1 as well as expression of SREBP-1c and its targets including ACC1, FAS, and SCD1 are reduced by DEPTOR overexpression. Notably, positive staining for SREBP-1 is primarily located in the nuclei of the hepatocytes of ethanol-fed mice (red arrows). (D,E) Overexpression of DEPTOR represses the expression and cytoplasmic translocation of lipin-1 in chronic-binge ethanol-fed mice. Immunofluorescent staining shows that strong staining for lipin 1 (green) was predominantly located in the nuclear (blue) of hepatocytes (red arrows) of pair-fed mice, but it was elevated and mainly present in the cytoplasm in hepatocytes (yellow arrows) surrounding central or portal veins of ethanol-fed mice. Notably, strong staining for lipin 1 is visualized mainly in the cytoplasm of hepatocytes (yellow arrows) of control mice on the ethanol diet, and this induction is inhibited by overexpression of DEPTOR. (F) Expression of key genes involving fatty acid oxidation such as PPARγ, CPT-1α, and PGC-1α is analyzed. Data are presented as mean ± SEM, n = 6-8. *P < 0.05 versus pair-fed mice, #P < 0.05 versus ethanol-fed mice with Ad-GFP injection. Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; EtOH, ethanol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; N, cleaved nuclear (~68 kDa) form of SREBP-1; P, precursor (~125 kDa) form of SREBP-1.
of hepatocytes in pair-fed mice, and it was markedly reduced by ethanol feeding (Fig. 1E). Strikingly, ethanol exposure decreased DEPTOR and increased phosphorylation of S6 and 4E-BP1 in a dose-dependent manner in AML-12 mouse hepatocytes (Fig. 1F). In contrast to hyperphosphorylated mTOR, hepatic DEPTOR levels were decreased ~50% in chronic-binge ethanol-fed mice. DEPTOR levels inversely correlated with mTOR phosphorylation in ethanol-fed mice, suggesting that alcohol-induced mTORC1 activation may be attributed to the down-regulation of DEPTOR. Although other mechanisms may contribute to alcohol-induced activation of mTORC1, impaired DEPTOR in hepatocytes may be a key determinant of early-stage ALD.

**ADENOVIRAL OVEREXPRESSION OF DEPTOR INHIBITS mTORC1 SIGNALING AND AMELIORATES ALCOHOLIC FATTY LIVER**

To gain direct evidence for the *in vivo* functional relevance of decreased DEPTOR to ALD pathology, we generated an adenoviral vector expressing DEPTOR (Ad-DEPTOR) and confirmed adenoviral overexpression of DEPTOR in human HepG2 cells (Fig. 2A). Adenovirus-mediated overexpression of DEPTOR was achieved by tail vein injection of Ad-DEPTOR into chronic-binge ethanol-fed mice, as reflected by a 3.7-fold increase in hepatic DEPTOR (Fig. 2B; Supporting Fig. S2A). We further determined whether alcohol-mediated hyperactivation of mTORC1 in the liver is a reversible process. Mice overexpressing DEPTOR displayed much less hepatic lipid vacuolization and triglyceride accumulation compared with control mice overexpressing green fluorescent protein (GFP). Hepatic and plasma levels of cholesterol, body weight, and food intake were comparable between the two groups (Fig. 2C; Supporting Fig. S2D,E). Because there were no significant changes in TSC2 and Raptor in ethanol-fed mice (Fig. 1D), the results indicate that excessive alcohol consumption inhibits DEPTOR, which may stimulate mTORC1 and promote hepatic steatosis.

Enforced expression of DEPTOR was sufficient to inhibit alcohol-induced activation of mTORC1, accompanied by reduced Ser2481 autophosphorylation of mTOR and Thr389 phosphorylation of S6K1, which is essential for mTOR-regulated activity of S6K1. Overexpression of DEPTOR also caused an approximately 50% reduction in phosphorylation of S6 and 4E-BP1 in ethanol-fed mice, largely owing to inactivation of mTOR and S6K1 kinases (Fig. 2D; Supporting Fig. S2B-C). The intensity and areas of positive staining for S6 phosphorylation present in lipid droplet–rich hepatocytes were reduced by DEPTOR overexpression (Fig. 2E). Increasing DEPTOR levels correlated with the reduction in S6 phosphorylation and triglyceride overproduction in chronic-binge ethanol-fed mice (Fig. 2F). Collectively, the dysregulation of DEPTOR–mTORC1 signaling represents a key determinant of the ethanol-sensing machinery that modulates lipid metabolism.

**HEPATIC OVEREXPRESSION OF DEPTOR SUPPRESSES ALCOHOL-INDUCED ACTIVATION OF SREBP-1 IN MICE**

SREBP-1, a critical transcription factor that activates the synthesis of fatty acids and triglycerides, has been implicated in hepatic steatosis in obesity-induced fatty liver. While the role of ethanol in SREBP-1 appears controversial, SREBP-1 null mice exhibit lowered hepatic triglyceride accumulation upon ethanol feeding. To delineate the mechanism by which overexpression of DEPTOR restores hepatic lipid homeostasis in ALD, the cleavage processing of SREBP-1 was examined by assessing amounts of the precursor (~125 kDa) and nuclear active (~68 kDa) forms of SREBP-1. The active, nuclear form of SREBP-1 was significantly elevated in chronic-binge ethanol feeding, accompanied by increased SREBP-1 precursor (Fig. 3A,B), as was seen in earlier studies. Immunohistochemical analysis further confirmed that expression and nuclear translocation of SREBP-1 were increased by ethanol feeding and down-regulated by DEPTOR overexpression. Notably, strong positive staining for SREBP-1 was primarily localized in the nucleus of lipid droplet–rich hepatocytes of ethanol-fed mice, and this effect was eliminated by DEPTOR overexpression. SREBP-1c has been demonstrated to be transcriptionally up-regulated by nuclear SREBP through a feed-forward mechanism because nuclear SREBP-1 can bind to the sterol regulatory element motif present on its own promoters. Intriguingly, accumulation of the active, nuclear form of SREBP-1 was suppressed by DEPTOR overexpression. The precursor form of SREBP-1 was slightly diminished, possibly owing to the reduction of SREBP-1c mRNA by
FIG. 4
suppressing its feed-forward loop. Moreover, ethanol-mediated up-regulation of SREBP-1c and its target genes, including acetyl-coenzyme A carboxylase (ACC1), fatty acid synthase (FAS), and stearoyl coenzyme A desaturase 1 (SCD1), was significantly decreased by DEPTOR overexpression. Consequently, ethanol-mediated induction of FAS was reduced by DEPTOR overexpression, which coincided with lowered triglyceride content in the liver of mice and in AML-12 hepatocytes (Figs. 2F and 3C; Supporting Fig. S3). Thus, hepatic overexpression of DEPTOR protects against alcohol-induced de novo lipogenesis and steatosis at least in part through the down-regulation of the proteolytic processing, nuclear translocation, and transcriptional activity of SREBP-1.

HEPATIC OVEREXPRESSION OF DEPTOR REDUCES ALCOHOL-INDUCED CYTOPLASMIC ACCUMULATION OF LIPIN 1 IN MICE

We next tested the hypothesis that DEPTOR might be functionally linked to the regulation of lipin 1, the key metabolic enzyme that dephosphorylates phosphatidic acid to form diacylglycerol (phosphatidic acid phosphatase activity) in the triglyceride synthetic pathway. Chronic-binge ethanol exposure caused a robust increase in expression of lipin 1 and the cytoplasmic localization of lipin 1 (Fig. 3D), consistent with increased phosphatidic acid phosphatase activity of cytoplasmic lipin 1 in the mouse model of chronic ethanol feeding. Notably, positive staining areas for lipin 1 were overlapped with those of lipid droplet–rich hepatocytes in ethanol-fed mice. Conversely, enforced expression of DEPTOR reduced the expression and cytoplasmic accumulation of lipin 1 caused by ethanol (Fig. 3E). Taken together, DEPTOR inhibits alcohol-induced lipogenesis at least partially by reducing cytoplasmic translocation of lipin 1.

HEPATIC OVEREXPRESSION OF DEPTOR STIMULATES EXPRESSION OF KEY GENES INVOLVED IN FATTY ACID OXIDATION IN CHRONIC-BINGE ETHANOL-FED MICE

Real-time PCR analysis showed that chronic-binge ethanol exposure caused a significant impairment in peroxisome proliferator–activated receptor alpha (PPARα)–mediated fatty acid oxidation, as evidenced by decreased expression of the nuclear receptor PPARα (Fig. 3F). Complementary to the up-regulation of PPARα by DEPTOR overexpression, expression of its major target gene, carnitine palmitoyl transferase 1α (CPT-1α), which is the key rate-limiting enzyme of fatty acid oxidation, was also induced. The lipid-lowering effect of DEPTOR was further supported by elevated PPARγ coactivator 1α (PGC-1α), a transcription cofactor known to activate PPARα (Fig. 3F). Collectively, alcoholic disruption of DEPTOR signaling causes a metabolic switch from fatty acid oxidation toward fatty acid synthesis, which can be reversed by overexpression of DEPTOR.

mTORC1 IS ESSENTIAL FOR ALCOHOL-INDUCED LIPID METABOLIC ABNORMALITIES AND HEPATIC STEATOSIS IN MICE

To explore the therapeutic potential of a pharmacological inhibitor of mTORC1 on ALD, we took advantage of rapamycin because pharmacological inhibitors of mTOR such as rapamycin are Food and Drug Administration–approved drugs for immunosuppression and anticancer treatment in humans. Rapamycin forms a complex with 12-kDa FK506-binding protein (FKBP12); this complex specifically binds to the

**FIG. 4.** Pharmacologic intervention with mTORC1 inhibition reduces hepatic lipogenesis, stimulates fatty acid oxidation, and ameliorates hepatic steatosis in chronic-binge ethanol–fed mice. (A) Schematic structure and rapamycin binding site of the mTOR kinase. The FRB domain is the docking site of the FKBP12-rapamycin complex. (B) Phosphorylation of mTOR and its downstream effectors was sensitive to treatment with rapamycin in ethanol–fed mice. (C) Positive staining for phosphorylated S6 was primarily localized in the cytoplasm of lipid-laden hepatocytes (yellow arrows) in vehicle control mice, and the staining intensity is reduced in rapamycin–treated mice. (D) The cleavage and nuclear translocation of SREBP-1 (red) and 4′,6-diamidino-2-phenylindole (blue) in liver sections of mice. (E,F) mTORC1 is required for alcohol to induce hepatic lipogenesis and to inhibit PPARα–mediated fatty acid oxidation in mice. Data are presented as the mean ± SEM, n = 6–8. *P < 0.05 versus ethanol-fed mice with vehicle administration. Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; EtOH, ethanol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; H&E, hematoxylin and eosin; N, cleaved nuclear (~68 kDa) form of SREBP-1; P, precursor (~125 kDa) form of SREBP-1; p, phosphorylation.
FKBP12-rapamycin binding (FRB) domain of mTORC1, which allosterically inhibits its kinase activity (Fig. 4A). The induction of Ser2481 phosphorylation of mTOR and Thr389 phosphorylation of S6K1 in ethanol-fed mice was almost completely blocked by rapamycin treatment (Fig. 4B). Suppression of hepatic mTOR and S6K1 kinases was evidenced by markedly decreased phosphorylation of 4E-BP1 and S6, respectively (Fig. 4B). Rapamycin administration attenuated hepatic steatosis with ~40% reduction in liver triglyceride content compared with vehicle-treated, ethanol-fed mice (Fig. 4C). No significant alterations in hepatic cholesterol content, body weight, and food intake were noted in rapamycin-treated, ethanol-fed mice (Supporting Fig. S4). Furthermore, the number and density of hepatocytes stained with S6 phosphorylation were much less in rapamycin-treated mice than in vehicle-treated mice (Fig. 4C). This suggests that ethanol stimulates S6K1 activity and hepatic steatosis by acting upstream of mTORC1.

Ethanol-mediated accumulation of nuclear SREBP-1 and induction of FAS were abolished in a rapamycin-sensitive manner (Fig. 4D). Immunofluorescence analysis revealed that ethanol-induced nuclear translocation of SREBP-1 was eliminated by rapamycin treatment (Fig. 4D). Newly generated SREBP-1 precursor was also down-regulated, possibly due to reduced SREBP-1c transcription. Moreover, expression and cytoplasmic location of lipin 1 were suppressed by rapamycin administration (Fig. 4E). Consequently, mRNA abundance of SREBP-1c and its target genes including ACC1, FAS, and SCD1 was reduced. The down-regulation of both SREBP-1 and lipin 1 and up-regulation of PPARα coincided with hepatic triglyceride reduction in ethanol-fed mice upon rapamycin treatment (Fig. 4F). Our studies with pharmacological and molecular inhibition of mTORC1 demonstrate that mTORC1 is necessary to activate lipogenesis and inhibit fatty acid oxidation in response to alcohol challenge.

HEPATIC S6K1 ACTIVITY IS REQUIRED FOR ALCOHOL TO induction SREBP-1-DEPENDENT LIPOGENESIS IN MICE

As mentioned above, enhanced phosphorylation and activity of S6K1, the major downstream kinase of mTORC1, are associated with hepatic lipid accumulation in ethanol-fed mice (Fig. 1B). If this kinase is important for the development of ALD, inactivation of S6K1 would mimic the protective effect of DEPTOR on ALD. To this end, a dominant negative form of S6K1 (DN-S6K1) containing the K100R mutation in the adenosine triphosphate binding site on the kinase domain (Fig. 5A), which is the catalytically inactive form of S6K1, was used. In vivo adenoviral gene transfer of DN-S6K1 into chronic-binge ethanol-fed mice was successfully accomplished through tail vein injection, as evidenced by an ~6-fold elevation of S6K1 and a remarkable reduction of S6 phosphorylation (Fig. 5B). No significant alteration in 4E-BP1 phosphorylation was noted (Fig. 5B). To define the functional consequence of DN-S6K1, mice expressing DN-S6K1 displayed less ethanol-induced steatosis, as revealed by liver histology and reduced liver triglyceride levels (Fig. 5C). No differences in hepatic cholesterol, body weight, and food intake between the two groups were evident (Supporting Fig. S5). Immunohistochemical staining showed significantly less positive staining for S6 phosphorylation in hepatocytes of mice expressing DN-S6K1 than in those of control mice expressing GFP (Fig. 5C). Furthermore, alcohol-induced accumulation of nuclear SREBP-1 and expression of key lipogenic genes were counteracted by overexpressing DN-S6K1 (Fig. 5D). Surprisingly, unlike the down-regulation of SREBP-1 by DN-S6K1, hepatic levels of lipin 1 appeared indistinguishable between the two groups (Fig. 5E-F), suggesting that alcohol up-regulates lipin 1 through an S6K-independent mechanism. These studies support a model in which uncontrolled mTORC1 activity by ethanol promotes lipid biosynthesis through S6K1-dependent and independent mechanisms.

MOLECULAR AND PHARMACOLOGICAL INHIBITION OF mTORC1 PROTECTS AGAINST ALCOHOL-INDUCED HEPATIC INFLAMMATION AND INJURY IN MICE

Hepatic mRNA expression of the proinflammatory cytokines and chemokines, including tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and monocyte chemotactic protein 1 (MCP-1), was significantly higher in chronic binge-fed mice than in pair-fed mice; such an inflammatory response was attenuated by DEPTOR overexpression (Fig. 6A). A
FIG. 5. Hepatic S6K1 activity is required for the proteolytic activation of SREBP-1 and its lipogenic process in mice after chronic-binge ethanol feeding. (A) Schematic structure and phosphorylation sites of p70S6K1. DN-S6K1 bears a mutation of lysine 100 to arginine (K100R) in the adenosine triphosphate binding site of the kinase domain. Immunoblots confirmed adenovirus-mediated overexpression of DN-S6K1 in HepG2 hepatocytes. (B, C) Overexpression of DN-S6K1 inhibits S6K1 activity and attenuates hepatic steatosis in ethanol-fed mice. Notably, strong positive staining for phosphorylated S6 in the cytoplasm of hepatocytes (yellow arrows) in ethanol-fed mice is markedly decreased by overexpression of DN-S6K1. (D, E) Overexpression of DN-S6K1 suppresses the accumulation of nuclear SREBP-1 and induction of lipogenic genes in response to ethanol feeding. Notably, positive staining for the nuclear translocation of SREBP-1 is presented in hepatocytes (red arrows) around central and portal veins in GFP-expressed mice. (F) Alcohol-mediated induction of lipin 1 is unaffected by overexpression of DN-S6K1. Data are presented as mean ± SEM, n = 6-8. *P < 0.05 versus ethanol-fed mice with Ad-GFP injection. Abbreviations: ATP, adenosine triphosphate; EtOH, ethanol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; H&E, hematoxylin and eosin; p, phosphorylation; TOS, TOR signaling.
minimal alteration in hepatic expression of the macrophage marker F4/80 was evident, similar to the minimal effect of ethanol on DEPTOR in RAW264.7 mouse macrophages (Supporting Fig. S6). However, consistent with neutrophil infiltration in human ALD, hepatic expression of neutrophil markers, such as Lymphocyte antigen 6 complex locus G (Ly6G), myeloperoxidase (MPO), and cluster of differentiation 11b (CD11b), was up-regulated by chronic-binge ethanol feeding (Fig. 6A), suggesting that neutrophil infiltration into liver tissue is a hallmark of local inflammation in mice and humans with early-stage ALD. Importantly, overexpression of DEPTOR suppressed alcohol-induced liver neutrophil accumulation with a minimal effect on hepatic macrophages (Fig. 6A). To further evaluate the effect of DEPTOR on alcohol-induced cell death, decreased phosphorylation of Akt, a major regulator of hepatocyte survival, and increased caspase-3 cleavage, a specific marker of apoptosis, were observed in mice after chronic-binge ethanol feeding; these pathological changes were prevented by DEPTOR overexpression (Fig. 6B, D). Accordingly, chronic-binge feeding–associated elevation of ALT levels was reduced. Hepatic DEPTOR levels were negatively correlated with plasma ALT levels in chronic-binge ethanol-fed mice (Fig. 6C). Moreover, rapamycin-treated mice were resistant to chronic-binge feeding–induced hepatocellular damage and inflammation, as reflected by stimulation of Akt-mediated survival signaling and inhibition of caspase-3 cleavage and neutrophil activation. Decreased levels of cleaved caspase-3 were correlated with lowered plasma ALT levels in rapamycin-treated mice (Fig. 6E). Finally, a similar reduction in hepatic apoptosis and plasma ALT levels was seen in mice expressing DN-S6K1 (Fig. 6F).

HEPATOCELLULAR DELETION OF SIRT1 DISRUPTS DEPTOR SIGNALING, PROMOTES mTORC1 ACTIVATION, AND EXACERBATES THE DEVELOPMENT OF ALCOHOLIC FATTY LIVER AND LIVER INJURY IN MICE

Our previous studies indicate that hepatic overexpression of SIRT1, a master nutrient sensor, inhibits mTORC1 activity and attenuates hepatic steatosis in obese ob/ob mice. However, there is little knowledge of the crosstalk between SIRT1 and mTORC1 signaling in ALD. Protein levels of SIRT1 were dramatically reduced in AML-12 mouse hepatocytes and in the livers of mice in response to ethanol exposure (Fig. 7A). These results, along with the inhibitory effect of ethanol on DEPTOR (Fig. 1E, F), suggest that ethanol causes the concomitant down-regulation of SIRT1 and DEPTOR in hepatocytes and in vivo.

To further investigate a causal relationship between SIRT1 and DEPTOR in the setting of ALD, SIRT1 LKO mice were used to investigate the role of genetic SIRT1 ablation in hepatocytes on alcoholic activation of mTORC1. SIRT1 LKO mice displayed a decrease in DEPTOR and a mild increase in basal mTORC1 activity when compared with pair-fed wild-type (WT) mice. Intriguingly, DEPTOR levels were reduced by chronic-binge ethanol feeding and further attenuated by hepatic SIRT1 deficiency. Consequently, phosphorylation of mTOR and its substrates, S6K1 and 4E-BP1, was enhanced by ethanol feeding; such an induction was further potentiated by hepatic SIRT1 deficiency (Fig. 7B).

To determine the in vivo functional consequence of hepatocyte–specific SIRT1 loss on ALD, hematoxylin and eosin staining revealed that SIRT1 LKO mice exhibited a higher degree of steatosis when compared to the WT mice that underwent the same ethanol treatment, although SIRT1 LKO mice were pheno-typically normal under a chow diet, similar to previous findings. Consistent with more severe hepatic steatosis, ethanol-fed SIRT1 LKO mice exhibited much higher hepatic and plasma triglyceride levels than WT mice without affecting hepatic and plasma cholesterol levels (Fig. 7C). Thus, hepatic ablation of SIRT1 disrupts DEPTOR function and exacerbates the development of alcoholic fatty liver.

To further understand the mechanisms by which SIRT1 LKO mice are more susceptible to developing alcoholic fatty liver, expression and activity of the major regulators controlling lipid metabolic pathways were examined. Accumulation of the active, nuclear form of SREBP-1 and expression of lipin 1 were significantly higher in ethanol-fed SIRT1 LKO mice than in WT controls. Accordingly, chronic ethanol feeding significantly elevated expression of key lipogenic enzymes including ACC1, FAS, and SCD1 in WT mice; these increases were more pronounced in ethanol-fed SIRT1 LKO mice. Moreover, SIRT1 loss further reinforced alcohol-mediated reduction of PPARα and PGC-1α (Fig. 7D; Supporting Fig. S7C). The more severe steatosis phenotype in SIRT1 LKO
mice likely results from elevated fatty acid synthesis and diminished fatty acid oxidation.

SIRT1 LKO mice also displayed higher ALT levels than WT mice after chronic-binge feeding (Fig. 7E). Hepatic expression of TNFα and IL-1β, two major proinflammatory cytokines that are associated with metabolic disease,\(^{23}\) were significantly increased in SIRT1 LKO mice. Notably, ethanol-fed SIRT1 LKO mice had increased neutrophil markers, while hepatic expression of the macrophage marker F4/80 was comparable between WT and SIRT1 LKO mice in both normal and ethanol-feeding conditions. More severe liver injury seen in ethanol-fed SIRT1 LKO mice was further characterized by reduced Akt activity and increased apoptosis (Fig. 7F). Collectively, the argument of alcoholic liver injury by SIRT1 loss is characterized by abnormal...
FIG. 7
hepatocellular fat accumulation, reduced cell survival, and increased inflammatory and apoptotic responses.

DISRUPTING SIRT1 AND DEPTOR SIGNALING IS A MOLECULAR SIGNATURE IN PATIENTS WITH ALD

To gain clinical evidence for the relationship among SIRT1, DEPTOR, and lipogenesis, liver samples from healthy subjects (n = 6) and patients with ALD (n = 8) were used as described.10,12 Protein levels of SIRT1 and DEPTOR were reduced by ~50% in liver tissues from patients with ALD compared to normal livers (Fig. 8A,B). In contrast to DEPTOR inhibition, mTORC1 autophosphorylation at Ser2481 was increased ~2.6-fold in patients with ALD. A >4-fold increase in S6 phosphorylation, as a marker of S6K1 activity, was also observed. Likewise, hepatic phosphorylation of 4E-BP1, the downstream target of mTORC1, was increased ~3-fold in patients with ALD (Fig. 8B). While emerging evidence shows much lower SIRT1 levels in patients with ALD,22 our animal and human studies provide additional evidence that aberrant inhibition of DEPTOR is associated with defective SIRT1 in the progression of ALD.

To test whether mTORC1 hyperactivation is clinically relevant to the lipogenic and apoptotic processes in ALD, we took advantage of our observation that the master lipogenic regulator SREBP-1 directly binds the FAS promoter and up-regulates its transcription, thereby promoting triglyceride synthesis in human hepatocytes.15 Hepatic nuclear SREBP-1 levels and FAS expression were increased in patients with ALD (Fig. 8C), aligned with elevated lipogenesis in patients with nonalcoholic fatty liver disease (NAFLD).24 Excess fat accumulation was also accompanied by the down-regulation of PPARx (Fig. 8D). Consistent with our finding that mTORC1 inhibition ameliorates liver injury in ethanol-fed mice (Fig. 6), mTORC1 activation was associated with excessive hepatocyte apoptosis in human ALD, as evidenced by increased cleaved caspase-3 (Fig. 8E). Collectively, the deregulation of SIRT1–DEPTOR–mTORC1 signaling and the imbalance of lipogenesis and fatty acid oxidation may represent major pathological features of human ALD.

Discussion

We have discovered that mTORC1 activity is enhanced in experimental animals and patients with ALD, characterized by an increase in mTORC1-mediated phosphorylation and activity of S6K1. Sustained mTORC1 activation by alcohol is likely attributed to defects of SIRT1 and DEPTOR. Mechanistically, mTORC1 is necessary for alcohol-induced lipogenesis, as reflected by the proteolytic activation, nuclear translocation, and transcriptional activity of the critical lipogenic transcriptional factor SREBP-1 and by the cytoplasmic accumulation of the key lipogenic regulator lipin 1. Pharmacological intervention with rapamycin protects against alcohol-induced lipogenesis and ameliorates steatosis, inflammation, and acute-on-chronic liver injury. Importantly, the concomitant reduction of SIRT1 and DEPTOR signaling is linked to elevated lipogenesis and decreased fatty acid oxidation in human liver specimens with ALD. These translational studies reveal that dysregulation of the SIRT1–DEPTOR–mTORC1 axis is a critical determinant of ALD pathology (Fig. 8F).

DEPTOR CONVERGES UPSTREAM OF mTORC1 SIGNALING AND ATTENUATES ALCOHOLIC HEPATIC STEATOSIS AND LIVER INJURY

mTORC1 has been implicated in NAFLD,7,25 but whether and how the mTORC1 pathway is activated by alcohol feeding have yet to be investigated. Here, using the murine model approximating human ALD,

FIG. 7. Hepatocyte-specific deletion of SIRT1 disrupts DEPTOR signaling, stimulates mTORC1 activity, and exacerbates the development of alcoholic fatty liver and liver injury in mice. (A) Protein levels of SIRT1 are measured in AML12 hepatocytes and in livers of WT mice and SIRT1 LKO mice under conditions of normal and ethanol exposure. (B) Hepatocyte-specific deletion of SIRT1 down-regulates DEPTOR and enhances mTORC1 activity in mice after chronic-binge ethanol feeding. (C) Genetic ablation of SIRT1 in the liver increases the susceptibility to alcohol-induced fatty liver. (D) Hepatic SIRT1 deficiency exacerbates alcohol-mediated abnormalities of lipid metabolism in mice. (E,F) Hepatic SIRT1 loss aggravates alcohol-induced inflammation, apoptosis, and liver injury. Data are presented as the mean ± SEM, n = 4-8. *P < 0.05, versus pair-fed WT mice, †P < 0.05, versus ethanol-fed WT mice. Abbreviations: EtOH, ethanol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; H&E, hematoxylin and eosin; MCP-1, monocyte chemotactic protein 1; N, cleaved nuclear (~68 kDa) form of SREBP-1; P, precursor (~125 kDa) form of SREBP-1; p, phosphorylation.
FIG. 8. Integrated down-regulation of SIRT1 and DEPTOR contributes to the pathogenesis in patients with ALD. (A–E) Representative immunoblots and densitometric quantification for SIRT1, mTORC1 signaling, lipid metabolic regulators, and apoptosis in normal liver tissues (n = 6) and liver tissues from patients with ALD (n = 8). Data are presented as the mean ± SEM. *P < 0.05 versus normal liver tissue. (F) Proposed model for the deregulation of the SIRT1–DEPTOR–mTORC1 axis in the pathogenesis of ALD in mice and humans. Chronic alcohol consumption causes SIRT1 suppression in hepatocytes, which is coupled to the down-regulation of DEPTOR and activation of mTORC1 and S6K1. Aberrant activation of mTORC1 by alcohol stimulates the proteolytic processing, nuclear translocation, and transcriptional activity of SREBP-1; promotes the cytoplasmic translocation of lipin 1; and inhibits the transcriptional activity of PPARα, which in turn increases fatty acid synthesis and down-regulates fatty acid oxidation. The alcohol-induced hepatic lipogenic process acts through parallel S6K1-dependent and independent pathways. Alcohol feeding acts largely through SIRT1 inhibition and mTORC1 activation to induce excess fat accumulation and apoptosis in hepatocytes. Hepatic lipotoxicity and inflammation likely contribute to the development of acute-on-chronic alcoholic liver injury. Hepatic loss of SIRT1 impairs DEPTOR function, stimulates mTORC1 and lipogenesis, and promotes triglyceride overproduction, thereby leading to inflammation and liver injury in ALD. DEPTOR-dependent inhibition of mTORC1 provides a potential druggable target for treating ALD in humans. Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; N, cleaved nuclear (∼68 kDa) form of SREBP-1; P, precursor (∼125 kDa) form of SREBP-1; p, phosphorylation; PPRE, PPAR response element; SRE, sterol regulatory element.
several lines of evidence suggest DEPTOR's function in hepatocytes. First, chronic-binge ethanol feeding leads to a robust increase in phosphorylation and activity of mTOR and its downstream kinase S6K1 in vivo. Alcohol-induced mTORC1 signaling mainly occurs in hepatocytes, consistent with the hepatocyte being a major cell type for ethanol metabolism. Second, DEPTOR is highly expressed in hepatocytes of normal mice and is markedly suppressed in mice after chronic-binge ethanol feeding, indicating a pathological role of DEPTOR impairment in ALD. This notion is further supported by the fact that gain of function of DEPTOR confers insensitivity of mTORC1 signaling to ethanol exposure in hepatocytes and in vivo. The clinical relevance of mTORC1 induction to ALD is evidenced by decreased DEPTOR and increased S6K1 signaling in patients with ALD. Third, it is being increasingly recognized that excess lipid accumulation in the liver promotes inflammatory cell infiltration, thereby stimulating local inflammation and eventually liver injury. Because decreased fat accumulation by mTORC1 inhibition enables us to examine its impact on hepatic inflammation, we find that pharmacological inhibition of mTORC1 not only protects against hepatosteatosis but also ameliorates alcoholic liver injury, possibly through lowered hepatic lipids, promoted hepatocyte survival, and reduced hepatic neutrophil infiltration and inflammation. Therefore, dysregulation of DEPTOR–mTORC1 signaling may represent a mechanism for the pathogenesis of ALD.

INHIBITION OF DEPTOR MAY REPRESENT A MOLECULAR MECHANISM RESPONSIBLE FOR ALCOHOL-INDUCED METABOLIC REPROGRAMMING

One of the most important findings of the present study is that mTORC1 is responsible for the alcohol-mediated metabolic switch from fatty acid oxidation toward fatty acid synthesis. The current study has used in vivo molecular and pharmacological approaches to manipulate DEPTOR–mTORC1 signaling: (1) gain of function of DEPTOR in livers of chronic-binge ethanol-fed mice, (2) pharmacological inhibition of mTORC1 with rapamycin, (3) loss of function of hepatic S6K1, and (4) SIRT1 LKO mice. These combined studies suggest that alcohol-induced de novo lipogenesis is, at least partially, mediated through the disruption of DEPTOR and activation of mTORC1 and S6K1. Our in vivo and in vitro data indicate that inhibition of mTORC1 by DEPTOR counteracts the ability of ethanol to stimulate SREBP-1-dependent lipogenesis. The present study identifies a functional connection between DEPTOR and SREBP-1 in hepatocytes at multiple levels. First, our critical finding with high clinical significance is that hepatic DEPTOR levels inversely correlate with SREBP-1 activity in patients with ALD, suggesting that individuals with lower levels of DEPTOR may be predisposed to the deleterious consequence of excess alcohol consumption. Conversely, DEPTOR-dependent inhibition of mTORC1 is sufficient to eliminate the proteolytic processing, nuclear translocation, and transcriptional activity of SREBP-1 in chronic-binge ethanol-fed mice, the mouse model that nearly resembles the pathological features of human ALD. Second, recent studies show that mTORC1 directly phosphorylates lipin 1, the key enzyme for diacylglycerol synthesis, and promotes nuclear exclusion and cytoplasmic accumulation of lipin 1 and that mTORC1 inhibition decreases SREBP-1 processing in a lipin 1-dependent fashion. As demonstrated in animals treated with rapamycin, mTORC1 is necessary for the cytoplasmic accumulation of lipin 1 and nuclear translocation of SREBP-1 in response to alcohol feeding. The regulation of SREBP-1 by DEPTOR is further supported by our findings that exogenous expression of DEPTOR reduces SREBP-1 activity, possibly by repressing S6K1. Interestingly, alcohol-mediated stimulation of lipin 1 acts through an S6K1-independent mechanism. Finally, because the effect of mTORC1 inhibition on SREBP-1 in ethanol-fed mice shares some features seen in mice with liver-specific knockout of SCAP, the major lipid sensor that regulates SREBP-1 processing, we suspect that SCAP may be involved in the effect of DEPTOR on SREBP-1. Therefore, the molecular mechanisms by which DEPTOR down-regulates SREBP-1 activity are more complex than originally envisioned. It is likely that DEPTOR
cooperates with mTORC1 and S6K1 to control SREBP-1 processing by modulating distinct regulators.

**DEFECTIVE SIRT1 IS FUNCTIONALLY LINKED TO DEPTOR INHIBITION IN MICE AND HUMANS WITH ALD**

An intriguing observation in the present study is that defective SIRT1 is coupled to the dysregulation of DEPTOR–mTORC1–S6K1 signaling in hepatocytes exposed to ethanol and in patients with ALD. We previously identified that SIRT1 stimulates adenosine monophosphate–activated protein kinase (AMPK) and lowers triglyceride accumulation in hepatocytes in insulin-resistant states. We have generated mice with overexpression of hepatic SIRT1 and liver-specific deletion of SIRT1 using the Cre/LoxP system. These mice show opposite phenotypes with regard to lipid homeostasis. Hepatic overexpression of SIRT1 attenuates fatty liver and glucose intolerance in obese, diabetic mice. In contrast, SIRT1 KO in hepatocytes of mice reduces the hepatocyte-derived hormone fibroblast growth factor 21 (FGF21) and accelerates the development of fatty liver caused by fasting. Because recent studies show that SIRT1 expression is reduced in patients with ALD, the current study further explores whether SIRT1 loss promotes lipid accumulation associated with ALD. SIRT1 LKO mice develop a more severe hepatic steatosis phenotype than control mice upon alcohol feeding, accompanied by diminished DEPTOR and activated mTORC1–S6K1 signaling. These defects converge to contribute to increased lipogenesis and impaired fatty acid oxidation, as evidenced by aberrant activation of SREBP-1 and lipin 1 as well as repression of PPARz and PGC-1z. In support of these findings, the overall triglyceride content of the liver, a well-established indicator of lipotoxicity, is profoundly increased in SIRT1 LKO mice; alterations in lipid metabolism could contribute to pathological features of ALD including hepatocyte death, neutrophil infiltration, and inflammation. Although recent studies by the You group indicate that ethanol-mediated impairment of hepatic SIRT1 signaling through lipin 1 contributes to the development of fatty liver, our studies provide additional evidence that loss of SIRT1 may further decrease alcohol-mediated reduction of DEPTOR levels to accelerate the development of ALD. While this regulation appears to contribute to alcohol-induced lipid metabolic disturbance, the precise mechanism for the link between SIRT1 and DEPTOR is not understood. The regulation of DEPTOR stability involves its phosphorylation, ubiquitination, and degradation. Given the fact that mTOR kinase regulates the phosphorylation and ubiquitin-proteasome degradation of DEPTOR, future studies are needed to elucidate the mechanistic link between SIRT1 and DEPTOR by determining whether SIRT1 directly binds to DEPTOR itself or whether SIRT1 affects DEPTOR function through these modulations or through a cofactor that functions cooperatively with DEPTOR.

In conclusion, the integrated defects of SIRT1 and DEPTOR may represent a mechanism responsible for the pathogenesis of ALD in mice and humans. Disruption of SIRT1 and DEPTOR plays a causal role in alcohol-induced mTORC1 activation, hepatocyte lipogenesis, and apoptosis. Therapeutically, pharmacological and molecular inhibition of mTORC1 with rapamycin and DEPTOR overexpression ameliorates alcoholic steatosis, inflammation, and acute-on-chronic liver injury. Therefore, targeting SIRT1 and DEPTOR could be of therapeutic potential for treating ALD in humans.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29849/suppinfo.