Aberrant deposition of fat including free fatty acids in the liver often causes damage to hepatocytes, namely lipotoxicity, which is a key pathogenic event in the development and progression of fatty liver diseases. This study demonstrates a pivotal role of sphingosine kinase 1 (SphK1) in protecting hepatocytes from lipotoxicity. Exposure of primary murine hepatocytes to palmitate resulted in dose-dependent cell death, which was enhanced significantly in SphK1-deficient cells. In keeping with this, expression of dominant-negative mutant SphK1 also markedly promoted palmitate-induced cell death. In contrast, overexpression of wild-type SphK1 profoundly protected hepatocytes from lipotoxicity. Mechanistically, the protective effect of SphK1 is attributable to suppression of ER stress-mediated pro-apoptotic pathways, as reflected in the inhibition of IRE1α activation, XBP1 splicing, JNK phosphorylation, and CHOP induction. Of note, SphK1 inhibited the IRE1α pathway by reducing IRE1α expression at the transcriptional level. Moreover, S1P mimicked the effect of SphK1, suppressing IRE1α expression in a receptor-dependent manner. Furthermore, enforced overexpression of IRE1α significantly blocked the protective effect of SphK1 against lipotoxicity. Therefore, this study provides new insights into the role of SphK1 in hepatocyte survival and uncovers a novel mechanism for protection against ER stress-mediated cell death.

Non-alcoholic fatty liver disease (NAFLD)5 has emerged as a substantial public health concern worldwide. The disease currently affects 20–35% of the general population in Western countries, and 10% of patients can progress to more severe conditions, including steatohepatitis, cirrhosis, and liver failure (1). Early-stage NAFLD features aberrant deposition of lipids in the liver. Specifically, the content of intracellular free fatty acids (FFAs) in hepatocytes correlates with the severity of NAFLD (2, 3). There is extensive evidence that the accumulation of intracellular FFAs is inherently toxic to hepatocytes, provoking endoplasmic reticulum (ER) stress and leading to cell death, namely lipotoxicity. Hepatic lipotoxicity is regarded as a key characteristic of liver injury during the development and progression of NAFLD (2, 3). In response to ER stress, cells activate a series of signaling pathways that are collectively termed the unfolded protein response (UPR). The UPR is initiated via three canonical ER stress biosensors, including PKR-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and transcription factor 6 (ATF6). Activation of the UPR culminates in either adaptive regulation that overcomes the stress or the deleterious outcome of apoptosis (4, 5).

IRE1α is an atypical ER resident transmembrane protein possessing both kinase and ribonuclease properties (6, 7). Upon ER stress, IRE1α is activated by its oligomerization and transautophosphorylation. Acting as a ribonuclease, the active IRE1α is able to excise the mRNA of transcription factor X-box DNA binding protein 1 (XBP1), resulting in transcriptional up-regulation of several sets of genes, including ER chaperones (e.g. GRP78), ER-associated degradation-regulated genes (e.g. EDEM1) and pro-death transcription factors such as CHOP (2, 8). In addition, by binding to tumor necrosis factor receptor-associated factor 2 (TRAF2), IRE1α can activate apoptosis signal-regulating kinase 1 (ASK1), leading to activation of the JNK-mediated pro-apoptotic pathways (2, 4). Therefore, activation of the IRE1α arm of the UPR can either alleviate ER stress, promoting cell survival, or induce cell death via activation of the JNK and CHOP pathways. Although substantial evidence suggests that activation of JNK and CHOP is a key mechanism responsible for hepatic lipotoxicity (2, 4), the role of IRE1α has yet to be defined.

The signaling enzyme sphingosine kinase (SphK) that catalyzes sphingosine phosphorylation to generate sphingosine 1-phosphate (S1P) has been implicated broadly in various diseases, including cancer, atherosclerosis, and metabolic disor-
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Cell Viability and Cell Death Assays—Cell viability was measured by the colorimetric MTS assay as described previously (22). For cell death assays, cells were stained with propidium iodide (Sigma) for 20 min (Life Technologies), followed by flow cytometry analysis.

Immunoblot Analysis—Immunoblot assays were conducted according to the standard protocol with the following primary antibodies: anti-IRE1α (catalog no. 3294), anti-phospho-eIF2α (catalog no. 3597), anti-total eIF2α (catalog no. 2103), anti-CHOP (catalog no. 5554), anti-PARP (catalog no. 9532), and anti-GRP78 (catalog no. 3177) (Cell Signaling Technology); anti-phospho-JNK (catalog no. SC-6254) and anti-total JNK (catalog no. SC-571) (Santa Cruz Biotechnology); anti-phospho-IRE1α (catalog no. ab124945) and anti-calnexin (catalog no. ab22595) (Abcam); and anti-FLAG (catalog no. F1804) and anti-β-actin (catalog no. P2103) (Sigma).

Real-time and Conventional PCR—Total RNA was extracted from cells using TRizol (Life Technologies) according to the protocol of the manufacturer. RNA concentration was estimated using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and 1 μg of total RNA was reverse-transcribed using high-capacity cDNA reverse transcription kits (Applied Biosystems). Quantification of mRNA levels was performed with a Rotor-Gene 6000 real-time PCR machine (Qiagen) using SYBR Green (Bio-Rad). Mouse Xbp1 was amplified from cDNA by conventional PCR with T100™ thermal cycler (Bio-Rad) using RBC TaqDNA polymerase (RBC Bioscience). For detection of Xbp1 mRNA splicing, PCR products were subjected to 2% agarose gel. For measuring the half-life (t1⁄2) of IRE1α mRNA, time course assays were performed in the presence of 5 μM actinomycin D (Calbiochem) as described previously (23).

Reporter Gene Assays—Huh7 hepatocytes were transfected with a luciferase reporter plasmid that was constructed with the 5′-flanking region (from −614 to +252) of the Ire1a gene (24) together with the Renilla luciferase vector pRLSV (Promega, Madison, WI), which served as an internal control for determining transfection efficiency. 24 h post-transfection, cells were washed, cultured for an additional 4 h in serum-free medium, and treated as indicated. For reporter assays, the treated cells were lysed using passive lysis buffer, and the reporter gene activity was determined by the Dual-Luciferase assay system (Promega) according to the instructions of the manufacturer instructions.

Measurement of Sphingolipids—Hepatocytes were homogenized in lipid extraction buffer containing isopropanol/water/ethyl acetate (30:10:60, v/v). Following the addition of an internal standard mixture (including C17-ceramide, C17-sphingosine, and C17-S1P) to homogenates, the organic solvent was evaporated in a SpeedVac system (Thermo). The dry lipid extracts were reconstituted in the HPLC mobile phase containing 1 mM ammonium formate and 0.2% (v/v) formic acid in a mixture of methanol and deionized water (80:20, v/v). The content of sphingolipids was quantified relative to external standards using HPLC-MS/MS as described previously (22).

Statistical Analysis—All data are expressed as mean ± S.D. and represent at least three independent experiments. Comparisons between multiple groups were analyzed with two-way...
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**Results**

**SphK1 Protects Hepatocytes against Lipotoxicity**—In agreement with previous reports (20, 25), lipotoxicity was clearly observed in primary murine hepatocytes exposed to palmitate for 24 h, as reflected in a dose-dependent increase in cell death (Fig. 1A). Notably, the primary hepatocytes isolated from Sphk1<sup>-/-</sup> mice exhibited a significant increase in palmitate-induced apoptosis compared with WT cells (Fig. 1A), suggesting a pro-survival effect of SphK1 in hepatocytes undergoing lipotoxic stress. To further address the protective effect of SphK1, we manipulated SphK1 expression in Huh7 hepatocytes stably overexpressing the gene encoding either WT SphK1 (SphK1<sup>WT</sup>) or a dominant-negative mutant, SphK1<sup>G82D</sup>. Remarkably, overexpression of SphK1<sup>WT</sup> significantly abrogated palmitate-induced cell death, whereas SphK1<sup>G82D</sup> profoundly potentiated cells to lipotoxicity compared with control cells transfected with an empty vector (Fig. 1B). Moreover, we applied a siRNA-based strategy to test whether the effect of SphK1 is isofrom-specific. As shown in Fig. 1C, the expression levels of SphK1 and SphK2 were effectively knocked down by siRNA targeting of each isoenzyme in Huh7 hepatocytes. In keeping with the data from Sphk1<sup>-/-</sup> hepatocytes, the siRNA-mediated knockdown of SphK1 significantly promoted cell death in palmitate-treated cells (Fig. 1D). In contrast, knockdown of SphK2 significantly attenuated palmitate-induced cell death (Fig. 1D), indicating an isoform-specific effect of SphK1. Taken together, these data demonstrate an important role of SphK1 in protecting hepatocytes against lipotoxicity.

**SphK1 Inhibits Lipotoxicity via Its Effect on the UPR**—The ER stress response, especially activation of the CHOP-dependent UPR pathway, is regarded as a key mechanism responsible for lipotoxicity in hepatocytes (2, 4). As expected, exposure of Huh7 cells to palmitate resulted in a time-dependent increase in CHOP expression and PARP cleavage (Fig. 2A). In line with this, treatment of Huh7 cells with palmitate resulted in a time-dependent increase in CHOP expression (Fig. 2A). Correspondingly, cleavage of PARP, an effector of apoptosis downstream of the CHOP pathway, occurred at the same time points of palmitate treatment (Fig. 2A). Notably, overexpression of SphK1<sup>WT</sup> significantly inhibited, whereas SphK1<sup>G82D</sup> enhanced, palmitate-induced CHOP expression and PARP cleavage (Fig. 2A), suggesting that the protective effect of SphK1 is attributable to suppression of the CHOP pathway.

Our previous study has demonstrated that cellular inhibitor of apoptosis protein 1 (cIAP1) is a specific E3 ligase promoting CHOP ubiquitination and degradation (26). In line with this, treatment of Huh7 hepatocytes with palmitate resulted in a significant down-regulation of cIAP1 expression (Fig. 2A). Consistent with the effect of SphK1 on CHOP expression, palmitate-induced reduction of cIAP1 was prevented by overexpression of SphK1<sup>WT</sup> but potentiated by SphK1<sup>G82D</sup> (Fig. 2A). The data reveal an association between cIAP1 and CHOP expression, which is regulated by SphK1 in hepatocytes under lipotoxic stress.
overexpression of SphK1WT slightly attenuated the palmitate-treatment caused a similar time-dependent increase in the level of total IRE1 expression was also increased by palmitate-induced phosphorylation of IRE1 was not significantly different in palmitate-treated cells with untreated cells. Remarkably, overexpression of SphK1WT prevented palmitate-induced increases in both total and phosphorylated IRE1α. In contrast, SphK1G82D markedly facilitated the effect of palmitate on up-regulation of IRE1α expression. The data indicate that SphK1 has little effect on activation of the PERK-eIF2α pathway, which is therefore unlikely to be responsible for the SphK1-induced suppression of CHOP.

SphK1 Suppresses the IRE1α Arm of the UPR—IRE1α is another key regulator of the UPR. As expected, palmitate-induced ER stress in hepatocytes resulted in a time-dependent increase in phosphorylation of IRE1α (Fig. 3A and B). Of note, the expression level of total IRE1α was also increased by palmitate treatment in a similar time-dependent manner. As such, the ratio of phosphorylated to total IRE1α was not significantly different in palmitate-treated cells compared with untreated cells (Fig. 3B). Remarkably, overexpression of SphK1WT suppressed XBP1 mRNA, a direct target of the ribonuclease IRE1α. In keeping with the changes in IRE1α, palmitate treatment resulted in a time-dependent increase in XBP1 mRNA splicing (Fig. 4A). The PALM-induced XBP1 splicing was suppressed profoundly by SphK1WT but potentiated by SphK1G82D (Fig. 4, A and B). The spliced XBP1 serves as an active transcription factor, promoting expression of a variety of ER function-related genes, including EDEM1 and CHOP. Consistent with the pattern of CHOP expression, palmitate treatment caused a similar time-dependent increase in the level of EDEM1 mRNA (Fig. 4C). Furthermore, overexpression
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FIGURE 5. SphK1 inhibits IRE1α expression. A and B, levels of IRE1α mRNA were evaluated by real-time quantitative RT-PCR in (A) Huh7 cells stably transfected with an empty vector (EV), SphK1WT, or SphK1G82D and (B) Sphk1−/− or WT primary murine hepatocytes treated with palmitate (PA, 500 μM) for the indicated time. C, the stably transfected Huh7 cells were treated with 5 μg/ml actinomycin D for 0, 1, 2, and 3 h in the presence or absence of palmitate (500 μM), and then IRE1α mRNA expression levels and its half-lives (t1/2) were analyzed. Veh, vehicle. D, Sphk1WT, Sphk1G82D, or control-transfected Huh7 cells were cotransfected with an ire1α luciferase reporter plasmid together with a Renilla control vector. 24 h after transfection, cells were treated with palmitate (500 μM) for an additional 8 h, and then reporter gene activity was determined by Dual-Luciferase assays and normalized relative to Renilla luciferase activity. Data are shown as mean ± S.D. (n = 3). *, p < 0.05; **, p < 0.01; ***, p < 0.001; n.d., no difference. Veh, vehicle; EV, empty vector.

FIGURE 6. SphK1 regulates sphingolipid metabolism in hepatocytes. A and B, Sphk1−/− or WT primary murine hepatocytes (A) and Sphk1WT, Sphk1G82D, or the control transfected Huh7 cells (B) were treated with 500 μM palmitate (PA) for 24 h. Then levels of ceramide mass, sphingosine, and S1P were determined by HPLC-MS/MS in the treated hepatocytes. Data are shown as mean ± S.D. (n = 3). *, p < 0.05; **, p < 0.01; ***, p < 0.001; n.d., no difference. Veh, vehicle; EV, empty vector.

SphK1WT significantly reduced, whereas Sphk1G82D promoted, palmitate-induced increases in Ire1α promoter activity compared with control-transfected cells (Fig. 5D). Taken together, these data demonstrate a role of SphK1 in suppression of IRE1α at the transcriptional level in stressed hepatocytes.

SphK1 Regulates Sphingolipid Metabolism in Hepatocytes—Because palmitate is a major precursor for de novo synthesis of sphingolipids, its lipotoxicity is suggested to be attributable to changes in sphingolipid metabolism (11, 27). To clarify whether the effect of SphK1 on palmitate-induced ER stress and lipotoxicity is linked through the sphingolipid metabolic pathway, we examined the production of several key metabolites related to SphK enzymatic activity in hepatocytes. Exposure of primary WT hepatocytes to palmitate resulted in a significant increase in contents of ceramide mass and, to less extent, S1P, but no change in sphingosine, compared with untreated cells (Fig. 6A). Notably, Sphk1−/− hepatocytes displayed a nearly 50% reduction of S1P and a slight increase in sphingosine content compared with WT cells treated with or without palmitate. However, there was no significant change in the levels of ceramides between WT and Sphk1−/− hepatocytes (Fig. 6A). In line with the observations from primary hepatocytes, Huh7 cells overexpressing Sphk1WT resulted in a significant increase in S1P production, along with reduced sphingosine content, compared with control-transfected cells in the presence or absence of palmitate (Fig. 6B). By contrast, Sphk1G82D significantly inhibited S1P production and marginally enhanced levels of ceramide and sphingosine compared with control Huh7 cells (Fig. 6B). Taken together, the data reveal a critical role of SphK1 in controlling sphingolipid metabolism in hepatocytes exposed to palmitate.

SIP Suppresses IRE1α Expression—Given the effect of SphK1 on the sphingolipid metabolic pathway in hepatocytes, we then...
tested whether SphK-related sphingolipid metabolites might regulate IRE1α expression. Treatment with S1P resulted in a significant reduction of IRE1α expression in both palmitate-treated and untreated hepatocytes. In contrast, ceramide significantly elevated IRE1α expression, whereas sphingosine had no effect (Fig. 7A), indicating a specific effect of S1P on the suppression of IRE1α. To further verify whether the effect of S1P is receptor-dependent or -independent, we utilized p-FTY720 and dihydro-S1P, two S1P analogues that activate all five members of S1P receptors (except p-FTY720 has no effect on S1P2) but have no significant intracellular effects (28). Interestingly, both p-FTY720 and dihydro-S1P had a similar effect as S1P on the suppression of IRE1α expression in palmitate-treated or untreated hepatocytes (Fig. 7B), suggesting a S1P receptor-mediated action. Moreover, S1P treatment profoundly prevented the elevated expression of IRE1α in Spkh1−/− hepatocytes in the presence or absence of palmitate (Fig. 7C), further demonstrating S1P to be critical for SphK1-regulated IRE1α expression.

**IRE1α Suppression Is Required for the Protective Effect of SphK1 Against Lipotoxicity**—Having demonstrated the effects of SphK1 on IRE1α expression and cell survival, we wanted to determine whether a causative relationship exists between these two related events. To this end, we manipulated IRE1α expression by enforced overexpression of the gene to compensate for the SphK1-induced reduction of IRE1α under lipotoxic stress. In keeping with the data shown in Fig. 2A, palmitate-induced CHOP expression and PARP cleavage were inhibited significantly in hepatocytes overexpressing SphK1WT (Fig. 8A), suggesting a SphK1-mediated action. Moreover, S1P treatment profoundly reversed the SphK1-induced suppression of CHOP expression and PARP cleavage in hepatocytes exposed to lipotoxic stress (Fig. 8B).
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Palmitate. Accordingly, although overexpression of SphK1\textsuperscript{WT} significantly inhibited palmitate-induced cell death, the protective effect was blocked markedly by overexpression of IRE1\textalpha (Fig. 8C). Collectively, the data indicate that SphK1-induced IRE1\textalpha suppression is responsible for protecting hepatocytes from ER stress-mediated lipotoxicity.

**Discussion**

In this study, we provided experimental evidence showing a potent protective effect of SphK1 against ER stress-mediated lipotoxicity in hepatocytes. Mechanistically, we demonstrated a critical role of IRE1\textalpha suppression for stressed cell survival, as reflected in the following findings. The toxic saturated FFA palmitate up-regulates IRE1\textalpha expression, which contributes to ER stress-mediated lipotoxicity. SphK1 suppresses palmitate-induced IRE1\textalpha expression and protects hepatocytes against lipotoxicity. Enforced overexpression of IRE1\textalpha abolishes the cytoprotective effect of SphK1. Therefore, we conclude that SphK1 protects hepatocytes from lipotoxicity by, at least in part, suppressing IRE1\textalpha expression.

The anti-apoptotic property of SphK1 has been well demonstrated in a wide variety of cell types (12, 29). We have reported recently that SphK1 protects pancreatic \( β \) cells against lipotoxicity both *in vitro* and *in vivo* (22). Congruent with this, SphK1 is also required for survival of hepatocytes undergoing lipotoxic stress. We found that SphK1-deficient primary hepatocytes exhibited a significantly higher susceptibility to lipotoxicity compared with wild-type cells (Fig. 1A). Furthermore, overexpression of SphK1\textsuperscript{WT} profoundly protected hepatocytes from palmitate-induced cell death. In contrast, the dominant-negative mutant SphK1\textsuperscript{G82D} or siRNA-mediated knockdown of SphK1 significantly sensitized hepatocytes to lipotoxicity (Fig. 1B). Collectively, these data clearly illustrate a protective effect of SphK1 against lipotoxicity in hepatocytes. It was noted that knockdown of SphK2 by its specific siRNA significantly rescued hepatocytes from palmitate-induced cell death (Fig. 1B), indicating an isoform-specific effect of SphK1 in promoting hepatocyte survival.

Lipotoxicity resulting from the accumulation of FFA in hepatocytes is regarded as a key pathogenic event in fatty liver diseases, including NAFLD. It is believed that hepatic lipotoxicity is mediated through ER stress and activation of the UPR pathways (2, 4). The UPR consists of multiple signaling pathways that are initiated via three major proximal sensors of ER stress, including PERK, IRE1, and ATF6, of which the IRE1 axis of the UPR is the most evolutionarily conserved (4, 30). In response to ER stress, IRE1\textalpha is activated upon its oligomerization and *trans*-autophosphorylation, leading to splicing of XBP1 mRNA and formation of active XBP1. The IRE1\textalpha-XBP1 axis transcriptionally up-regulates several sets of genes that are implicated in protein folding, secretion and translocation, resulting in adaptation to ER stress (31, 32). On the other hand, the active form of XBP1 induces CHOP expression, promoting cell death under certain conditions (30, 33). In addition, activation of IRE1\textalpha can trigger apoptosis by activation of JNK (34) or its interaction with Bax/Bak (35). Because of these simultaneous actions, the role of IRE1\textalpha has not been fully elucidated for the survival of hepatocytes undergoing ER stress.

This study demonstrates that palmitate induces activation of the IRE1\textalpha axis of the UPR in hepatocytes, as evidenced by the increases in phosphorylation of IRE1\textalpha, splicing of XBP1 mRNA, phosphorylation of JNK, and up-regulation of EDEM1 and CHOP expression. Of note, besides the increased level of IRE1\textalpha phosphorylation, total levels of IRE1\textalpha expression were also increased significantly by palmitate treatment, and, therefore, the ratio of phosphorylated to total IRE1\textalpha was unchanged (Fig. 3). This finding suggests that, in addition to the posttranscriptional regulation (*e.g.* phosphorylation) of IRE1\textalpha, transcriptional regulation of the gene expression is another important event for activation of the IRE1\textalpha pathway. Indeed, up-regulation of IRE1\textalpha in the liver undergoing ER stress has been demonstrated by several experimental models both *in vivo* and *in vitro*. Intraperitoneal injection of a chemical ER stressor, tunicamycin, induces a considerable increase in IRE1\textalpha protein expression in the liver (36). Treatment with a chemotherapeutic agent, sorafenib, elevates IRE1\textalpha expression in a variety of human hepatocellular carcinoma lines, including MHCC97-L, PLC/PRF/5, and HepG2 (37). In addition, it has been reported that levels of IRE1\textalpha mRNA and protein expression are increased in palmitate-treated HepG2 cells (38, 39). In this study, we were able to confirm that palmitate treatment induces a significant up-regulation of IRE1\textalpha expression in both human Huh7 cells and primary murine hepatocytes (Figs. 3 and 5). Of importance is that we found that suppression of IRE1\textalpha expression is associated with the protective effect of SphK1 against lipotoxicity in hepatocytes. Overexpression of SphK1\textsuperscript{WT} completely abrogated, whereas SphK1\textsuperscript{G82D} significantly potentiated, the up-regulation of IRE1\textalpha in hepatocytes exposed to palmitate (Figs. 3 and 5). Moreover, Sphk1 deficient primary hepatocytes that are more sensitive to lipotoxicity exhibited a higher level of IRE1\textalpha expression in response to palmitate treatment compared with WT hepatocytes (Fig. 5B), further supporting the role of SphK1 in regulating IRE1\textalpha expression. Notably, neither SphK1\textsuperscript{WT} nor SphK1\textsuperscript{G82D} significantly altered the half-lives \((t_{1/2})\) of IRE1\textalpha mRNA (Fig. 5C), implying that SphK1 regulates the transcription, but not the stability, of IRE1\textalpha mRNA. Indeed, the data from *Ire1α* gene reporter assays (Fig. 5D) clearly demonstrated an effect of SphK1 in the transcriptional regulation of *Ire1α* gene expression.

The signaling of SphK1 relies chiefly on its product, S1P, which functions mainly through the receptors, including S1P\textsubscript{1}, S1P\textsubscript{2}, S1P\textsubscript{3}, S1P\textsubscript{4}, and S1P\textsubscript{5} (9, 11). On the other hand, SphK1 acts as a key enzyme in the control of sphingolipid metabolism, which also contributes to the biological function of SphK1. In keeping with previous reports (40, 41), we found that treatment of hepatocytes with palmitate resulted in a significant increase in levels of ceramide mass and S1P but no change in sphingosine. A study using \(^3\)C labeling methods (41) revealed that a palmitate-induced increase in ceramide mass results directly from the enhanced *de novo* synthesis pathway, whereas SphK1 activity appears to be responsible for the increased S1P in palmitate-treated cells. Indeed, we found that, despite considerable alterations in ceramide mass and sphingosine levels in cells either overexpressing or deficient in SphK1, the predominant change induced by SphK1 manipulation is S1P produc-
tion (Fig. 6), which is consistent with our previous report (22). Moreover, although S1P significantly reduces IRE1α expression, ceramide increases its mRNA levels, and sphingosine has no effect, demonstrating a specific role of S1P responsible for the SphK1-mediated suppression of IRE1α expression. Furthermore, the findings that both dihydro-S1P and p-FTY720 are capable of mimicking S1P to suppress IRE1α expression indicate a S1P receptor-dependent effect. Although the precise role of S1P receptors and the specific receptor involved in IRE1α regulation remain to be identified, our findings uncover a new signaling role of the SphK1/S1P axis in regulating IRE1α expression in hepatocytes under lipotoxic stress.

Given the effect of SphK1 in suppressing IRE1α expression and protecting against lipotoxicity, we have further elucidated their mechanistic connections. The findings that overexpression of IRE1α significantly attenuated the protective effect of SphK1 on palmitate-induced CHOP expression, PARP cleavage, and cell death (Fig. 8) indicate that IRE1α suppression is critical for the cytotoxic effect of SphK1. It was, however, noted that IRE1α overexpression failed to completely block SphK1-mediated protection, suggesting that other alternative pathways that transduce ER stress to cellular demise are also influenced by SphK1. Indeed, our prior study has reported that cIAP1 functions as an E3 ubiquitin ligase, promoting CHOP ubiquitination and degradation, thereby protecting ER stress-mediated pancreatic β cell death (26). Moreover, have we reported that the protective effect of SphK1 against lipotoxicity in β cells is attributable, at least partially, to its up-regulation of cIAP1 (22). In line with this finding, we found that cIAP1 was degraded, accompanied by palmitate-induced cell death in hepatocytes, which was prevented by overexpression of SphK1WT but promoted by SphK1G82D (Fig. 2A), supporting the role of cIAP1 in SphK1-promoted cell survival under lipotoxic stress. Furthermore, activation of the PERK axis of the UPR pathway has been shown to potentiate palmitate-induced apoptosis in pancreatic β cells (42). In keeping with this finding, we also observed a significant reduction of eIF2α phosphorylation by SphK1 overexpression in stressed hepatocytes, whereas SphK1G82D had no effect (Fig. 2B). Therefore, it appears that besides suppressing IRE1α, SphK1 inhibits a subset of pro-apoptotic signaling events, protecting hepatocytes from ER stress-mediated lipotoxicity. Nevertheless, when considering a critical role of lipotoxicity in the development and progression of NAFLD, it is reasonably postulated that the pro-survival effect of SphK1, as demonstrated in this study, would help to pave a new way for the management of fatty liver diseases.

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