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Inhibition of SH2-domain-containing inositol 5-phosphatase (SHIP2) ameliorates palmitate induced-apoptosis through regulating Akt/FOXO1 pathway and ROS production in HepG2 cells

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ABSTRACT

The serine–threonine kinase Akt regulates proliferation and survival by phosphorylating a network of protein substrates; however, the role of a negative regulator of the Akt pathway, the SH2-domain-containing inositol 5-phosphatase (SHIP2) in apoptosis of the hepatocytes, remains unknown. In the present study, we studied the molecular mechanisms linking SHIP2 expression to apoptosis using overexpression or suppression of SHIP2 gene in HepG2 cells exposed to palmitate (0.5 mM). Overexpression of the dominant negative mutant SHIP2 (SHIP2-DN) significantly reduced palmitate-induced apoptosis in HepG2 cells, as these cells had increased cell viability, decreased apoptotic cell death and reduced the activity of caspase-3, cytochrome c and poly (ADP-ribose) polymerase. Overexpression of the wild-type SHIP2 gene led to a massive apoptosis in HepG2 cells. The protection from palmitate-induced apoptosis by SHIP2 inhibition was accompanied by a decrease in the generation of reactive oxygen species (ROS). In addition, SHIP2 inhibition was accompanied by an increased Akt and FOXO-1 phosphorylation, whereas overexpression of the wild-type SHIP2 gene had the opposite effects. Taken together, these findings suggest that SHIP2 expression level is an important determinant of hepatic lipoapotosis and its inhibition can potentially be a target in treatment of hepatic lipoapoptosis in diabetic patients.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common liver disease that contributes to a wide range of liver diseases including a simple steatosis to non-alcoholic steatohepatitis (NASH) [1,2]. Hepatic neutral lipid accumulation mainly triacylglycerol is the hallmark of NAFLD. This excessive lipid accumulation in hepatocytes promotes lipotoxicity-induced apoptosis (lipoapoptosis) [3]. In particular, several studies have suggested that saturated free fatty acids (FFAs) such as palmitic acid can induce endoplasmic reticulum (ER) stress and mitochondria mediated lipoapoptosis in hepatocytes. In addition, enhanced hepatic lipoapoptosis is severely associated with infiltration of inflammatory cells and macrophages, inflammatory and fibrotic cytokine release, and fibrogenesis [4]. Therefore, increased hepatocyte apoptosis by saturated FFAs appears to be the major contributor to the development and progression of NAFLD and NASH.

Activation of the phosphoinositide 3-kinase (PI3-K) is a pivotal step in signaling pathways which control many biological functions including anti-apoptosis, cytoskeletal reorganization, chemotaxis, and cell proliferation [5]. Active PI3-K phosphorylates phosphatidylinositol diphosphate (PIP2) and converts it to phosphatidylinositol triphosphate (PIP3). Following stimulation with growth factors or cytokines, enhanced PIP3 levels recruit Akt from the cytosol to the plasma membrane, where it is phosphorylated by PDK1 and another kinase on Thr 308 and Ser 473, respectively [6]. Akt activation leads to phosphorylation of several downstream substrates such as Bcl-2-associated death protein (BAD), glycogen synthase kinase-3 and forkhead transcription factors. These activated proteins can then suppress the apoptotic pathways through multiple downstream effectors [7].

The level of PIP3 is a critical point to regulate several metabolic and non-metabolic events in different cells. PIP3 level is determined by a balance between the generation by PI3-K and degradation by lipid phosphatases such as phosphatase and tensin homolog (PTEN)

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and SH2-domain-containing inositol 5-phosphatase (SHIP2). These lipid phosphatases dephosphorylate PIP3 and convert it to PIP2 [8]. SHIP2 acts as a negative regulator of the insulin signaling both in vitro and in vivo [9]. An increased expression of SHIP2 has been reported from the skeletal muscle and adipose tissues of db/db mice [10,11]. Several in vivo and in vitro studies have demonstrated that the alterations of SHIP2 expression have a major impact on the insulin signaling by modulating the activity of Akt [9,12,13]. More importantly we have recently shown that SHIP2 modulation affects lipid and lipoprotein metabolism via changes in the activity of the insulin signaling pathway [14]. Therefore, given the important role of Akt in regulating both the insulin signaling and cell survival pathways, we in this study proposed that SHIP2 as a modulator of Akt could be involved in regulating apoptosis in hepatocytes. Accordingly, in this study we investigated the role of SHIP2 in apoptosis induced by palmitate in HepG2 cells.

2. Materials and methods

2.1. Cell culture

HepG2 and HEK T293 packaging cells were purchased from the Pasteur Institute of Iran. Cells were maintained at 37 °C (in an atmosphere of 5% CO_2) in DMEM containing 10% FBS, 2 mM glutamine, and 1% penicillin–streptomycin. For palmitate treatment, appropriate amount of palmitae was first dissolved in 50% ethanol at 54 °C and then this solution was added to DMEM containing 1% BSA. In order to conjugate palmitate with BSA, this media was gently shacked at 37 °C for 2 h. After filtration, HepG2 cells were treated with this media for 24 h. BSA (1%) was used as control.

2.2. Retroviral infection

T293 packaging cells were transfected with pBABE-SHIP2 construct (SHIP2-WT), pBABE-dominant negative form of SHIP2 gene (SHIP2-DN) (these vectors were a kind gift from Dr. Christophe Erneux) and pBABE-GFP vector simultaneous with the helper vectors such as PMD2 and PUMVC. Viral supernatants were collected during 48–96 h after transfection. HepG2 cells were infected with the viral supernatants in the presence of 10 μg/ml of polybrene. Infected cells were selectively stabled with 2 μg/ml pouromycin.

2.3. Cell viability assays

Cell viability was measured using the MTT assay, which is based on the conversion of MTT to formazan crystals by mitochondrial dehydrogenases. Additionally, we assessed the cell toxicity using the dead-cell stain propidium iodide (PI). Briefly, for PI assay, the cells were washed twice with PBS and then were stained with PI (7 μM) for 1 h in the dark at room temperature. Thereafter, the samples were analyzed using a FACS can flow cytometery (Becton Dickinson). Data from 10,000 events were analyzed.

2.4. Measurement of intracellular reactive oxygen species (ROS) level

ROS production was assessed after staining the cells with 5-(and 6)-chloromethyl-2′, 7′-dichlorodihydro fluorescein diacetate, acetyl ester (CM-H2-DCFDA). To distinguish living from the dead cells, we used PI as a second indicator dye. Briefly, the cells were grown to reach 50–75% confluence in 6-well plates. After washing the cells with PBS, CM-H2-DCFDA (0.25 μM) and PI (3 mg/ml) was added to each well. Cells were incubated for 10 min at 37 °C and then ROS level and cell viability was simultaneously analyzed by flow cytometry. Only cells negative for PI staining were analyzed for DCFDA-dependent green fluorescence. A total of 10,000 PI negative cells were analyzed for each strain.

2.5. Western blot analysis

Western-blot analysis was carried out according to our previous report [15]. The antibodies used were SHIP2 (R&D system), p-Akt (S473), Akt, p-FOXO-1, cleaved poly (ADP-ribose) polymerase (PARP) [Cleaved PARP (Asp214), Cat# 9541S], cleaved caspase-3, cytochrome c (Cell Signaling Technology, Beverly, MA, USA), and β-actin (Abcam, Cambridge, MA, USA).

2.6. Real-time-PCR

Real time PCR was carried out according to our previous report [16].

2.7. Statistical analyses

All statistical analyses were performed using SPSS18.0 (SPSS, Chicago, IL, USA). Comparisons among all groups were performed with the one-way analysis of variance. Values of $p < 0.05$ were considered statistically significant. Results are expressed as the mean ± SEM.

3. Results

3.1. Generation of functionally active SHIP2 modulated cells

In order to study the role of SHIP2 in hepatocytes apoptosis, we generated SHIP2 stable HepG2 cells which overexpress the wild type (SHIP2-WT) and the dominant negative mutant (SHIP2-DN) of SHIP2 genes. Our results showed that the SHIP2-WT and SHIP2-DN cells had around 2.8 and 3.3 fold more mRNA expression compared to the GFP control cells, respectively (Fig. 1 Supplementary file). Data of mRNA level were confirmed at protein level, as SHIP2-WT and SHIP2-DN cells had 3.5 and 3.8 fold more protein levels compared to control cells, respectively (Fig. 1 Supplementary file).

3.2. Palmitate decreases cell viability and induces apoptosis in HepG2 cells

Because previous studies have reported that palmitate induces apoptosis in various cell types [17–19], we first determined the appropriate dose of palmitate for inducing apoptosis in HepG2 cells. The results of the MTT assay revealed that lower than 0.5 mM of palmitate did not significantly suppressed the viability of HepG2 cells (Fig. 1A), while higher concentrations drastically reduced the cell viability. Therefore, we chose 0.5 mM palmitate for the next experiments. After 24 h of palmitate treatment, the cell viability was reduced by approximately 50% in comparison with the vehicle treated (BSA) control group (Fig. 1A). In addition, 0.5 mM palmitate induced a significant increase (47%) in the apoptotic cell death characterized by PI staining (Fig. 1B).

3.3. SHIP2 inhibition ameliorates palmitate-induced apoptosis in HepG2 cells

Several studies have documented that Akt as a survival factor regulates apoptosis in different cells [20,21]. Since the activity of Akt is controlled by lipid phosphatases such as SHIP2, we then tested whether SHIP2 inhibition affects palmitate-induced apoptosis in HepG2 cells. Our data showed that the inhibition of

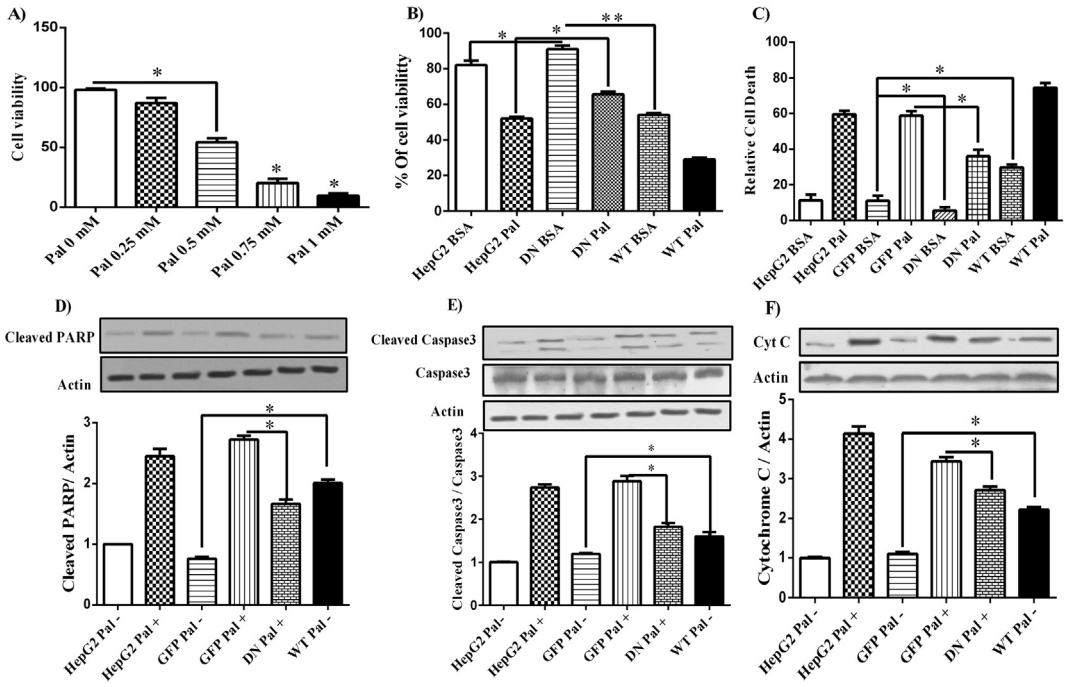

Fig. 1. Analysis of cell viability and apoptotic cell death in SHIP2 modulated cells. A: Cell viability of HepG2 cells after treatment with different concentrations of palmitate for 24 h. HepG2 cell viability was assessed by MTT assay. B: Assessment of the viability of the SHIP2 modulated cells at the presence of 0.5 mM palmitate. C: Determination of the cells death by PI staining. D–F: The effect of SHIP2 modulation on the cleaved PARP, cleaved caspase-3 and cytochrome C protein level. Values are mean ± SEM of at least three independent experiments. *$p < 0.05$ and **$p < 0.001$ vs BSA treated cells. GFP: Green fluorescent protein, Pal: 0.5 mM Palmitate, DN: overexpressed dominant negative SHIP2 gene, WT: overexpressed wild type SHIP2 gene.

SHIP2 via the dominant negative expression significantly protected palmitate-induced reduction in HepG2 cell viability (Fig. 1C). SHIP2 inhibition also could ameliorate palmitate-induced apoptotic cells death by 39% in HepG2 cells (Fig. 2B). On the other hand, SHIP2-WT overexpression per se reduced the cell viability and increases the rate of apoptotic cell death by 30% and 76%, respectively (Fig. 1B–C). These findings suggest that the expression level of SHIP2 is a determinant factor in control of the apoptotic cell death in a lipotoxic condition.

3.4. SHIP2 inhibition attenuates palmitate-induced apoptosis through reduction of caspase-3, cytochrome c and PARP activation

In the subsequent experiments, we analyzed the cleaved caspase-3, the cleavage of PARP and cytochrome c, three well-established hallmarks of apoptosis. Immunoblot results showed that the expression of PARP cleavage significantly increased after 24 h treatment with 0.5 mM palmitate (Fig. 1D). The analysis results of cleaved caspase-3 and cytochrome c were similar to that of the cleaved PARP (Fig. 1E–F).

To address the impact of SHIP2 inhibition on palmitate-induced activation of caspase-3, cytochrome c and PARP, we evaluated the levels of these three proteins in SHIP2 modulated cells. We observed that SHIP2 inhibition significantly reduces the cleaved PARP, cleaved caspase-3 and cytochrome c levels at the presence of 0.5 mM palmitate by 59%, 61% and 32%, respectively (Fig. 1D–F). Interestingly, overexpression of SHIP2-WT could significantly increase the levels of the cleaved PARP (2.5 fold), cleaved caspase-3 (1.6 fold) and cytochrome c (2.0 fold) in comparison with the GFP control cells. Taken together, these data suggest that the effect of SHIP2 modulation on apoptosis is mediated through regulating the activation of the caspase-3, cytochrome c and PARP in HepG2 cells.

3.5. SHIP2 inhibition attenuates palmitate-induced apoptosis through reduction of ROS generation

It has been previously shown that palmitate induces oxidative stress in different cells [18,22]. To investigate whether the effects of SHIP2 on apoptosis is mediated through the ROS production, HepG2 cells were co-incubated with 0.5 mM palmitate and 50 μM ascorbic acid as a ROS scavenger. Ascorbic acid co-treatment reduced ROS level (Fig. 2A) and prevented palmitate-induced apoptosis of HepG2 cells (Fig. 2B). In addition, ascorbic acid co-treatment led to an improvement in the viability of HepG2 cells treated with palmitate (Fig. 2C). As shown in Fig. 2E, the SHIP2-DN cells treated with palmitate had significantly lower ROS production (39%), compared to the control cells. To study the impact of ROS in the apoptotic cell death of the SHIP2-WT cells, we measured the death of these cells by PI staining at the presence of 50 μM ascorbic acid. Interestingly, ascorbic acid could significantly reduce the apoptotic cell death of the SHIP2-WT cells (Fig. 2F) compared to the GFP control cells.

3.6. SHIP2 inhibition prevents palmitate induced-apoptosis via increase in phosphorylation of Akt and FOXO-1

Previous studies have shown a role for the transcription factor forkhead box protein O-1 (FOXO-1) in mediating palmitate-induced apoptosis in hepatocytes [23] and skeletal muscle cells [18]. Akt activation has been shown to increase the FOXO-1 phosphorylation, which results in inhibition of FOXO-1 entry into the nucleus [23]. According to these observations, we first evaluated the Akt phosphorylation in palmitate treated cells. Treatment of HepG2 cells with palmitate resulted in a 41% reduction in phospho-Akt level compared to the controls (Fig. 3A). SHIP2 inhibition reverted this effect; whereas overexpression of the SHIP2-WT resulted in a

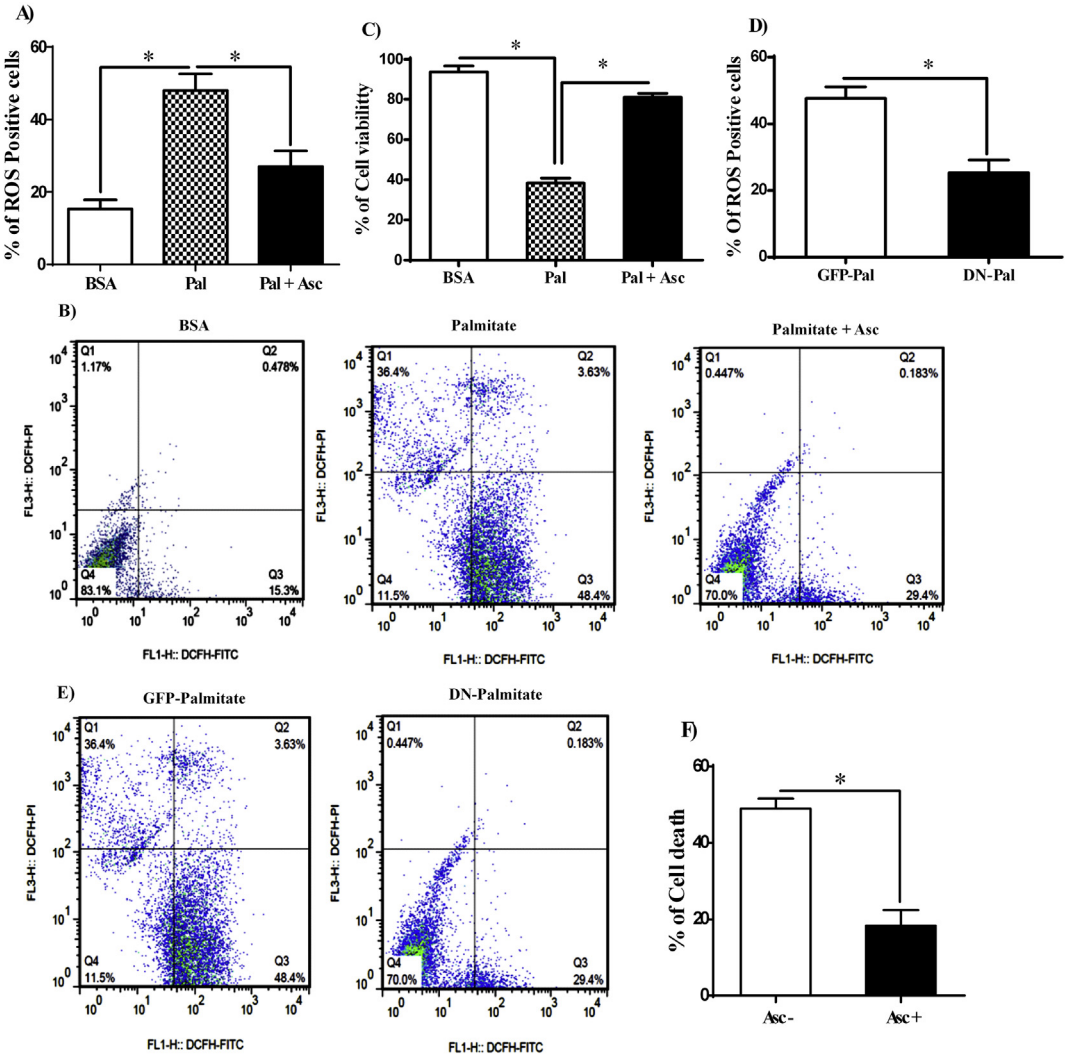

Fig. 2. Determination of ROS level at the presence of palmitate. A: Detection of ROS level after co-treatment of HepG2 cells with 0.5 mM palmitate and 50 μM ascorbic acid (Asc). B: The flow cytometric analysis of ROS production in HepG2 cells using CM-H2-DCFDA and PI double staining. Lower left quadrant (DCFDA−/PI−) represents viable cells with low ROS, lower right quadrant (DCFDA+/PI−) represents viable cells with high ROS, upper left quadrant (DCFDA−/PI+) represents dead cells with low ROS, and upper right quadrant (DCFDA+/PI+) represents dead cells with high ROS. C) Analysis of the cell viability by MTT assay. D: Determination of ROS level in SHIP2-DN cells treated with palmitate, E: The flow cytometric analysis of ROS production in the SHIP2-DN cells after treatment with palmitate. F: Analysis of the death of the SHIP2-WT cells at the presence of ascorbic acid. Values are mean ± SEM of at least three independent experiments. *$p < 0.05$ vs BSA treated cells. GFP: Green Fluorescent Protein, Pal: 0.5 mM Palmitate, DN: overexpressed dominant negative SHIP2 gene, WT: overexpressed wild type SHIP2 gene, Asc: Ascorbic acid.

decrease in phospho-Akt (52%) compared to control cells. Next, we investigated the role of FOXO-1 in palmitate-induced apoptosis by evaluating its phosphorylation in HepG2 cells. We found that palmitate reduces the FOXO-1 phosphorylation in HepG2 cells. Interestingly, SHIP2 inhibition prevented palmitate-reduced FOXO-1 phosphorylation, whereas overexpression of the SHIP2-WT decreased the FOXO-1 phosphorylation in HepG2 cells by 52% (Fig. 3B).

4. Discussion

Non-alcoholic steatohepatitis (NASH), the most extreme form of NAFLD, is a progressive liver disease that is characterized by steatosis and liver inflammation leading to liver cirrhosis and hepatocellular carcinoma. Lipoapoptosis induced by the excessive fat accumulation is the underlying factor leading to the infiltration of inflammatory cells, the release of inflammatory and fibrotic cytokines, and fibrogenesis in the liver [24]. Thus, therapeutic strategies that combat these lipotoxic events could have the beneficial effects on the progression of simple steatosis to NASH. In the present study, we provided evidence that SHIP2 as a negative regulator of the Akt pathway can be considered as a potential target for attenuating the lipoapoptosis in hepatocytes.

A few studies have addressed the role of SHIP2 in the susceptibility of the cells to apoptosis [25,26]. However, the role of SHIP2 in palmitate-induced apoptosis in HepG2 hepatocytes is not well understood. In this study using different approaches we were able to demonstrate that SHIP2 inhibition could prevent palmitate-induced apoptosis in hepatocytess. By contrast, overexpression of the SHIP2-WT gene led to a massive apoptosis in HepG2 cells. Our results are in the line with the data obtained from previous studies in brain [26] and podocytes [25]. Soed et al. have shown that the number of apoptosis-positive cells was increased in cerebral cortex of the transgenic mice overexpressing SHIP2 [26]. In another study, it was reported that overexpression of SHIP2 in cultured podocytes promotes apoptosis via reduction in Akt phosphorylation [25]. By contrast, SHIP2 inhibition in INS1E insulinoma cells did not prevent palmitate-induced apoptosis in spite of an increased Akt activity

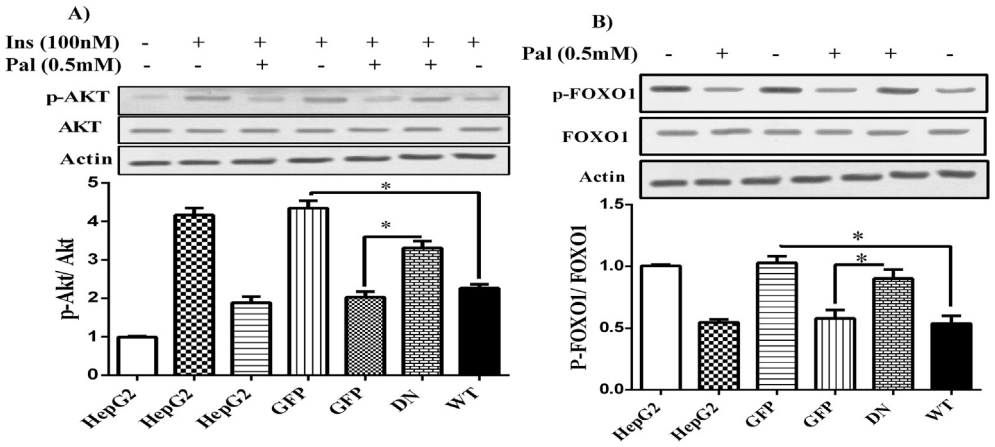

Fig. 3. Effect of SHIP2 modulation on Akt and FOXO-1 phosphorylation. A: The phosphorylation levels of Akt. B: The phosphorylation levels of FOXO-1. Values are mean ± SEM of at least three independent experiments. *p < 0.001 vs BSA treated cells. GFP: Green Fluorescent Protein, Pal: 0.5 mM Palmitate, DN: overexpressed dominant negative SHIP2 gene, WT: overexpressed wild type SHIP2 gene.

[27]. In addition, it has been reported that SHIP2 did not induce apoptosis of OPM2 human myeloma line [28]. The reasons for discrepancies between the studies are not clear, but it could be attributed to different cell types and/or inducers of apoptosis used.

The mechanism by which SHIP2 influences lipoapoptosis in HepG2 cells is most likely regulated via the changes in the activity of the Akt/FOXO-1 pathway. Akt, a serine/threonine kinase, is phosphorylated by PI3-K [18]. Once phosphorylated and activated, Akt can regulate a wide range of cellular processes involved in protein synthesis, cell survival, proliferation, and metabolism [29]. Akt regulation of cell survival involves the direct inhibition (phosphorylation) of pro-apoptotic signals such as Bad and FOXOs. Phosphorylation by Akt inactivates FOXOs resulting in their accumulation in the cytoplasm. Therefore, we in this study hypothesized that the effects of SHIP2 inhibition on palmitate-induced apoptosis may be exerted through regulating this pathway. In the present study SHIP2 inhibition prevented palmitate-induced reduction of phospho-Akt, confirming that SHIP2 acts as an upstream regulator of the Akt in these cells. This finding is consistent with previous studies in different cell types such as 3T3-L1 adipocytes, L6 myotubes, C3A hepatoma and INS1E [12,27,30]. All these studies have demonstrated an enhancing Akt phosphorylation following the inhibition of SHIP2. We also observed an increased FOXO-1 phosphorylation in parallel with increased Akt phosphorylation in SHIP2-DN cells treated with palmitate. These data imply that SHIP2 inhibition could prevent the apoptosis of HepG2 cells by regulating the activity of the Akt/FOXO-1 pathway. These events ultimately lead to a decreased nuclear accumulation of FOXO-1 protein resulting in a decreased expression of apoptotic genes in HepG2 cells. According to our data, apoptosis induced by overexpression of the SHIP2-WT appears to be mediated through the modulation of the Akt/FOXO-1 pathway. Overexpression of SHIP2-WT resulted in a decreased FOXO-1 phosphorylation in parallel with decreased Akt phosphorylation in HepG2 cells.

Another mechanism linking SHIP2 to apoptosis is oxidative stress. A growing body of evidence suggests that the oxidative stress due to excessive production of ROS is the main contributor to the pathophysiology of diabetic complications [22,31]. Increasing ROS production has been demonstrated to account for apoptosis in response to palmitate in numerous cell lines [32,33]. However, the role of ROS in stimulating lipoapoptosis appears to be cell-type dependent as palmitate-treated neonatal cardiomyocytes undergo apoptosis independently of the oxidative stress [34]. In the current study, ROS production was identified at increased levels following palmitate-induced apoptosis in HepG2 cells, consistent with previous studies [22,33,35]. We also found that ascorbic acid co-treatment simultaneously normalized palmitate-induced ROS accumulation and the apoptotic cell death indicating that apoptosis initiation is dependent on ROS accumulation in HepG2 cells. In addition, SHIP2 modulation was able to affect the status of the oxidative stress in HepG2 cells. Overexpression of the SHIP2-DN gene led to a reduction in ROS level in the cells treated with palmitate. Furthermore, ascorbic acid, a ROS scavenger, could prevent the apoptotic death of HepG2 cells overexpressing the SHIP2-WT gene. These findings imply that the alterations in the oxidative status and ROS production may be the link between SHIP2 modulation and apoptosis in HepG2 cells.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.134.

Transparency document

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References

[1] G.C. Farrell, C.Z. Larter, Nonalcoholic fatty liver disease: from steatosis to cirrhosis, Hepatology 43 (2006) S99–S112.
[2] R. Meshkani, K. Adeli, Mechanisms linking the metabolic syndrome and cardiovascular disease: role of hepatic insulin resistance, J. Tehran Univ. Heart Cent. 4 (2009) 77–84.
[3] S.H. Ibrahim, Y. Akazawa, S.C. Cazanave, S.F. Bronk, N.A. Elmi, N.W. Werneburg, D.D. Billadeau, G.J. Gores, Glycogen synthase kinase-3 (GSK-3) inhibition attenuates hepatocyte lipoapoptosis, J. Hepatol. 54 (2011) 765–772.
[4] B. Vick, A. Weber, T. Urbanik, T. Maass, A. Teufel, P.H. Krammer, J.T. Opferman, M. Schuchmann, P.R. Galle, H. Schulze-Bergkamen, Knockout of myeloid cell leukemia-1 induces liver damage and increases apoptosis susceptibility of murine hepatocytes, Hepatology 49 (2009) 627–636.
[5] D.A. Fruman, R.E. Meyers, L.C. Cantley, Phosphoinositide kinases, Annu. Rev. Biochem. 67 (1998) 481–507.

[6] D.R. Alessi, M. Andjelkovic, B. Caudwell, P. Cron, N. Morrice, P. Cohen, B. Hemmings, Mechanism of activation of protein kinase B by insulin and IGF-1, EMBO J. 15 (1996) 6541.
[7] L. Wang, P. Gai, R. Xu, Y. Zheng, S. Lv, Y. Li, S. Liu, Shikonin protects chondrocytes from interleukin-1beta-induced apoptosis by regulating PI3K/Akt signaling pathway, Int. J. Clin. Exp. Pathol. 8 (2015) 298.
[8] M. Vinciguerra, M. Foti, PTEN and SHIP2 phosphoinositide phosphatases as negative regulators of insulin signalling, Arch. Physiol. Biochem. 112 (2006) 89–104.
[9] M.W. Sleeman, K.E. Wortley, K.-M.V. Lai, L.C. Gowen, J. Kintner, W.O. Kline, K. Garcia, T.N. Stitt, G.D. Yancopoulos, S.J. Wiegand, Absence of the lipid phosphatase SHIP2 confers resistance to dietary obesity, Nat. Med. 11 (2005) 199–205.
[10] H. Hori, T. Sasaoka, H. Ishihara, T. Wada, S. Murakami, M. Ishiki, M. Kobayashi, Association of SH2-containing inositol phosphatase 2 with the insulin resistance of diabetic db/db mice, Diabetes 51 (2002) 2387–2394.
[11] S. Gorgani-Firuzjaee, S. Ahmadi, R. Meshkani, Palmitate induces SHIP2 expression via the ceramide-mediated activation of NF-κB, and JNK in skeletal muscle cells, Biochem. Biophys. Res. Commun. 450 (2014) 494–499.
[12] T. Wada, T. Sasaoka, M. Funaki, H. Hori, S. Murakami, M. Ishiki, T. Haruta, T. Asano, W. Ogawa, H. Ishihara, Overexpression of SH2-containing inositol phosphatase 2 results in negative regulation of insulin-induced metabolic actions in 3T3-L1 adipocytes via its 5′-phosphatase catalytic activity, Mol. Cell. Biol. 21 (2001) 1633–1646.
[13] S. Kagawa, Y. Soeda, H. Ishihara, T. Oya, M. Sasahara, S. Yaguchi, R. Oshita, T. Wada, H. Tsuneki, T. Sasaoka, Impact of transgenic overexpression of SH2-containing inositol 5′-phosphatase 2 on glucose metabolism and insulin signaling in mice, Endocrinology 149 (2008) 642–650.
[14] S. Gorgani-Firuzjaee, K. Adeli, R. Meshkani, SH2 domain containing inositol 5-phosphatase (SHIP2) regulates de-novo lipogenesis and secretion of apoB100 containing lipoproteins in HepG2 cells, Biochem. Biophys. Res. Commun. (2015) (in press).
[15] S. Vakili, S.S. Ebrahimi, A. Sadeghi, S. Gorgani-Firuzjaee, M. Beigy, P. Pasalar, R. Meshkani, Hydrodynamic-based delivery of PTP1B shRNA reduces plasma glucose levels in diabetic mice, Mol. Med. Rep. 7 (2013) 211–216.
[16] G. Taheripak, S. Bakhtiyari, M. Rajabibazl, P. Pasalar, R. Meshkani, Protein tyrosine phosphatase 1B inhibition ameliorates palmitate-induced mitochondrial dysfunction and apoptosis in skeletal muscle cells, Free Radic. Biol. Med. 65 (2013) 1435–1446.
[17] D. Laybutt, A. Preston, M. Åkerfeldt, J. Kench, A. Busch, A. Biankin, T. Biden, Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes, Diabetologia 50 (2007) 752–763.
[18] G. Taheripak, S. Bakhtiyari, M. Rajabibazl, P. Pasalar, R. Meshkani, Protein tyrosine phosphatase 1B inhibition ameliorates palmitate-induced mitochondrial dysfunction and apoptosis in skeletal muscle cells, Free Radic. Biol. Med. 65 (2013) 1435–1446.
[19] R. Meshkani, A. Sadeghi, G. Taheripak, M. Zarghooni, S. Gerayesh-Nejad, S. Bakhtiyari, Rosiglitazone, a PPARgamma agonist, ameliorates palmitate-induced insulin resistance and apoptosis in skeletal muscle cells, Cell Biochem. Funct. 32 (2014) 683–691.
[20] J. Downward, PI 3-kinase, Akt and Cell Survival, Seminars in Cell & Developmental Biology, Elsevier, 2004, pp. 177–182.
[21] H.-P. Gerber, A. McMurtrey, J. Kowalski, M. Yan, B.A. Keyt, V. Dixit, N. Ferrara, Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway requirement for Flk-1/KDR activation, J. Biol. Chem. 273 (1998) 30336–30343.
[22] C.D. Wei, Y. Li, H.Y. Zheng, Y.Q. Tong, W. Dai, Palmitate induces H9c2 cell apoptosis by increasing reactive oxygen species generation and activation of the ERK1/2 signaling pathway, Mol. Med. Rep. 7 (2013) 855–861.
[23] Á. González-Rodriguez, Ó. Escribano, J. Alba, C.M. Rondinone, M. Benito, Á.M. Valverde, Levels of protein tyrosine phosphatase 1B determine susceptibility to apoptosis in serum-deprived hepatocytes, J. Cell. Physiol. 212 (2007) 76–88.
[24] H.Y. Choi, S.Y. Hwang, C.H. Lee, H.C. Hong, S.J. Yang, H.J. Yoo, J.A. Seo, S.G. Kim, N.H. Kim, S.H. Baik, Increased selenoprotein p levels in subjects with visceral obesity and nonalcoholic fatty liver disease, Diabetes Metabol. J. 37 (2013) 63–71.
[25] M.E. Hyvönen, P. Saurus, A. Wasik, E. Heikkilä, M. Havana, R. Trokovic, M. Saleem, H. Holthöfer, S. Lehtonen, Lipid phosphatase SHIP2 downregulates insulin signalling in podocytes, Mol. Cell. Endocrinol. 328 (2010) 70–79.
[26] Y. Soeda, H. Tsuneki, H. Muranaka, N. Mori, S. Hosoh, Y. Ichihara, S. Kagawa, X. Wang, N. Toyooka, Y. Takamura, The inositol phosphatase SHIP2 negatively regulates insulin/IGF-I actions implicated in neuroprotection and memory function in mouse brain, Mol. Endocrinol. 24 (2010) 1965–1977.
[27] R. Grempler, S. Leicht, I. Kischel, P. Eickelmann, N. Redemann, Inhibition of SH2-domain containing inositol phosphatase 2 (SHIP2) in insulin producing INS1E cells improves insulin signal transduction and induces proliferation, FEBS Lett. 581 (2007) 5885–5890.
[28] Y. Choi, J. Zhang, C. Murga, H. Yu, E. Koller, B.P. Monia, J.S. Gutkind, W. Li, PTEN, but not SHIP and SHIP2, suppresses the PI3K/Akt pathway and induces growth inhibition and apoptosis of myeloma cells, Oncogene 21 (2002) 5289–5300.
[29] G. Tzivion, M. Dobson, G. Ramakrishnan, FoxO transcription factors; Regulation by AKT and 14-3-3 proteins, Biochim. Biophys. Acta (BBA) Mol. Cell Res. 1813 (2011) 1938–1945.
[30] T. Sasaoka, H. Hori, T. Wada, M. Ishiki, T. Haruta, H. Ishihara, M. Kobayashi, SH2-containing inositol phosphatase 2 negatively regulates insulin-induced glycogen synthesis in L6 myotubes, Diabetologia 44 (2001) 1258–1267.
[31] Y. Sato, S. Fujimoto, E. Mukai, H. Sato, Y. Tahara, K. Ogura, G. Yamano, M. Ogura, K. Nagashima, N. Inagaki, Palmitate induces reactive oxygen species production and β-cell dysfunction by activating nicotinamide adenine dinucleotide phosphate oxidase through Src signaling, J. Diabetes Invest. 5 (2014) 19–26.
[32] N. Lin, H. Chen, H. Zhang, X. Wan, Q. Su, Mitochondrial reactive oxygen species (ROS) inhibition ameliorates palmitate-induced INS-1 beta cell death, Endocrine 42 (2012) 107–117.
[33] S. Nakamura, T. Takamura, N. Matsuzawa-Nagata, H. Takayama, H. Misu, H. Noda, S. Nabemoto, S. Kurita, T. Ota, H. Ando, Palmitate induces insulin resistance in H4IIEC3 hepatocytes through reactive oxygen species produced by mitochondria, J. Biol. Chem. 284 (2009) 14809–14818.
[34] D.L. Hickson-Bick, G.C. Sparagna, L.M. Buja, J.B. McMillin, Palmitate-induced apoptosis in neonatal cardiomyocytes is not dependent on the generation of ROS, Am. J. Physiol. Heart Circulatory Physiol. 282 (2002) H656–H664.
[35] T.A. Miller, N.K. LeBrasseur, G.M. Cote, M.P. Trucillo, D.R. Pimentel, Y. Ido, N.B. Ruderman, D.B. Sawyer, Oleate prevents palmitate-induced cytotoxic stress in cardiac myocytes, Biochem. Biophys. Res. Commun. 336 (2005) 309–315.