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Journal of Nutritional Biochemistry 54 (2018) 35–47

Journal of Nutritional Biochemistry

# Down-regulation of stearoyl-CoA desaturase-1 increases susceptibility to palmitic-acid-induced lipotoxicity in human trophoblast cells

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Received 11 July 2017; received in revised form 12 September 2017; accepted 11 November 2017

**Abstract**

In early pregnancy, adequate dietary factors are important for the growth of human trophoblast cells, followed by placental development. Although stearoyl-CoA desaturase 1 (SCD1) is expected to relieve palmitic acid (PA)-induced lipotoxicity by regulating diacylglycerol and ceramide, its function is unclear in human trophoblast cells. The aim was to investigate inhibitory effects of SCD1 activity on PA-induced trophoblast cell death. PA induces cell death and inhibits the invasion of human trophoblast cells (HTR8/SVneo). In addition, we demonstrate that SCD1 has a protective role against PA in human trophoblast cells by regulating AKT-mediated signaling pathway and mitochondrial membrane potential. The knockdown of SCD1 enhances the proapoptotic activity of PA in HTR8/SVneo cells. Lastly, we investigated microRNA expression predicted to target SCD1 and diacylglycerol O-acyltransferase 1 (DGAT1) by PA. Collectively, the results suggest potential roles of SCD1 and DGAT1 in alleviating the toxicity of PA and maintaining lipid homeostasis for normal placentation.


*Keywords:* Trophoblast; Palmitic acid; Apoptosis; SCD1; Mitochondria

## 1. Introduction

Normal growth and invasion of trophoblast are important for the development of functional placenta. Elevated apoptosis and decreased motility of trophoblast are closely associated with several pregnancy-related disorders, including preeclampsia, intrauterine growth restriction and spontaneous abortion [1,2]. Maternal exposure to environmental risk factors affects functional characteristics and alters gene expression in trophoblast cells. The toxicity of environmental factors that include air pollution, organic pollutants and heavy metal contaminations induces permanent damage to the placentation procedure [3,4]. Numerous items of evidence suggest that dietary factors also cause cytotoxicity in various human organs. Also, it is well known that maternal nutrient availability and placental transport of nutrients from the mother to the fetus are essential for fetal development. In parallel, maternal nutrient status affects the growth and gene expression of placenta [5]. In response to low-protein diet in postimplantation mice, trophoblast growth and phenotype are newly programmed [6]. In addition, maternal obesity can result in increased risk of maternal complications in pregnancy and birth outcomes [7]. Moreover, it was reported that maternal obesity altered gene expression involved in mitochondrial and lipid metabolism in human umbilical vein endothelial cells and lipid profile in infant cord blood [8]. Therefore, it is necessary to identify whether excessive exposure to specific lipid type induces malfunction of trophoblast.

Excessive consumption of saturated fatty acid (SFA) induces obesity, hyperlipidemia, insulin resistance and cardiovascular diseases. These harmful effects are mainly accompanied by lipotoxicity, which is nonadipose cellular dysfunction induced by accumulated lipids. Palmitic acid (PA), the most widely distributed SFA, is found in meat, dairy and some plant oils, such as palm and coconut oils. Also, several cosmetics contain PA as a detergent and emollient. PA-induced lipotoxicity has been reported in various cell types. In cardiomyoblast, exposure to PA leads to cell death through endoplasmic reticulum (ER) stress accompanied by diacylglycerol (DAG) accumulation in ER [9]. Also, conversion of PA to monounsaturated fatty acids (MUFAs) can protect pancreatic β-cells from PA-induced ER stress [10]. Moreover, the PA induces intracelular levels of inflammatory cytokines, including interleukin-1β (IL-1β), IL-6 and IL-8, and reactive oxygen species (ROS) production, leading to the apoptosis of human placental cells [11]. However, the PA-mediated signal transduction has not yet been elucidated in human placental cells.

Stearoyl-CoA desaturase 1 (SCD1) is a rate-limiting enzyme involved in the unsaturation of SFA. Expression of SCD1 is elevated in the placentas of obese women compared to lean women [12]. Also, SCD1 expression is elevated in response to PA in hepatoblastoma cells

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https://doi.org/10.1016/j.jnutbio.2017.11.005
0955-2863/

[13]. In addition, SCD1 has a protective role against external stress and maintains ER homeostasis. Several studies describe SCD1 protecting nonadipose cells from SFA-induced lipotoxicity through reducing ceramide and DAG accumulation [10,14]. Hence, SCD1 activation is regarded as a novel therapy for alleviating metabolic disease, such as diabetes mellitus, related to high concentration of SFA [15]. Maternal fat mass increases from the first trimester, peaking in the second trimester; but the functional role of SCD1 in trophoblast development and placentation has not been understood.

In the present study, we demonstrated that PA induces trophoblast cell death by modulating mitochondrial membrane potential, and lipotoxicity caused by PA is reinforced by the inhibition of SCD1 activity. Moreover, knockdown of SCD1 using small interfering RNA (siRNA) regulates PA-induced gene expression involved in fatty acid (FA) metabolism. Collectively, we suggest that SCD1 has a protective role against the down-regulation of cell viability and invasion promoted by SFA in human trophoblast cells, therefore maintaining normal trophoblast development and placentation.

## 2. Materials and methods

### 2.1. Chemicals

PA was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Ruthenium red and CAY10566 were obtained from Abcam (Cambridge, UK). 2-Aminoethyl diphenylborinate and N-acetyl-L-cysteine were obtained from Sigma-Aldrich, Inc., and BAPTA-AM was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). LY294002 was obtained from Cell Signaling Technology (Beverly, MA, USA), and U0126 was obtained from Enzo Life Science (Farmingdale, NY, USA). The antibodies against phosphorylated Bcl-2 ($Ser^{70}$), Bad ($Ser^{112}$), eIF2α ($Ser^{51}$), AKT ($Ser^{473}$), GSK3β ($Ser^{9}$), P70S6K ($Thr^{421}$/$Ser^{424}$), S6 ($Ser^{235}$/$Ser^{236}$), ERK1/2 ($Thr^{202}$/$Tyr^{204}$) and P90RSK ($Thr^{573}$), and Bcl-xL, IRE1α, total Bcl-2, Bad, eIF2α, AKT, GSK3β, P70S6K, S6, ERK1/2 and P90RSK were purchased from Cell Signaling Technology. The antibodies against GRP78, ATF6α and TUBA were purchased from Santa Cruz Biotechnology Inc.

### 2.2. Cell culture

HTR8/SVneo cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in RPMI-1640 with 2.05 mM L-Glutamine (Cat. No. SH30027.01, HyClone, Carlsbad, CA, USA) with 5% fetal bovine serum (FBS) at 37 °C in a $CO_2$ incubator. PA was conjugated to fatty-acid-free BSA (Sigma, A7030) before treatment. PA was dissolved in 0.1 M NaOH at 70°C to make 100 mM of stock solution and mixed with 15% fatty-acid-free BSA for 2 h to generate 5 mM PA–BSA conjugate solution. In each assay, 15% fatty-acid-free BSA solution mixed with 0.1 M NaOH (19:1) was used as vehicle.

### 2.3. Proliferation assay

Proliferation assays were conducted using the Cell Proliferation ELISA, BrdU kit (Cat. No. 11,647,229,001, Roche, Indianapolis, IN, USA), according to the manufacturer's recommendations [16].

### 2.4. Annexin V and propidium iodide (PI) staining

Apoptosis of HTR8/SVneo cells was analyzed using fluorescein isothiocyanate Annexin V apoptosis detection kit I (BD Biosciences, Franklin Lakes, NJ, USA) as described previously [16].

### 2.5. Cell cycle analysis

To examine the distribution of subG1, G0/G1, S and G2/M phases of PA-treated cells, we stained HTR8/SVneo cells with PI (BD Biosciences) in the presence of 100 μg/ml RNase A (Sigma). Fluorescence was analyzed using a flow cytometer (BD Biosciences).

### 2.6. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

The TUNEL assay of HTR8/SVneo cells treated with PA (800 μM) was performed as described previously [16]. The cells were subjected to TUNEL staining mixture using the *In Situ* Cell Death Detection kit, TMR red (Roche). Relative fluorescence intensity was quantified by red/DAPI ratio using MetaMorph software (Molecular Devices).

### 2.7. Matrigel invasion assay

Cell invasion was performed in 8-μm pore Transwell inserts (Cat. No. 3422, Corning, Inc., Corning, NY, USA) coated with Matrigel for 2 h at 37°C. HTR8/SVneo cells ($1\times10^5$ cells per 200 μl) in serum-free medium containing PA (800 μM) were plated onto the upper chamber, while medium containing 5% FBS was added to the lower wells. Invaded cells were counted in five nonoverlapping locations that covered approximately 70% of the insert membrane growth area using DM3000 (Leica, Wetzlar, Germany) microscopy.

### 2.8. Western blot analysis

Western blot analysis of HTR8/SVneo cells treated with PA and other treatments was performed as described previously [16].

### 2.9. Determination of cellular ROS

Intracellular ROS production was estimated using 2′,7′-dichlorofluorescin diacetate (Sigma), which is converted to fluorescent 2′,7′-dichlorofluorescin (DCF) in the presence of peroxides as described previously [16].

### 2.10. Measurement of intracellular free $Ca^{2+}$ concentration

Intracellular concentrations of free $Ca^{2+}$ in HTR/SVneo cells were determined as described previously [16]. Briefly, cells were stained with 3 μM fluo-4 AM (Invitrogen) for 20 min. Fluorescent intensity was analyzed using a flow cytometer (BD Bioscience). To determinate mitochondrial free $Ca^{2+}$ levels, Rhod-2 AM (Cat. No. R1244, Invitrogen) was used, which is accumulated in mitochondria. After HTR8/SVneo cells were stained with 200 nM MitoTracker (Cat. No. M7514, Invitrogen) for 30 min, the cells were then stained with Rhod-2 AM staining for 30 min. Fluorescence was detected by LSM710 confocal microscopy. Relative fluorescence intensity was quantified by red/green ratio using MetaMorph software.

### 2.11. Lipid peroxidation assay

The lipid peroxidation assay was performed using a dClick-iT lipid peroxidation imaging kit (Cat. No. C10446, Invitrogen) according to the manufacturer's recommendation. Relative fluorescence intensity was quantified by green/DAPI ratio using MetaMorph software.

### 2.12. JC-1 mitochondrial membrane potential assay

The JC-1 mitochondrial membrane potential was determined using a mitochondria staining kit (Cat. No. CS0390, Sigma-Aldrich) as described previously [16].

### 2.13. siRNA knockdown experiment

For mRNA interference against *SCD1* and *DGAT1*, HTR8/SVneo cells ($5\times10^5$ cells) were seeded in six-well plates and transfected with nontargeting control siRNA (siCTR) (Cat. No. SR30004, OriGene, Rockville, MD, USA), siRNA directed against SCD1 (siSCD1) (Cat. No. SR304248, OriGene) and siRNA directed against DGAT1 (siDGAT1) (Cat. No. sc-40,487, Santa Cruz Biotechnology Inc) using transfection reagent Lipofectamine 2000 according to the manufacturer's instructions. Briefly, cells were cultured with siRNA and Lipofectamine 2000 diluted in Opti-MEM reduced serum medium (Cat. No. 32,985,070, Gibco). After 6-h incubation at 37°C in a $CO_2$ incubator, media were removed, and media were added containing 800 μM PA or vehicle for 18 h at 37°C.

### 2.14. Quantitative RT-PCR analysis

Target gene expression was determined as described previously [17]. The primer sets are listed in Table 1.

### 2.15. Migration assay

Cell migration was evaluated using Ibidi migration culture dish inserts, according to the manufacturer's instruction (Ibidi, Germany). A 70-μl suspension of HTR8/SVneo cells ($2\times10^5$ cells/ml) was seeded into each well of the culture inserts and grown overnight to full confluence. Then, cells were transfected with 10 nM siCTR or siSCD1 in the presence of either PA (800 μM) or vehicle. After 12-h incubation at 37°C in a $CO_2$ incubator, the migration of cells into the defined cell-free gap (500 μm) was observed, and light microscopy images of the gap field were acquired by DM3000 (Leica). For assay analyses, gap closure was computed.

### 2.16. Quantification RT-PCR analysis for microRNAs

microRNA (miRNA) first-strand cDNA was synthesized from total RNA using a miRNA first-strand cDNA synthesis kit (Agilent Technologies, Santa Clara, CA, USA). The expression of miRNAs was measured using the High-Specificity miRNA QPCR Core Reagent Kit (Applied Biosystems) following guidelines from the vendor. For