Inhibition of lipogenesis and induction of apoptosis by valproic acid in prostate cancer cells via the C/EBP α /SREBP-1 pathway

Bo Pang

Tianjin Medical University

Juanjuan Zhang

Tianjin Medical University

Jihong Yuan

Tianjin Medical University

Yanan Shi

Tianjin Medical University

Ling Qiao (✉ qiaoling@tjmu.edu.cn)

Tianjin Medical University <https://orcid.org/0000-0003-1160-9738>

Research

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Abstract

Background

Lipid metabolism reprogramming is now accepted as a new hallmark of cancer. Hence, targeting the lipogenesis pathway may be a promising avenue for cancer treatment. Valproic acid (VPA) emerges as a promising drug for cancer therapy, however, the underlying mechanisms are not yet fully understood. This study aimed to investigate the effects and mechanism of VPA on cell viability, lipogenesis, and apoptosis in human prostate cancer PC-3 cells.

Methods

The effects of VPA on the viability of PC-3 cells were investigated using MTT assay and wound-healing assay. Oil Red O staining was used to examine lipid droplets, and DAPI staining assay and Annexin V-PI staining assay were used to measure the extent of cell apoptosis. Quantitative real-time PCR and Western blotting were used to determine the expression of lipogenesis and apoptosis genes. Statistical and analytical data with SPSS 17.0 Software, statistical significance was set at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ levels.

Results

The results showed that VPA significantly reduced lipid accumulation and induced apoptosis of PC-3 cells. Moreover, the expression of CCAAT/enhancer-binding protein α (C/EBP α), as well as sterol regulatory element-binding protein 1 (SREBP-1) and its downstream effectors, including fatty acid synthase (FASN), acetyl CoA carboxylase 1 (ACC1), and antiapoptotic B cell lymphoma 2 (Bcl-2), markedly decreased in PC-3 cells after VPA administration. Mechanistically, the overexpression of C/EBP α rescued the levels of SREBP-1, FASN, ACC1, and Bcl-2, enhanced lipid accumulation and attenuated apoptosis of VPA-treated PC-3 cells. Conversely, C/EBP α knockdown by siRNA further decreased the levels of SREBP-1, FASN, ACC1, and Bcl-2 and lipid accumulation and enhanced apoptosis. In addition, SREBP-1a and 1c enhanced the expression of FASN and ACC1, but only SREBP-1a had a significant effect on Bcl-2 expression in VPA-treated PC-3 cells.

Conclusions

On the whole, it is concluded that VPA significantly inhibits cell viability via inhibiting lipogenesis and inducing apoptosis by the C/EBP α /SREBP-1 pathway in PC-3 cells. Hence, VPA, which targets lipid metabolism and apoptosis, is a promising candidate for PCa chemotherapy.

Background

Over the past decades, an increase in the incidence of various metabolic disorders, including type 2 diabetes mellitus, obesity, nonalcoholic fatty liver disease, atherosclerosis, thromboembolism, and cancer, has been strongly associated with dysregulated lipid metabolism. Noticeably, the alteration of lipid

metabolism has been increasingly recognized as a hallmark of cancer cells. Therefore, targeting lipid metabolic reprogramming is a potential cancer treatment strategy [1, 2]. Prostate cancer (PCa) is a common malignant tumor in the urinary system and the third leading cause of cancer-related death in men worldwide. PCa is well recognized as a lipid-enriched tumor, an important biological feature of PCa progression is the dysregulation of lipid metabolism [3–5]. Previous studies indicated that inhibiting de novo lipogenesis via deleting calcium/calmodulin-dependent protein kinase kinase 2, activating AMP-activated protein kinase, or selectively inhibiting fatty acid synthase (FASN) reduced cell growth in human PCa cells [6, 7].

Valproic acid (VPA), a histone deacetylase inhibitor, is known as an anticonvulsant and mood-stabilizing drug for more than 40 years. Previous study indicates that VPA suppresses adipogenesis and decreases the expression levels of peroxisome-proliferator-activated receptor γ , CCAAT/enhancer-binding protein α (C/EBP α), and FASN, which are key regulators of adipogenesis in adipocytes [8]. Treatment of ob/ob mice with VPA for 14 days results in decreased hepatic fat accumulation [9]. Recent studies have shown that VPA can suppress the malignancy of various cancers, including prostate cancer, glioblastoma, and melanoma, because it inhibits tumor growth and metastasis, induces differentiation and apoptosis, and enhances chemotherapy sensitivity [10–12]. Although study indicates that VPA pretreatment suppresses PCa cell viability [13], its potential roles and, more importantly, the underlying mechanisms of its actions have not been extensively studied.

The present study aimed to gain insights into the antitumor mechanisms of VPA in PCa cells. The results showed that VPA inhibited cell viability in PC-3 cells through suppressing lipid accumulation and inducing cell apoptosis. It involved the key regulators of lipogenesis, including C/EBP α and sterol regulatory element-binding protein-1 (SREBP-1) and their target genes. These findings might provide a therapeutic insight for PCa targeting lipogenesis and apoptosis.

Methods

Cell culture and pharmacological intervention

The human prostate cancer PC-3 cells (obtained from Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center of Cancer, Tianjin, China) were cultured in RPMI 1640 medium (Gibco, CA, USA) with 10% (v/v) fetal bovine serum (Gibco, CA, USA) containing antibiotics (100 U/mL penicillin G and 100 mg/mL streptomycin) and incubated at 37°C in 5% CO₂ humidified incubator. As per experimental requirements, the PC-3 cells were treated with valproic acid (VPA, purchased from Sigma-Aldrich Chemicals, MO, USA) and then subjected to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay, wound-healing assay, Oil-Red O staining, 4', 6'-diamidino-2-phenylindole (DAPI) staining assay, Annexin V-propidium iodide (PI) staining assay, quantitative real-time polymerase chain reaction (qRT-PCR), and Western blot analysis.

MTT cell viability assay

PC-3 cells were seeded in 96-well plates at a density of 2×10^4 cells/well and incubated overnight, followed by treatment with the indicated concentrations of VPA. At the endpoint of treatment, 10 μ L of 5 mg/mL MTT in phosphate-buffered saline (PBS, purchased from Amresco Inc., OH, USA) was added to each well and incubated in the dark for 4 h at 37°C. The cell culture medium was replaced with 150 μ L of dimethyl sulfoxide, and the mixture was stirred for 10 min. The optical density (OD) values for samples were measured using a Pan-wavelength microplate reader at 570 nm. Triplicate wells were used for each sample, and the experiments were repeated at least three times to get means and standard deviations. Each test should include a blank containing a complete culture medium without cells.

Wound-healing assay

The cell migration ability was examined using the wound-healing assay. PC-3 cells in each group were seeded into a six-well plate. When the confluence reached 60%-80%, a wound was built afterward across the cell monolayer using a BioClean 1000 μ L plastic pipette tip. The cells were rinsed with PBS three times and incubated with fresh medium containing different dosages of VPA for another 4 days or 2.0 mM VPA for another 2, 4, and 6 days. The cell migration into the wound area was photographed under an inverted microscope (Leica, Germany) with 100 $\times$ magnification. The relative migration rate was calculated using the following formula: relative migration rate = (distance between the gap at 0 h - distance between the gap at each time point)/distance between the gap at 0 h $\times$ 100%.

Oil-Red O staining

PC-3 cells were seeded in a 6-well plate containing a glass coverslip bottom. The cells attached to the coverslip were fixed in 4% paraformaldehyde for 15 min and then analyzed with an Oil-Red O stain kit (Nanjing Jiancheng Bioengineering Institute, China) according to the supplier's instructions. The lipid droplets stained with Oil-Red O were visualized with an Olympus BX53 microscope (Olympus Corporation, Tokyo, Japan) equipped with a DP72 microscope digital camera and Image-Pro Plus 7.0 software. Light absorbance of the extracted dye was measured at 520 nm.

Western blot analysis

The cultured cells were collected and solubilized using protein lysis buffer. Lysates were cleared by centrifugation, and proteins were separated by gel electrophoresis. The membranes were blocked in PBS containing 0.1% Tween 20 and 5% (w/v) milk for 2 h at room temperature. The blots were first incubated with primary antibodies: anti-C/EBP α , anti-SREBP-1 (Abcam, Cambridge, UK), anti-Flag (Sigma-Aldrich), anti-FASN, anti-acetyl CoA carboxylase 1 (ACC1), anti-B cell lymphoma 2 (Bcl-2), or anti- β -Actin (Proteintech, Wuhan, China), followed by incubation with the appropriate secondary antibody, that is, goat anti-rabbit IgG (H + L)-HRP or goat anti-mouse IgG (H + L)-HRP (RayBiotech, Beijing, China). The detection was performed using an enhanced chemiluminescence kit (Advansta, CA, USA).

DAPI staining assay

PC-3 cells (4×10^5 per well) were cultured in a six-well plate, and nuclear morphology was tested using a DAPI staining assay. Following treatment with VPA (0, 1.0, 2.0, 5.0, and 10.0 mM) for 4 days, the cells were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 2 min. Then, they were stained with 10 μ g/mL DAPI (purchased from Roche Corporation, Switzerland) at 37 °C in the dark for 10 min. The nuclear morphology was viewed under ultraviolet light, and the images were captured using an inverted fluorescence microscope (Leica, Germany). Apoptotic cells were identified on the basis of characteristic changes, including nuclear condensation, fragmentation, and presence of apoptotic bodies [14].

Annexin V-PI staining assay

The cells (4×10^5 per well) were seeded in a six-well plate overnight and treated with varying concentrations of VPA for 4 days. To measure the extent of cell apoptosis, an Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit was used according to the manufacturer's instructions (Sungene Biotech, Tianjin, China). In brief, the cells were harvested and suspended in $1 \times$ binding buffer. Then, 100 μ L of the cell suspension was incubated with 5 μ L of Annexin V-FITC for 10 min, followed by incubation with 5 μ L of PI solution for another 5 min. The labeled cells were then assessed by flow cytometry and analyzed using Cellquest 6.0 (BD Biosciences, NJ, USA) [15].

Cell transfection

In brief, the PC-3 cells seeded in a 6-well, 12-well, or 96-well plate were transfected using Lipofectamine 3000 transfection reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. The following plasmids were used: pcDNA3-C/EBP α (a gift from Dr. Hong Yin), pcDNA3.1-2xFlag-SREBP-1a, and pcDNA3.1-2xFlag-SREBP-1c (from Addgene). The siRNA against C/EBP α (in short, Si-C/EBP α) and the negative control siRNA (in short, Si-Control) were designed and synthesized by RiboBio Company (Guangzhou, China). The siRNA against C/EBP α used in the present study was as follows: sense: 5'-GGAGCUGACCAGUGACAAUdTdT-3'; and antisense: 5'-AUUGUCACUGGUCAGCUCCdTdT-3'.

Quantitative real-time PCR

Total RNA was extracted from PC-3 cells after transfection with C/EBP α -expressing plasmid or Si-C/EBP α and/or treatment with VPA (2.0 mM) using an E.Z.N.A. HP Total RNA Extraction Kit (Omega Bio-Tek, GA, USA). Reverse transcription reactions were performed with 2.0 μ g total RNA using a RevertAid First Strand cDNA Synthesis Kit and oligo (dT) primers (Fermentas Inc., Canada) according to the manufacturer's protocol. The abundance of mRNA was detected using an SYBR Green real-time PCR kit according to the manufacturer's instructions (Sangon Biotech, Shanghai, China). The transcript quantities of SREBP-1a and SREBP-1c were normalized to those of β -Actin and measured by the comparative Ct ($2^{-\Delta\Delta C_t}$) method. The measurement was performed in three independent experiments and each with three replicates. The sequences of the primer pairs were as follows: SREBP-1a: 5'-CGGCGCTGCTGACCGACATC-3' and 5'-CCCTGCCCCACTCCCAGCAT-3'; SREBP-1c: 5'-GCGCAGATCGCGGAGCCAT-3' and 5'-

CCCTGCCCCACTCCCAGCAT-3'; and β -Actin: 5'-CCAGAGATGGCCACGGCTGCT-3' and 5'-TCCTTCTGCATCCTGTCTGGCA-3'.

Statistical analysis

The experiments were repeated at least three times, and data were analyzed using SPSS (version 17.0; SPSS, Inc., IL, USA). All data were expressed as the mean $\pm$ SD. A statistical analysis between two groups was performed using the Student's t test. One-way analysis of variance was used for the bar graphs containing three or more groups. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ indicate significant differences, whereas the nonsignificant difference is denoted by NS.

Results

The effect of VPA on PC-3 cell viability

The incidence of PCa presents an increasing trend in recent years. Previous studies indicated that VPA was a potential suppressor of the growth of PCa cells [10, 13, 16]. To test whether the growth inhibition was dose dependent, the PC-3 cells were treated with different concentrations of VPA (ranging from 0.5 to 10.0 mM) for 4 days, followed by the MTT cell viability assay. Our results showed that the growth of PC-3 cells was slow after VPA administration. The growth ability decreased in a dose-dependent manner, ranging from 7.52% for the low VPA concentration (0.5 mM) up to 76.7% for the high VPA concentration (10.0 mM) compared with the control cells (Fig. 1a, left). Meanwhile, PC-3 cells were treated with 2.0 mM VPA over a time course of 1, 2, 4, and 6 days. As shown in Fig. 1a (right), VPA inhibited the growth of PC-3 cells significantly in a time-dependent manner, ranging from 9.73% on day 1 up to 64.4% on day 6 compared with the control cells. The inhibitory effect of VPA on PC-3 cell growth was further confirmed by the wound-healing assay. Consistently, similar results were observed, and showed that VPA markedly delayed the wound closure of PC-3 cells (Fig. 1b and 1c). Together, the results demonstrate that VPA remarkably prevents the PC-3 cell viability.

The effect of VPA on lipogenesis in PC-3 cell

Cancer is a disorder characterized by increased metabolic activity, leading to enhanced cell growth and proliferation. Alterations in lipid metabolism are one of the main features in cancer cells [5, 17, 18]. Therefore, the functional effect of VPA on lipogenesis and apoptosis in PC-3 cells were determined in this study. PC-3 cells were treated with 0, 0.5, 1.0, 2.0, 5.0, and 10.0 mM VPA for 4 days, followed by Oil-red O staining and Western blot analysis. The results showed that VPA decreased lipid deposition in PC-3 cells in a dose-dependent manner, as evidenced by Oil-red O staining (Fig. 2a, b). The expression levels of FASN and ACC1, the key enzymes of de novo lipogenesis, were significantly suppressed in a dose-dependent manner when PC-3 cells were cultured in a medium containing VPA (Fig. 2c, d).

The effect of VPA on apoptosis in PC-3 cell

VPA is now a promising anticancer compound due to its pharmacological action on lipogenesis and apoptosis [8–10]. Therefore, the degree of apoptosis for VPA-treated PC-3 cells was analyzed by DAPI staining assay and Annexin V-PI staining assay. DAPI staining showed that the nuclear morphology of VPA-treated cells presented more and brighter blue fluorescence, including the rippled surface of nuclei, chromatin condensation, and nuclear fragmentation, compared with the control cells, suggesting the typical characteristics of apoptosis (Fig. 3a). To further investigate the apoptosis of PC-3 cells induced by VPA, the apoptotic cells were quantified with a flow cytometer using Annexin V-FITC and PI double-staining. As shown in Fig. 3b and 3c, the percentage of apoptotic cells treated with VPA exhibited a significant rise compared with the control cells (from 8.89–22.73%). Consistent with these results, Western blot analysis showed that the expression level of Bcl-2 in VPA-treated PC-3 cells significantly decreased in a dose-dependent manner (Fig. 3d, e). Collectively, the results above suggest that VPA suppresses the growth of PC-3 cells by inhibiting lipogenesis and inducing apoptosis.

C/EBP α regulated lipogenesis and apoptosis-related genes in VPA-treated PC-3 cells

C/EBP α and SREBP-1 play a pivotal role in lipid metabolism by inducing the transcription of genes related to lipogenesis [8, 19]. Recent studies have revealed that C/EBP α and SREBP-1 are markedly upregulated in human cancers, providing the mechanistic link between lipid metabolism alterations and malignancies [18, 20, 21]. To identify the metabolic events occurring during VPA-induced antitumor progression, the expression levels of C/EBP α and SREBP-1 were determined by Western blot analysis. As shown in Fig. 4a and 4b, the expression levels of C/EBP α and SREBP-1 were significantly suppressed in PC-3 cells treated with VPA in a dose-dependent manner. Furthermore, the ectopic expression of C/EBP α inverted the downregulation of SREBP-1, FASN, ACC1, and Bcl-2 caused by VPA, as shown by Western blot analysis (Fig. 4c, d). The siRNA strategy was used to specifically knockdown C/EBP α . The Western blot analysis results confirmed the dramatic C/EBP α downregulation in PC-3 cells. As expected, decreasing the protein levels of SREBP-1, FASN, ACC1, and Bcl-2 were enhanced in PC-3 cells transfected with siRNA sequences specifically against C/EBP α (in short, Si-C/EBP α) compared with the cells transfected with scrambled control siRNA (in short, Si-Control) (Fig. 4e, f). These results indicate that C/EBP α play an important role in expression of SREBP-1, FASN, ACC1, and Bcl-2 in VPA-treated PC-3 cells. Hence, it is presumed that the C/EBP α /SREBP-1 pathway might be involved in the suppression of lipogenesis and induction of apoptosis in PC-3 cells after VPA administration.

Effects of SREBP-1a and SREBP-1c on expression of lipogenesis and apoptosis-related genes in VPA-treated PC-3 cells

The changes in cell metabolism and growth are closely linked through SREBPs. The SREBP family consists of three subtypes: SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1a and SREBP-1c are derived from a single gene through the use of alternative transcription start sites. In general, SREBP-1c seems to act more specifically on the genes involved in fatty acid synthesis, while SREBP-1a has an influence on a large number of genes involved in the regulation of lipid metabolism, cell proliferation, differentiation, and death [19–22]. Importantly, our previous study indicated that C/EBP α enhanced SREBP-1a activation at

the transcriptional level [23]. Hence, the present study examined the effect of C/EBP α on the transcriptional levels of SREBP-1a and SREBP-1c in PC-3 cells in the presence of VPA. PC-3 cells were transfected with C/EBP α -expressing plasmid or Si-C/EBP α for 48 h in the presence of VPA (2.0 mM), and the transcriptional levels of SREBP-1a and SREBP-1c were tested by qRT-PCR analysis. The results demonstrated that ectopic expression of C/EBP α rescued the suppression of SREBP-1a and SREBP-1c activation, while Si-C/EBP α exerted an enhanced inhibitory effects in PC-3 cells exposed to VPA (Fig. 5a). In addition, the ectopic expression of SREBP-1a rescued the expression of FASN and ACC1, as well as the expression of Bcl-2 in VPA-treated cells (Fig. 5b, c). Differently from SREBP-1a, the ectopic expression of SREBP-1c rescued the expression of FASN and ACC1, but had no effect on Bcl-2 expression in VPA-treated cells (Fig. 5d, e).

Effects of C/EBP α /SREBP-1 pathway on lipogenesis and apoptosis in VPA-treated PC-3 cells

Subsequently, the effects of C/EBP α and SREBP-1 on PC-3 cell viability, lipogenesis, and apoptosis were examined. MTT cell viability assay showed an apparent increase in C/EBP α - or SREBP-1-expressing cells and a sharp decrease in Si-C/EBP α cells compared with the control cells (Fig. 6a). The ectopic expression of C/EBP α or SREBP-1 led to a significant increase in lipid accumulation in PC-3 cells treated with VPA compared with the control cells. The loss of lipids and smaller lipid droplets as a result of C/EBP α knockdown was also confirmed by Oil-red O staining (Fig. 6b, c). Enhancing C/EBP α or SREBP-1 expression partially suppressed and Si-C/EBP α enhanced VPA-induced apoptosis in PC-3 cells (Fig. 6d, e). Taken together, it is concluded that VPA treatment significantly decreases lipid accumulation and induces apoptosis as a result of the inhibition of C/EBP α , as well as SREBP-1 and its target gene expression. VPA-triggered suppression of C/EBP α /SREBP-1 pathway might provide mechanistic insights and offer a novel therapeutic strategy for PCa.

Discussion

Metabolic reprogramming not only promotes cancer cell plasticity but also provides novel insights for treatment strategies. Altered lipid metabolism is increasingly recognized as a signature of cancer cells, and the proteins involved in this process can be excellent chemotherapeutic targets for cancer treatment [24–27]. A link between PCa progression and lipogenesis has been investigated in the last few decades. Several recent studies suggest that the activation of de novo lipogenesis and cholesterologenesis induces PCa cell proliferation and promotes PCa development and progression. Hence, targeting the lipogenesis pathway may be a promising avenue for PCa treatment [26–29].

VPA is known as a histone deacetylase inhibitor and an anticonvulsant and mood-stabilizing drug. It has been reported as a potent and promising anticancer drug candidate [10–12]. In the present study, the anticancer effects of VPA on human prostate cancer PC-3 cells were detected using MTT and wound-healing assay. The results indicated that VPA inhibited cell growth significantly in a dose-dependent and time-dependent manner. Moreover, VPA significantly inhibited lipogenesis and induced cellular apoptosis in PC-3 cells. Further assays were performed to analyze the underlying molecular mechanisms. C/EBP α

and SREBP-1 are crucial factors controlling lipogenesis. It is reported that VPA suppresses the accumulation of intracellular triacylglycerol and decreases the expression level of C/EBP α [8]. In addition, a growing amount of evidence suggests that aberrant SREBP-1 activity can contribute to cancer [30]. A previous study indicated that SREBP-1 was overexpressed and involved in the transcriptional regulation of fatty acid synthesis through the altered expression of FASN in prostate cancer cells [19]. Hence, the present study concentrated on C/EBP α and SREBP-1. Our results showed that the expression levels of C/EBP α and SREBP-1, as well as their target genes FASN, ACC1, and Bcl-2, decreased significantly after VPA treatment.

An adapted “metabolic switch” accompanies most physiological and pathological changes in cellular functions. Lipid metabolism is essential for cancer cells and is associated with the regulation of a variety of key cellular processes and functions [31–33]. The cellular metabolites can directly or indirectly regulate the apoptotic machinery. Metabolism is emerging as one of the key factors contributing to the evasion of apoptosis in cancer [34]. The suppression of C/EBP α inhibits cell proliferation by inducing G1-phase arrest and apoptosis [20]. Emerging evidence indicates that SREBP-1a couples lipid synthesis to cell progression and apoptosis. A recent study shows that SREBP-1a regulates antiapoptotic factor apoptosis inhibitor 6 (Api6) besides regulating lipid metabolism in response to nutrient levels [28, 35]. Consistently, our results revealed that the antiapoptotic Bcl-2 expression regulated by SREBP-1a might be involved in the apoptosis of VPA-treated PC-3 cells. Our study suggests distinct roles of the SREBP-1 isoforms. SREBP-1a might play a more important role in VPA-induced apoptosis in PC-3 cells.

Conclusions

Together, the present study indicate that VPA inhibits prostate cancer growth via the C/EBP α /SREBP-1 pathway targeting lipogenesis and apoptosis. Therefore, blocking fatty acid biosynthesis could be a potential treatment for PCa.

Abbreviations

ACC1:acetyl-coenzyme A carboxylase 1; Bcl-2:antiapoptotic B cell lymphoma 2; Api6:antiapoptotic factor apoptosis inhibitor 6; C/EBP α :CCAAT/enhancer-binding protein α ; DAPI:4', 6'-diamidino-2-phenyl-indole; FASN:fatty acid synthase; FITC:fluorescein isothiocyanate; MTT:3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; OD:optical density; PBS:phosphate-buffered saline; PI:propidium iodide; PCa:prostate cancer; qRT-PCR:quantitative real-time PCR; SREBP-1:sterol regulatory element-binding protein 1; SREBP-1a:sterol regulatory element-binding protein-1a; SREBP-1c:sterol regulatory element-binding protein-1c; VPA:valproic acid

Declarations

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Authors' contributions

BP performed experiments, analyzed data and helped write the manuscript. JZ, JY and YS helped to design and perform experiments. LQ conceived the project, designed the overall research, wrote the manuscript, mentored and supervised participants. All authors have read, commented on, and approved the final manuscript.

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Availability of data and materials

The authors confirm that all materials described in the manuscript are fully available to any scientist wishing to use them, without restriction.

Ethics approval

Not applicable.

Consent for publication

All authors agree to publish this article in the journal of Lipids in Health and Disease.

Competing interests

The authors declare that they have no competing interests

Author details

NHC Key Laboratory of Hormones and Development (Tianjin Medical University), Tianjin Key Laboratory of Metabolic Diseases, Tianjin Medical University Chu Hsien-I Memorial Hospital & Tianjin Institute of Endocrinology, Tianjin Medical University, Tianjin 300134, China.

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Figures

Figure 1

Effects of VPA on PC-3 cell viability. (a) Viability of PC-3 cells after treatment with increasing concentrations of VPA (0, 0.5, 1.0, 2.0, 5.0, and 10.0 mM) for 4 days was measured using the MTT assay. VPA significantly inhibited cell growth in a dose-dependent manner (left). The viability of PC-3 cells with VPA treatment (2.0 mM) was measured at various time points (0, 1, 2, 4, and 6 days). VPA significantly inhibited cell growth in a time-dependent manner (right). (b) Representative PC-3 cell wound healing width images. The migration of PC-3 cells treated with various concentrations of VPA for 4 days (up) or VPA (2.0 mM) for 0, 2, 4, and 6 days (down) was assessed using the wound-healing assay. (c) The PC-3 cell migration rate was calculated according to the width from 0 to 6 days. Data are expressed as mean $\pm$ SD, $n = 3$. Statistically significant differences are indicated: NS (no significance), * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 2

The effect of VPA on lipogenesis in PC-3 cell. (a) Effect of VPA on lipid storage in PC-3 cells was detected by Oil-Red O staining. (b) Oil-Red O extracted with isopropanol was measured at OD520. Scale bar = 100 μ m. (c) Western blot detection of the lipogenic gene expression of PC-3 cells after treatment with various concentrations of VPA. β -Actin was used as a loading control. (d) A semi-quantitative analysis of lipogenic gene expression was performed using ImageJ.

Figure 3

The effect of VPA on apoptosis in PC-3 cell. (a) PC-3 cells were incubated with various concentrations of VPA for 4 days, and the changes in nuclear morphology were evaluated using DAPI staining. (b) Effects of VPA on the apoptosis of PC-3 cells. PC-3 cells were treated with 0 (control), 0.5, 1.0, 2.0, and 5.0 mM VPA. The apoptotic rate was measured by flow cytometry with Annexin V-FITC/PI staining. PI, Propidium iodide. (c) Summary of the percentage of apoptotic cells treated with various concentrations of VPA. (d) Level of Bcl-2 in PC-3 cells was analyzed by Western blot analysis. β-Actin was used as a loading control. (e) The fold-change was calculated based on the densitometric analysis of the band intensities. All

experiments were performed three times. Data are expressed as mean $\pm$ SD. Statistically significant differences are indicated: NS (no significance), * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 4

C/EBP α regulated lipogenesis and apoptosis-related genes in VPA-treated PC-3 cells (a and b) Expression of C/EBP α and SREBP-1 in PC-3 cells treated with various concentrations of VPA was analyzed by Western blot analysis. The levels of C/EBP α and SREBP-1 were quantified by densitometry and

normalized with those of β -Actin. (c and d) PC-3 cells were transfected with C/EBP α -expressing plasmid, followed by treatment without or with 2.0 mM VPA for 48 h. The levels of C/EBP α , SREBP-1, FASN, ACC1, and Bcl-2 were measured by Western blot analysis. The fold-change was calculated based on a densitometric analysis of the band intensities. Mock: The cells were transfected with empty plasmid. (e and f) PC-3 cells were transfected with Si-C/EBP α , followed by VPA treatment. The levels of C/EBP α , SREBP-1, FASN, ACC1, and Bcl-2 were measured by Western blot analysis. The fold-change was calculated based on the densitometric analysis of the band intensities. Mock: The cells were transfected with scrambled control si-RNA (Si-Control). Statistically significant differences are indicated: NS (no significance), *P < 0.05, **P < 0.01, and ***P < 0.001.

A

B

C

D

E

Figure 5

Effects of SREBP-1a and SREBP-1c on expression of lipogenesis and apoptosis-related genes in VPA-treated PC-3 cells (a) qRT-PCR analysis of the mRNA levels of SREBP-1a and 1c in PC-3 cells after transfection with C/EBPα or Si-C/EBPα and treatment without or with VPA. β-Actin was used for normalization. (b and c) Cells were transfected with SREBP-1a-expressing plasmid, followed by treatment without or with 2.0 mM VPA. The protein expression in treated cells was determined by Western blot

analysis. β -Actin was used as an internal reference. (d and e) Expression levels of SREBP-1, FASN, ACC1, and Bcl-2 were analyzed by Western blot analysis in VPA-treated PC-3 cells after transfection with SREBP-1c.

Figure 6

Effects of C/EBPα/SREBP-1 pathway on lipogenesis and apoptosis in VPA-treated PC-3 cells (a) PC-3 cells were transfected with an expressing plasmid encoding C/EBPα or SREBP-1a/c, Si-Control, or Si-

C/EBP α , followed by treatment without or with 2.0 mM VPA. The cell viability was determined using MTT assay. (b) Representative microscopic images of Oil-Red O staining of PC-3 cells transfected with C/EBP α - or SREBP-1-expressing plasmid or Si-C/EBP α following treatment with VPA (2.0 mM) for 48 h. (c) Oil-Red O extracted with isopropanol was measured at OD520. (d) Representative dot plots of flow cytometric cell viability measurement on PC-3 cells under different treatments. (e) Percentages of apoptotic cells were analyzed by flow cytometry. The values represent the mean $\pm$ SD of three experiments. Statistically significant differences are indicated: NS (no significance), *P < 0.05, **P < 0.01, and ***P < 0.001.