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Research Article

Potential Pathways Involved in Elaidic Acid Induced Atherosclerosis in Human Umbilical Vein Endothelial Cells

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Researches have demonstrated that trans-fatty acids are related to the progression of atherosclerosis, but the underlying mechanism is not clear till now. In the presented study, two-dimensional electrophoresis based proteomics was used to discover the role of elaidic acid in atherosclerosis. In human umbilical vein endothelial cells (HUVEC), twenty-two and twenty-three differentially expressed proteins were identified in low (50 μmol/L) and high (400 μmol/L) concentration elaidic acid simulated groups, respectively, comparing with the control group. The expressions of some selected proteins ($PSME_3$, $XRCC_5$, $GSTP_1$, and $GSTO_1$) were validated by qRT-PCR analysis. Western blotting analysis further confirmed that elaidic acid downregulated the expression of $PSME_3$ and $XRCC_5$. Moreover, P53, the downstream protein of $PSME_3$, was further investigated. Results demonstrated that a variety of proteins, many of which were related to oxidative stress, apoptosis, and DNA damage, were involved in the elaidic acid induced atherosclerosis. Furthermore, P53 was demonstrated to regulate the atherosclerosis through cell cycle arrest and apoptosis pathway.

1. Introduction

Trans-fatty acids (TFA) refer to unsaturated fatty acids that contain at least one double bond in the trans-configuration [1]. It was reported that the dietary TFA contribute 2.6% of total energy and 7.4% of fat energy in the USA [2]. The Food and Drug Administration made a preliminary determination that the major source of trans-fats, partially hydrogenated oils, was no longer "generally recognized as safe."

TFA is considered to be correlated positively with coronary heart disease, especially atherosclerosis (AS) [3]. So those countries with high TFA intake, such as Northern European countries, had higher coronary heart disease [4]. Fischer et al. found that diet containing linolelaidic acid would increase the proportion of LDL/HDL by 8.1% [5]. Another TFA, elaidic acid, could influence arachidonic acid metabolism, playing an important role in formation of athermanous plaque [6].

The potential mechanism by which TFA promotes AS has been widely studied. Our previous studies have shown that elaidic acid decreased lactate dehydrogenase leakage, content of secretory NO, and the activity of NO synthase in endothelial cells [7]. The activity of caspase-3 was increased by elaidic acid and a caspase inhibitor could protect endothelial cells against trans-C18:1 [8]. As reviewed by Smith et al., there is a positive correlation between TFA levels and AS, and the effects of TFA might be mediated through transcriptional regulation of NF-κB and PPARγ [9]. TFA regulate gene transcription, such as peroxisome-proliferator-activated receptors, liver X receptor, and sterol regulatory element-binding protein-1.4. In animal studies, the consumption of trans-fat altered the expression in adipocytes of genes for PPARγ, resistin, and lipoprotein lipase [10], compounds with central roles in the metabolism of fatty acids and glucose [11]. However, more proteins involved in TFA induced AS need

to be known; the physiological roles of these protein and relationship among them still remain to be characterized.

In recent years, proteomics as a useful means to detect the changes of the protein expression has been applied to identify proteins involved in atherogenesis [12, 13]. It has been reported that many proteins related to calcium-mediated processes, migration of VSMCs (vascular smooth muscle cells), MMP (Motor Mount Pump) activation, and regulation of proinflammatory cytokines were involved in AS [14].

In this study, elaidic acid was used to stimulate endothelial cells, the first interface between circulating blood and the underlying tissues. As a critical metabolic organ, endothelial cells are involved in the generation and regulation of most physiological and pathological processes, such as hemostasis, inflammation, and AS [15]. The two-dimensional electrophoresis- (2-DE-) based proteomics was used to detect these proteins related to elaidic acid induced AS. Among all these proteins, we concentrated on $PSME_3$ and its downstream proteins P53, to discuss the potential mechanism of elaidic acid in AS.

2. Materials and Methods

2.1. Cell Culture. HUVEC (provided by College of Medical Sciences, Nanchang University) were cultured as described by Frost and Lane [15]. Briefly, the cells were incubated in DMEM containing 10% fetal bovine serum at 37°C in a humidified incubator (5% CO_2 at 37°C). The cells were treated with 50 μmol/L (low concentration group) or 400 μmol/L (high concentration group) of elaidic acid (Sigma, St. Louis, USA) for 48 h. The cells cultured in the medium without elaidic acid served as a control group.

2.2. Sample Preparation and Quantification. After being incubated with elaidic acid for 48 h, HUVEC were harvested by centrifugation at 4°C (1000 ×g, 5 min). The supernatants were discarded and the pellets were washed three times with PBS. Then the cells were solubilized in a cell lysis buffer containing 7 M urea, 2 M thiourea, 4% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 0.5% IPG buffer, and 1 mM PMSF. The cell lysates were centrifuged at 15,000 ×g for 60 min and the supernatants were collected and stored at −80°C. Protein concentration was quantified by the Bradford assay [16].

2.3. Effects of Elaidic Acid on Cell Viability. The MTT assay shows an index of cell viability because of the good relationship between cell numbers and absorbance. HUVECs were treated with various concentrations of elaidic acid (50 μmol/l, 200 μmol/l, and 400 μmol/l) for 48 h compared with the solvent group (200 μmol/l of sodium hydroxide) and the control so as to determine the influence of elaidic acid on the viability. MTT dye dissolved in PBS was added to each well for 4 h before the incubation period came to end. The reactions were stopped by adding solubilization reagent (DMSO); then the absorbance was measured at a wavelength of 490 nm with an enzyme-labeling meter (Thermo, Finland). Absorbance of medium without cells was set as the blank control. The viability inhibition was calculated according to the following formula: the viability inhibition ratio (%) = [(the absorbance of elaidic acid group − the absorbance of blank control group)/(the absorbance of control group − the absorbance of blank control group)] × 100%.

2.4. 2-Dimensional Electrophoresis (2DE). Protean IEF cell and Protean II xi cell (Bio-Rad, California, USA) were used to perform 2-DE analysis. The procedure was performed according to manufacturer's instruction. Briefly, nonlinear IPG strips (17 cm, pH 3–10 NL) were rehydrated with samples (190 μg of protein) for 18 h at 20°C. IEF was started at 150 V and the voltage was progressively increased to 5000 V until a maximum of 10000 V. Then, the strips were equilibrated 15 min in equilibration buffer (6 M urea, 2% SDS, and 20% glycerol) twice, with the addition of 20 mg/mL dithiothreitol (DTT) for the first treatment and 2.5% iodoacetamide for the second treatment. Then strips were loaded on 12.5% SDS-PAGE gel and electrophorised for 30 min at 15 mA/gel and then at 30 mA/gel until the bromophenol blue reached the bottom of the gels. After SDS-PAGE, gels were fixed with 10% acetic acid and 40% ethanol overnight, followed by a silver staining according to Blum's method [17]. The analysis of protein spots on the gels was performed with PDQuest software (Version 7.1, Bio-Rad Laboratories); the sophisticated analysis tool reveals subtle differences among 2D gels. Powerful automatching algorithms quickly and accurately match gels with little manual intervention. All protein spots that showed differential changes in expression of 2-fold or greater were considered statistically significant.

2.5. MALDI-TOF Mass-Spectrometric Analysis. Differentially expressed spots were excised from the gel and the gel pieces were destained with 30 mM $K_3Fe(CN)_6$: 100 mM $Na_2S_2O_3$ (1 : 1) and washed 2 × 15 min with 50% ACN and 25 mM NH_4HCO_3 [18]. Then the gel pieces were dehydrated in 100% ACN for 15 min before being dried in a vacuum freeze-drier. The dried gel pieces were digested with trypsin overnight at 37°C and then mixed with a-4-hydroxy cinnamic acid (Sigma, USA) for MALDI-TOF/TOF analysis (ABI, USA). Briefly, 1 μL of peptide was spotted onto a target plate with an equal volume of matrix solution (10 mg/mL α-cyano-4-hydroxycinnamic acid in 70% (v/v) acetonitrile, 1% (v/v) TFA). Mass spectra were acquired in the mass: charge range of 875–3500 *m/z* on a QSTAR XL mass spectrometer equipped with an oMALDI source (Applied Biosystems Inc., Foster City, CA, USA) [19]. The monoisotopic peak masses were subjected to database searching against the NCBI database using MASCOT software (Matrix Science, UK). MASCOT identifies proteins by interpreting mass spectrometry data. This method for protein identification is a bottom-up approach, where a protein sample dissociated to form smaller peptides. MASCOT then compares these molecular weights against NCBI database of known peptides, identifying the protein.

2.6. Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) Analysis. Total RNA was isolated and purified using PrimeScript RT Reagent kit (TaKaRa Bio). RNA was converted into cDNA using superscript III kit (Invitrogen, USA). qRT-PCR was performed using 7300 system according to a

standard program. After an initial hold of 10 min at 95°C, the samples were cycled 40 times at 95°C for 15 sec and 60°C for 60 sec. The expression level of the target transcript was normalized to that of the internal control gene, GAPDH, and the amplification data were analyzed with SDS 2.3 analysis software and calculated using ΔCt.

2.7. Western Blotting Analysis. Following cell protein quantitative analysis, 40 μg of total protein was separated by 10% SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membrane at 40 Ma/cm^2 for 45 min. The PVDF membrane was blocked with 5% (w/v) fat-free milk powder in Tris buffered saline with Tween (TBST) overnight at 4°C. After washing with TBST, the PVDF membranes were probed for 1 h with mouse monoclonal $XRCC_5$ antibody, rabbit polyclonal $PSME_3$ antibody, and rabbit polyclonal CDK4 antibody (Abcam, USA) and then incubated for 1 h with HRP-linked anti-mouse antibody (Abcam, USA). The protein was visualized with an Emitter Coupled Logic detection system. The analysis of protein spots on the gels was performed with PDQuest software version 7.1. The amount of selected proteins was normalized with internal standard β-actin.

2.8. Statistical Analysis. All data were presented as means ± SEM. Statistical assessments were performed with SPSS 13.0 software. Statistical differences between groups under different conditions were analyzed with ANOVA and the Student's t-test. Significance was established at a level of $p < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Elaidic Acid Inhibited the Viability of HUVECs. The viabilityof HUVECs treated with different concentrations of elaidic acid for 48 h was examined by MTT assays. There was no statistical significance between the cell viability of control and that of the solvent group ($p > 0.05$). But viabilities of elaidic acid treated groups were significantly reduced ($p < 0.05$). This result showed that elaidic acid decreased the viability of HUVECs in a dose-dependent manner (Figure 1).

3.1.2. Changes of Protein Expression in Endothelial Cells Induced by Elaidic Acid. In this study, 2DE was used to examine proteins expressed in normal HUVEC group and cells treated with elaidic acid groups. Figure 2 showed the 2DE results in elaidic acid of 50 μmol/L treated group. Twenty-two differently expressed proteins were found in the low concentration group, of which 16 proteins showed increase and 6 proteins showed decrease. In high concentration elaidic acid (400 μmol/L) treated group, 23 differently expressed proteins were found (Figure 3). Among these proteins, the expressions of 17 proteins were downregulated and only 6 proteins were upregulated.

3.1.3. Identification of Differently Expressed Proteins. All proteins that showed different expressions were identified by mass spectroscopy. In the elaidic acid of 50 μmol/L treated group, the expressions of $GSTP_1$, $COTL_1$, $HMGB_1$, DIABLO, and $PSMB_7$ were upregulated; the expressions of $TXNRD_1$ and LTA_4H were downregulated (Table 1). However, after being treated with 400 μmol/L elaidic acid, the expression of $COTL_1$ increased; the expressions of $XRCC_5$, $PSME_3$, SAE_1, $GSTO_1$, SRM, NNMT, EIF_3K, and ETFB decreased (Table 2). These differently expressed proteins play vital roles in cell physiology such as oxidative stress, inflammation, apoptosis, and chromosome damage.

3.1.4. Validation of Differential Gene Expression by qRT-PCR. To confirm the outcome of 2DE, mRNA expression levels of proteins such as PSME3, XRCC5, $GSTP_1$, and $GSTO_1$ were

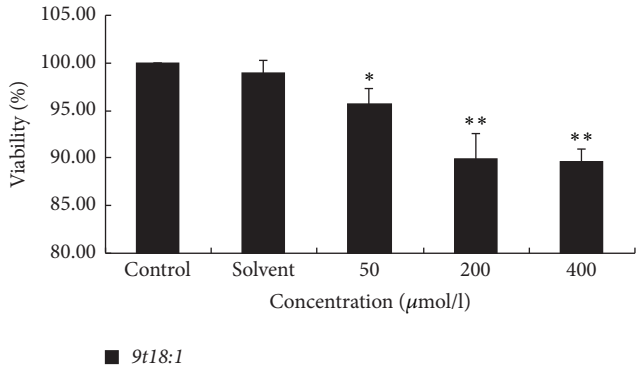

Figure 1: Effect of elaidic acid on cell viability. Control: medium without cells; Solvent: HUVEC treated with 200 μmol/l sodium hydroxide; 50: HUVEC treated with 50 μmol/L elaidic acid; 200: HUVEC treated with 200 μmol/L elaidic acid; 400: HUVEC treated with 400 μmol/L elaidic acid. $*$ indicated a significant difference ($p < 0.05$), $**$ indicated a significant difference ($p < 0.01$).

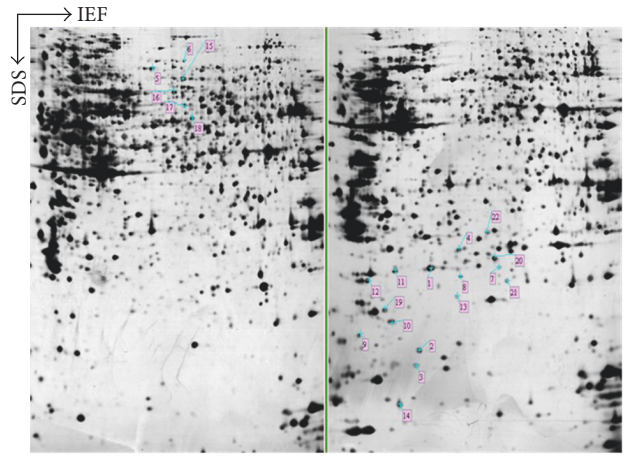

Figure 2: Two-dimensional electrophoresis (2DE) map of HUVEC. (a) Normal HUVEC; (b) HUVEC treated with 50 μmol/L elaidic acid. First dimension: IPG 3–10, 17 cm, NL; second dimension: 12.5% SDS-PAGE gels. Among the 22 different expression proteins, 20 spots were identified by peptide mass fingerprinting and MS/MS analysis. Corresponding identifications are reported in Table 1.

Table 1: List of identified proteins differentially expressed in HUVEC treated with 50 μmol/L elaidic acid.

Spot number	Identification(s)	SWISS-PROT entry name	PI observed	Mass (kDa)	Expression level
(1)	Glutathione S-transferase P ($GSTP_1$)	P09211	5.43	23341	↑
(2)	Adenine phosphoribosyltransferase(APRT)	P07741	5.78	19595.4	↑
(3)	Stathmin 1 ($STMN_1$)	P16949	6.75	9858.3	↑
(4)	Proteasome subunit beta type-7($PSMB_7$)	Q99436	7.57	29946.2	↑
(5)	ATP-dependent DNA helicase 2subunit 2 ($XRCC_5$)	P13010	5.55	82652.3	↓
(6)	Isoform Short of Cold shockdomain containing protein E1($CSDE_1$)	O75534	5.84	85693.4	↓
(7)	Neighbor of COX4 (COX_4NB)	O43402	5.92	23757.7	↑
(8)	cDNA FLJ52710, highly similarto Abhydrolase domain containingprotein 14B ($ABHD_{14}B$)	B4DKK0	5.94	19784.2	↑
(9)	DIABLO homolog, mitochondrialPrecursor (DIABLO)	Q9NR28	9.38	35822.5	↑
(10)	Isoform 1 of ATP synthasesubunit d, mitochondrial (ATP_5H)	O75947	5.21	18479.5	↑
(11)	Isoform 1 of Ubiquitin conjugating enzyme E2 K (UBE_2K)	P61086	5.33	22392.6	↑
(12)	SET nuclear oncogene (SET)	B2REB7	4.14	30974.2	↑
(14)	Coactosin-like protein ($COTL_1$)	Q14019	5.54	15935	↑
(15)	Putative uncharacterized proteinGARS (GARS)	Q75MN1	5.88	77481.3	↓
(16)	Isoform 1 of Leukotriene A-4Hydrolase (LTA_4H)	P09960	5.8	69241.2	↓
(17)	Dihydropyrimidinase-related protein 2 ($DPYSL_2$)	Q16555	5.95	62254.6	↓
(18)	Isoform 5 of Thioredoxin reductase 1, cytoplasmic ($TXNRD_1$)	Q16881	6.07	54671.9	↓
(19)	Isoform 1 of RNA-binding protein 8A (RBM_8A)	Q9Y5S9	5.5	19876.7	↑
(20)	Proteasome subunit beta type-3($PSMB_3$)	P49720	6.14	22933.4	↑
(21)	Isoform 2 of high mobility groupprotein B1 ($HMGB_1$)	P09429	5.62	24878.2	↑
(22)	Isoform 2 of 3,2-trans-enoyl-CoAisomerase, mitochondrial (DCI)	P42126	9.07	30876.3	↑

Table 2: List of identified proteins differentially expressed in HUVEC treated with 400 μmol/L elaidic acid.

Spot number	Identification(s)	SWISS-PROT entry name	PI observed	Mass (kDa)	Expression level
(1)	Eukaryotic translation initiation factor 3 subunit K (EIF_3K)	Q9UBQ5	4.81	25043.4	↓
(2)	Coactosin-like protein ($COTL_1$)	Q14019	5.54	15935	↑
(3)	Isoform 2 of proteasome activator complex subunit 3 ($PSME_3$)	P61289	5.79	30867.2	↓
(4)	Glutathione S-transferase omega-1 ($GSTO_1$)	P78417	6.23	27548	↓
(6)	Platelet-activating factor acetylhydrolase IB subunit gamma ($PAFAH_1B_3$)	Q61205	6.33	25718.2	↑
(7)	S-formylglutathione hydrolase (ESD)	P10768	6.54	31442.5	↓
(8)	DTYMK protein (DTYMK)	Q6FGU2	8.87	21051	↓
(9)	Proteasome subunit alpha type-2 ($PSMA_2$)	P25787	6.92	25882.3	↓
(10)	Transgelin-2 ($TAGLN_2$)	P37802	8.41	22377.2	↑
(11)	Isoform 1 of electron transfer flavoprotein subunit beta (ETFB)	P38117	8.24	27826.2	↓
(12)	EF-hand domain containing protein D2 ($EFHD_2$)	Q96C19	5.15	26680.5	↓
(13)	Nicotinamide N-methyl-transferase (NNMT)	P40261	5.56	29555.1	↓
(14)	Putative uncharacterized protein H-N1 (HN_1)	P54920	5.23	33211.3	↑
(15)	Glyoxylate reductase/hydroxypyruvate reductase (GRHPR)	Q9UBQ7	7.05	43565.6	↓
(16)	Diphosphomevalonate decarboxylase (MVD)	P53602	6.79	43377.1	↓
(17)	cDNA FLJ42145 fis, clone TESTI-4000228, highly 21 similar to Mus musculus ubiquitin family domain containing 1 ($UBFD_1$)	B3KW52	5.69	32619.5	↑
(18)	Alpha-soluble NSF attachment protein (NAPA)	P54920	5.23	33211.3	↓
(19)	Spermidine synthase (SRM)	P19623	5.3	33802.7	↓
(20)	SUMO-activating enzyme subunit 1 (SAE_1)	Q9UBE0	7.6	33363.2	↓
(21)	Isoform 4 of Heterogeneous nuclear ribonucleoprotein D0 (HNRNPD)	Q14103	8.53	30653.1	↑
(22)	ATP-dependent RNA helicase (DDX3X)	O00571	6.73	73198	↓
(23)	ATP-dependent DNA helicase 2 subunit 2 ($XRCC_5$)	P13010	5.55	82652.3	↓

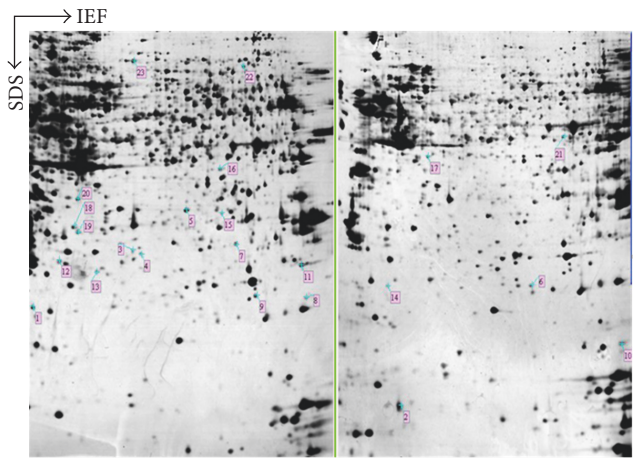

Figure 3: Two-dimensional electrophoresis (2DE) map of HUVEC. (c) Normal HUVEC; (d) HUVEC treated with 400 μmol/L elaidic acid. First dimension: IPG 3–10, 17 cm, NL; second dimension: 12.5% SDS- PAGE gels. Among the 23 different expression proteins, 22 spots were identified by peptide mass finger printing and MS/MS analysis. Corresponding identifications are reported in Table 2.

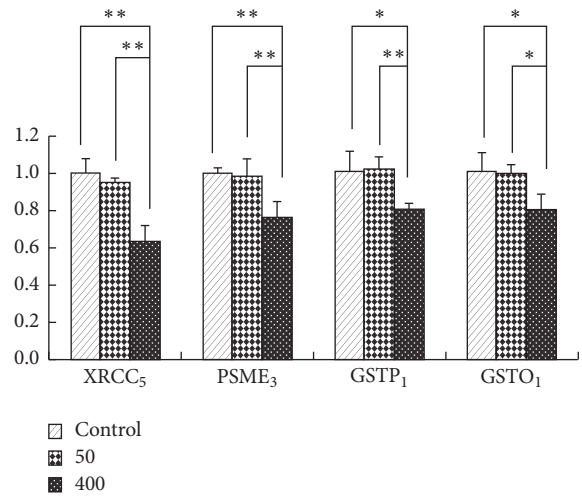

Figure 4: Effect of elaidic acid on $XRCC_5$, $PSME_3$, $GSTP_1$, and $GSTO_1$ mRNA expression. $*$ indicated a significant difference ($p < 0.05$), $**$ indicated a significant difference ($p < 0.01$).

analyzed through qRT-PCR. The expression of downstream gene P53 was also detected. To further investigate the p53 pathway, the expressions of some relative gene, such as p21, p53, $PSME_3$, CDK4, $CyclinD_1$, and Bcl-2 were detected at the same time.

Figure 4 showed that mRNA levels of $XRCC_5$, $PSME_3$, $GSTP_1$, and $GSTO_1$ were decreased significantly in 400 μmol/L elaidic acid group ($p < 0.05$). All of these genes were benefit to the HUVECs proliferation or DNA repair. Obviously, 400 μmol/L elaidic acid can inhibit HUVECs viability. Therefore, both of the tumor suppressor genes, p21 and p53, were increased (Figure 5) in mRNA levels significantly, whereas $PSME_3$, CDK4, CyclinD1, and Bcl-2 were decreased significantly in high concentration group ($p < 0.05$). All of these results indicated that 400 μmol/L elaidic acid can increase the DNA damage and strengthen the cell cycle arrest of HUVECs.

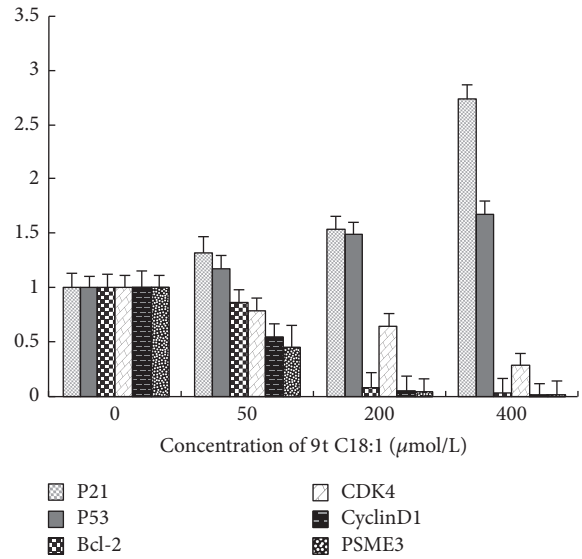

Figure 5: Effect of TFA on p21, p53, Bcl-2, CDK4, CyclinD1, and PSME3 mRNA expression in HUVEC.

3.1.5. Protein Expression Confirmed by Western Blotting. Figures 6 and 7 displayed that the protein expression levels in the elaidic acid treated groups of oncogene, XRCC5, were significantly reduced. And the cell cycle regulation related PSME3 and CDK4 were also significantly decreased comparing to the control groups, which were consistent with the results of mRNA levels and the 2DE results. It was obvious that the 400 μmol/L elaidic acid can promote the HUVECs apoptosis and AS development.

3.2. Discussions. According to 2DE-based proteomic, a variety of proteins showed differential expression in elaidic acid induced HUVEC. Many of those proteins are involved in oxidative stress, cell apoptosis, and DNA damage in atherosclerosis progression.

3.2.1. Elaidic Acid Induced the Downregulation of Antioxidative Proteins. Both randomized trials and observational studies showed that increased intake of TFA was associated with a higher risk of cardiovascular disease [20, 21] and previous studies have clearly demonstrated that oxidative damage plays a major role in the pathogenesis of cardiovascular diseases including dysfunction and pathological cardiac remodeling [22]. Dhibi et al. [23] observed rats fed with oxidized soybean oil diet and found the relationship between the consumption of TFA and nonalcoholic fatty liver disease. The results had shown that high TFA levels in the oxidized oils may represent a direct source of oxidative stress for the organism.

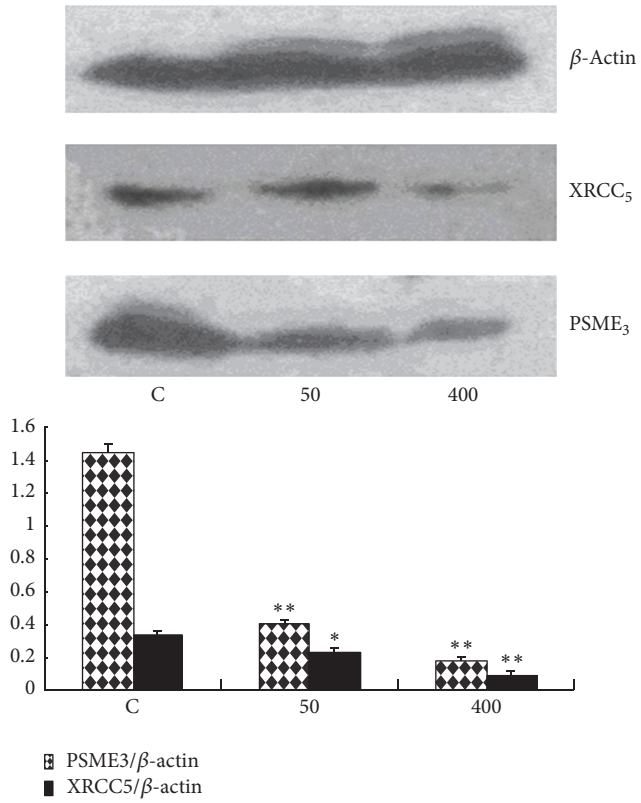

Figure 6: Effect of elaidic acid on expression of $XRCC_5$ and $PSME_3$ proteins. C: normal HUVEC as control; 50: HUVEC treated with 50 μmol/L elaidic acid; 400: HUVEC treated with 400 μmol/L elaidic acid. $*$ indicated a significant difference ($p < 0.05$); $**$ indicated a very significant difference ($p < 0.01$), when compared to the control group.

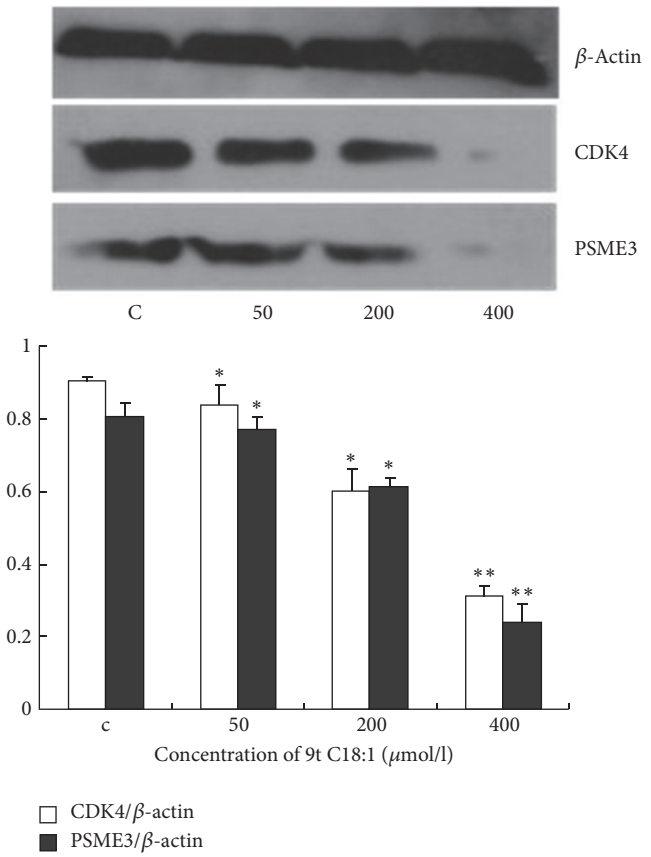

Figure 7: Effect of elaidic acid on expression of CDK_4 and $PSME_3$ proteins. C: normal HUVEC as control; 50: HUVEC treated with 50 μmol/L elaidic acid; 200: HUVEC treated with 200 μmol/L elaidic acid; 400: HUVEC treated with 400 μmol/L elaidic acid. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared to the control group.

In the results of our experiment, elaidic acid treatment caused a decreased expression of some antioxidative proteins, including $TXNRD_1$ (Table 1, spot (18)) and SRM (Table 2, spot (19)). Thioredoxin reductase (TXNRD), maintains a high ratio of reduced to oxidized thioredoxin, a primary intracellular thiol with redox-buffering capacity [24]. $TXNRD_1$ regulates the levels of intracellular reactive oxygen species and modulates intracellular oxidative states [25]. The downregulation of $TXNRD_1$ expression inhibits antioxidative ability of cells, thus increasing the risk of AS. Li et al. [26] have demonstrated that Nrf2 coordinates a group of antioxidant genes, including GPx, HO-1, NQO-1, Txn-1, Txnrd-1, SOD-2, and SOD-3, to suppress oxidative stress in the heart serving as a negative regulator of maladaptive cardiac remodeling and dysfunction. Cho et al. [27] suggested that a substantial portion of NRF2-mediated pulmonary protection against hyperoxic injury was via cellular defense enzymes associated with thiol metabolism and homeostasis pathways. Based on our knowledge, we supposed that TFA might lead to oxidative damage through Nrf2 pathway, mediating the expression of downstream gene $TXNRD_1$. In addition, it was reported that TFA presented in our diet had a direct proinflammatory effect, which promotes leukocyte adhesion to the endothelium through ROS-dependent NF-κB activation [28]. Therefore, the mechanism of TFA induced oxidative damage could be owed to these two pathways, Nrf2 pathway and NF-κB pathway.

Spermidine synthase (SRM) was found decreased in 400 μmol/L elaidic acid group. Its product, spermidine, is predominant polyamine in eukaryotic cells [29]. It plays beneficial roles in cell activity, such as regulating cytosolic Ca^{2+} homeostasis and reducing lipid peroxidation [30]. Spermidine and nitric oxide were both key points of L-Arginine metabolic pathway. Spermidine and spermine, both polyamines, were mediators derived from the arginase product L-ornithine which have also been shown to inhibit NO synthesis in activated J774.2 and rat alveolar macrophages [31]. Spermine suppressed the expression of iNOS. Our previous study had demonstrated that TFA induced cell inflammation through NO-NOS pathway [7], and TFA could increase the expression of iNOS in smooth muscle cells [32]. Taken together, the nitric oxide pathway was involved in the mechanism of TFA induced cell injury and it plays an important role in AS [33]. It is suggested that elaidic acid affected the process of AS by decreasing the expression of some antioxidative proteins.

3.2.2. Effect of Elaidic Acid on Cell Apoptosis Pathway. The presented study also indicated that elaidic acid inhibited the viability of HUVECs in a dose-dependent manner and affected the expressions of some proteins involved in cell apoptosis, such as the DIABLO (Table 1, spot (9)), $GSTO_1$ (Table 2, spot (4)), and $PSME_3$ (Table 1, spot (20), and Table 2, spot (3)). DIABLO is a novel protein that can promote apoptosis by binding to inhibitor of apoptosis protein [34]. $GSTO_1$, a member of phase II detoxification enzymes, was reported to play an antiapoptotic role in cell. Overexpression of these proteins is associated with activation of survival pathways (AKt and ERK1/2) and inhibition of apoptotic pathways (JNK_1) [35]. In addition, $PSME_3$ has been showed to implicate in the regulation of cell cycle progression and apoptosis. Murata et al. [36] observed that $PSME_3$ deficient mice grew more slowly and were around 10% smaller than wild-type mice at maturity. $PSME_3$ deficient embryonic fibroblasts demonstrated spontaneous apoptosis and G1 arrest [37].

P53 is a downstream protein of $PSME_3$, which was found to have increased expression. $PSME_3$ can regulate p53 by enhancing its MDM_2-mediated degradation [38]. Exposure to cellular stress would trigger the p53 tumor suppressor and then induce cell cycle arrest (at G1 and/or G2 phase) or apoptosis [39]. In our experiment, elaidic acid treatment increased mRNA expressions of p53 and p21 and decreased Cyclin D_1 and CDK_4 mRNA expressions, leading to the blocking of G0/G1 transition. P21, the transcription product of CIP/WAF1, is the direct cause of G1 phase retardation. Complexes consisting of Cyclin D_1, CDK_4, and CDK_2 are suggested to regulate the transformation of G_1/S phase. P21 gene was reported to inhibit the activity of CDKs kinase and further regulate the cell cycle [40–44]. On the other hand, it was reported that Bcl-2, likely acting downstream of p53, blocked p53-dependent apoptosis [45]. Bcl-2 regulated and controlled apoptosis through mitochondrial regulation pathway [46]. Bin et al. [47] reported that TFA induced apoptosis of HUVEC by caspase pathway and the mitochondria. Moreover, Rao et al. [48] have demonstrated that p53 expression increased after TFA treatment mediated by lipid rafts and Fas/FasL pathway. We concluded that elaidic acid increased p53 and decreased Bcl-2 mRNA expression, which contributed to the apoptosis of HUVEC. A potential molecular mechanism involved in elaidic acid induced apoptosis and cell cycle arrest via p53 activation in HUVEC were proposed (Figure 8).

3.2.3. Effect of Elaidic Acid on DNA Damage. In 50 μmol/L of elaidic acid stimulated HUVEC group, the expression levels of $GSTP_1$ and $PSMB_7$ were increased (Table 1). $GSTP_1$ (spot (1)) is a member of the glutathione s-transferase superfamily. It has been reported that the inactivation of $GSTP_1$ indicated the induction of DNA damage [49]. The overexpression of $GSTP_1$ in cancer cells enhanced their resistance; then tumors often develop toward anticancer agents [49]. $PSMB_7$ (spot (4)), the proteasome subunit, is associated with anthracycline resistance. The patients with high $PSMB_7$ expression had a significantly shorter duration than that of the patients with low expression ($p < 0.001$) [50]. Both of GSTP1 and PSMB7

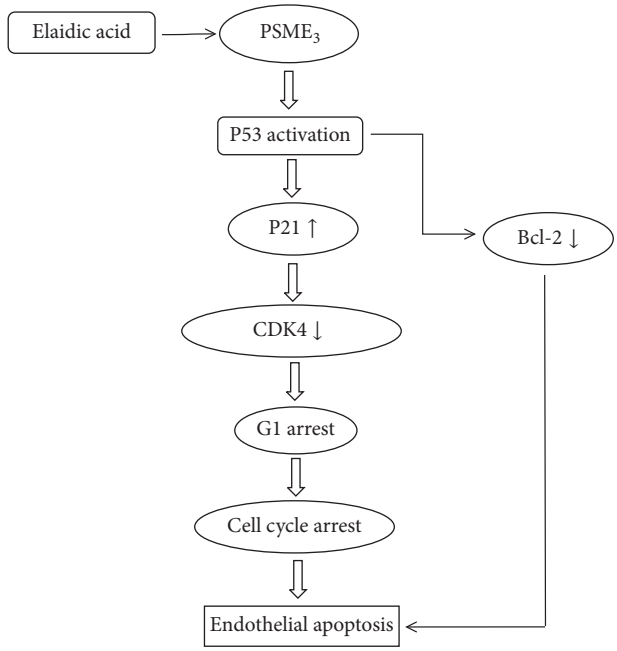

Figure 8: Schematic diagram showing signaling pathway of elaidic acid treatment in endothelial apoptosis.

could protect cells from damage. So the results indicated that HUVEC performed self-protection function to resist damage in low concentration of elaidic acid treatment.

However, elaidic acid of high concentration could induce cells damage. As found in the study, $GSTO_1$ (spot (4)) expression was decreased in HUVEC treated with 400 μmol/L of elaidic acid. The overexpression of $GSTO_1$ was associated with anticancer therapies resistance [51]. Some other proteins related to DNA damage were also downregulated in HUVEC treated with 400 μmol/L elaidic acid, such as $XRCC_5$ (spot (23)), $PSME_3$ (spot (3)), and SAE_1 (spot (20)). $XRCC_5$, a subunit of the Ku heterodimer protein, played a major role in cellular resistance to radiation [52]. However, the expression of P53 was increased (Figure 5); this indicated that the DNA damage level was increased. It is to keep the cell from progressing through the cell cycle, if there is damage to DNA presented. Of course, the expression of p21 was also enhanced (Figure 5); this indicated the severe damage of DNA and that HUVECs may have entered apoptosis. Furthermore, the upregulation of P21 was related to the PSME3 for its degradation depended on the content of PSME3. $PSME_3$, a proteasome activator, is involved in the degradation of many proteins that regulate cell cycle. $PSME_3$ was reported to play a vital role in the maintenance of chromosomal stability exposed to DNA damaging agents [53]. Moreover, SAE_1 is implicated in signal transduction for DNA damage and repair [54].

4. Conclusion

Elaidic acid was used to stimulate endothelial cells; two-dimensional electrophoresis- (2DE-) based proteomics was

used to detect these proteins related to elaidic acid induced AS, to find that expression of $PSME_3$ and its downstream proteins P53 changed. The study indicated that elaidic acid could affect the process of AS by variety means inducing oxidative stress, cell apoptosis, and DNA damage. Furthermore, proteomic analysis by 2DE could be applied on the identification and characterization of signaling pathways involved in trans-fatty acids induced atherogenesis.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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