Article

Xanthigen Suppresses Preadipocyte Differentiation and Adipogenesis through Down-regulation of PPARγ and C/EBPs and Modulation of SIRT-1, AMPK, and FoxO Pathways

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ABSTRACT: Xanthigen is a source of punicic acid and fucoxanthin derived from pomegranate seed and brown seaweed, respectively with recognized triacylglycerol-lowering effects in humans, yet the mechanism remains to be fully elucidated. The present study investigated the inhibitory effects of Xanthigen, fucoxanthin, and punicic acid (70% in pomegranate seed oil) on the differentiation of 3T3-L1 preadipocytes. Xanthigen potently and dose-dependently suppressed accumulation of lipid droplets in adipocytes compared to its individual components, fucoxanthin and pomegranate seed oil. Western blot analysis revealed that Xanthigen markedly down-regulated the protein levels of key adipogenesis transcription factors peroxisome proliferator-activated receptor (PPAR)γ, CCAAT/enhancer binding protein (C/EBP) β, and C/EBPδ as well as a key enzyme involved in adipogenesis, fatty acid synthase (FAS). Xanthigen up-regulated the NAD⁺-dependent histone deacetylases (SIRT1) and activated AMP-activated protein kinase (AMPK) signaling in differentiated 3T3-L1 adipocytes. In addition, Xanthigen may also stimulate insulin trigger signaling and result in Akt-dependent phosphorylation of forkhead/winged helix O (FoxO)1 and FoxO3a. These results indicate that Xanthigen suppresses adipocyte differentiation and lipid accumulation through multiple mechanisms and may have applications for the treatment of obesity.

KEYWORDS: Xanthigen, 3T3-L1 adipocyte, differentiation, SIRT1, PPARγ, AMPK, FoxO, C/EBPs

■ INTRODUCTION

Obesity is a highly prevalent condition related to metabolic disorders worldwide. Excessive fat stored in adipose tissue caused by environmental factors, lifestyles, and genetic factors is associated with numerous metabolic diseases such as cardiovascular disease, insulin resistance, type 2 diabetes, hypertension, fatty liver disease, and increase mortality.[1] As caloric intake increases, adipocytes store energy in the form of triacylglycerols that result in enhanced adipogenesis, increased adipose tissue mass, and consequently obesity.[2] In addition to fat storage, adipose tissue is a major endocrine and metabolic organ secreting adipocytokines, cytokines, growth factors, and hormones that are involved in host immunity, energy homeostasis, systemic insulin sensitivity, and tissue regeneration. In obesity, dysfunction of adipose tissue contributes to abnormal cytokine and hormone production in adipocytes and results in metabolic dysfunction.[2]

Adipocyte differentiation is a pivotal biological process during adipogenesis in adipose tissue that is characterized by alteration of cellular properties. Differentiation of preadipocytes undergoes morphological and biochemical transition from growth arrest, clonal expansion, and terminal differentiated to mature adipocytes, followed by changes in genetic programming for lipid synthesis and storage.[3] This process is controlled by a set of transcription factors such as C/EBPs and ligand-dependent nuclear receptor PPARγ. Once initiated by

adipogenic signal, C/EBPβ and C/EBPδ are rapidly expressed in the early phase of differentiation.[4] The induction of C/EBPβ and C/EBPδ functions to up-regulate PPARγ and C/EBPα in terminal differentiation that induces a series of adipocyte-specific gene expression involved in adipogenesis, insulin signaling, and glucose and lipid metabolism.[3,5] A number of studies have documented that C/EBPs and PPARγ play central roles in mediating adipocyte differentiation. Studies have shown that overexpression of C/EBPβ or C/EBPδ in preadipocytes promoted lipid accumulation.[6] A reduced droplet accumulation is occurred in mice lacking C/EBPβ or C/EBPδ.[7] In addition, activation of PPARγ is sufficient to promote adipose differentiation in fibroblastic cells.[8]

Besides C/EBPs and PPARγ, insulin and insulin-like growth factor-1 (IGF-1) signaling are also involved in the regulation of adipocyte differentiation.[9] FoxO transcription factors are important for cellular metabolism and tissue homeostasis by transcription of genes involved in the regulation of glucose metabolism, reactive oxygen species (ROS) detoxification, cell cycle arrest, apoptosis, autophagy, and DNA repair.[10] In the insulin/IGF-1-dependent signaling cascade, phosphorylation of

FoxOs by Akt promotes their exclusion from the nucleus that results in their inactivation.[11,12] Among the isoform of FoxOs, FoxO1 has been reported to play an essential role in coupling insulin signaling to adipocyte differentiation.[13] AMPK acts as a nutrient sensor and central regulator of cellular energy homeostasis. Studies indicate that activation of AMPK leads to the inhibition of adipocyte differentiation and decreases adipogenesis.[14,15] Phosphorylation of metabolic enzymes acetyl-CoA carboxylase 1 (ACC1) and HMG-CoA reductase (HMGCR) by AMPK promotes fatty acid oxidation and reduces cholesterol synthesis.[16,17]

It is believed that a combination of decreased caloric intake and physical exercise is effective for the prevention of obesity,[18] but it is difficult for people to change lifestyles and control their diet. Some antiobesity drugs have been used to treat overweight or obese patients, however, with less efficacy and safety concerns.[19] Nowadays, researchers have become increasingly interested in searching for natural products from dietary and edible plants that possess antiobesity activity. A large body of literature shows that many phytochemicals and natural products are used to treat obesity as metabolic stimulates, appetite suppressants, and starch blockers and by regulation of glucose and lipid metabolism as well as targeting adipocyte differentiation.[20]

Xanthigen is a source of punicic acid and fucoxanthin derived from pomegranate seed oil and brown seaweed, respectively. Fucoxanthin, a major marine carotenoid molecule of edible brown seaweed, has been found to have anticancer, antioxidant, and anti-inflammatory properties.[21−23] Punicic acid is a conjugated linolenic acid, rich in the seeds of *Punica granatum*, which is reported to inhibit cancer cell proliferation, neutrophil hyperactivation, and colonic inflammation in vivo.[24−26] Recent research has also demonstrated the antiobesity potential of both fucoxanthin and punicic acid, evidenced in the suppression of adipocyte differentiation and high-fat-diet induced obesity and insulin resistance in vivo.[27−29] Although dietary intake of Xanthigen promotes weight loss and reduces body and liver fat in obese women,[30] the effect of Xanthigen at the cellular level remains unclear. In this study, we investigated the inhibitory effects of pomegranate seed oil (PSO), fucoxanthin, and Xanthigen on differentiation of 3T3-L1 preadipocytes. We found that Xanthigen was more potent on suppression of 3T3-L1 adipocyte differentiation than pomegranate seed oil and fucoxanthin, through down-regulation of C/EBPβ, C/EBPδ, and PPARγ, up-regulation of SIRT-1, and modulation of FoxOs and AMPK signalings.

■ MATERIALS AND METHODS

Materials. Dulbecco's modified Eagle's medium (DMEM), penicillin−streptomycin, fetal calf serum (FCS), and fetal bovine serum (FBS) were from Gibco BRL (Grand Island, NY). Insulin, 3-isobutylmethylxanthine (IBMX), and dexamethasone (DEX) were purchased from Sigma Chemical Co. (St. Louis, MO). Anti-PPARγ, anti-C/EBPβ, and anti-C/EBPδ antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β-actin antibody was purchased from Sigma Chemical Co. Other antibodies used in this study were purchased from Cell Signaling Technology (Beverly, MA). Xanthigen, fucoxanthin, and pomegranate seed oil were provided from PoliNat Inc., Canary Islands, Spain. Xanthigen is a mixture of 300 mg of pomegranate seed oil standardized for 80% punicic acid and 300 mg of brown seaweed extract containing 2.4 mg of fucoxanthin.

Cell Culture and Adipocyte Differentiation. Mouse 3T3-L1 preadipocytes purchased from the American Type Culture Collection (Rockville, MD) were grown in DMEM supplemented with 2 mM glutamine (GIBCO BRL), 1% penicillin/streptomycin (10000 units of penicillin/mL and 10 mg streptomycin/mL), and 10% fetal bovine serum at 37 °C under a humidified 5% CO_2 atmosphere. For differentiation of 3T3-L1 preadipocytes, cells were seeded into a 96-well (2×10^4/mL) plate or a 10 cm dish and cultured as described above. Two days after confluence (defined as day 0), cells were incubated in differentiation medium containing 1.7 μM insulin, 0.5 mM 3-isobutylmethylxanthine (IBMX), and 1 μM dexamethasone (DEX) in DMEM containing 10% fetal bovine serum (FBS) for 48 h. The medium was then replaced by DMEM containing 10% FBS and insulin (1.7 μM) with or without PSO, fucoxanthin, and Xanthigen, which was replaced every 2 days. After 8 days (at day 10), the cells were harvested, and then total protein was extracted for Oil Red O staining and Western blot analysis.

Cell Culture and Adipocyte Differentiation. The effects of PSO, fucoxanthin, and Xanthigen on cell viability were assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, cells were seeded into a 96-well plate (2×10^4/mL) overnight and treated with a series of concentrations of PSO, fucoxanthin, and Xanthigen for 48 h. The final concentrations of dimethyl sulfoxide (DMSO) in the culture medium were <0.05%. At the end of treatment, MTT (200 μg/mL) was added, and cells were incubated for a further 4 h at 37 °C. After removal of the liquid, the MTT formazan were dissolved by DMSO. Cell viability was determined by scanning with an ELISA reader with a 570 nm filter.

Oil Red O Staining. To quantify lipid accumulation, Oil Red O staining was performed on day 10. Briefly, cells were washed with phosphate-buffered saline (PBS) twice, fixed with 10% formalin for 60 min, stained with 0.5% Oil Red O in 2-propanol for 1 h at room temperature, and washed twice with distilled water. The stained lipid droplets within cells were visualized by light microscope and photographed with a digital camera at 100× magnification. The stained lipid droplets were dissolved in 2-propanol and quantified for absorbance (540 nm) measurement.

Western Blotting. The total proteins were extracted via addition of gold lysis buffer (50 mM Tris-HCl, pH 7.4; 1 mM NaF; 150 mM NaCl; 1 mM EGTA; 1 mM phenylmethanesulfonyl fluoride; 1% NP-40; and 10 μg/mL leupeptin) to the cell pellets on ice for 30 min, followed by centrifugation at 10000g for 30 min at 4 °C. The total proteins were measured by Bio-Rad Protein Assay (Bio-Rad Laboratories, Munich, Germany). The samples (50 μg of protein) were mixed with 5× sample buffer containing 0.3 M Tris-HCl (pH 6.8), 25% 2-mercaptoethanol, 12% sodium dodecyl sulfate (SDS), 25 mM EDTA, 20% glycerol, and 0.1% bromophenol blue. The mixtures were boiled at 100 °C for 5 min and were subjected to 10% SDS−polyacrylamide minigels at a constant current of 20 mA. Electrophoresis was then carried out on SDS−polyacrylamide gels. Proteins on the gel were electrotransferred onto an immobile membrane (PVDF; Millipore Corp., Bedford, MA) with transfer buffer composed of 25 mM Tris-HCl (pH 8.9), 192 mM glycine, and 20% methanol. The membranes were blocked with blocking solution containing 20 mM Tris-HCl and then immunoblotted with primary antibodies including SIRT-1 and β-actin (Transduction Laboratories, Lexington, KY). The blots were rinsed three times with PBST buffer for 10 min each. Then blots were incubated with 1:5000 dilution of the horseradish peroxide (HRP)-conjugated secondary antibody (Zymed Laboratories, San Francisco, CA) and then washed again three times with PBST buffer. The transferred proteins were visualized with an enhanced chemiluminescence detection kit (ECL; Amersham Pharmacia Biotech, Buckinghamshire, U.K.).

Statistical Analysis. Data were presented as the mean ± SE for the indicated number of independently performed experiments. One-way ANOVA was used to assess the statistical significance between the differentiated 3T3-L1 adipocytes and PSO-, fucoxanthin-, and Xanthigen-treated cells, respectively. A P value of <0.05 was considered to be statistically significant.
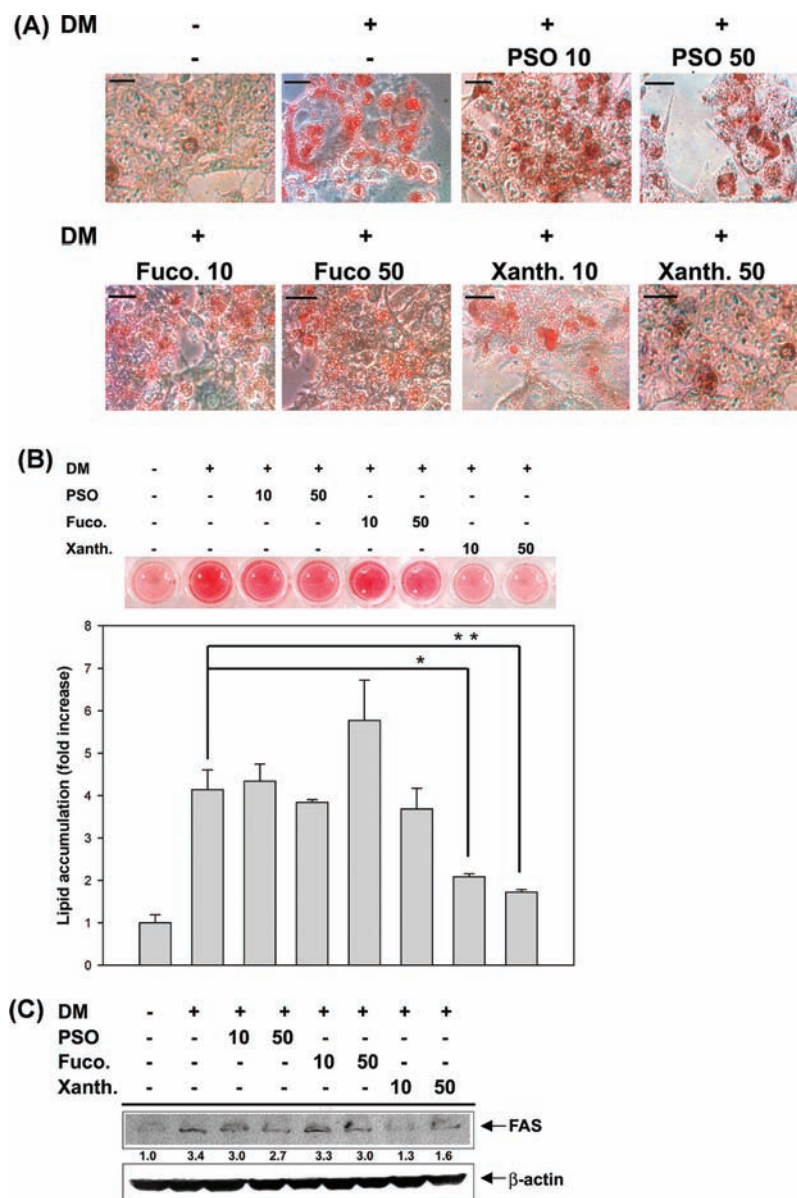
Figure 1. Effects of PSO, fucoxanthin, and Xanthigen treatment on 3T3-L1 adipocyte differentiation. 3T3-L1 preadipocytes were incubated with IBMX, DEX, and insulin with or without PSO, fucoxanthin, and Xanthigen, respectively, for 2 days and then replaced with DMEM containing insulin and PSO, fucoxanthin, and Xanthigen for 8 days. (A) Cells were stained with Oil Red O and photographed. Scale bars = 20 μm. (B) Oil Red O stained cells were extracted by 2-propanol and lipid content was quantified by spectrophotometric analysis at 540 nm. ($*$) $P < 0.01$ and ($***$) $P < 0.001$ indicate statistically significant differences from the differentiated 3T3-L1 adipocytes. (C) Protein level of FAS in cell lysates was analyzed by Western Blotting. β-Actin was used as a loading control. This experiment was repeated three times with similar results.

RESULTS

Cytotoxicity Effect of PSO, Fucoxanthin, and Xanthigen in 3T3-L1 Preadipocytes. To investigate the cytotoxic effect of PSO, fucoxanthin, and Xanthigen, we estimated the cell viability of 3T3-L1 preadipocytes after treatment with different concentrations of PSO, fucoxanthin, and Xanthigen by the MTT-tetrazolium assay. We found treatment with PSO and fucoxanthin at 100 μg/mL for 48 h significantly decreased cell viability by 68 ± 6.9 and 77.2 ± 4.5% ($P < 0.01$), respectively, whereas Xanthigen showed no significant cytotoxicity (91.8 ± 5.3%). To compare the effects of PSO, fucoxanthin, and Xanthigen on adipocyte differentiation, lower concentrations of 10 and 50 μg/mL were used for the following study according to the weaker cytotoxicity effect, which showed no significant

effect on cell viability of 3T3-L1 preadipocytes (data not shown).

Effect of PSO, Fucoxanthin, and Xanthigen on Lipid Accumulation in 3T3-L1 Adipocytes. To investigate the effects of PSO, fucoxanthin, and Xanthigen on adipocyte differentiation, 3T3-L1 preadipocytes were treated with differentiated medium (DM) and insulin with or without PSO, fucoxanthin, and Xanthigen. Ten days after the initiation of differentiation, lipid accumulation was measured by Oil Red O staining. Figure 1A shows the result of Oil Red O staining under a microscope. Treatment of 3T3-L1 adipocytes with PSO and fucoxanthin at 10 and 50 μg/mL did not show significantly decreased lipid droplet accumulation. However, Xanthigen potently reduced lipid accumulated in 3T3-L1 adipocytes in a dose-dependent manner. Quantitative results

are shown in Figure 1B: PSO and fucoxanthin (50 μg/mL) treatment only slightly decreased lipid accumulation, whereas the treatment with fucoxanthin at 10 μg/mL showed an increased lipid content that was consistent with a previous study,[31] indicating fucoxanthin enhanced lipid accumulation when applied at early stage of differentiation. On the other hand, Xanthigen treatment displayed a notable and dose-dependent decreased lipid accumulation in differentiated adipocytes (decreases of 49.6 and 63.3% at 10 and 50 μg/mL, respectively). We also found that the protein expression of fatty acid synthase (FAS), a central enzyme in adipogenesis, was markedly reduced by Xanthigen treatment compared with PSO and fucoxanthin (Figure 1C). Because Xanthigen is rich in both PSO and fucoxanthin, these results demonstrated the potential effect of Xanthigen on suppression of 3T3-L1 adipocyte differentiation.

Xanthigen Inhibited PPARγ and C/EBPs and Up-regulated SIRT-1 Expression in 3T3-L1 Adipocytes. PPARγ and C/EBPs are master transcription factors in the regulation of adipogenesis in adipocytes.[5,8] Hence, we next investigated the expression of PPARγ, C/EBPβ, and C/EBPδ to characterize the effect of Xanthigen on 3T3-L1 adipocyte differentiation. As shown in Figure 2A, the protein expression
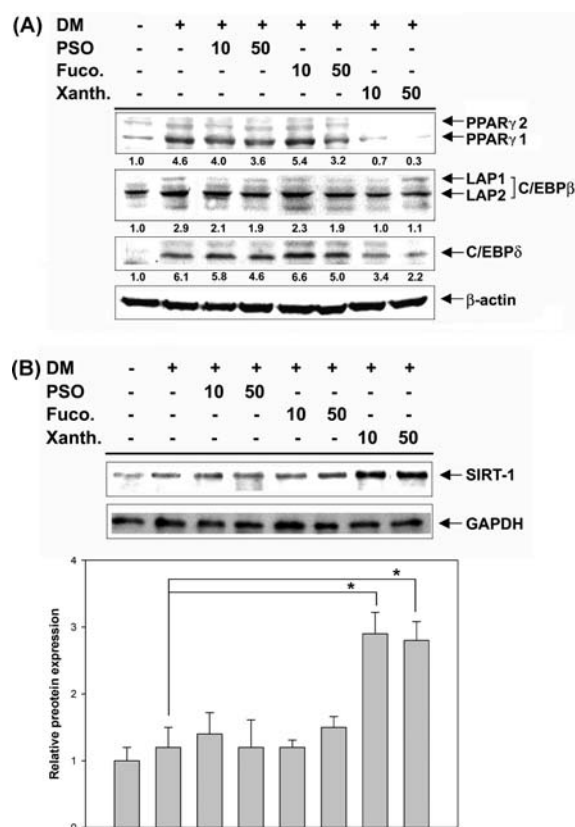

Figure 2. Effects of Xanthigen on PPAR, C/EBPs, and SIRT1 protein expressions in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were treated as described previously. After 8 days of treatment, total protein was extracted and the protein expressions of (A) PPARγ, C/EBPβ, and C/EBPδ and (B) SIRT1 were determined by Western blot analysis. The relative protein expression of SIRT1 was performed by densitometric analysis. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. ($\ast$) $P < 0.01$ indicates statistically significant difference from the differentiated 3T3-L1 adipocytes. This experiment was repeated three times with similar results.

of PPARγ was increased in differentiated adipocytes compared to undifferentiated cells. It was found that treatment with PSO and fucoxanthin both slightly decreased PPARγ protein levels, whereas fucoxanthin at a concentration of 10 μg/mL increased PPARγ protein expression. Notably, treatment with Xanthigen at a concentration of 10 μg/mL almost completely inhibited PPARγ protein expression. In addition, C/EBPβ and C/EBPδ have been reported to be essential and critical regulators of adipocyte differentiation through up-regulating PPARγ and C/EBPα[4] We found that the protein levels of C/EBPβ and C/EBPδ in PSO- and fucoxanthin-treated groups revealed a similar pattern of PPARγ protein. However, treatment with Xanthigen effectively attenuated both C/EBPβ and C/EBPδ protein expression in differentiated adipocytes (Figure 2A).

SIRT-1 has been reported to play a central role in adipogenesis.[32] Up-regulated SIRT-1 is found in the period of adipocyte differentiation.[33] In the results of Western blot analysis (Figure 2B), the protein level of SIRT-1 was slightly increased after 10 days of differentiation (1.2-fold increased compared to undifferentiated cells). Remarkably, Xanthigen significantly up-regulated SIRT-1 protein expression compared to PSO and fucoxanthin (2.9- and 2.8-fold increases at 10 and 50 μg/mL, respectively). These results indicated that Xanthigen suppressed adipocyte differentiation through inhibition of PPARγ, C/EBPβ, and C/EBPδ and up-regulation of SIRT-1 protein expression.

Effect of Xanthigen on PI3K/Akt-Dependent FoxOs Signaling in 3T3-L1 Adipocytes. FoxOs represent central regulators of metabolism in insulin-responsive tissues such as liver, adipose, and muscle cells.[10] The activity of FoxOs is negatively regulated by insulin and growth factor stimulation through Akt-dependent phosphorylation and nuclear exclusion.[11,12] To determine whether Xanthigen can modulate FoxOs signaling pathways, the potential involvements of FoxO1 and FoxO3a were evaluated by using anti-phospho-specific antibody in Western blot analysis. In differentiated adipocytes, both protein levels of FoxO1 and FoxO3a were increased compared to undifferentiated cells (Figure 3A). Slightly elevated phosphorylated FoxO1 and FoxO3a were found in PSO-treated cells but not in fucoxanthin-treated cells. On the other hand, in the Xanthigen-treated group, both phosphorylated FoxO1 and FoxO3a were dramatically increased (Figure 3). These results of immunoblot analysis suggested that Xanthigen might promote insulin signaling-mediated phosphorylation of FoxOs, which results in inactivation of FoxOs and suppressed 3T3-L1 adipocyte differentiation.

Xanthigen-Activated AMPK Signaling in 3T3-L1 Adipocytes. Recent studies suggest that AMPK is involved in the regulation of glucose and lipid metabolism and acts as a regulator for adipocyte differentiation.[14,15] To clarify the inhibitory effect of Xanthigen on suppression of 3T3-L1 adipocyte differentiation, we examined whether Xanthigen can modulate AMPK signaling. As shown in Figure 4A, increased phosphorylated AMPKα, a catalytic subunit, occurred in both PSO- and fucoxanthin-treated cells, whereas no change was observed in fucoxanthin treatment at a concentration of 10 μg/mL. Moreover, phosphorylation of AMPKβ, a regulatory subunit, was also elevated in PSO- and fucoxanthin-treated cells. Treatment with Xanthigen resulted in a more robust increase in both phosphorylation of AMPKα and AMPKβ than in individual PSO and fucoxanthin treatments. ACC is a key enzyme involved in adipogenesis that controls the synthesis of
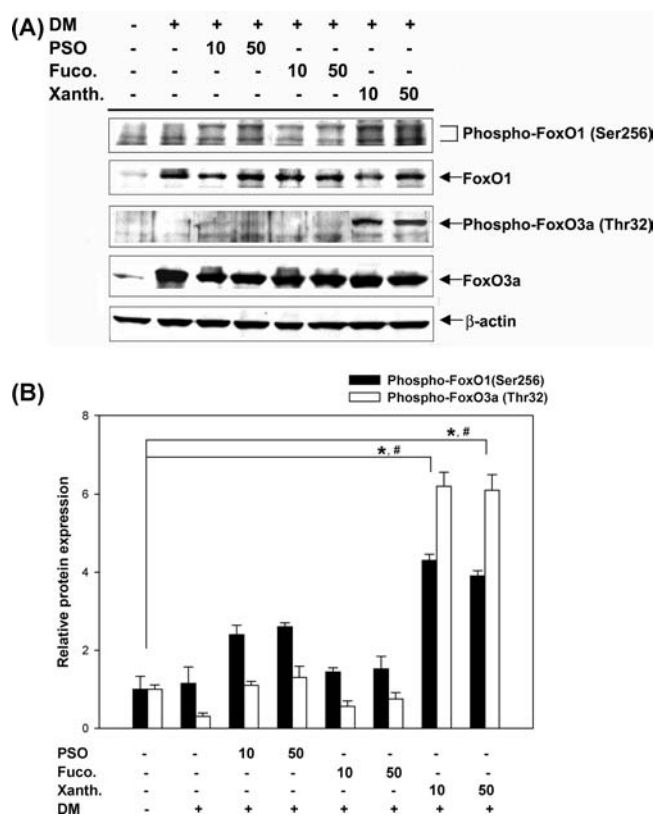
Figure 3. Effects of Xanthigen on FoxOs signaling in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were treated as described previously. (A) Phospho-FoxO1 (Ser256), phospho-FoxO3a (Thr132), total FoxO1, and FoxO3a were examined by Western blot analysis. (B) Densitometric and quantification analysis of phospho-FoxO1 (Ser256) and phospho-FoxO3a (Thr132). (∗) $P < 0.01$ indicates statistically significant difference from the differentiated 3T3-L1 adipocytes. This experiment was repeated three times with similar results.
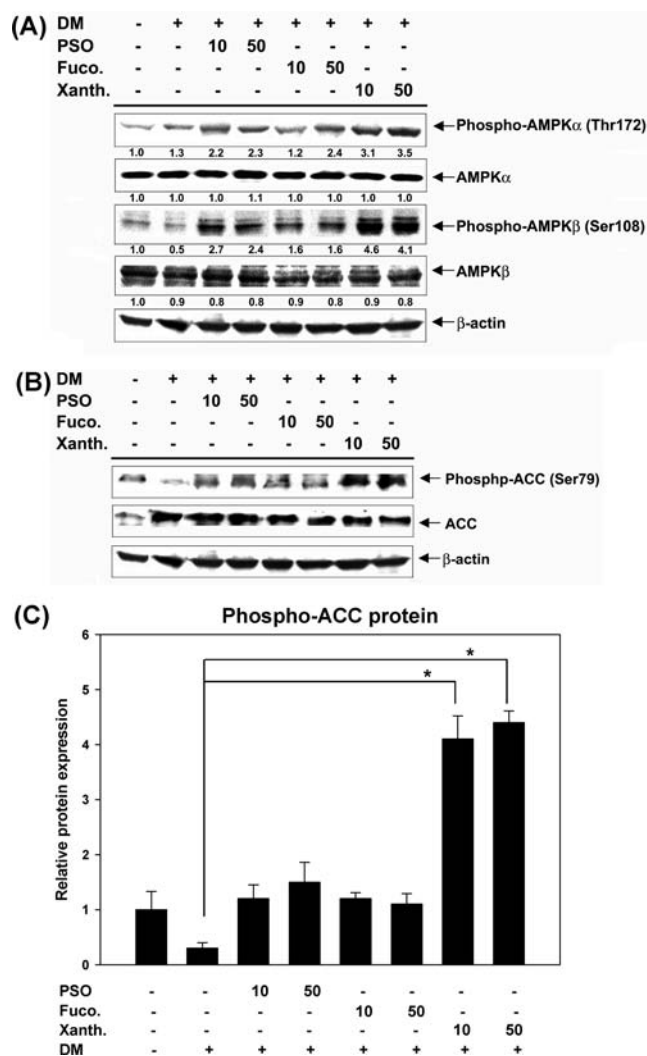

Figure 4. Effects of Xanthigen on activation of AMPK signaling in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were treated as described previously. (A) Phospho-APMKα (Thr172), phospho-AMPKβ (Ser108), total AMPKα, and AMPKβ and (B) Phospho-ACC (Ser 79) and total ACC were examined by Western blot analysis. (C) Densitometric and quantification analysis of phospho-ACC (Ser 79). (∗) $P < 0.05$ indicates statistically significant difference of phospho-APMKα (Thr172), and (#) $P < 0.01$ indicates statistically significant difference of phospho-AMPKβ (Ser108) from the differentiated 3T3-L1 adipocytes. This experiment was repeated three times with similar results.

malonyl-CoA from acetyl-CoA.[34] Phosphorylation of ACC by AMPK has been found to inhibit enzyme activity of ACC.[35] Therefore, we also determined whether ACC is an important target for Xanthigen subsequent to AMPK activation. Figure 4B shows that elevated protein levels of ACC were observed in differentiated adipocytes and PSO-, fucoxanthin-, and Xanthigen-treated cells. However, the phosphorylated ACC was insignificantly increased in both PSO- and fucoxanthin-treated cells compared to the differentiated adipocytes. Again, Xanthigen was also found to cause a greatly increased phosphorylated ACC compared with PSO and fucoxanthin (4.1- and 4.4-fold increases at 10 and 50 μg/mL, respectively) that displayed similar results in the activation of AMPK (Figure 4B,C). Taken together, these results suggested that Xanthigen suppressed 3T3-L1 adipocyte differentiation not only through inhibition of PPARγ and C/EBPs but also through activation of AMPK signaling.

■ DISCUSSION

Numerous studies have documented that adipocytes are a potential target for antiobesity strategy, and many dietary natural products are considered to be attractive antiobesity drugs through inhibition of differentiation, regulation of lipid and glucose metabolism, and induction of apoptosis and cell cycle arrest in adipocyte.[20] Xanthigen is a source of PSO and fucoxanthin, derived from *Punica granatum* and edible brown seaweed, respectively, both reported to have antiobesity property.[28,29,31] In this study, the effects of PSO, fucoxanthin, and Xanthigen on the suppression of 3T3-L1 adipocyte differentiation were investigated. For the first time, we found that Xanthigen revealed a marked reduced lipid accumulation in differentiated 3T3-L1 adipocytes through modulation of multiple molecular pathways.

In our results, we also found that Xanthigen inhibited FAS expression, a key enzyme that catalyzes the synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA,[34] a promising therapeutic target of obesity.[36] However, treatment with fucoxanthin, one of the components in Xanthigen, displayed an enhanced effect of lipid accumulation (Figure 1). A previous study has shown that treatment of fucoxanthin at the later stage of clonal expansion inhibited adipocyte

differentiation via down-regulation of PPARγ.[29] Another recent study demonstrated a differential effect of fucoxanthin on adipocyte differentiation by application in various stages and suggested a promoted effect when applied at early stages by elevated PPARγ.[31] Our result is consistent with the above paper but occurred only at lower concentration (10 and 50 μg/mL) and not at high concentration, which may be due to higher cytotoxicity in 3T3-L1 preadipocytes (data not shown).

PPARγ has been suggested as a master regulator of adipogenesis and is necessary for adipocyte differentiation.[8,37] At the early stage of the differentiation process, rapid activations of C/EBPβ and C/EBPδ are initial events that subsequently lead to transcriptional up-regulation of PPARγ.[6,38] Activation of PPARγ immediately leads to induction of C/EBPα along with C/EBPβ and C/EBPδ and subsequently transcripts a series of adipocyte-specific genes responsible for terminal differentiation. In our present study, we found that Xanthigen dramatically decreased PPARγ expression accompanied by the reduced protein levels of C/EBPβ and C/EBPδ (Figure 2A). However, it is not clear that decreased PPAR-γ is a direct effect of Xanthigen through attenuation of C/EBPβ and C/EBPδ levels. The detailed mechanism should be further investigated. These results indicated Xanthigen inhibited C/EBPβ and C/EBPδ expression and, thus, secondarily resulted in PPAR-γ expression in adipocytes.

Growing research has exhibited the role of Sirtuins in the modulation of many biological functions, such as longevity, stress resistance, and cellular metabolism.[39] Several recent studies have documented the function of Sirtuins in the regulation of adipocyte differentiation. Overexpression of SIRT1 in white adipocytes is found to attenuate adipogenesis through repression of PPARγ and C/EBPα.[32] Moreover, in differentiated fat cells, up-regulated SIRT1 promotes lipolysis and loss of fat mass.[32] SIRT1 is found to control lipolysis via deacetylation of FoxO1 in adipocytes.[40] Here, we showed that Xanthigen markedly up-regulated SIRT1 protein expression in differentiated 3T3-L1 adipocytes (Figure 2B). According to the role of SIRT1 in adipocytes, up-regulation of SIRT1 by Xanthigen may provide a possible mechanism for decreasing lipid accumulation. Accumulating studies have demonstrated that insulin and IGF-1 signaling promote adipocyte differentiation through activation of PI3K/Akt and downstream transcription factor FoxOs.[13] A previous study shows that overexpression of FoxO1 inhibits adipocyte differentiation at clonal expansion stage.[13] However, a recent study demonstrates that FoxO1 is important in the early stage of terminal adipocyte differentiation. Knockdown FoxO1 presents a decreased lipid droplet accumulation that accompanied reduced PPARγ and C/EBPα in 3T3-L1 adipocytes.[41] Akt-dependent phosphorylation of FoxOs results in their exclusion from the nucleus to cytoplasm by binding to 14-3-3 protein, thus blocking the binding ability of FoxOs to DNA and subsequent downstream gene transcription.[11] Our results revealed that phosphorylation of FoxO1 at Ser256 and FoxO3a at Thr32 were both enhanced by Xanthigen (Figure 3), indicating Xanthigen may promote insulin-triggered PI3K/Akt signaling that caused inactivation of FoxOs. However, the detailed mechanism remains to be elucidated.

Emerging studies suggest that AMPK is a master metabolic regulator responsible for modulating cellular metabolism and as a promising target for metabolic disorder such as obesity and type 2 diabetes.[42] AMPK is involved in modulation of adipose tissue metabolism through mediation of glucose uptake, β-oxidation of fatty acid, lipolysis, and adipokine secretion.[43] AMPK is also found to inhibit adipocyte differentiation and directly phosphorylate downstream target ACC at Ser79, which inhibits the enzymatic activity and subsequent decrease in fatty acid synthesis.[15,17] Here we also explore the effects of Xanthigen on AMPK signaling. Our findings showed that Xanthigen significantly induced phosphorylation of AMPKα and AMPKβ as well as phosphorylated ACC (Figure 3), which contributed to decreased lipid accumulation in differentiated 3T3-L1 adipocytes. Furthermore, it has been reported that phosphorylation of AMPKα at Thr172 and AMPKβ at Ser108 by upstream kinase liver kinase B (LKB) 1 is required for AMPK enzyme activation.[44−46] This information indicates that Xanthigen may activate AMPK through modulation of upstream signaling, but it needs to be further elucidated.

In conclusion, in the present study we demonstrated that the Xanthigen, a source of PSO and fucoxanthin, significantly suppressed 3T3-L1 adipocyte differentiation and lipid accumulation. Xanthigen exerts an antiadipogeneic effect through down-regulation of C/EBPβ, C/EBPδ, and PPARγ, modulation of multiple signaling pathways including Akt-dependent FoxOs signaling, and activation of the AMPK signaling cascade as well as induction of SIRT1 (Figure 5). These results provided the
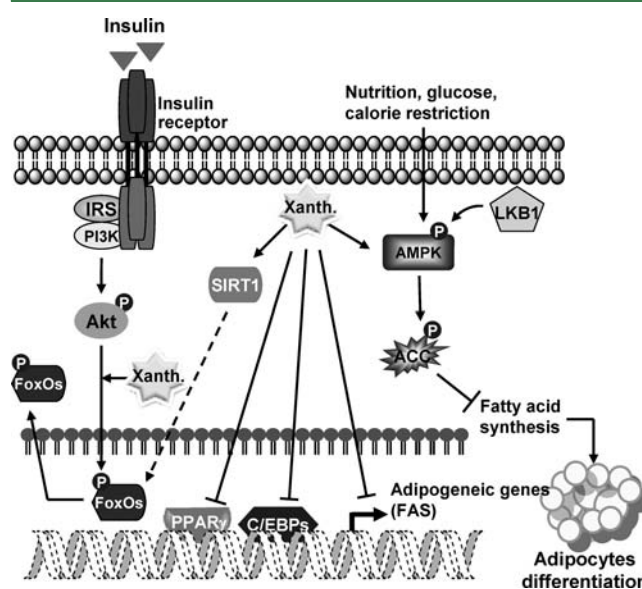

Figure 5. Possible mechanism of Xanthigen on suppression of 3T3-L1 adipocytes differentiation.

molecular mechanism of Xanthigen on suppression of adipocyte differentiation. On the basis of these findings, we suggest Xanthigen to have great potential as a novel agent for the treatment of obesity.

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ABBREVIATIONS USED

ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; C/EBP, CCAAT/enhancer binding protein; FAS, fatty acid synthase; FoxO, forkhead/winged helix O; HMGCR, HMG-CoA reductase; IGF-1, insulin-like growth factor 1; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; TZD, thiazolidinedione.

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