

## RESEARCH ARTICLE

# Inhibitory Effects of Onion (*Allium cepa* L.) Extract on Proliferation of Cancer Cells and Adipocytes via Inhibiting Fatty Acid Synthase

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### Abstract

Onions (*Allium cepa* L.) are widely used in the food industry for its nutritional and aromatic properties. Our studies showed that ethyl acetate extract of onion (EEO) had potent inhibitory effects on animal fatty acid synthase (FAS), and could induce apoptosis in FAS over-expressing human breast cancer MDA-MB-231 cells. Furthermore, this apoptosis was accompanied by reduction of intracellular FAS activity and could be rescued by 25 mM or 50 mM exogenous palmitic acids, the final product of FAS catalyzed synthesis. These results suggest that the apoptosis induced by EEO occurs via inhibition of FAS. We also found that EEO could suppress lipid accumulation during the differentiation of 3T3-L1 adipocytes, which was also related to its inhibition of intracellular FAS activity. Since obesity is closely related to breast cancer and obese patients are at elevated risk of developing various cancers, these findings suggested that onion might be useful for preventing obesity-related malignancy.

**Keywords:** Fatty acid synthase - onion - MDA-MB-231 cells - 3T3-L1 adipocytes - apoptosis

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### Introduction

Allium vegetables have been employed for a long time in traditional medical practice to treat a variety of diseases. Onion (*Allium cepa* L.), one of the representative Allium vegetables, has been used for centuries for its pungency, flavoring value, and medicinal properties. The bulb of onion is used medicinally and has been consumed as seasoning food for many centuries (Sengupta et al., 2004). Phytochemical research has proved that onion is rich in flavonols and organosulfur compounds, which have exhibited tumor inhibitory properties in laboratory studies (Virtanen and Matikkala, 1976; Block, 1985; Arabbi et al., 2004).

High intakes of onions have been directly associated with the management and prevention of obesity (Lee et al., 2008). Onion extract supplementation reduced the amounts of mesenteric fat and influenced the adipokine production at a transcriptional level in the high-fat induced obese animal model (Kim et al., 2012). Onion extracts reduced blood low-density lipoprotein cholesterol and increased high-density lipoprotein cholesterol of high-fat feeding Sprague-Dawley rats (Lee et al., 2012). Some researchers investigated the anti-obesity activity of a 70% ethanol extract from *Allium fistulosum* L. (commonly known as Chinese onion) in high-fat induced obese mice. The extracts significantly reduced body weight, white adipose tissue weight and adipocyte size of the treated mice compared to high-fat induced control mice (Sung et al., 2011).

In recent years, extensive research has focused on the anticarcinogenic potential of onion and its constituents. Onion and its organosulfur constituents are studied extensively for their chemopreventive potential against cancer (Le Bon and Siess, 2000). In a French epidemiological study, higher onion intake was correlated with lower risk of breast cancer (Challier et al., 1998). Furthermore, many experimental studies have demonstrated that organosulfur compounds and Allium extracts have inhibitory effects on carcinogenesis in animals.

Numerous studies suggest that obesity and excess weight can play a prominent role in the incidence and progression of various cancers (Prieto-Hontoria et al., 2011). Obesity has been associated with a higher risk and a poor prognosis of breast cancer in multiple studies (Tarter et al., 1981; van den Brandt et al., 2000; Lahmann et al., 2004; Kroenke et al., 2005; Caan et al., 2008; Dawood et al., 2008). According to an American Cancer Society study, obesity can increase the mortality of patients with cancers of the breast, kidneys, liver, etc. (Calle et al., 2003).

Fatty acid synthase (FAS, EC 2.3.1.85), a metabolic enzyme that catalyzes the synthesis of long-chain fatty acids, is expressed at high levels in adipose tissues and a variety of human cancers, including breast (Alo et al., 1996; Milgraum et al., 1997), prostate (Epstein et al., 1995; Swinnen et al., 2002), endometrium (Pizer et al., 1998), ovary (Gansler et al., 1997), colon (Rashid et al., 1997), lung (Visca et al., 2004; Orita et al., 2008),

and pancreas cancer (Alo *et al.*, 2007). Although the mechanism of FAS over expression is unknown, it seems to be up-regulated during the early stages of tumorigenesis (Rashid *et al.*, 1997; Kuhajda, 2000). This differential expression between normal and neoplastic tissues makes FAS a potential diagnostic tumor marker (Walter *et al.*, 2009). The fatty-acid synthesis as a target pathway for chemotherapy has been identified by the studies with FAS inhibitors (Kuhajda, 2006).

Onion is well-known for its benefits to weight control and cancer prevention. However, there has no “cross-talk” between its anti-obesity and cancer prevention activities. This study therefore aimed to examine whether the anti-cancer activity of onion is related to its anti-obesity effect. Our previous study has found that crude extracts of nine species of *Allium* vegetables including onion showed high inhibitory activity on FAS (Sun *et al.*, 2009). In the present study, we further investigated the inhibitory effect of onion extracts on FAS over-expressed cancer cells and adipocytes.

## Materials and Methods

### *Reagents and antibodies*

Acetyl-CoA, Malonyl-CoA, Insulin (INS), Dexamethasone (DEX), Oil red O, NADPH, MTT dye, Hoechst-33258, 3-isobutyl-1-methylxanthine (IBMX), palmitic acid, EDTA and DTT were purchased from Sigma. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from GIBCO BRL. Antibodies against FAS and GAPDH were purchased from Cell Signaling Technologies.

### *Preparation of plant extracts*

Dried bulb of onion (467.5 g) was extracted successively with petroleum (3 × 1500 ml, 30 min) and 50% ethanol (3 × 1500 ml, 30 min) at room temperature, to give 0.81g and 80.0 g of extract, respectively. Ethanol extract (80.0 g) was dissolved in 500 ml of water and then partitioned successively at room temperature with ethyl acetate (3 × 500 ml) to give 5.92 g of ethyl acetate extracts (EEO). The aqueous phase contained 72.69 g of residue. And then, 100 mg of EEO was dissolved in 20 ml DMSO to make a 5 mg/ml stock solution for long storage at -20 °C.

### *Cell culture*

Mouse 3T3-L1 preadipocytes and human breast cancer MDA-MB-231 cells were both obtained from the Cell Culture Center of the Institute of Basic Medical Sciences (IBMS), Chinese Academy of Medical Sciences (Beijing, China). 3T3-L1 preadipocytes were incubated in DMEM (low glucose) and MDA-MB-231 cells in DMEM (high glucose), 10% fetal bovine calf serum, 100 U/ml penicillin-streptomycin.

### *Cell lysis and immunoblotting*

Cells were lysed as previously described (Uddin *et al.*, 2004). Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane (Immobilon; Millipore, Billerica, MA). Immunoblotting

was done with different antibodies and visualized by the enhanced chemiluminescence (Amersham, Piscataway, NJ) method.

### *Differentiation of 3T3-L1 preadipocytes*

Two days after confluence (Day 0, d0), the cell differentiation was induced in DMEM containing 10% FBS, 0.5 mM IBMX, 1 mM DEX, and 1.7 mM INS for two days (Day 2, d2, early stage), and another two days (Day 4, d4, middle stage) in DMEM containing 10% FBS and 1.7 mM INS. Cells were maintained in DMEM containing 10% FBS every other day for the following four days (Day 6–8, d6–8, late stage). All experiments were strictly carried out on cell lines between 5–20 passages. All cell culture condition was 37 °C in a humidified 5% CO<sub>2</sub> incubator.

### *MTT assay*

3T3-L1 preadipocytes were seeded in 96-well plate (5 × 10<sup>3</sup> cells/well) and then treated with EEO in different concentrations for the next 24 h. Thereafter, 20 ml of MTT solution (5 mg MTT/ml in PBS) was added into each well of a microtiter plate and incubated for 4 h at 37 °C. The resultant formazan product was dissolved in 200 ml DMSO/well, and its concentration was measured at 492 nm by a microplate spectrophotometer (Multiskan, MK3).

### *Hoechst-33258 staining*

3T3-L1 preadipocytes were seeded in 12-well culture dishes (5 × 10<sup>4</sup> cells/well). After experimental treatment, cells were washed twice with PBS, and stained with Hoechst-33258 (5 mg/ml) for 5 min in the dark, and then followed by extensive washes. Nuclear staining was examined under the fluorescence microscope and images were captured using ImagePro Plus software (MediaCybernetics, Silver spring, MD).

### *Intracellular fatty acids assay*

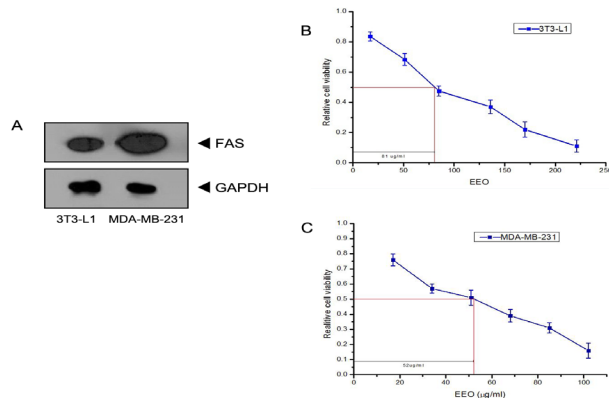
The amount of intracellular fatty acid was determined by Fatty Acid Assay Kit. Briefly, 3T3-L1 preadipocytes were seeded in 100 mm cell culture dishes. After experimental treatment, cells were washed twice with PBS, and then extracted by homogenization with 200 ml of chloroform-Triton X-100 (1% Triton X-100 in pure chloroform) in a microhomogenizer. Then spinned the extract 5–10 min at top speed in a microcentrifuge. Collected organic phase (lower phase), air dried at 50 °C to remove chloroform. Vacuum dried 30 min to remove trace chloroform. Dissolved the dried lipids in 200 ml of Fatty Acid Assay Buffer by vortexing extensively for 5 min. Added 2 ml ACS Reagent into all sample wells and incubated the reaction at 37 °C for 30 min. Added 50 ml of the Reaction Mix containing 44 ml Assay Buffer, 2 ml Fatty Acid Probe, 2 ml Enzyme Mix and 2 ml Enhancer to the test samples. Incubated the reaction for 30 min at 37 °C, protected from light. The colorimetric assay was measured at 570 nm by a microplate spectrophotometer.

### *Oil Red O staining*

To investigate the effects of EEO on lipid accumulation in 3T3-L1 preadipocytes, the cells were differentiated

**Table 1. The Inhibitory Activity of the Four Fractions Isolated from Onion Against FAS**

Fractions	Mass (g)	FAS inhibitory activity IC <sub>50</sub> (µg/ml)
Petroleum ether	0.81	150±7
Ethanol	3.15	90±4
Ethyl acetate (EEO)	5.92	2.4±0.3
Water	72.69	50±2



**Figure 1. Inhibitory Effects of EEO on FAS Over-expressed MDA-MB-231 and 3T3-L1 Cells.** (A) FAS expression in MDA-MB-231 cells was higher than 3T3-L1 cells. (B) Cells were incubated with 0-250 µg/ml EEO for 24 h at 37 °C in humidified 5% CO<sub>2</sub> incubator. (C) Cells were incubated with 0-110 µg/ml EEO for 24 h at 37 °C in humidified 5% CO<sub>2</sub> incubator. Results were expressed as percentages of cell viability as compared with untreated control (means ± S.D., n = 8). The experiments were repeated in twice. The IC<sub>50</sub> of EEO on MDA-MB-231 cells was 52 µg/ml. As well as the IC<sub>50</sub> of 3T3-L1 cells was 81 µg/ml

in the presence of EEO at various concentrations. Intracellular lipid accumulation was determined by Oil Red O staining at day 8. Cells were washed twice with PBS and stained with Oil Red O (six parts 0.6% Oil Red O dye in isopropanol and four parts water) for 1 h. After washed three times with distilled water, cells were photographed under the microscope. Lipid and Oil red O were dissolved in isopropanol and absorbance was measured by the microplate spectrophotometer (Multiskan, MK3) at the wavelength of 492 nm.

#### FAS preparation and activity assays

The preparation, storage and use of FAS isolated from chicken liver were performed as described previously (Soulié et al., 1984). The final purified enzyme was determined to be homogeneous by polyacrylamide gel electrophoresis (PAGE) analysis in the presence and absence of SDS. FAS activity was determined by monitoring the decrease in absorbency at 340 nm resulting from the oxidation of NADPH using an Amersham Pharmacia Ultrospec 4300 pro UV-Vis spectrophotometer at 37 °C. The assay mixture contained 100 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 2.5 µM acetyl-CoA, 10 µM malonyl-CoA, 32 µM NADPH, and 10 µg chicken liver FAS in a total volume of 2.0 ml (Tian et al., 1985).

#### FAS inhibition assays

The fast-binding inhibition was determined by adding

inhibitor to the reaction system before FAS initiated the reaction. The activity of FAS in the presence of inhibitor was designated as A<sub>i</sub>, and the control activity of FAS in the absence of inhibitor was designated as A<sub>0</sub>. A<sub>i</sub>/A<sub>0</sub> was the remaining activity (R.A.) of FAS that was less than 1 for the inhibition of FAS. Generally, this inhibition is induced by non-covalent loading of inhibitor on the enzyme, which is reversible. The concentrations of inhibitor required for 50% inhibition (IC<sub>50</sub>) were obtained from the dose-response curves of inhibition. It had been checked that the DMSO of less than 2.5 % in the assay nearly did not affect on the activity of FAS.

#### Cell FAS activity assay

FAS activity in cells was assessed as described previously with appropriate modifications (Menendez et al., 2004). After cells were harvested, pelleted by centrifugation, resuspended in cold assay buffer (100 mM potassium phosphate buffer, 1 mM EDTA, 0.6 mM PMSF and 1 mM dithiothreitol, pH 7.0) ultrasonically disrupted and centrifuged at 12000 rpm for 30 min at 4 °C, the supernatant was collected for the overall reaction assay. 25 µl supernatant was added into the reaction mix contained 25 mM KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> buffer, 0.25 mM EDTA, 0.25 mM dithiothreitol, 30 mM acetyl-CoA, 100 mM malonyl-CoA, 350 mM NADPH (pH 7.0) in a total volume of 200 µl. Protein content in the supernatant was determined using a bicinchoninic acid (BCA) assay (Pierce) and results were expressed as the specific activity of FAS at the same protein concentration.

## Results

#### Inhibitory effect of EEO on FAS in vitro

Four fractions (petroleum ether, ethanol, ethyl acetate and water) of onion were tested to determine their inhibitory activities on FAS. It indicated that EEO showed the highest activity to inhibit FAS with IC<sub>50</sub> of 2.4±0.3 µg/ml (Table 1). So EEO was chosen for the further cell level research.

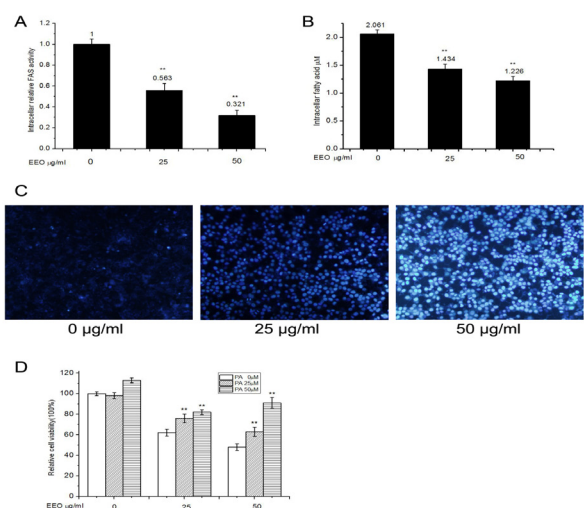
#### Expression of FAS in MDA-MB-231 cells and 3T3-L1 adipocytes

FAS levels of MDA-MB-231 and 3T3-L1 cells were determined by Western blotting analysis with specific antibodies against the targeted proteins and GAPDH as control. As shown in Figure 1A, both the two cells expressed FAS in high levels. And FAS was higher expressed in MDA-MB-231 cells than in 3T3-L1 cells.

#### EEO inhibited intracellular FAS activity in MDA-MB-231 cells and 3T3-L1 adipocytes

Compared with control, EEO significantly inhibited the intracellular FAS activity with a dose-dependent manner. As shown in Figure 2A, after treated with EEO at the concentrations of 25 µg/ml and 50 µg/ml for 24 h, the intracellular FAS activities of MDA-MB-231 cells were reduced to 56.3% and 32.1%, separately, compared with control. Figure 3A showed that 20, 40 and 60 µg/ml EEO inhibited 37.7%, 69.8% and 73.6% intracellular FAS activities in 3T3-L1 adipocytes, respectively.





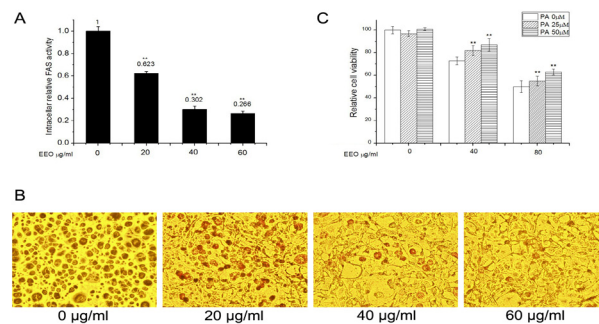
**Figure 2. Apoptotic Effects of EEO on MDA-MB-231 Cells Via Inhibiting FAS Activity.** (A) Effect of EEO on FAS activity in MDA-MB-231 cells. MDA-MB-231 cells were treated with EEO in the indicated concentrations for 24 h. FAS specific activity was determined by Cell FAS activity assay. Data were expressed as means  $\pm$  S.D. (n = 3). \*\* p<0.01 significantly different from control (0  $\mu$ g/ml) (B) MDA-MB-231 cells were treated with EEO at various concentrations (0, 25, 50  $\mu$ g/ml) for 24 h. And then the amount of intracellular fatty acid was determined by Fatty Acid Assay Kit. Data were expressed as means  $\pm$  S.D. (n = 3). \*\* p<0.01 significantly different from respective control. (C) Effect of EEO with 0  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml on nuclear chromatin morphology with Hoechst 33258 staining: original magnification  $\times$ 200; exposure times: 100 s. (D) The effect of exogenous palmitic acid on MDA-MB-231 cells. The relative cell viability was determined by MTT. Data were expressed as means  $\pm$  S.D. (n = 3). \*\* p<0.01 significantly different from respective control

#### *Inhibitory effects of EEO on viability of MDA-MB-231 cells and 3T3-L1 adipocytes in vitro*

To identify whether EEO influence the survival of MDA-MB-231 and 3T3-L1 cells, cells were treated with 0-250  $\mu$ g/ml EEO, and after that cell viability was examined by MTT assay. As shown in Figure 1B, MDA-MB-231 cell viability was reduced to 76% with 17  $\mu$ g/ml EEO and to 31% with 85  $\mu$ g/ml EEO. Cell growth was dramatically suppressed by 84% after treating with 102  $\mu$ g/ml EEO, when compared to the negative control (0  $\mu$ g/ml). EEO showed high inhibition of cell population growth in a dose-dependent manner with 50% growth inhibitory concentration ( $IC_{50}$ ) value of 52  $\mu$ g/ml. As shown in Figure 1C, The  $IC_{50}$  of EEO on 3T3-L1 adipocytes was 81  $\mu$ g/ml.

#### *EEO reduced intracellular fatty acids in MDA-MB-231 cells*

The amount of intracellular fatty acids in MDA-MB-231 cells treated with 25  $\mu$ g/ml and 50  $\mu$ g/ml EEO were measured. Results (Figure 2B) showed that the level of intracellular fatty acids in treated cells decreased by 30.6% and 40.8%, compared with the control (2.06  $\mu$ M). 3.6. EEO reduced lipid accumulation in 3T3-L1 adipocytes Oil Red O staining to visualize lipid after 3T3-L1 cells were incubated with EEO. Mature adipocytes store a lot of lipids, which can be stained red by Oil Red O. Differentiated adipocytes were stained in red, while



**Figure 3. Inhibitory Effect of EEO on Intracellular Lipid Accumulation of 3T3-L1 Adipocytes.** (A) 3T3-L1 preadipocytes were treated with EEO at the indicated concentrations for 24 h. FAS specific activity was determined by Cell FAS activity assay. Data were expressed as means  $\pm$  S.D. (n = 3). \*\* p<0.01 significantly different from control (0 mM). (B) The intracellular lipid content was measured by Oil Red O staining. 3T3-L1 preadipocytes were treated with EEO at the indicated concentrations for 24 h. (C) The effect of exogenous palmitic acid on 3T3-L1 cells. And the relative cell viability was determined by MTT. Data were expressed as means  $\pm$  S.D. (n = 3). \*\* p<0.01 significantly different from respective control

undifferentiated cells were not. The representative images demonstrated that the 3T3-L1 cells treated with EEO obviously reduced their lipid accumulation, since they were less stained than differentiated cells (Figure 3B).

#### *EEO induced MDA-MB-231 cells apoptosis*

In order to examine whether the inhibitory effect of EEO on MDA-MB-231 cells was due to apoptotic cell death, apoptotic events of Hoechst-33258 staining was tested. After exposed to three concentrations of EEO (0  $\mu$ g/ml, 25  $\mu$ g/ml and 50  $\mu$ g/ml) for 24 h, apoptosis of MDA-MB-231 cells was demonstrated by Hoechst-33258 staining, revealed cell membrane permeability increasement and nuclear condensation (Figure 2C).

#### *Palmitic acid rescued cells apoptosis induced by EEO*

To confirm that the cell apoptosis induced by EEO was related to FAS inhibition, MDA-MB-231 and 3T3-L1 cells were exposed for 24 h to different concentrations of EEO (0  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml) in presence of exogenous palmitic acid (0  $\mu$ M, 25  $\mu$ M, 50  $\mu$ M), the end product of FAS reaction. Palmitic acid reduced the cytotoxic effects of EEO, as the cell viabilities were restored significantly and with dose-dependent manner (Figure 3A).

## Discussion

Obesity is a growing health problem in both developed nations and some developing countries like China. It is linked with several health disorders such as hypertension, cardiovascular diseases, type 2 diabetes and certain cancers. Obesity-related cancers of the breast, prostate and colon are the leading cancers in the industrialized countries.

Recent emerging epidemiological data further reveal that obesity is also associated with poor prognosis in patients with breast cancer. It was reported that 48% of breast cancers are diagnosed among obese women and

that 23% of these breast cancers are attributable to obesity (Colditz, 1992). Excess adipose tissue leads to metabolic changes such as reduced high-density lipoprotein cholesterol, elevated triglycerides, hypertension, and insulin resistance (Khandekar et al., 2011). Obesity-induced esophageal reflux, hypertension, insulin resistance, and hormone alternations could contribute to an increased risk in esophageal, kidney, colorectal, and breast cancers, respectively (Wang and Dubois, 2012).

Although studies are inconclusive at this time, preliminary evidence suggests that onion may play a role in decreasing the risk of obesity-related cancers. Several epidemiological studies have shown a reduction of the risk of different cancers via consumption of onion (Hsing et al., 2002; Setiawan et al., 2005; Galeone et al., 2006). The supporting mechanisms including inhibition of carcinogen formation, modulation of carcinogen metabolism, inhibition of mutagenesis and genotoxicity, inhibition of cell proliferation and increase of apoptosis, inhibition of angiogenesis, and immune system enhancement. These mechanisms, however, didn't include the fatty acid synthesis pathway.

FAS may play a junction role between obesity and cancer risk: on the one hand, increased de novo lipogenesis contributes to increased fat mass (Lenhard, 2011), on the other hand, high expression of FAS in human breast, colorectal, prostate, endometrial, ovary, and thyroid cancers supports the hypothesis that FAS is essential for generating cell membranes during tumor cell proliferation (Menendez and Lupu, 2007). In this study, we found that EEO not only showed a high inhibitory activity on FAS (Table 1), but also influenced the normal life cycle of both cancer and fat cells (Figure 1B, 1C). These results suggested that FAS, the target for cancer and obesity, was also an acting site of EEO.

The reduction of intracellular FAS activity (Figure 2A) and fatty acids amount (Figure 2B) in MDA-MB-231 cells revealed that FAS was the target where EEO acted on. The activity of FAS in cells was important to the amount of intracellular fatty acids because the FAS plays the key role of de novo fatty acid biosynthesis. It was reported that most normal human tissues, except liver and adipose tissue, exhibit low levels of FAS expression. However, the expression of FAS is surprisingly high in a variety of human cancers, such as cancer of the breast, prostate, ovary and lung (Shurbaji et al., 1996; Gansler et al., 1997; Alo et al., 1999; Kuhajda, 2000; Visca et al., 2004). Figure 1A showed that high levels of FAS expression in MDA-MB-231 and 3T3-L1 cells. Interestingly, the level of FAS expression had close relation to the cell viabilities treated with EEO. EEO reduced the viabilities of both MDA-MB-231 and 3T3-L1 cells. But the  $IC_{50}$  of 3T3-L1 cells was higher than that of MDA-MB-231 cells. It revealed that EEO had lower toxicity on normal 3T3-L1 cells (relatively low level of FAS expression) than on cancer MDA-MB-231 cells. So EEO would prevent cancer and have no cytotoxic to normal cells in an appropriate concentration. This result provided the possibility for the utility of onions to explore effective drugs for preventing and treating cancer. In this study, EEO was obtained from dried bulb, the edible parts, of onions. Considering that

onion has been used as a food source since prehistoric times, we believe that high intake of EEO is safe.

Like reported FAS inhibitors such as C75 and cerulenin (Kuhajda, 2006), EEO could induce apoptosis in cancer cells (Figure 2C). Previous studies suggested that the mechanism of the apoptosis through inhibiting FAS could be explained by the accumulation of malonyl-CoA, which was likely to trigger cancer cell death and induce apoptosis (Pizer et al., 2000; Zhou et al., 2003). It was presented that some signal pathways in cell apoptosis had close relation to the inhibition of FAS, which might be an ideal reason for why FAS inhibitors could treat cancer cells.

Some research, however, showed that palmitic acid, the final product of FAS, was important for the formation of cell membrane. So the reduction of synthesized palmitic acid might be another reason to explain why the inhibition of FAS could induce apoptosis. In this study, we found that the EEO treated cell viabilities could be rescued by adding exogenous palmitic acid, which provided strong evidence for the cell membrane thesis (Figure 2D and Figure 3C).

In mature adipocytes, the accumulation of fatty acids significantly reduced after adding EEO (Figure 3B), along with the declined FAS activity (Figure 3A). Since the size of fat cells depended on the lipid accumulation, adipose tissue mass could be reduced by preventing lipid accumulation inside adipocytes. Although low concentration (20  $\mu$ g/ml) of EEO failed to influence the viability of 3T3-L1 cells, it did reduce the intracellular lipid accumulation. It revealed that EEO had the application potential in preventing and/or treating obesity.

Obesity-related health problems consume about 7% of the US health-care budget in direct medical costs and about 1-5% in Europe (Pan et al., 2004). Obesity represents a major avoidable contribution to the costs of illness in the developed countries. Onion, as a worldwide edible plant, is cheap and convenient to get. If EEO is developed into a new drug for treating obesity-related cancers, the money saved will be considerable.

In conclusions, EEO could induce MDA-MB-231 cells apoptosis and reduce intercellular lipid accumulation of 3T3-L1 adipocytes via inhibiting intracellular FAS activity. The result of palmitic acid rescued EEO induced apoptosis in cancer cells confirmed that the apoptosis was related to inhibition of FAS. Since EEO showed potent inhibition on the proliferation of MDA-MB-231 and 3T3-L1 cells, it had the potential to be developed into a drug candidate for treating obesity-related cancers.

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## References

Alo PL, Amini M, Piro F, et al (2007). Immunohistochemical expression and prognostic significance of fatty acid synthase

- in pancreatic carcinoma. *Anticancer Res*, **27**, 2523-27.
- Alo PL, Visca P, Marci A, et al (1996). Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. *Cancer*, **77**, 474-82.
- Alo PL, Visca P, Trombetta G, et al (1999). Fatty acid synthase (FAS) predictive strength in poorly differentiated early breast carcinomas. *Tumori*, **85**, 35-40.
- Arabbi PR, Genovese MI, Lajolo FM. (2004). Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. *J Agr Food Chem*, **52**, 1124-31.
- Block E (1985). The chemistry of garlic and onions. *Sci Am*, **252**, 114-9.
- Caan BJ, Kwan ML, Hartzell G, et al (2008). Pre-diagnosis body mass index, post-diagnosis weight change, and prognosis among women with early stage breast cancer. *Cancer Cause Control*, **19**, 1319-28.
- Calle EE, Rodriguez C, Walker-Thurmond K, et al (2003). Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *New Engl J Med*, **348**, 1625-38.
- Challier B, Perarnau JM, Viel JF. (1998). Garlic, onion and cereal fibre as protective factors for breast cancer: a French case-control study. *Eur J Epidemiol*, **14**, 737-47.
- Colditz GA (1992). Economic costs of obesity. *Am J Clin Nutr*, **55**, S503-7.
- Dawood S, Broglio K, Gonzalez-Angulo, et al (2008). Prognostic value of body mass index in locally advanced breast cancer. *Clin Cancer Res*, **14**, 1718-25.
- Epstein JI, Carmichael M, Partin AW. (1995). OA-519 (fatty acid synthase) as an independent predictor of pathologic state in adenocarcinoma of the prostate. *Urology*, **45**, 81-6.
- Galeone C, Pelucchi C, Levi F, et al (2006). Onion and garlic use and human cancer. *Am J Clin Nutr*, **84**, 1027-32.
- Gansler TS, Hardman W, Hunt DA, et al (1997). Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. *Hum Pathol*, **28**, 686-92.
- Hsing AW, Chokkalingam AP, Gao YT, et al (2002). Allium vegetables and risk of prostate cancer: A population-based study. *J Natl Cancer I*, **94**, 1648-51.
- Khandekar MJ, Cohen P, Spiegelman BM. (2011). Molecular mechanisms of cancer development in obesity. *Nature Rev Cancer*, **11**, 886-95.
- Kim OY, Lee SM, Do H, et al (2012) Influence of quercetin-rich onion peel extracts on adipokine expression in the visceral adipose tissue of rats. *Phytother Res*, **26**, 432-7.
- Kroenke CH, Chen WY, Rosner B, et al (2005). Weight, weight gain, and survival after breast cancer diagnosis. *J Clin Oncol*, **23**, 1370-8.
- Kuhajda FP. (2000). Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition*, **16**, 202-8.
- Kuhajda FP. (2006). Fatty acid synthase and cancer: new application of an old pathway. *Cancer Res*, **66**, 5977-80.
- Lahmann PH, Hoffmann K, Allen N, et al (2004). Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Int J Cancer*, **111**, 762-71.
- Le Bon AM, Siess MH. (2000). Organosulfur compounds from allium and the chemoprevention of cancer. *Drug Metabol Drug Interact*, **17**, 51-79.
- Lee KH, Kim Y, Park E, et al (2008). Effect of onion powder supplementation on lipid metabolism in high fat-cholesterol fed SD Rats. *J Food Sci Nutr*, **13**, 1371-6.
- Lee SM, Moon J, Do HJ, et al (2012). Onion peel extract increases hepatic low-density lipoprotein receptor and ATP-binding cassette transporter A1 messenger RNA expressions in Sprague-Dawley rats fed a high-fat diet. *Nutr Res*, **32**, 210-7.
- Lenhard JM. (2011). Lipogenic enzymes as therapeutic targets for obesity and diabetes. *Curr Pharm Design*, **17**, 325-31.
- Menendez JA, Lupu R. (2007). Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nature Rev Cancer*, **7**, 763-77.
- Menendez JA, Mehmi I, Atlas E, et al (2004). Novel signaling molecules implicated in tumor-associated fatty acid synthase-dependent breast cancer cell proliferation and survival: Role of exogenous dietary fatty acids, p53-p21WAF1/CIP1, ERK1/2 MAPK, p27KIP1, BRCA1, and NF-kappaB. *Int J Oncol*, **24**, 591-608.
- Milgram LZ, Witters LA, Pasternack, GR, et al (1997). Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma. *Clin Cancer Res*, **3**, 2115-20.
- Orita H, Coulter J, Tully E, et al (2008). Inhibiting fatty acid synthase for chemoprevention of chemically induced lung tumors. *Clin Cancer Res*, **14**, 2458-64.
- Pan SY, Johnson KC, Ugnat AM, et al (2004). Association of obesity and cancer risk in Canada. *Am J Epidemiol*, **159**, 259-68.
- Pizer ES, Lax SF, Kuhajda FP, et al (1998). Fatty acid synthase expression in endometrial carcinoma: correlation with cell proliferation and hormone receptors. *Cancer*, **83**, 528-37.
- Pizer ES, Thupari J, Han WF, et al (2000). Malonyl-coenzyme A is a potential mediator of cytotoxicity induced by fatty-acid synthase inhibition in human breast cancer cells and xenografts. *Cancer Res*, **60**, 213-8.
- Prieto-Hontoria PL, Pérez-Matute P, Fernández-Galilea M, et al (2011). Role of obesity-associated dysfunctional adipose tissue in cancer: a molecular nutrition approach. *BBA-Bioenergetics*, **1807**, 664-78.
- Rashid A, Pizer ES, Moga M, et al (1997). Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. *Am J Pathol*, **150**, 201-8.
- Sengupta A, Ghosh S, Bhattacharjeethe S. (2004). Allium vegetables in cancer prevention: An overview. *Asian Pac J Cancer Prev*, **5**, 237-45.
- Setiawan VW, Yu GP, Lu QY, et al (2005). Allium vegetables and stomach cancer risk in China. *Asian Pac J Cancer Prev*, **6**, 387-95.
- Shurbaji MS, Kalbfleisch JH, Thurmond TS. (1996). Immunohistochemical detection of a fatty acid synthase (OA-519) as a predictor of progression of prostate cancer. *Hum Pathol*, **27**, 917-21.
- Soulié JM, Sheplock GJ, Tian WX, et al (1984). Transient kinetic studies of fatty acid synthetase. A kinetic self-editing mechanism for the loading of acetyl and malonyl residues and the role of coenzyme A. *J Biol Chem*, **259**, 134-40.
- Sun XB, Tian WX. (2009). Inhibitory effects of Allium vegetable extracts on fatty acid synthase. *Food Sci Technol Res*, **15**, 343-6.
- Sung YY, Yoon T, Kim SJ, et al (2011). Anti-obesity activity of Allium fistulosum L. extract by down-regulation of the expression of lipogenic genes in high-fat diet-induced obese mice. *Mol Med Report*, **4**, 431-5.
- Swinnen JV, Roskams T, Joniau S, et al (2002). Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. *Int J Cancer*, **98**, 19-22.
- Tartert PI, Papatestas AE, Ioannovich J, et al (1981). Cholesterol and obesity as prognostic factors in breast cancer. *Cancer*, **47**, 2222-7.
- Tian WX, Hsu RY, Wang YS. (1985). Studies on the reactivity of the essential sulfhydryl groups as a conformational probe for the fatty acid synthetase of chicken liver. Inactivation

- by 5,5'-dithiobis-(2-nitrobenzoic acid) and intersubunit cross-linking of the inactivated enzyme. *J Biol Chem*, **260**, 11375-87
- Uddin S, Ah-Kang J, Ulaszek J, et al (2004). Differentiation stage-specific activation of p38 mitogen-activated protein kinase isoforms in primary human erythroid cells. *P Natl Acad Sci USA*, **101**, 147-52.
- van den Brandt PA, Spiegelman D, Yaun SS, et al (2000). Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. *Am J Epidemiol*, **152**, 514-27.
- Virtanen AL, Matikkala EJ. (1976). The isolation of S-methyl-L-cysteine sulphoxide and S-n-propyl-L-cysteine from onion (*Allium cepa*) and the antibiotic activity of crushed onion. *Acta Chem Scand*, **13**, 1898-900.
- Visca P, Sebastiani V, Botti C, et al (2004). Fatty acid synthase (FAS) is a marker of increased risk of recurrence in lung carcinoma. *Anticancer Res*, **24**, 4169-73.
- Walter K, Hong SM, Nyhan S, et al (2009). Serum fatty acid synthase as a marker of pancreatic neoplasia. *Cancer Epidem Biomar*, **18**, 2380-5.
- Wang D, Dubois RN. (2012). Associations between obesity and cancer: the role of fatty acid synthase. *J Natl Cancer I*, **104**, 343-5.
- Zhou W, Simpson PJ, McFadden JM, et al (2003). Fatty acid synthase inhibition triggers apoptosis during S phase in human cancer cells. *Cancer Res*, **63**, 7330-7.