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Concomitant supplementation of lycopene and eicosapentaenoic acid inhibits the proliferation of human colon cancer cells☆

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Abstract

Several studies indicated that people who live in the Mediterranean region have very low rates of chronic diseases such as cardiovascular disease and cancer. It is well known that Mediterranean-style diet is rich in vegetables, tomato, fruit, fish and olive oil. These important dietary components may contribute to lower risk of cancer. Lycopene, a major component in tomato, exhibited potential anticarcinogenic activity. Previous studies showed that consumption of fish containing eicosapentaenoic acid (EPA) correlated with reduced risk of cancer. However, the combined effects of lycopene and EPA on the proliferation of human colon cancer have not been studied well yet. Thus, we investigated the anticancer properties and therapeutic potential of lycopene and EPA in human colon cancer HT-29 cells.

In this study, we determined the combined effects of lycopene and EPA on the proliferation of human colon cancer HT-29 cells. We demonstrated that low concentration of lycopene and EPA could synergistically inhibit the proliferation of colon cancer cells. The inhibitory mechanism was associated with suppression of phosphatidylinositol 3-kinase/Akt signaling pathway. Furthermore, treatment of lycopene and EPA also synergistically blocked the activation of downstream mTOR molecule. Immunocytochemical staining results revealed that lycopene and EPA could also up-regulate the expression of apoptotic proteins such as Bax and Fas ligand to suppress cell survival.

In conclusion, our novel findings suggest that lycopene and EPA synergistically inhibited the growth of human colon cancer HT-29 cells even at low concentration. The inhibitory effects of lycopene and EPA on cell proliferation of human colon cancer HT-29 cells were, in part, associated with the down-regulation of the PI-3K/Akt/mTOR signaling pathway.

Keywords: Lycopene; Eicosapentaenoic acid; Akt; mTOR; Proliferation; Colon cancer cells

1. Introduction

Colorectal cancer is one of the leading causes of cancer death in Western countries, including North America. In the United States alone, nearly 56,000 deaths are attributed to this cancer annually [1]. The phosphatidylinositol 3-kinase (PI-3K)/Akt pathway has been shown to be the predominant growth-factor-activated pathway in the tumorogenesis of many types of cancer, including colon cancer [2–7]. Akt is activated by extracellular stimuli in a PI-3K dependent manner and has uncovered essential roles in the control of transcription and protein translation, which impact on cell growth, survival and cell cycle progression [8]. Consequently, antiapoptotic signals transduced by PI-3K and downstream mTOR molecules have become a focus of recent drug discovery research. Activation of Akt signaling pathway would phosphorylate proapoptotic Bad protein, which promotes association of Bad with 14-3-3 adaptor protein and allows excess Bcl-2 or Bcl-XL to out compete Bax, thereby preventing apoptosis [3]. Cumulative evidence indicates that the constitutively active Akt induces cell survival and malignant transformation, whereas inhibition of

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Akt activity stimulates apoptosis in a range of mammalian cells [9]. Suppression of Akt would induce the activation of proapototic Bax protein and cellular apoptosis. Furthermore, activation of Akt would also phosphorylate the FOXO3 (FKHR [Forkhead in rhabdomyosarcoma]) transcriptional factor and suppress the expression of apoptotic Fas ligand protein [10]. Thus, inhibition of the PI-3K/Akt pathway could prevent the uncontrolled cellular proliferation.

The mTOR protein is a viable target for chemotherapeutic intervention with the PI-3K/Akt pathway. The mTOR protein is an activator of the cell cycle and, thus, a candidate tumor activator [11]. Up-regulation of Akt/mTOR signaling pathway could enhance cell cycle progression. An increase in mTOR activity, due to activation of Akt signaling pathway, occurs in colorectal carcinoma and correlates with aggressive, high-grade tumors and poor prognosis. However, suppression of mTOR would induce the arrest of cell cycle progression [12]. Thus, inhibition of PI-3K/Akt/mTOR pathway is a promising approach for discovery of novel chemotherapeutic agents.

Epidemiological studies have shown that consumption of traditional Mediterranean diets with good amounts of vegetables especially tomato, fruits, olive oil, grains, beans and fish has lower rates of chronic diseases such as heart disease and cancer. Lycopene, a major component in tomato, exhibited potential anticarcinogenesis activity in many types of cancer [13–15]. Epidemiologic studies reported statically significant inverse associations between tomato consumption and risk of several types of cancer such as lung, prostate and colon cancer [16–20].

Diets enriched in n-3 polyunsaturated fatty acids (PUFA) have been shown to suppress the tumor growth [21–24]. PUFA with five double bonds eicosapentaenoic acid (EPA) has been shown to be more effective in suppression of cell growth than PUFA with six double bonds of docosahexenoic acid [21]. EPA, a major component in fish oil, was also demonstrated as an anticancer compound [25–28]. Animal study showed that consumption of tomato has been associated with reduced malignant lesion in a rodent model [15]. Experimental data demonstrated that anticarcinogenic effect of carotenoids such as β-carotene in vitro is different from lycopene mainly in the latter's ability to inhibit cellular growth. Most of the reports concerning the anticarcinogenic activity of carotenoids are based on their ability to be converted into vitamin A, which has been associated with differentiation and cancer regression [29]. Recent study even showed that beta-carotene could antagonize the effects of EPA on the growth of colorectal adenocarcinoma cells due to their scavenging effects of free radicals [30]. Peroxisome proliferators-activated receptor γ (PPARγ) is a ligand-activated transcription factor belonging to the steroid/thyroid receptor superfamily and plays a critical role in the control of adipogenesis [31, 32]. PPARγ has also been shown to suppress cell proliferation and tumorigenesis of various types of cancer [33–36]. N-3 PUFA might modulate PPARγ expression and mediate cell death [37,38]. In our previous study, we already demonstrated that lycopene could inhibit the growth of human colon cancer cells in a dose dependent manner (0, 2, 5 and 10 μM). At a concentration of 10 μM, lycopene could effectively suppress the proliferation of colon cancer cells up to 47% during the 24-h period. At a concentration of 2 μM, lycopene could effectively suppress the proliferation of colon cancer cells up to 20% during the 24-h period [39]. It suggested that lycopene could suppress proliferation of human colon cancer cells via modulation of cell signaling pathways. Normal range of human serum lycopene level is around 0.1–2 μM [40]. Normal range of human plasma EPA is around 30–80 μM [41]. However, up to date, the synergistic effects of lycopene and EPA on blockade of colorectal cancer have not been demonstrated yet. Lack of results across numerous studies may not be able to demonstrate the physiological concentration of lycopene and EPA against human colon cancer.

Therefore, in this study, we determined the inhibitory effect of lycopene and EPA on Akt/mTOR signaling pathways in human colon cancer HT-29 cells.

2. Materials and methods

2.1. Reagents and antibodies

Lycopene was purchased from Extrasynthese, Genay, France. EPA was purchased from Cayman Chemical (Ann Arbor, MI, USA). Antiphosphorylation Akt polyclonal antibody, anti-Akt polyclonal antibody, Anti-PPARγ monoclonal antibody and antiphosphorylation mTOR monoclonal antibody were purchased from R and D Systems (Minneapolis, MN, USA). Anti-β-actin antibody, wortmanin, troglitazone and THF (tetrahydrofuran) were purchased from Sigma (St. Louis, MO, USA). Human colon cancer cells HT-29 was purchased from American Type Culture Collection (Walkersville, MD, USA). McCoy's medium and phosphate-buffered saline (PBS) were purchased from GIBCO. Lycopene was dissolved in THF at a concentration of 10 mM and stored at −20°C. EPA was dissolved in ethanol at a concentration of 250 mM and stored at −20°C. Immediately before the experiment, the stock solution was added to the cell culture medium.

2.2. Cell culture

Briefly, HT-29 colon cancer cells were cultured in a 37°C humidified incubator with 5% CO_2 and grown to confluency using fetal bovine serum (FBS) supplemented McCoy's media. Cells used in different experiments have the similar passage number. McCoy's medium was supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and 1.5 g/L sodium bicarbonate.

2.3. Supplementation with lycopene and EPA

HT-29 colon cancer cells were incubated with different concentrations (0 and 2 μM) of lycopene or EPA (0 and

25 μM) for variable time points. For efficient uptake of lycopene by HT-29 colon cancer cells, lycopene or EPA were incorporated into FBS for 30 min and mixed with medium. In control groups, cells were incubated with equivalent volume of solvent THF containing 0.025% butylated hydroxytoluene as an antioxidant.

2.4. Assessment of cell proliferation

3-[4,5-dimethhylthiaoly]-2,5-diphenyltetrazolium bromide (MTT) assay was conducted to detect the cell proliferation. HT-29 colon cancer cells were seeded in 24-well plates, each well containing 5×104 cells. After 24 h, the culture medium was replaced by media with lycopene (0 and 2 μM) and EPA (0 and 25 μM), respectively. To investigate the effects of PI-3K inhibitor or PPARγ agonist, the culture medium was replaced by media with wortmanin (10 μM) and troglitazone (10 μM), respectively. These were triplicate for each concentration. After incubation of 24 h, plate was taken out and fresh MTT (final concentration 0.5 mg/ml in PBS) was added to each well.

After 2 h of incubation, the culture media were discarded, 200 μl of acidic isopropanol was added to each well and vibrated to dissolve the depositor. The optical density was measured at 570 nm with a microplate reader.

2.5. Western blotting analysis

HT-29 human colon cancer cells were cultured in 10% FBS culture media with lycopene (0 and 2 μM) and EPA (0 and 25 μM) for various lengths of time. Cells were lysed in a buffer containing: 1× PBS, 1% Ipegal CA-630 (Sigma), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS) with 100 μM of phenylmethylsulfonyl fluoride, aprotinin and specific phosphatase inhibitors, and sodium orthovanadate. Nuclear proteins were prepared as briefly described in the previous study [33]. Cellular proteins (100 μg) were fractioned on 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membrane, and blotted with antiphosphorylation Akt polyclonal antibody, according to the manufacturer's instructions. Nuclear proteins (100 μg) were fractioned on 10% SDS-PAGE, transferred to nitrocellulose membrane, and blotted with anti-PPARγ monoclonal antibody, according to the manufacturer's instructions. The blots were stripped and reprobed with either total-Akt or β-actin antibody as loading control. Level of phosphorylated mTOR was measured by using the same procedure described above.

2.6. Quantum dot-based immunofluorescence and imaging techniques

Human colon cancer cells, cultured in McCoy's medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. Cells were cultured on a glass eight-well Tek Chamber and treated with various concentration of lycopene (0 and 2 μM) or EPA (0 and 25 μM) for 24 h. At the end of experiment, cells were fixed in 4% paraformaldehyde in 20 mM of HEPES and 150 mM of NaCl for 20 min, permeabilized in 0.01% Triton X-100 in PBS for 10 min, blocked with 1% bovine serum albumin (BSA)/PBS for 1 h and then incubated at room temperature for 1 h with anti-Fas ligand and anti-Bax primary antibody at 1:50 in blocking solution. At the end of incubation, cells were washed with PBS and incubated with Quantum dot (Q-dot) 525 and 655 secondary antibody for another 1 hr in 1.5% BSA/PBS. Images were acquired on an Olympus BX-51 microscope using the Olympus DP-71 digital camera and imaging system. Images of cells were randomly documented from 10 different fields. Mean integrated fluorescence of Bax and Fas ligand were measured by accessory software. Similar procedures were repeated from three independent experiments.

2.7. Statistical analysis

The quantitative methodology was used to determine whether the difference in the cell viability between experimental sets of colon cancer cells. In brief, statistical analyses of the differences in viability among triplicate sets of experimental conditions were performed using SPSS. Confirmation of difference in cell viability as being statistically significant requires rejection of the null hypothesis of no difference between mean viability indices obtained from replicate sets at the P=.05 level with the one-way analysis of variance.

3. Results

3.1. Combined effects of lycopene and EPA on the proliferation of human colon cancer HT-29 cells

Our previous studies indicated that lycopene at supplemental levels inhibited growth of human colon cancer cell HT-29. Several studies also demonstrated that fish oil as an anticancer compound [25–28]. However, the effects of lycopene and EPA on proliferation of cancer cells have not been demonstrated yet. Here, we examined the combined effects of lycopene and EPA on proliferation of human colon cancer HT-29 cells. As shown in Fig. 1A, the proliferation of human colon cancer HT-29 cells was blocked by treatment of lycopene (2 μM) or EPA (25 μM) up to 18% and 20%, respectively. Surprisingly, results showed that lycopene (2 μM) and EPA (25 μM) synergistically inhibited cell proliferation of human colon cancer cell HT-29 by almost 70%. It suggested that combination of lycopene and EPA could be an effective way to suppress human colon cancer cell proliferation and survival. To investigate whether Akt or PPARγ signaling pathway plays an important role in determining survival of human colon cancer cells, we used PI-3K specific inhibitor,

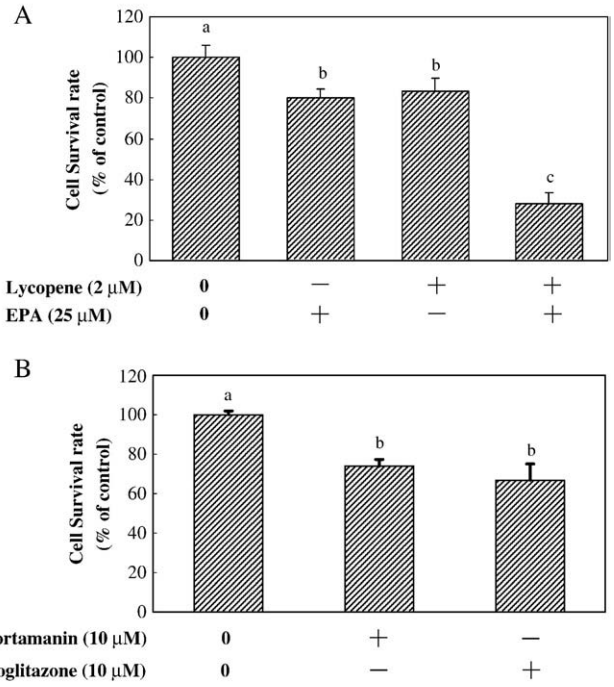

Fig. 1. Combined effects of lycopene and EPA on the proliferation of human colon cancer HT-29 cells. Human colon cancer cells, cultured in McCoy's medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. Approximately 50,000 cells were seeded on each well of 24-well plate. The human colon cancer cells were then cultured in McCoy's medium with lycopene (2 μM) or/and EPA (25 μM) for 24 h until measurement of cell proliferation. To investigate the effects of PI-3K inhibitor or PPARγ agonist, the culture medium was replaced by media with wortmanin (10 μM) or troglitazone (10 μM) for 24 h until measurement of cell proliferation. The incubation was stopped at different time points and measured with MTT assays for cell proliferation. The analysis of cell proliferation was described in Materials and methods. Data from three separate experiments are shown as the mean±S.E.M. Similar results were observed from three independent experiments. Different letters represent statistically significant difference ($P<.05$).

wortmanin, or PPARγ agonist, troglitazone, to investigate the molecular mechanism of action.

As shown in Fig. 1B, 10 μM of wortmanin and troglitazone effectively inhibited the proliferation of human colon cancer HT-29 cells up to 25% and 33 %, respectively. These results suggest that PI-3K and PPARγ could play crucial roles in determining survival of human colon cancer cells.

3.2. Synergistic effect of lycopene and EPA on consecutive activation of Akt molecule

In our previous studies, we also demonstrated that Akt is constitutively activated and plays an important role in the proliferation of human colon cancer cells [39]. It is well known that PI-3K/Akt/mTOR signaling pathway plays an important role in tumor progression. Thus, we examined the inhibitory effect of lycopene and EPA on the activation of PI-3K/Akt signaling pathway by western blotting assay in this study. As shown in the Fig. 2A, combination of lycopene and EPA significantly suppressed the activation of Akt compared with untreated colon cancer HT-29 cells. Concomitant supplementation of lycopene and EPA could significantly inhibit the proliferation of human colon cancer HT-29 cells (Fig. 2B). However, at a concentration of 25 μM, EPA alone or combination of lycopene and EPA could moderately induce the expression of PPAR-γ in human colon cancer HT-29 cells (Fig. 2C). Thus, one plausible mechanism by which lycopene and EPA synergistically inhibits cell survival in

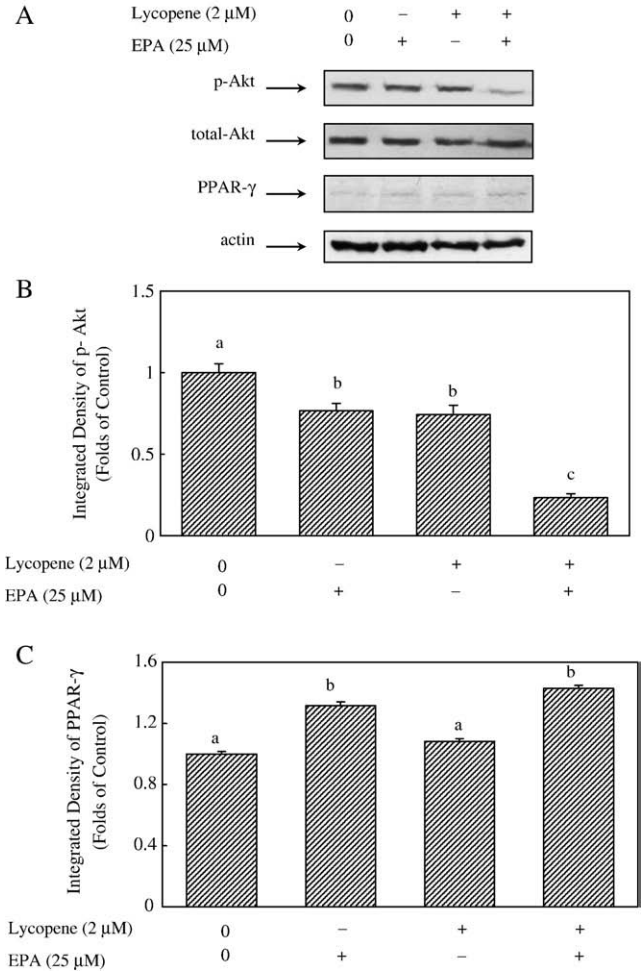

Fig. 2. Synergistic effect of lycopene and EPA on consecutive activation of Akt molecule. Human colon cancer cells, cultured in McCoy's medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation, and resuspended in the same medium. After washing out the media, human colon cancer cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with lycopene (2 μM) or/and EPA (25 μM) for 24 h. Total cell lysates were blotted with anti-phosphorylation-Akt antibody as described in Materials and Methods. Nuclear proteins were blotted with anti-PPARγ antibody, as described in Materials and methods. The levels of detection in cell lysate represent the amount of phosphorylated-Akt or PPARγ in human colon cancer cells. The blots were stripped and reprobed with either anti-actin or anti-total Akt polyclonal antibody as loading control. The results presented are representative of three different experiments. The immunoreactive bands are noted with arrow.

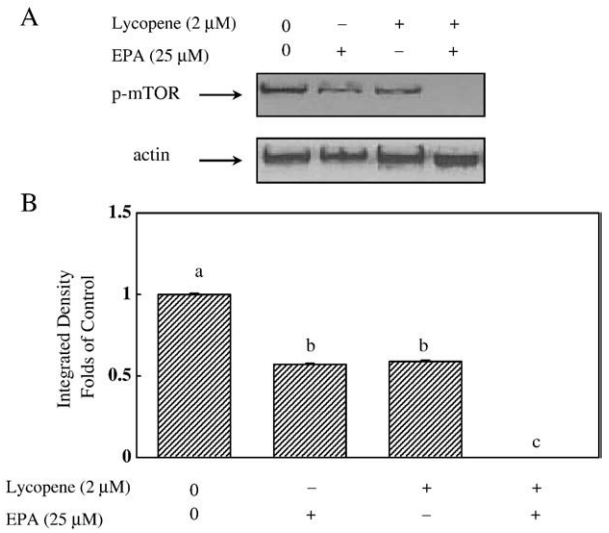

Fig. 3. Lycopene and EPA synergistically inhibit the activation of mTOR molecule. Human colon cancer cells, cultured in McCoy's medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation, and resuspended in the same medium. After washing out the media, human colon cancer cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with lycopene (2 μM) or/and EPA (25 μM) for 24 h. Total cell lysates were blotted with antiphosphorylated mTOR antibody, as described in Materials and methods. The levels of detection in cell lysate represent the amount of phosphorylated-mTOR in human colon cancer cells. The blots were stripped and reprobed with anti-actin polyclonal antibody as loading control. The results presented are representative of three different experiments. The immunoreactive bands are noted with arrow.

colon cancer cells is a major inhibition to PI-3K/Akt/mTOR signaling pathway.

3.3. Lycopene and EPA synergistically inhibit the activation of mTOR molecule

Since PI-3K/Akt has been found to be an important mediator of cell proliferation and translation via mTOR molecule, we examined the effect of lycopene and EPA on activity of downstream mTOR molecule in human colon cancer HT-29 cells. As shown in Fig. 3, lycopene and EPA synergistically suppressed the activity of mTOR compared to unstimulated HT-29 cells. These results were consistent with the combined effects of lycopene and EPA on suppression of cell proliferation.

3.4. Combined effect of lycopene and EPA on expression of apoptotic Bax protein

To investigate whether lycopene and EPA suppress survival of human colon cancer HT-29 cells, we analyzed the expression of Bax by using immunocytostaining analysis. Distribution of Bax protein was shown in Fig. 4A–H. As shown in Fig. 4A, B, rare Bax protein was expressed in human colon cancer HT-29 cells. In Fig. 4C, D, low concentration of lycopene had little effects on expression of Bax protein.

In Fig. 4E–F, low concentration of EPA had moderate effects on expression of Bax protein. However, in Fig. 4 G–H,

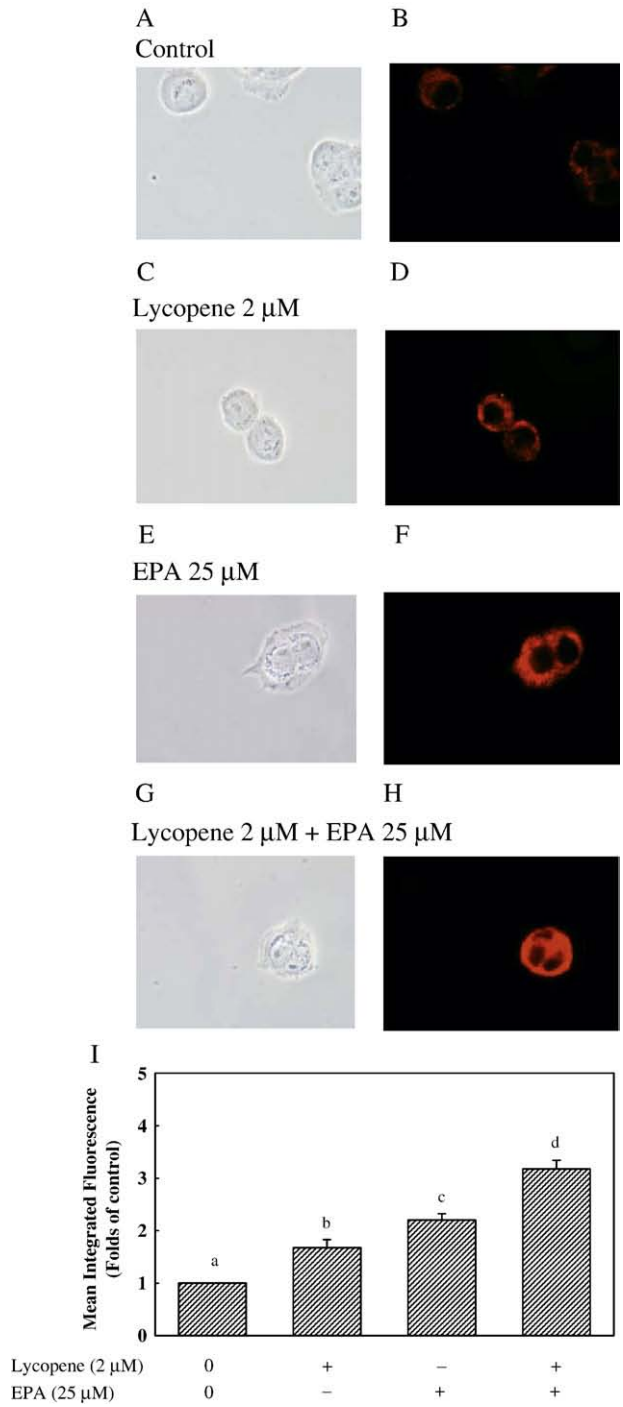

Fig. 4. Combined effect of lycopene and EPA on expression of apoptotic Bax protein. Human colon cancer cells were incubated in McCoy's medium with 10% FBS in an eight-well Tek Chamber with lycopene (2 μM) or/and EPA (25 μM) for 24 h. At the end of incubation, cells were blotted with anti-Bax primary antibody and Q-dot 655 secondary antibody, as described in Materials and methods. The levels of detection in cell represent the amount and localization of Bax protein in human colon cancer cells. Images (1000×) were acquired using Olympus DX-51 fluorescent stereomicroscope and DP-71 imaging system. (A, C, E, G) Images taken in light fields. (B, D, F, H) Images taken in dark fields. (I) The amount of Bax expression (mean density of integrated fluorescence) in human colon HT-29 cancer cells. Similar results were observed from three independent experiments. Different letters represent statistically significant difference ($P<.05$).

combined effects of lycopene and EPA could significantly induce the expression of Bax protein. As shown in Fig. 4I, lycopene and EPA could synergistically increase mean integrated fluorescence (MIF) of Bax protein in comparison with control cells (MIF: 3.17 folds of control cells). It suggested that lycopene and EPA could synergistically induce the expression of Bax protein and suppress the cell survival.

3.5. Combined effect of lycopene and EPA on expression of Fas ligand protein

To investigate whether lycopene and EPA suppress survival of human colon cancer HT-29 cells, we analyzed the expression of Fas ligand by using immunocytostaining analysis. Distribution of Fas ligand protein was shown in Fig. 5 (A–H). As shown in Fig. 5 A, B, rare Fas ligand protein was expressed in human colon cancer HT-29 cells. In Fig. 5 C, D, low concentration of lycopene had little effects on expression of Fas ligand protein. In Fig. 5 E, F, low concentration of EPA had little effects on expression of Fas ligand protein. However, in Fig. 5 G, H, combined effects of lycopene and EPA could significantly induce the expression of Fas ligand protein.

As shown in Fig. 5I, lycopene and EPA could synergistically increase MIF of Fas ligand protein in comparison with control cells (MIF: 10.8 folds of control cells). It suggested that lycopene and EPA could synergistically induce the expression of Fas ligand protein and suppress the cell survival.

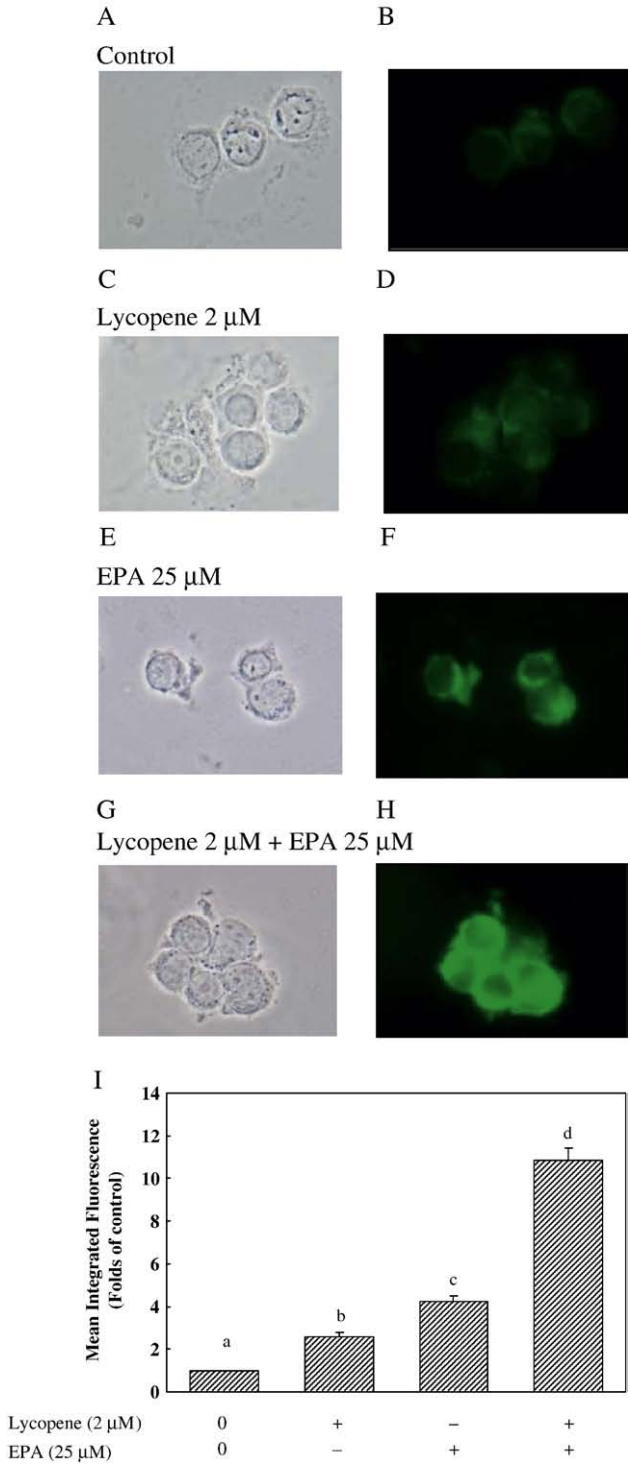

Fig. 5. Combined effect of lycopene and EPA on expression of Fas ligand protein. Human colon cancer cells were incubated in McCoy's medium with 10% FBS in an eight-well Tek Chamber with lycopene (2 μM) or/and EPA (25 μM) for 24 h. At the end of incubation, cells were blotted with anti-Fas ligand primary antibody and quantum dot 525 secondary antibody as described in Materials and methods. The levels of detection in cell represent the amount and localization of Fas ligand protein in human colon cancer cells. Images (1000×) were acquired using Olympus DX-51 fluorescent stereomicroscope and DP-71 imaging system. (A, C, E, G) Images taken in light fields. (B, D, F, H) Images taken in dark fields. (I) Amount of Fas ligand expression (mean density of integrated fluorescence) in human colon HT-29 cancer cells. Similar results were observed from three independent experiments. Different letters represent statistically significant difference ($P<.05$).

4. Discussion

Most of the reports concerning the anticarcinogenic activity of carotenoids are based on their ability to be converted into vitamin A which has been associated with differentiation and cancer regression [29]. However, over-activated signaling pathways maybe play crucial roles in the development of cancer. For example, a variety of alteration result in activation of the PI3 kinase/Akt/mTOR pathway in cancer cells [3]. Several components of the PI-3K-Akt pathways are dysregulated in a wide spectrum of human cancers. Constitutive activation of PI-3K/Akt induces not only tumor angiogenesis but also the progression of tumor development. Recent studies reported that mutation of regulatory subunit p85-α and constitutively activated Akt molecules support the proliferation of colorectal cancer cells

[7]. PI-3K and Akt have come onto to the scene and are reaching central status as an important regulator of mammalian cell proliferation and survival during tumor development. Increasing evidence implicates inhibition of PI-3K pathways would hinder the growth of tumor.

Phytochemicals may block these activated signaling pathways beyond their potent antioxidant activity. Interestingly, a previous study indicated that beta-carotene could antagonize the effects of EPA on cell growth and lipid peroxidation in colon adenocarcinoma cells [30]. At the high concentration of 250 μM, EPA inhibited the growth of neoplastic WiDr cells. Beta-carotene (1–50 μM) could antagonize the EPA-mediated cell death. These observations were based on the emphasis of their free radical scavenging capability. In our previous study, we already demonstrated that lycopene could inhibit the growth of human colon cancer cells in a dose dependent manner (0, 2, 5 and 10 μM). At a concentration of 10 μM, lycopene could effectively suppress the proliferation of colon cancer cells up to 47% during the 24-h period [39]. At a concentration of 2 μM, lycopene could effectively suppress the proliferation of colon cancer cells up to 20% during the 24-h period [39]. It suggested that lycopene could suppress cell growth via modulation of cell signaling pathways. Recent study indicated that EPA inhibited the expression of vascular endothelial growth factor and prostaglandin E_2 in human colon cancer HT-29 cells [28]. These evidences suggest that either lycopene or EPA could modulate cell signaling pathways to suppress growth of cancer cells. In the present study, we speculated that lycopene and EPA block the active site of Akt and prevent its downstream signaling pathways required for tumor growth. Lycopene and EPA inhibited the proliferation of human colorectal cancer HT-29 cells (Fig. 1). At 24-h time point, combined supplementation of lycopene (at a concentration of 2 μM) and EPA (at a concentration of 25 μM) suppressed cell proliferation up to 70%. These results demonstrated that combination of lycopene and EPA effectively blocks the growth and survival of human colorectal cancer cells. Furthermore, we found that lycopene and EPA significantly inhibited Akt activity in colorectal cancer cells (Fig. 2). In our previous study, we already identify that Akt is overactivated in human colon cancer cells. Here, we furthermore demonstrated that lycopene and EPA synergistically suppressed the survival of colon cancer cells via inhibition of Akt kinase activity. These results indicated the importance of lycopene and EPA on the proliferation of colorectal cancer cells.

Recent studies have demonstrated that the signals of serine phosphorylation in Akt complex markedly enhanced the phosphorylation of mTOR. Thus, it is conceivable that the changes in phosphorylation of Akt/mTOR are associated with the changes in the cell cycle. We further demonstrated that lycopene and EPA effectively inhibit the activity of mTOR, a downstream molecule of PI-3K/Akt signaling pathways (Fig. 3). Suppression of Akt/mTOR correlated with decreased cell survival and enhanced apoptosis of cancer cells.

These results indicated that the blockade of Akt and mTOR activation by lycopene and EPA treatment is the major modulator for their effect on cell signaling pathway. In the present study, we demonstrated for the first time that lycopene and EPA effectively inhibited the serine

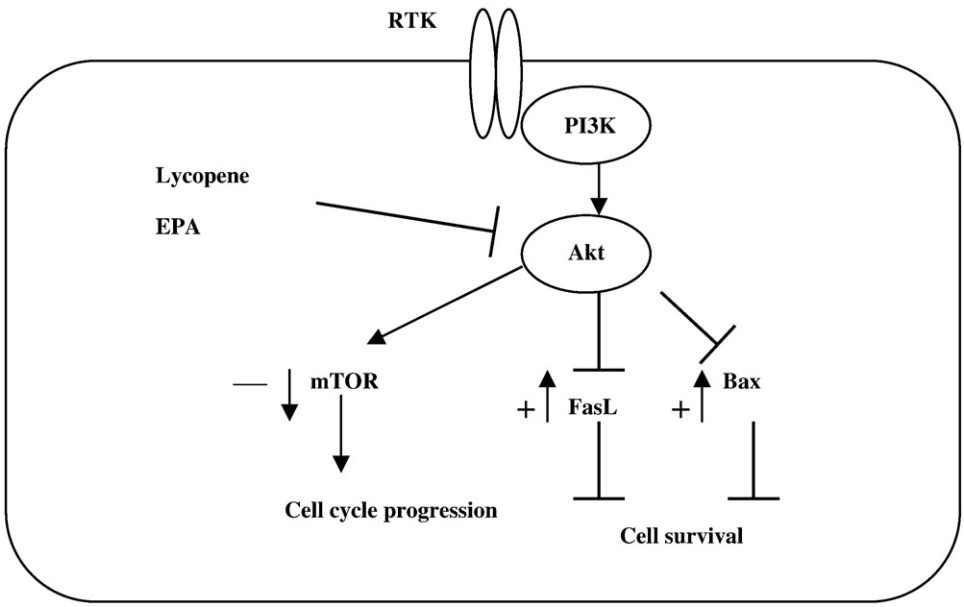

Fig. 6. Proposed mechanisms of lycopene and EPA on growth of human colon cancer. HT-29 cells. Proposed mechanisms of lycopene and EPA on proliferation of human colon cancer HT-29 cells. PI-3K/Akt/mTOR signaling pathways play important roles in the proliferation and survival of human colon cancer cells. In conclusion, lycopene and EPA synergistically inhibited the activity of Akt and mTOR molecules. Suppression of Akt by treatment of lycopene and EPA would enhance the accumulation of Bax and Fas ligand proteins to block cell survival.

phosphorylation of Akt and mTOR in colon cancer cells. To examine the exact mechanism of this inhibition, we speculate this inhibitory effect to be evolved with lycopene and EPA, in a possible model of antisurvival signal transduction. As previously suggested, Bax and Fas ligand proteins are targeted molecules of Akt and reported to be important for anti-survival effects.

Bax and Fas ligand protein are important suppressors of cell survival. To further investigate possible mechanisms of action, we measured the expression of Fas ligand molecule in human colon cancer HT-29 cells. As shown in Fig. 4, 5, lycopene and EPA significantly enhanced the expression and distribution of Bax and Fas ligand proteins. It suggested that lycopene and EPA might have inhibitory on the colon cancer cells via modulation of several anti-survival molecules such as Bax and Fas ligand proteins.

In this study, we first demonstrated that lycopene and EPA may specifically inhibit PI-3K/Akt/mTOR phosphorylation cascade during tumor progression and suppress cell proliferation. Peroxisome proliferators-activated receptor-γ (PPARγ) are nuclear receptor of EPA and play important roles in cell death [37]. We also identified the stimulatory effect of EPA on the activation of PPARγ (Fig. 2). PPARγ signaling pathway has been demonstrated as an important mediator of cell apoptosis. Furthermore, lycopene and EPA increased protein level of Bax and Fas ligand proteins to suppress the cell survival. Increasing protein level of Bax and Fas ligand proteins leads to the blocking of cell survival. In conclusion, one of the mechanisms by which lycopene and EPA may exert their anti-tumorgenic effect, is in part through inhibition of Akt phosphorylation. Lycopene and EPA may also inhibit cell proliferation by suppression of mTOR signaling pathways. These findings provide a novel mechanistic insight into the inhibitory effects of lycopene and EPA on the growth of human colorectal cancer. Palozza et al. [30] showed that high concentration of PUFA generates lipid peroxidation and inhibits cell proliferation. These findings were based on the PUFA-mediated generation of free radicals. It has been demonstrated that beta-carotene could antagonize the effects of EPA on the growth of colorectal adenocarcinoma cells due to their scavenging effects of free radicals [30]. However, low level of EPA (25 μM) and β-carotene (1 μM) mildly suppressed the proliferation of human colon cancer cells [30]. These results suggest that EPA could induce cell death at high concentration but probably inhibit cell proliferation at low concentration. Therefore, it is plausible that EPA or lycopene could modulate the signaling pathways to suppress cell proliferation. Here, we proposed a model of synergistic effects of lycopene and EPA on the growth of human colon cancer HT-29 cells (Fig. 6). EPA alone or cotreatment of EPA/lycopene could induce PPARγ expression and inhibit cell proliferation. It suggests that EPA-dependent PPARγ signaling pathway could, in part, involve in the suppression of cell growth. However, the molecular mechanisms of action could be mainly due to their capabilities to block cellular PI-3K/Akt/mTOR signaling transduction pathways in human colon cancer HT-29 cells. Although we only test the novel action of lycopene and EPA on transformed human colon cancer HT-29 cells, it could provide a direction to study the concomitant effects of these dietary factors.

Taken together, lycopene and EPA inhibited the PI-3 K/Akt/mTOR activities, which play crucial roles in the control of human colon cell proliferation. Decreased activity of Akt enhanced the Bax and Fas ligand proteins leading to blockade of cell survival. Although the differentiated effects of fish oil and lycopene on human colon cancer have not been investigated completely yet, we are currently examining the detailed mechanisms of these dietary components.

In conclusion, lycopene and EPA may effectively inhibit the proliferation and progression of transformed colonocytes in a synergistic way by suppression of PI-3K/Akt/mTOR signal transduction pathways. Due to the crucial role of Akt/mTOR activation in colon carcinogenesis, lycopene and EPA effectively suppresses the activity of Akt/mTOR and may prove to be a potent new anticancer compound with improved selectivity toward transformed cells.

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