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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