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Research Article

Lycopene inhibits growth of human colon cancer cells *via* suppression of the Akt signaling pathway

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The aberrant regulation of the phosphoinositide 3-kinase/Akt survival signaling pathway in cancer has prompted significant interest in suppression of this pathway to treat cancer. Previous studies identified an important role for phosphoinositide 3-kinase/Akt in colon cancer progression. Lycopene, a major component in tomato, exhibited potential anti-carcinogenic activity. Consumption of tomato has been associated with reduced risk of several types of human cancer. However, the inhibitory mechanisms of lycopene on the proliferation of human colon cancer have not been studied well yet. Thus we investigated the inhibitory effects of lycopene on the Akt signaling pathway in human colon cancer HT-29 cells. Lycopene inhibited cell proliferation in human colon cancer HT-29 cells with a IC_{50} value of 10 μM. Lycopene treatment suppressed Akt activation and non-phosphorylated β-catenin protein level in human colon cancer cells. Immunocytochemical results indicated that lycopene increased the phosphorylated form of β-catenin proteins. These effects were also associated with reduced promoter activity and protein expression of cyclin D1. Furthermore, lycopene significantly increased nuclear cyclin-dependent kinase inhibitor $p27^{kip}$ abundance and inhibited phosphorylation of the retinoblastoma tumor suppressor protein in human colon cancer cells. In conclusion, lycopene inhibited cell proliferation of human colon cancer cells *via* suppression of the Akt signaling pathway and downstream targeted molecules.

Keywords: Akt / Colon cancer cells / Cyclin D1 / Lycopene / Quantum dot

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1 Introduction

Colorectal cancer is one of the leading causes of cancer death in Western countries, including North America. In the US alone, thousands of deaths are attributed to this cancer annually [1]. Animal studies showed that consumption of tomato has been associated with reduced malignant lesion in a rodent model [2, 3]. Epidemiologic studies reported statistically significant inverse association between tomato consumption and colon cancer risk for men and women [4–8]. Lycopene, a major component in tomato, exhibited potential anti-carcinogenic activity in many types of cancer [9–11]. However, to date it has not been possible to clearly demonstrate the benefits of lycopene supplementation in preventing human colon cancer.

During carcinogenesis, the Akt/protein kinase B (PKB) molecule plays an important role [12]. Akt represents a subfamily of serine/theronine kinase. Under extracellular stimuli, Akt is activated in a phosphatidylinositol 3-kinase (PI3K) dependent manner [12]. Suppression of the Akt signaling pathway could block the development of several types of tumors including colon cancer [13, 14]. One possible mechanism could be mediated by downregulation of cyclin D1 expression, since downstream signaling pathways of Akt could affect nuclear translocation of β-catenin and uncover essential roles in the transcriptional control of cyclin D1, which has an impact on cell growth and cell cycle progression. Furthermore, inactivation of the Akt signaling pathway could induce phosphorylation of β-catenin and ubiquitination-dependent proteolysis [15].

Several proteins under the control of Akt are known to control the cell cycle and play crucial roles in cancer development. One notable example is the cyclin-dependent kinase inhibitor, $p27^{kip}$, which is an inhibitor of the cell cycle,

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Abbreviations: FBS, fetal bovine serum; **MTT,** 3-[4,5-dimethhylthiaoly]-2,5-diphenyltetrazolium bromide; **PI3K,** phosphoinositide 3-kinase; **PKB**, protein kinase B; **Rb**, retinoblastoma protein

and thus a candidate tumor suppressor. Increasing levels of $p27^{kip}$ could inhibit activity of cyclin-dependent kinase4/6/cyclin D complex, and reduce phosphorylation of retinoblastoma protein (Rb). Reduced phosphorylation or hypophosphorylation of Rb leads to inactivation of the E2F transcription factor and suppression of the S-phase cyclin A. Thus, accumulation of nuclear $p27^{kip}$ could up-regulate the activity of Rb tumor suppressor and block proliferation of cancer cells [16]. A decrease in $p27^{kip}$ levels due to $p27^{kip}$ protein degradation occurs in roughly half of the cases of carcinoma [17–19] and correlates with aggressive, high-grade tumors and poor prognosis [20–22]. However, certain carcinomas of the breast, thyroid, esophagus or colon contain normal levels of $p27^{kip}$ but the protein has shifted location in these cancers [23]. Studies indicated that $p27^{kip}$ mainly resides in the cytoplasm rather than in the nucleus of those malignant cancer cells [23]. It has been suggested that the activated kinase Akt/PKB disables the nuclear translocation of $p27^{kip}$ in those malignant cancer cells.

Thus, in the current study, we determined the inhibitory effect of lycopene on the Akt signaling pathway and cell cycle progression in human colon cancer HT-29 cells.

2 Materials and methods

2.1 Reagents and antibodies

Lycopene was purchased from Extrasynthese (Genay, France). Anti-phosphorylation Akt polyclonal antibody and anti-$p27^{kip}$ antibody were purchased from R&D Systems (Minneapolis, MN). Anti-phosphorylation β-catenin monoclonal antibody was purchased from Cell Signaling Technology (Danvers, MA). Anti-phosphorylation Rb polyclonal antibody, anti-β-catenin and anti-cyclin D1 polyclonal antibody were purchased from Santa Cruz Biotech (Santa Cruz, CA). Quantum dot 565 secondary antibody was purchased from Invitrogen (Carlsbad, CA). Anti-β-actin antibody and wortamannin were purchased from Sigma (St. Louis, MO). Human colon cancer cells HT-29 were purchased from American Type Culture Collection (Walkersville, MD). THF, containing 0.025% butylated hydroxytoluene as an antioxidant, was purchased from Sigma. McCoy's medium and PBS were purchased from GIBCO. Lycopene was dissolved in THF at a concentration of 10 mM stock solution and stored at −20°C. Immediately before the experiment, the stock solution was added to the cell culture medium, as described in Section 2.3.

2.2 Cell culture

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 were supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine and 1.5 g/L sodium bicarbonate.

2.3 Supplementation with lycopene

HT-29 colon cancer cells were incubated with different concentrations (0, 2, 5, and 10 μM) of lycopene for various time points. For efficient uptake of lycopene by HT-29 colon cancer cells, lycopene was mixed with THF and incorporated into FBS for 30 min and mixed with the medium. In control groups, cells were incubated with equivalent volumes of solvent THF (final concentration: 0.005% v/v) as a carrier vehicle.

2.4 Assessment of cell proliferation

3-[4,5-Dimethylthiaoly]-2,5-diphenyltetrazolium bromide (MTT) assay was conducted to detect cell proliferation. HT-29 colon cancer cells were seeded in 24-well plates, each well containing 1×10^5 cells. After 24 h, the culture medium was replaced by media in which lycopene concentrations were 0, 2, 5 and 10 μM, respectively. There were triplicates for each concentration. From 1 to 3 days, one of the plates was taken out and fresh MTT (final concentration 0.5 mg/mL in PBS) was added to each well.

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

2.5 Western blot analysis

HT-29 human colon cancer cells were cultured in 10% FBS culture media in the presence or absence of lycopene for various lengths of time. Cells were lyzed in a buffer containing 1 × PBS, 1% Ipegal CA-630 (Sigma), 0.5% sodium deoxycholate, 0.1% SDS with 100 μM of PMSF, aprotinin and specific phosphatase inhibitors as well as sodium orthovanadate. Cell lysates were cleared by centrifugation. Cellular proteins were fractionated using 10% SDS-PAGE, transferred to nitrocellulose membrane, and blotted with anti-phosphorylated Akt polyclonal antibody, according to the manufacturer's instructions. The blots were stripped and reprobed with β-actin antibody as loading control. Protein levels of β-catenin, cyclin D1, $p27^{kip}$, and level of phosphorylated Rb were measured by using the same procedure.

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 trypsinized, pelleted by centrifugation, and resuspended in the same medium. To measure the stability of β-catenin under the

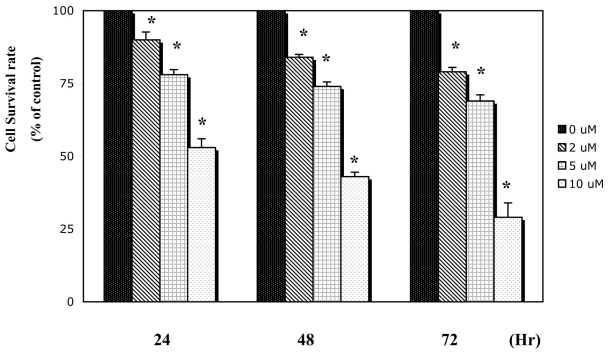


Figure 1. Inhibitory effects of lycopene on the proliferation of colon cancer cells. Approximately 30 000 cells were seeded in each well of the 24-well plate. Cells were then cultured in McCoy's medium with different concentrations of lycopene (0, 2, 5, 10 μM) for 24, 48, and 72 h. The cell proliferation was measured by MTT assay as described in Section 2.4. Data are mean ± SEM of three independent experiments. Asterisks represent statistically significant difference compared to the control group, $p < 0.05$.

treatment of lycopene (0, 2, and 10 μM), cells were cultured on a glass 8-well Tek Chamber 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% BSA/PBS for 1 h, and then incubated at room temperature for 1 h with anti-phosphorylated β-catenin primary antibody at 1:50 in blocking solution. At the end of incubation, the cells were washed with PBS and incubated with Quantum dot 565 secondary antibody for 1 h in 1.5% BSA/PBS. Images were obtained on an Olympus BX-51 microscope using the Olympus DP-71 digital camera and imaging system.

2.7 Transient transfection and luciferase reporter assays

To investigate whether lycopene affects the gene expression of cyclin D1, cyclin D1 promoter construct −1745 CDLUC and pAL3 basic construct were transiently transfected into HT-29 cells using Lipofectamine (Invitrogen). Luciferase reporter assays (Promega) were performed in triplicate according to the manufacturer's instructions. Luciferase fluorescence activity was measured in a luminometer (DRL ready TD 20/20).

2.8 Statistical analysis

The biostatistic methodology was used to determine whether there is a difference in the cell viability among 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 statistical software. 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 $p = 0.05$ level with the one-way ANOVA analysis.

3 Results

3.1 Inhibitory effects of lycopene on the proliferation of colon cancer cells

Lycopene inhibited proliferation of human colon cancer HT-29 cells in a dose-dependent manner (0, 2, 5 and 10 μM; Fig. 1). At a concentration of 10 μM, lycopene effectively suppressed the proliferation of colon cancer cells up to 47, 57 and 71% after 24, 48, and 72 h, respectively (Fig. 1). These results suggest that lycopene could effectively suppress proliferation and survival of human colon cancer HT-29 cells.

3.2 Lycopene inhibited the consecutive activation of Akt in human colon cancer cells

To investigate whether activation of Akt plays an important role in determining survival of human colon cancer cells, we used the PI3K specific inhibitor, wortamannin, to investigate the molecular mechanism of lycopene action. The cell survival rate of HT-29 cells in the presence of wortmannin decreased in a dose-dependent manner (0, 10, 30, 50 μM) in human colon cancer cells (Fig. 2A), indicating that blockade of Akt activation may suppress the survival of HT-29 cells.

Thus we examined the inhibitory effect of lycopene on the activation of PI3K/Akt using Western blot analysis. We found that the Akt molecule is consecutively activated in human colon cancer HT-29 cells (Fig. 2B). However, lycopene treatment still significantly suppressed the activation of Akt compared to untreated colon cancer HT-29 cells in carrier vector (THF only). Thus, it suggested that lycopene could interrupt the PI3K/Akt signaling pathway in part by interfering in the phosphorylation activation and inhibiting cell survival in human colon cancer HT-29 cells.

3.3 Lycopene modulated the cytoplasmic levels of phosphorylated β-catenin

To investigate whether lycopene affected the Akt/β-catenin pathways, we determined the level of the β-catenin molecule by measuring the protein level and cytoplasmic localization. As shown in Fig. 3A, lycopene inhibited the cytoplasmic levels of β-catenin in human colon cancer HT-29 cells. We also analyzed phosphorylated β-catenin *via* quantum dot immunocytostaining. As shown in Fig. 3 (B–G), lycopene dose-dependently (0, 2, 10 μM) enhanced the accumulation of phosphorylated β-catenin.

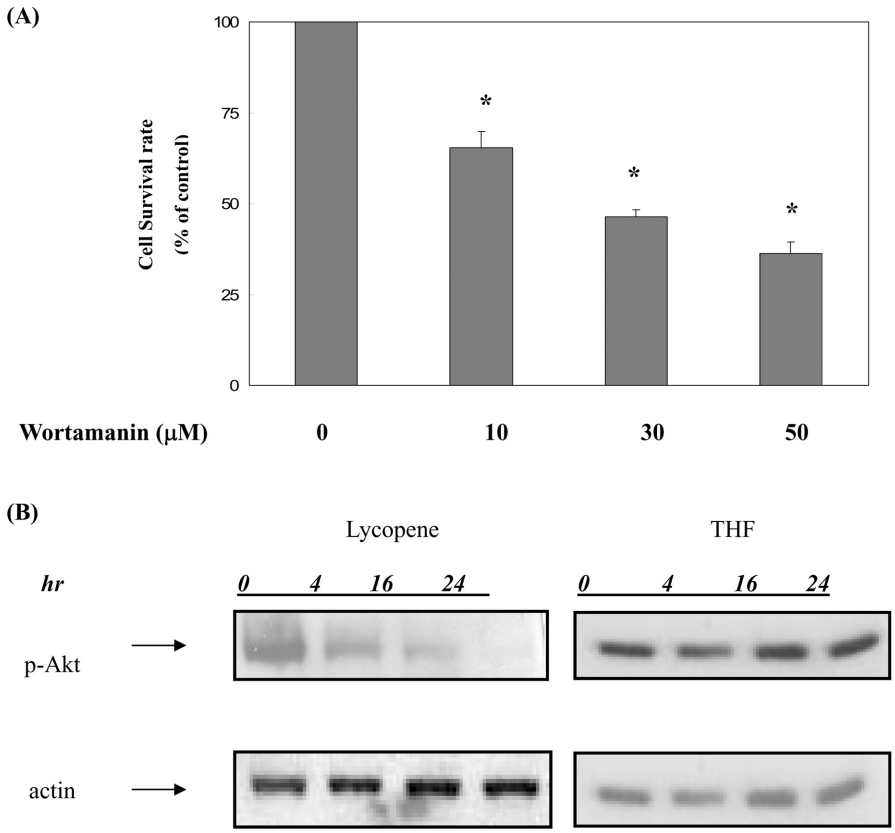


Figure 2. Lycopene inhibited consecutive activation of Akt in human colon cancer cells.
(A) Approximately 30 000 cells were seeded in each well of the 24-well plate. Human colon cancer HT-29 cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish at various concentrations (0, 10, 30, 50 μM) of wortamanin for 24 h. The cell proliferation was measured by MTT assay as described in Section 2.4. Data are mean ± SEM of three independent experiments. Asterisks represent statistically significant differences compared to the control group, $p < 0.05$.
(B) Human colon cancer HT-29 cells were seeded on a 10 cm tissue culture dish with 10% FBS McCoy's medium. Total cell lysates were blotted with anti-phosphorylated Akt antibody as described in Section 2.5. The levels of detection in cell lysates represent the amount of phosphorylated Akt in human colon cancer cells. The blots were stripped and reprobed with anti-actin antibody as loading control. The immunoreactive bands are noted with arrows.

3.4 Lycopene suppressed the cyclin D1 promoter activity and protein expression in human colon cancer cells

To determine whether lycopene suppressed the gene expression of cyclin D1, we used cyclin D1-luciferase construct (–1745 CD1) and basic construct (pAL3 basic). As shown in Fig. 4A, lycopene inhibited the promoter activity of cyclin D1 gene. No promoter activities were detected in the group of basic construct. Thus, it is plausible that lycopene could inhibit the promoter activity of cyclin D1.

To determine whether lycopene could further block downstream Akt signaling pathways, we examined the expression of cyclin D1 proteins. As shown in Fig. 4B, expression of cyclin D1 protein is suppressed under the treatment of lycopene in a time-dependent manner. These results suggest that lycopene might suppress Akt signaling pathways and expression of downstream cyclin D1 protein.

3.5 Lycopene induced the accumulation of nuclear $p27^{kip}$ protein and activity of Rb in colon cancer cells

Since PI3K/Akt has been found to be an important mediator of cell proliferation *via* $p27^{kip}$ translocation, we tested the effect of lycopene on nuclear localization of $p27^{kip}$ in colon cancer cells. As shown in Fig. 5, lycopene significantly induced the nuclear localization of $p27^{kip}$ compared to unstimulated HT-29 cells. Furthermore, lycopene indirectly blocked the phosphorylation of Rb and enhanced its activity.

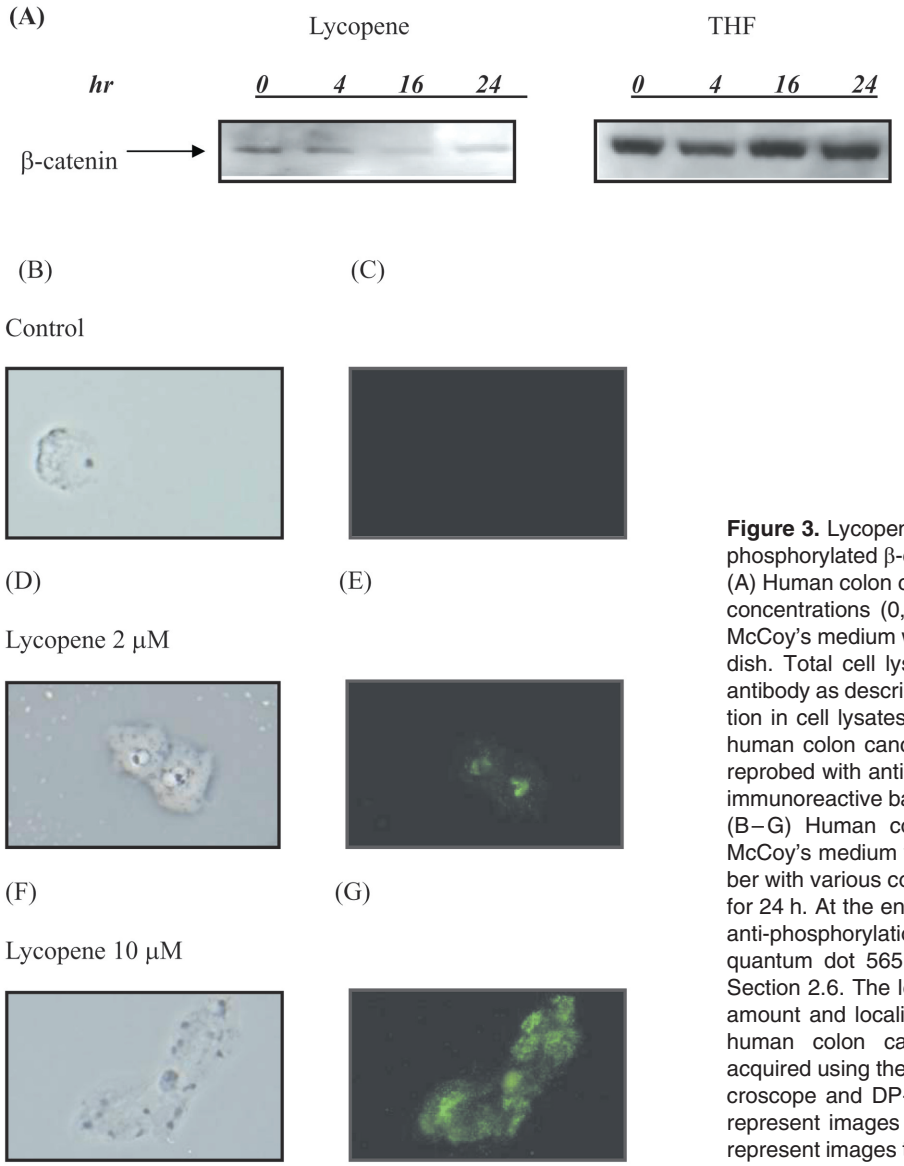
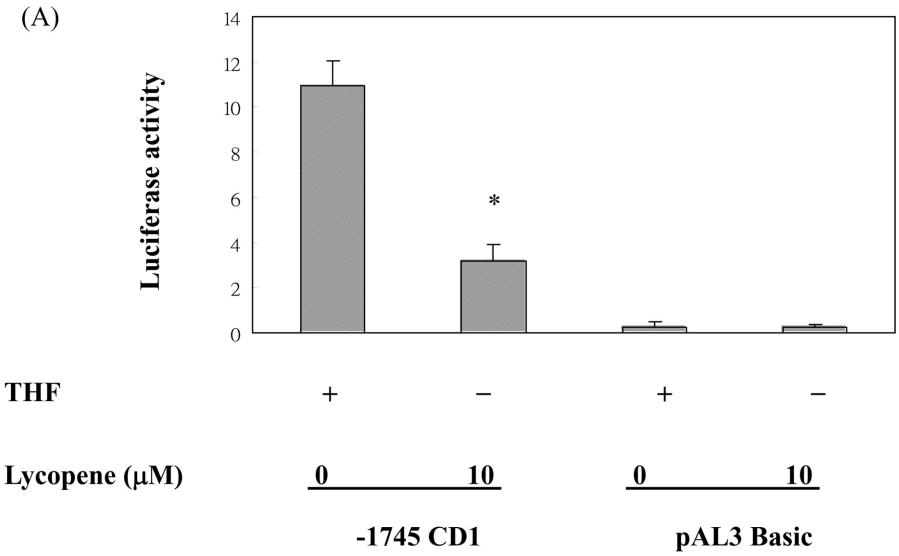


Figure 3. Lycopene modulated the cytoplasmic levels of phosphorylated β-catenin.
(A) Human colon cancer cells supplemented with various concentrations (0, 10 μM) of lycopene were cultured in McCoy's medium with 10% FBS in a 10 cm tissue culture dish. Total cell lysates were blotted with anti-β-catenin antibody as described in Section 2.5. The levels of detection in cell lysates represent the amount of β-catenin in human colon cancer cells. The blots were stripped and reprobed with anti-actin antibody as loading control. The immunoreactive bands are noted with arrows.
(B–G) Human colon cancer cells were incubated in McCoy's medium with 10% FBS in an 8-well Tek Chamber with various concentrations (0, 2, 10 μM) of lycopene for 24 h. At the end of incubation, cells were blotted with anti-phosphorylation β-catenin primary antibody and quantum dot 565 secondary antibody as described in Section 2.6. The levels of detection in cell represent the amount and localization of phosphorylation β-catenin in human colon cancer cells. Images (1000×) were acquired using the Olympus DX-51 fluorescent stereomicroscope and DP-71 imaging system. (B), (D), and (F) represent images taken in light fields. (C), (E), and (G) represent images taken in dark fields.

These findings suggest that lycopene could inhibit cell growth by reduced phosphorylation levels of Akt and Rb proteins.

4 Discussion

In this study, we used an *in vitro* model to demonstrate the inhibitory effects of lycopene on human colon cancer cells. Our results indicated that lycopene effectively inhibits the proliferation of human colon cancer cells in a dose-dependent manner. These findings provide important insights into the molecular mechanisms of lycopene in cancer prevention through the potential suppression of tumor growth. For the first time, our results demonstrated the inhibitory effects of lycopene on the growth of human colon cancer cells. PI3K and Akt have come onto the scene as an important regulator of mammalian cell proliferation and survival during tumor development. Several components of the PI3K-Akt pathways are dysregulated in a wide spectrum of human cancers [24, 25]. The inhibitory regulatory subunit of PI3 kinase is mutated in some colon cancer cells [26]. Recent studies reported that mutation of the regulatory subunit p85-α and constitutively activated Akt molecules support the proliferation of colorectal cancer cells [27]. Constitutive activation of PI3K/Akt induces not only tumor angiogenesis but also progression of tumor development [28–30]. Increasing evidence indicates that inhibition of PI3K pathways would inhibit tumour growth. Thus therapeutic strategies which target the PI3K/Akt pathway are now under development [31]. In the present study, we speculated whether lycopene could block the active site of



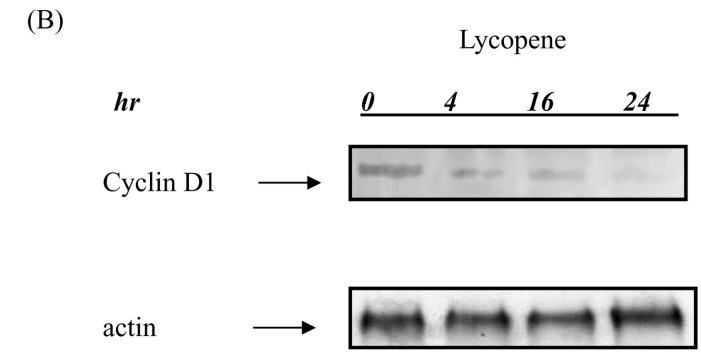


Figure 4. Lycopene suppressed cyclin D1 promoter activity and protein expression in human colon cancer cells.
(A) 90%-confluent human colon cancer HT-29 cells were treated with lycopene at different time points. Cyclin D1 luciferase reporter construct (1 μg) and pAL3 basic construct were transiently transfected into HT-29 cells, respectively. Triplicate experiments were performed at 24 h after treatment with or without lycopene (10 μM). Asterisks represent statistically significant difference compared to the control group, $p < 0.05$. The data shown are representative of three independent experiments.
(B) Human colon cancer cells, cultured in McCoy's medium with 10% FBS in a tissue culture dish, were trypsinized, 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 10 μM of lycopene at different time points (0, 4, 16, 24 h). Total nuclear cell lysates were blotted with anti-cyclin D1 antibody as described in Section 2.5. The levels of detection in nuclear cell lysates represent the amount of cyclin D1 in human colon cancer cells. The blots were stripped and reprobed with anti-actin polyclonal antibody as loading control. Each experiment was repeated three times with very similar results. The immunoreactive bands are noted with arrows.

Akt and prevent activation of its downstream signaling pathways required for tumor growth.

We also demonstrated that Akt is consecutively activated in human cancer cells (Fig. 2.). However, lycopene treatment effectively suppressed Akt activity. Lycopene significantly suppressed the survival of colon cancer cells *via* inhibition of Akt kinase activity suggesting that lycopene could be an effective natural compound with anti-cancer activity.

In colorectal carcinoma cells with intact APC genes, β-catenin was shown to contribute to the activation of the wingless/wnt pathway [32, 33]. Accumulation of β-catenin in the nucleus and the uncontrolled activation of target gene expression is believed to contribute to tumor progression [34, 35]. Previous studies suggested the cyclin D1 gene to be a target for the β-catenin/LEF-1 complex [36, 37]. Increased levels of β-catenin in colon cancer cells result in the activation of cyclin D1 gene promoter through a heterodimeric complex formation between β-catenin and LEF-1 which in turn results in the elevation of cyclin D1 gene expression, protein level, and cell cycle progression [36]. These data indicate that β-catenin may mediate carcinoma

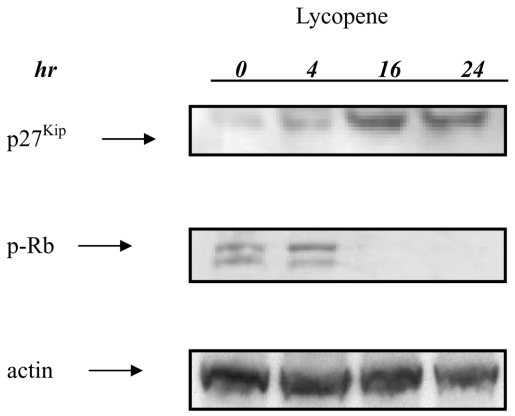


Figure 5. Lycopene-induced accumulation of nuclear p27^{kip} protein and activity of Rb in colon cancer cells.
Confluent human colon cancer cells were incubated with 10 μM of lycopene at 37°C at different time points (0, 4, 16, 24 h). After washing out the media, cytoplasma cell lysates and nuclear extracts of human colon cancer cells were prepared as described in the Section 2.4. (A) Total nuclear cell lysates were blotted with anti-p27^{kip} antibody as described in Section 2.5. The levels of detection in nuclear cell lysate represent the amount of p27^{kip} in human colon cancer cells. The levels of detection in nuclear extracts represent the amount of p27^{kip} in human colon cancer cells. The immunoreactive bands are noted with arrows. (B) Total nuclear cell lysates were blotted with anti-phosphorylated-Rb antibody as described in Section 2.5. The levels of detection in nuclear cell lysate represent the amount of tyrosine phosphorylated-Rb in human colon cancer cells. The immunoreactive bands are noted with arrows.

and suggest activation of the wnt signaling pathway in up to 80% of human colorectal carcinomas, and also suggest that activation of this pathway is the main initial event in colorectal tumorigenesis [38–41]. Recently, cyclin D1 was identified as a target of the β-catenin pathway in HT29 cells [32]. However, the effects of lycopene on genetic targets of this signaling pathway and their contribution to the neoplastic process have not yet been characterized. Thus, one of the purposes of the present work is to investigate whether lycopene inhibits cell cycle through suppression of the target genes in the development of human colorectal carcinomas. Our results showed that lycopene inhibited the protein levels of β-catenin in human colorectal cancer HT-29 cells. It is suggested that lycopene might block the Akt signaling pathways and protein level of downstream β-catenin molecule. Recent studies indicated that an increased phosphorylated level of β-catenin is correlated with downregulation of the Wnt/β-catenin pathway due to β-catenin proteosomal degradation [15]. While we did not measure degradation of phosphorylated β-catenin, our results demonstrate that lycopene treatment does dependently suppress β-catenin, which is prone to degradation. To further investigate the effects of lycopene on the regulation of β-catenin, we used an immunocytostaining assay conjugated with quantum dot techniques to identify the cytoplasmic localization of phosphorylated β-catenin protein. As shown in this study, lycopene treatment dose-dependently enhanced the cytoplasmic accumulation of phosphorylated β-catenin. Since serine phosphorylation of β-catenin leads to its ubiquitin degradation, it is plausible that lycopene could indirectly modulate the cytoplasmic level of β-catenin by an increased phosphorylation level of β-catenin. We further investigated the effects of lycopene on the promoter activity of cyclin D1. Results showed that lycopene significantly inhibited promoter activity and protein expression of cyclin D1. These results suggest that lycopene could block cell cycle progression by modulation of cyclin D1.

A decrease in p27^{kip} levels due to p27^{kip} protein degradation occurs in roughly 50% of the cases of carcinoma [17–19] and correlates with aggressive, high-grade tumors and poor prognosis [20–22]. Certain carcinomas of the breast, thyroid, esophagus or colon contain normal levels of p27^{kip} but, strangely, the protein is found in the cytoplasm rather than in the nucleus in these cancers. The activated kinase Akt/PKB pathway in these cells disables p27 and thus, p27^{kip} can no longer rein in cell division, providing the tumor cell with growth advantage and contributing to genome instability. Similar to the loss of p27^{kip}, this accumulation of cytoplasmic Akt-phosphorylated p27^{kip} correlated with tumor aggressiveness. Collectively, the current evidence indicates that Akt activation is an important component in cell proliferation. Recent studies have demonstrated that signals of serine phosphorylation in Akt complex markedly enhanced the phosphorylation of p27^{kip} and prevented its nucleus translocation [23, 42]. Thus, it is conceivable that the changes in serine phosphorylation of Akt are associated with changes in the cell cycle. Therefore, to further examine other possible mechanisms, we examined the effects of lycopene on cell cycle modulators. While p27^{kip}, through dephosphorylation signaling, would induce a pleiotropic response allowing cell cycle arrest, our data indicate that lycopene induced this process leading to the inhibition of cell growth. It suggested that the abundance of nuclear p27^{kip} and activity of Rb were increased with lycopene treatment.

These results indicated that the blockade of Akt activation by lycopene treatment is the major modulator for their effect on cell proliferation. In the present study, we demonstrated for the first time that lycopene inhibited the serine phosphorylation of Akt and decreased β-catenin protein levels in human colon cancer HT-29 cells. We also found that lycopene enhanced the phosphorylation level of β-catenin protein which leads to proteosomal degradation. To examine the exact mechanism of this inhibition, we speculate that this inhibitory effect involves lycopene, in a possible model of signal transduction *via* the cyclin changes. As previously suggested, cyclin D1 is reported to be important for progression of cell cycle, therefore changed levels of cyclins are likely to be a consequence of a cascading signal

through Akt signaling pathways. In this study, we demonstrated that lycopene may specifically inhibit PI3K/Akt phosphorylation cascade during tumor progression and suppress cyclin D1 promoter activity and protein level. Furthermore, lycopene increased nuclear $p27^{kip}$ protein but suppressed the phosphorylation of Rb protein. Increased protein level of $p27^{kip}$ and activity of Rb leads to the blocking of cell cycle progression.

In conclusion, one of the mechanisms by which lycopene may exert an antitumorgenic effect, is in part through inhibition of Akt phosphorylation as needed for cell cycle arrest during tumor growth. These findings provide a novel mechanistic insight into the inhibitory effects of lycopene on the growth of human colorectal cancer. The normal range of human serum lycopene level is 0.1 – 2 μM. However, 10 μM of human serum lycopene level could be reached by dietary supplementation. Here we only showed the *in vitro* effects of lycopene on human colon cancer HT-29 cell lines, our results are also consistent with the concept that consumption of tomato, in which lycopene is the major polyphenol, is associated with reduced risk of cancer development as evident from population studies and animal experiments showing suppression of tumor progression and cancer development. Thus it is suggested that lycopene could be a potent anti-cancer compound in chemopreventive and therapeutic applications in future preclinical studies.

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The authors have declared no conflict of interest.

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