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# LYCOPENE INDUCES APOPTOSIS BY INHIBITING NUCLEAR TRANSLOCATION OF β-CATENIN IN GASTRIC CANCER CELLS

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Reactive oxygen species (ROS) promote the development and progression of cancer by their effects on several signaling pathways. Lycopene, a major carotenoid natural product, is known to display antioxidant activity and to induce apoptosis of cancer cells. The aim of the present study was to investigate the mechanism by which lycopene induces apoptosis of the human gastric cancer AGS cells. In the present study, we showed that lycopene reduces the viability of AGS cells by inducing DNA fragmentation and increasing the Bax/Bcl-2 ratio. To determine the mechanistic basis for these effects, studies were conducted to assess the effects of this carotenoid on activation and nuclear translocation of β-catenin, and the expression of β-catenin target genes in AGS cells. The results showed that lycopene reduces the levels of ROS. It also inhibits activation of β-catenin signaling by changing the Wnt/β-catenin multi-protein complex such as a reduction in phosphorylation of glycogen synthase kinase 3β [GSK3β] and an increase in adenomatous polyposis coli [APC] and β-transducin repeats-containing proteins [β-TrCP]). It suppresses nuclear translocation of β-catenin and the expression of the β-catenin target survival genes c-myc and cyclin D1. Lycopene induces apoptosis by reducing ROS levels and suppressing β-catenin-c-myc/cyclin D1 axis. Thus, lycopene induces apoptosis of gastric cancer cells by disrupting nuclear translocation of β-catenin and expression of key cell survival genes.

Key words: *apoptosis, β-catenin, gastric cancer cells, lycopene, survival genes, reactive oxygen species, glycogen synthase kinase 3β, cyclin D1*

## INTRODUCTION

Gastric cancer is the most often diagnosed cancer in Korea (1). Although the incidence and mortality of gastric cancer has decreased, it is still the fourth most common type of cancer in the world and the second most common cancer in Asia (2). Yang *et al.* (3) performed a meta-analysis of epidemiologic studies to examine the possible association between tomato products consumption and gastric cancer. They systematically searched Medline and Embase to identify potential studies published from January 1966 to June 2012. They suggested that consumption of large amounts of tomato products is associated with a reduced risk of gastric cancer. Other epidemiological studies also show that consumption of tomato products and lycopene directly reduces the risk of gastric cancer (4-6). High serum levels of lycopene were significantly associated with reduced risk of developing gastric cancer (7).

It is known that cancer cells produce more reactive oxygen species (ROS) than do normal cells (8). Several studies have shown that ROS mediate transformations and tumorigenesis of human cancer cells through their effects on several signaling pathways such as janus kinase/signal transducers and activators of transcription (JAK/STAT) and the Wnt/β-catenin pathways (9-11). Therefore, substances that inhibit ROS production have the potential of inhibiting growth and inducing apoptosis of cancer cells. Recently, Cheng *et al.* (12) suggested that Wnt/β-catenin signaling pathway has potential value as a therapeutic target in the treatment of cancers.

Wnt/β-catenin signaling plays an important role in cell proliferation and cancer development (13). Misregulation of β-catenin signaling pathway is shown in gastric cancer (14). The adenomatous polyposis coli (APC) tumor suppressor is a negative regulator in β-catenin signaling pathway, and induces proteasomal degradation of β-catenin (15). APC interacts with β-catenin in a multi-protein complex containing β-transducin repeats-containing proteins (β-TrCP) to regulate the level of β-catenin (16). Phosphorylation of β-catenin by axis inhibition protein (AXIN) -glycogen synthase kinase 3β (GSK3β) complex targets β-catenin for ubiquitination-degradation. Phosphorylated β-catenin is specifically recognized by β-TrCP for proteosomal degradation. Therefore, β-TrCP plays a central role in recruiting phosphorylated β-catenin for degradation (17).

Because APC is part of the complex that induces destruction of β-catenin, loss of APC function leads to an accumulation of β-catenin in the cytosol and the nucleus (18). In its stable form, β-catenin accumulates in the nucleus where it binds to TCF. The β-catenin/TCF complex serves as a transcriptional regulator (19) to induce expression of cyclin D1 and c-myc, which is important for cancer cell survival and progression (20). Recent study showed that gonadotropin-releasing hormone (GnRH)-neuron-mediated network activity alters transcription of nuclear receptor subfamily 5 group by modulation of β-catenin in the female rat pituitary gland *in vivo* (21). Rybicka *et al.* (22) showed that macrophages have an impact on cancer stem-like cells increasing secretion of pro-angiogenic factors. Cancer stem-like cells, which were cocultured with macrophage, induced overexpression of genes involved in non-canonical Wnt pathway. This study suggest the role of Wnt/β-catenin signaling on cancer development.

Lycopene is a naturally synthesized carotenoid and an active component of tomatoes and other red fruits and vegetables such as grapefruits, red watermelon, and guava. It is a highly unsaturated acyclic isomer of β-carotene; its hydrocarbon chain contains 11 conjugated and 2 nonconjugated bonds (23). Due to its highly unsaturated acyclic structure, lycopene quenches singlet oxygen, scavenges free radicals, and prevents the oxidative damage of DNA, resulting in protecting transformation of normal cells to cancer cells (24, 25). Lycopene plays a potential anticancer activity against many types of cancers including prostate, pancreatic, breast and gastric cancer (26-28). The proposed anticancer mechanisms of lycopene are 1) modulation of gene functions, carcinogen-metabolizing enzymes, and apoptosis (24, 25, 29), 2) increasing gap junction communications, anti-proliferation, and antioxidant and anti-lipid peroxidation activities which are shown in oral cancer and precancerous lesions (24), and inhibition of G0/G1 and S-phase of the cell cycle (25, 30). Lycopene inhibited the growth of cancer cells by inactivation of β-catenin signaling in prostate cancer cells (31) and phosphoinositide 3-kinase/Akt survival signaling in colon cancer cells (32). Studies on gastric cancer models, lycopene reduced lipid peroxidation and oxidative injury by increasing reduced glutathione (GSH ) levels and the activities of GSH-dependent enzymes in gastric cancer rats (33, 34). ROS promote activation of Wnt/β-catenin signaling, which was shown in podocyte injury (35), renal arteriolar dysfunction in diabetic mice (36), and *Helicobacter pylori*-infected gastric epithelial cells (37). WNT/β-catenin signaling is upregulated in inflammation and oxidative stress and in many cancers (38). Therefore, reducing ROS by lycopene may inhibit Wnt/β-catenin signaling and oncogene expression, which may lead to apoptosis of gastric cancer cells.

The aim of the present study was to explore the mechanism by which lycopene induces apoptosis of the human gastric cancer AGS cells. For this purpose, the apoptotic effect of lycopene on growth of gastric cancer cells was first assessed by determining the apoptotic indices, including cell viability, DNA fragmentation, annexin V-positive cells, and Bax/Bcl-2 ratio, and the levels of ROS. Next, the effects of lycopene on activation of Wnt/β-catenin as well as the expression of β-catenin targeted c-myc and cyclin D1 genes were determined.

## MATERIALS AND METHODS

### *Cell lines and culture conditions*

The human gastric cancer cell line AGS (gastric adenocarcinoma, ATCC CRL 1739, Rockville, MD, USA) was purchased from the American Type Culture Collection (Rockville, MD, USA). Cells were grown in complete medium consisting of RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA). The cells were cultured at 37ºC under a humidified atmosphere consisting of 95% air and 5% $CO_2$.

### *Experimental protocol*

AGS cells ($5 \times 10^4$/mL) were incubated with lycopene (L9879, Sigma-Aldrich) dissolved in tetrahydrofuran (THF) (final concentration of 0.5, 1 or 2 μM) for 3 hours for the determination of ROS, and for 6 hours to determine the levels of Wnt/β-catenin multi-protein complex molecules (APC, β-TrCP, phospho- and total forms of GSK3β, and β-catenin). Following a 24-hour incubation period, cell viability, DNA fragmentation, the levels of Bax and Bcl-2, the ratio of Bax/Bcl-2, and expression of c-myc and cyclin D1 (both mRNA and protein) were analyzed in the cells treated with 0.5, 1 or 2 μM of lycopene. Non-treated cells (control) received THF (less than 0.3%) alone instead of lycopene.

For the determination of annexin V/PI-stained cells, 2 μM lycopene were treated to the cells for 24 hours. The levels of phospho- and total forms of Bcl-2 and Bax in the cells treated with 2 μM lycopene were measured at 0, 6, 12, and 24 h-culture. In separate experiment, the cells were treated with 2 μM lycopene for 6 hours for determining nuclear β-catenin using immunofluorescence staining.

### *Determination of cell viability*

The cells were incubated for 3 hours with MTT (thiazolyl blue; Sigma-Aldrich, St. Louis, MO, USA) dissolved in phosphate-buffered saline (PBS). The cells were lysed and viable cells were measured spectrophotometrically (37).

### *Annexin V - fluorescein isothiocyanate/propidium iodide staining*

AGS cells were seeded on glass cover slip coated with poly-L-lysine. Cells were treated with 2 μM lycopene and incubated for 24 hours. After incubation period, cells were washed with PBS, and stained with binding buffer containing 1% fluorescein isothiocyanate (FITC) annexin V and 1% propidium iodide (PI) solution. The cells were incubated, washed, and mounted. The cells were observed under fluorescence microscope using a dual filter set for FITC-annexin V (green) and PI (red) (39).

### *DNA fragmentation*

The degree of DNA fragmentation was assessed by determining the amount of oligonucleosome bound DNA contained within the cell extracts by using the sandwich ELISA assay (Cell Death Detection ELISA$^{PLUS}$ kit; Roche, Indianapolis, IN, USA) (40).

### *Measurement of intracellular reactive oxygen species levels*

AGS cells were treated with 10 μg/mL of 2',7'-dichlorofluorescein diacetate (DCF-DA; Sigma-Aldrich). Intensities of DCF were measured with a Victor5 multi-label counter (PerkinElmer, Boston, MA, USA) (35).

### *Real-time PCR analysis*

Cellular RNA was isolated by using the reagent TRI (Molecular Research Center, Inc., Cincinnati, OH, USA). The RNA was converted to cDNA by reverse transcription using a random hexamer as template, MuLV reverse transcriptase (Promega, Madison, WI, USA) as catalyst and the reaction conditions: 23ºC for 10 min, 37ºC for 60 min and 95ºC for 5 min (41). The cDNA was used for real-time PCR with specific primers for c-myc, cyclin D1, and β-actin. The primers used in this study are described in *Table 1*. The cDNA was amplified by using 45 repeat denaturation cycles at 95ºC for 30 s, annealing at 55ºC for 30 s and extension at 72ºC for 30 s. During the first cycle, the 95ºC step was extended to 3 min. The β-actin gene was amplified in the same manner to serve as the reference gene.

### *Western blot analysis*

Whole-cell extracts were prepared by the method previously described (41). Aliquots from whole-cell extracts were loaded onto 7 – 10% SDS polyacrylamide gels (20 – 40 μg protein/lane) and subjected to electrophoresis under reducing conditions. The isolated proteins were transferred onto nitrocellulose membranes.

The transfer of protein was verified using reversible staining with Ponceau S. The nitrocellulose membranes were blocked using 3% non-fat dry milk in TBS-T (Tris-buffered saline and 0.2% Tween 20). The proteins were detected using antibodies for β-catenin (610154, BD Transduction Laboratories, Franklin Lakes, NJ, USA), APC (sc-896, Santa Cruz Biotechnology, Dallas, TX, USA), β-TrCP (sc-8862, Santa Cruz Biotechnology), p-GSK3β (#5558, Cell Signaling Technology), GSK3β (sc-7291, Santa Cruz Biotechnology), Bcl-2 (sc-492, Santa Cruz Biotechnology), phospho-Bcl-2 (#2827, Cell Signaling Techmology), Bax (sc-526,

*Table 1.* Primers for RT-PCR.

| Target gene | Primer | Sequence (5'-3') |
|---|---|---|
| c-myc | forward primer | GGACGACGAGACCTTCATCAA |
| | reverse primer | CCAGCTTCTCTGAGACGAGCTT |
| cyclin D1 | forward primer | ACAAACAGATCATCCGCAAACAC |
| | reverse primer | TGTTGGGGCTCCTCAGGTTC |
| β-actin | forward primer | ACCAACTGGGACGACATGGAG |
| | reverse primer | GTGAGGATCTTCATGAGGTAGTC |

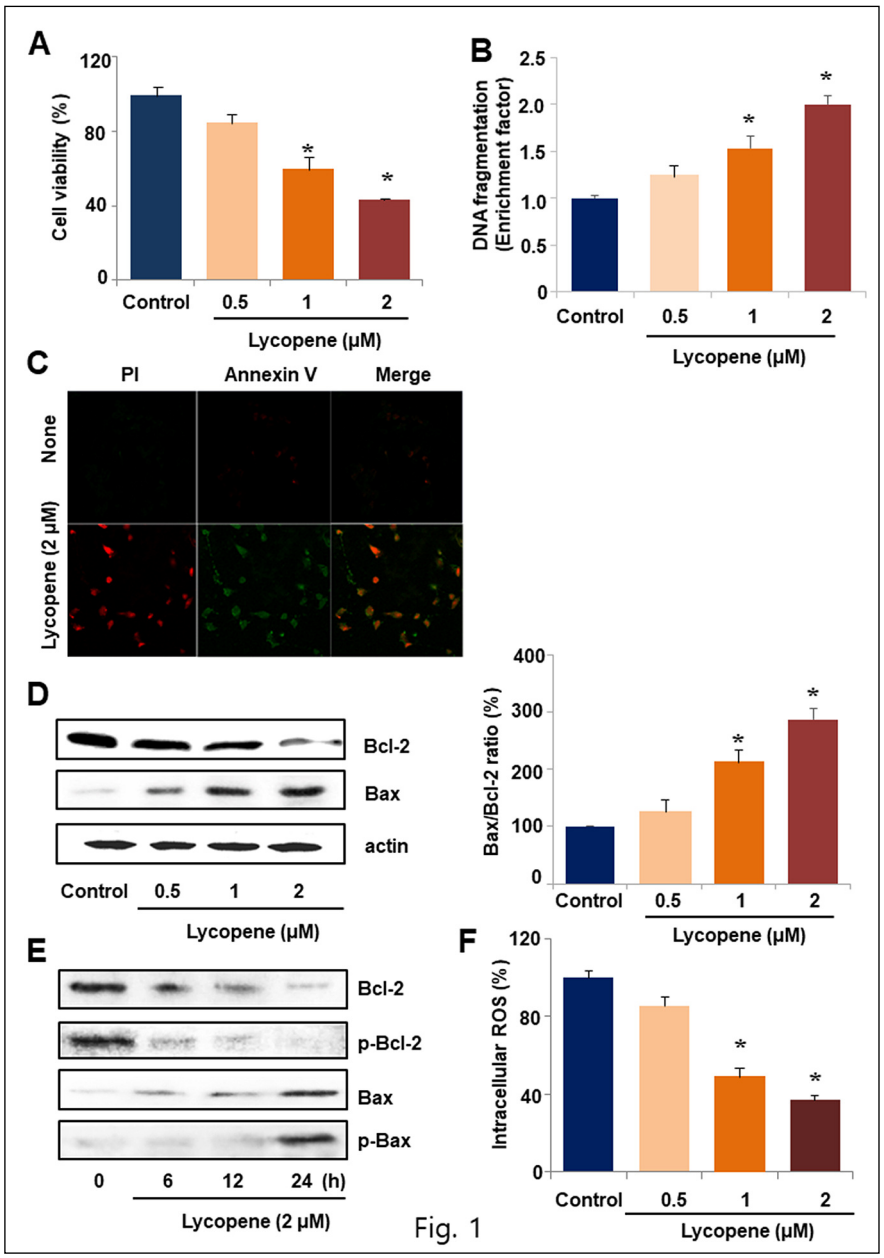


Fig. 1

*Fig. 1.* The effect of lycopene on cell viability, annexin V/PI-stained cells, DNA fragmentation, protein levels of phospho- and total forms of Bcl-2 and Bax, and intracellular ROS levels in AGS cells. Cells were treated with the indicated concentrations of lycopene for 24 h for determining cell viability, annexin V/PI-stained cells, DNA fragmentation, protein levels of phospho- and total forms of Bcl-2 and Bax and the Bax/Bcl-2 ratios, and for 3 h for measurements of intracellular ROS levels. (A) Cell viability measured by MTT assay. *P < 0.05 versus the corresponding Control. Control corresponds to the untreated cells; 0.5, 1 and 2 correspond to the cells treated with 0.5, 1, and 2 μM lycopene, respectively. (B) DNA fragmentation determined by the amount of oligonucleosome-bound DNA. *P < 0.05 versus Control. (C) Fluorescent microscopic image of annexin V-FITC/PI staining. AGS cells were stained with annexin V and PI after incubation with 2 μM lycopene for 24 h, and examined under fluorescence microscope. (D) Levels of Bcl-2 and Bax in whole-cell extracts determined by Western blot analysis. The ratio of Bax/Bcl-2 determined from the Bax and Bcl-2 protein band densities. *P < 0.05 versus Control. The Bax/Bcl-2 ratio for Control was set at 100. (E) Levels of phospho- and total forms of Bcl-2 and Bax determined by Western blot analysis. (F) Intracellular ROS level measured by DCF-DA fluorescent dye. *P < 0.05 versus. Control.

Santa Cruz Biotechnology), phospho-Bax (ab111391, Abcam, Cambridge, MA, USA), c-myc (sc-40, Santa Cruz Biotechnology), cyclin D1 (sc8396, Santa Cruz Biotechnology), and actin (sc-1615, Santa Cruz Biotechnology) in TBS-T solutions containing 3% dry milk and incubating overnight at 4ºC. After washing the membranes with TBS-T, the primary antibodies were detected using horseradish peroxidase-conjugated secondary antibodies (anti-mouse, anti-rabbit, anti-goat), and visualized by exposure to BioMax MR film (Kodak, Rochester, NY, USA) using an enhanced chemiluminescence detection system (Santa Cruz Biotechnology). Actin served as a loading control. Bax/Bcl-2 ratios were determined using the densities of the protein-bands for Bax and Bcl-2. The values obtained from four different experiments are expressed as mean ± S.E.

*Immunofluorescence staining for β-catenin*

ASG cells ($3 \times 10^4$ cells) seeded on a slide glass (a 6-well plate with Lab-Tek chamber slide) were treated with 2 μM lycopene for 6 hours. β-catenin was detected using confocal microscopy. For this purpose, the cells were fixed with cold methanol and then blocked by treating with blocking solution (1% BSA, 0.1% gelatin) for 1 hour, and then incubated with β-catenin antibody for 1 hour. After washing with PBS, the cells were treated with FITC-conjugated mouse anti-goat IgG antibody (Santa Cruz Biotechnology) for 1 hour. Five μg/mL of 4',6-diamidino-2-phenylindole (DAPI) in blocking solution was added (for nucleus detection) before subjecting the cells to laser scanning confocal microscope analysis (Zeiss LSM510, Carl Zeiss AG Corporate, Oberkochen, Germany).

*Statistical analysis*

Statistical analysis was performed using one-way ANOVA, followed by Newman-Keul's *post hoc* test. All data are reported as the mean ± S.E. of four independent experiments. A P-value of 0.05 or less was considered statistically significant.

## RESULTS

*Lycopene induces apoptosis and reduces reactive oxygen species in AGS cells*

Cell viabilities were measured using the MTT assay to determine the effect of lycopene on the growth of AGS cells. As shown in *Fig. 1A*, the viability of cells treated with lycopene decreases in a dose-dependent manner in contrast to those not exposed to lycopene. To assess the apoptotic effect of lycopene, DNA fragmentation was probed by measuring the amount of nucleosome-bound DNA in lycopene-treated versus untreated cells. *Fig. 1B* demonstrates that the degree of DNA fragmentation increases in response to lycopene in a dose-dependent manner. To further assess whether the decrease in cell viability is due to apoptotic effect of lycopene, AGS cells were stained with annexin V and PI after incubation with 2 μM lycopene for 24 hours, and examined under fluorescence microscope. Annexin V-FITC stain binds to phosphatidylserine (PS) exposed on the membrane of apoptotic cells. PI stain binds to the damaged nucleus of cells. Live cells stain weak for both annexin V and PI. Fluorescent image in *Fig. 1C* shows that lycopene increased annexin V and PI-stained cells. The results indicate that lycopene induced apoptosis of the cells. Lycopene also induced a dose-dependent increase in the level of pro-apoptotic Bax and a decrease in the level of anti-apoptotic Bcl-2 (*Fig. 1D*), which resulted in an increase in the Bax/Bcl-2 ratio (*Fig. 1E*). Lycopene (2 μM) increased levels of phospho- and total Bax, and decreased levels of phospho- and total Bcl-2 at 24 hours (*Fig. 1E*). Intracellular ROS levels of AGS cells reduced by lycopene treatment in a dose-dependent manner (*Fig. 1F*).

*Lycopene suppresses activation of Wnt/β-catenin signaling molecules and decreases nuclear β-catenin levels in AGS cells*

To explore the inhibitory effect of lycopene on activation of Wnt/β-catenin signaling molecules in AGS cells, levels of multi-protein complex molecules (APC, β-TrCP, phospho- and total GSK3β, β-catenin) were determined by using Western blotting. The results show that lycopene treatment causes an increase of APC and β-TrCP levels while it decreases the levels of p-GSK3β, and β-catenin (*Fig. 2A*). Total GSK3β was not changed by lycopene treatment (*Fig. 2A*). The effect of lycopene on the nuclear level of β-catenin was determined by using immunofluorescence staining. The results show that AGS cells treated with lycopene contain lower levels of β-catenin in their nuclei than non-treated cells (control) (*Fig. 2B*). This finding indicates that lycopene inhibits activation of Wnt/β-catenin signaling in AGS cells.

*Lycopene decreases expression of c-myc and cyclin D1 in AGS cells*

c-Myc and cyclin D1 are the proteins involved in cell proliferation and survival. To examine the effect of lycopene on the expression of the c-myc and cyclin D1 genes, AGS cells were treated with lycopene for 24 hours before determining the levels

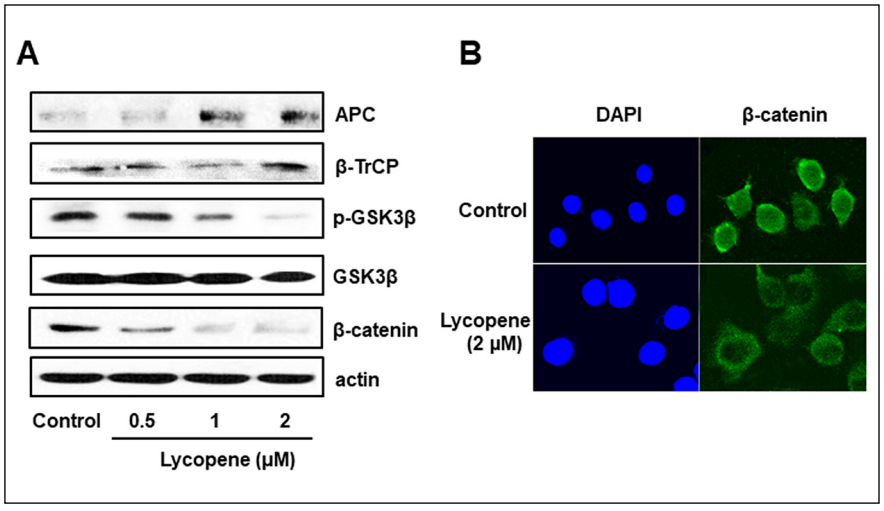


*Fig. 2.* Effects of lycopene on levels of Wnt/β-catenin signaling molecules and nuclear β-catenin in AGS cells. Cells were treated with the indicated concentrations of lycopene for 6 h. (A) Western blot analysis of APC, β-TrCP, phospho- and total GSK3β, and β-catenin present in untreated cells (column Control), and cells pre-treated with 0.5, 1 and 2 μM lycopene, respectively (columns 0.5, 1 and 2). (B) Confocal microscope images. Fixed cells were stained with β-catenin antibody to identify β-catenin, and with DAPI to locate the nucleus. The row of images labeled Control corresponds to untreated cells and that labeled Lycopene corresponds to the cells treated with 2 μM lycopene.

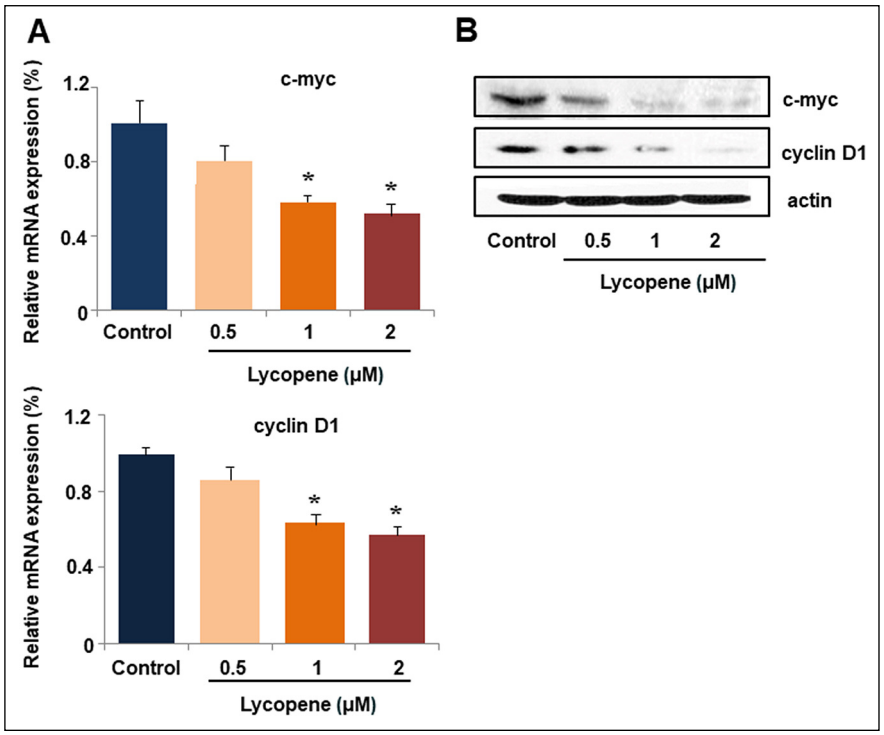


*Fig. 3.* Effects of lycopene on c-myc and cyclin D1 expression in AGS cells. Cells were treated with the indicated concentrations of lycopene for 24 h for assessing c-myc and cyclin D1 expression (A, B). (A) The relative levels of c-myc and cyclin D1 mRNA, determined by using real-time PCR analysis. *P < 0.05 versus Control. Column Control corresponds to untreated cells; columns 0.5, 1 and 2 correspond to the cells treated with 0.5, 1, and 2 μM lycopene, respectively. (B) Western blot detection of c-myc and cyclin D1.

of mRNA and protein. Cells treated with lycopene expressed lower levels of mRNAs as well as proteins (*Fig. 3A* and *3B*). The results demonstrate that lycopene induces apoptosis of AGS cells by suppressing expression of c-myc and cyclin D1.

## DISCUSSION

The abnormal activation of Wnt/β-catenin signal pathway is one of the critical mechanisms of human tumorigenesis. Overexpression of β-catenin is the main manifestation of the activation of this signal pathway (42). Several studies demonstrated that suppression of Wnt/β-catenin signaling pathway, by bioactive compounds curcumin and resveratrol, inhibited the growth of gastric cancer cells (43, 44). These studies suggested that abnormal activation of Wnt/β-catenin signaling is associated with gastric cancer development. Moreover, β-catenin target genes cyclin D1 and c-myc play important roles in cell proliferation and are correlated with the incidence of many tumors (45).

In the present study, we found that lycopene reduced cell viability in gastric cancer AGS cells. Moreover, lycopene induced apoptosis by increasing the Bax/Bcl-2 ratio and the level of DNA fragmentation and annexin V/PI-positive cells. In addition, lycopene increased the levels of APC and β-TrCP while it decreased total and active β-catenin levels and the nuclear β-catenin in AGS cells. In addition, lower level of nuclear β-catenin was shown in lycopene-treated cells. In general, levels of β-catenin are controlled by complex proteins including the tumor suppressor gene, APC. APC serves as a negative regulator of β-catenin/TCF transcriptional activity by inducing degradation of β-catenin in the cytoplasm. APC interacts with β-catenin in a multi-protein complex containing β-TrCP, which phosphorylates β-catenin for degradation (46, 47). Thus, a reduction in the levels of APC causes accumulation of β-catenin and consequent tumor growth.

Since lycopene increased the levels of APC and β-TrCP in the present study, we hypothesize that lycopene may induce phosphorylation and degradation of β-catenin, resulting in decreasing nuclear translocation of β-catenin in gastric cancer cells. This hypothesis was proven by the results showing that AGS cells treated with lycopene contain lower β-catenin in their nuclei than non-treated cells, determined by using immunofluorescence staining. The results showed that lycopene inhibits activation of Wnt/β-catenin signaling and nuclear translocation of β-catenin in gastric cancer AGS cells.

Here, we showed that lycopene down-regulated the β-catenin target genes c-myc and cyclin D1 in AGS cells. These observations clearly demonstrate that lycopene causes an increase in the levels of the β-catenin destruction complex including APC and β-TrCP and, as a result, it inhibits expression of c-myc and cyclin D1 in AGS cells. Therefore, lycopene induces apoptosis of gastric cancer cells by suppressing the Wnt/β-catenin signaling pathway.

The excess amounts of cellular ROS in cancer cells stimulate carcinogenesis-related signaling pathways, leading to progression of cancer (48). Among carcinogenesis-related signaling pathways, Wnt/β-catenin signaling is known to be associated with proliferation and oncogenesis of cancer cells including gastric cancer cells (49, 50). In present study, we demonstrated that lycopene reduces ROS levels. Since ROS activate Wnt/β-catenin signaling in various cells (35-38), lycopene may suppress Wnt/β-catenin signaling by reducing ROS in gastric cancer cells. Further studies are needed to determine the precise mechanism how antioxidants modify activation of Wnt/β-catenin signaling molecules.

In the present study, we found that lycopene showed potent effect at a concentration of 2 μM for reducing ROS, cell viability, and expression of c-myc and cyclin D1, and for increasing DNA fragmentation and the Bax/Bcl-2 ratio, and for changing the Wnt/β-catenin multi-protein complex (such as a reduction in β-catenin and phosphorylation of GSK3β, and an increase in APC and β-TrCP) in AGS cells. Thus, we used 2 μM of lycopene for the studies on annexin V/PI-stained cells, the levels of phospho- and total forms of Bax, and nuclear β-catenin in AGS cells. We also demonstrated that 2 μM of lycopene significantly reduced annexin V/PI-stained

cells, the levels of phospho- and total forms of Bax as well as nuclear localization of β-catenin in AGS cells. Palozza *et al.* (51) showed that lycopene (2 μM) inhibited cell growth by arresting cell cycle progression and by promoting apoptosis in immortalized fibroblasts exposed to cigarette smoke condensate. Salman *et al.* (26) reported that lycopene (2 μM) induces apoptosis of prostate, lung, and digestive tract cancer cells. Therefore, 2 μM of lycopene can be used for further studies to investigate anti-cancer mechanism of lycopene in various cancer cells.

In the present study, statistical analysis was performed using one-way ANOVA, followed by Newman-Keul's *post hoc* test. To fully understand group differences in an ANOVA, researchers must conduct tests of the differences between particular pairs of experimental group and control group. The most commonly used multiple comparison analysis statistics include Tukey, Newman-Keuls, Scheffee, Bonferroni and Dunnett. However, the Newman Keuls statistic is appropriate for studies in which even very small differences are important to find (52). Thus, we used Newman-Keul's *post hoc* test in the present study.

The limitation of the present study is that we only used one gastric cancer cell line. Further study should be performed using more gastric cancer cell lines to determine the inhibitory effect of lycopene on activation of Wnt/β-catenin signaling and cell survival in gastric cancer cells.

In conclusion, lycopene-induced apoptosis is accompanied by an increase in DNA fragmentation and a decrease in the ratio of anti-apoptotic protein Bcl-2 to pro-apoptotic protein Bax in gastric cancer cells. Lycopene inhibits activation of the β-catenin signalling, which down-regulates the expression of β-catenin targeted survival genes c-myc and cyclin D1 genes in gastric cancer cells. Taken together, lycopene induces apoptosis of gastric cancer cells by inhibiting Wnt/β-catenin signalling, disrupting nuclear translocation of β-catenin, and suppressing expression of key cell survival genes.

*List of abbreviations:* APC, adenomatous polyposis coli; DCF-DA, dichlorofluorescein diacetate; FITC, fluorescein isothiocyanate; GSK3β, glycogen synthase kinase 3beta; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide; ROS, reactive oxygen species; β-TrCP, beta-transducin repeats-containing proteins.

*Author' contributing:* Hyeyoung Kim (H. Kim) conceived and designed the experiments; Joo Weon Lim (J.W. Lim) assisted in experimental design; Mijung Kim (M. Kim) and Suhn Hyung Kim (S.H. Kim) performed the experiments; H. Kim and J.W. Lim analyzed the data; M. Kim wrote the paper; H. Kim reviewed and edited the paper. All authors agree with the edited version.

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REFERENCES

1. Shin A, Kim J, Park S. Gastric cancer epidemiology in Korea. J. *Gastric Cancer* 2011; 11: 135-140.
2. Global Cancer Observatory. International Agency for Research on Cancer, World Health Organization. http://globocan.iarc.fr/
3. Yang T, Yang X, Wang X, Wang Y, Dong Z. The role of tomato products and lycopene in the prevention of gastric cancer: a meta-analysis of epidemiologic studies. *Med Hypotheses* 2013; 80: 383-388.
4. Nouraie M, Pietinen P, Kamangar F, *et al.* Fruits, vegetables, and antioxidants and risk of gastric cancer among male smokers. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 2087-2092.
5. De Stefani E, Correa P, Boffetta P, Deneo-Pellegrini H, Ronco AL, Mendilaharsu M. Dietary patterns and risk of gastric cancer: a case-control study in Uruguay. *Gastric Cancer* 2004; 7: 211-220.
6. Kim JH, Lee J, Choi IJ, *et al.* Dietary carotenoids intake and the risk of gastric cancer: a case-control study in Korea. *Nutrients* 2018; 10: E1031. doi: 10.3390/nu10081031
7. Yuan JM, Ross RK, Gao YT, Qu YH, Chu XD, Yu MC. Prediagnostic levels of serum micronutrients in relation to risk of gastric cancer in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2004; 13: 1772–1780.
8. Kumari S, Badana AK, G MM, G S, Malla R. Reactive oxygen species: a key constituent in cancer survival. *Biomark Insights* 2018; 13: 1177271918755391. doi: 10.1177/1177271918755391
9. Simon AR, Rai U, Fanburg BL, Cochran BH. Activation of the JAK-STAT pathway by reactive oxygen species. *Am J Physiol* 1998; 275: C1640-C1652.
10. Yu JH, Kim H. Role of Janus kinase/signal transducers and activators of transcription in the pathogenesis of pancreatitis and pancreatic cancer. *Gut Liver* 2012; 6: 417-422.
11. Zhang Z, Wang X, Cheng S, *et al.* Reactive oxygen species mediated arsenic induced cell transformation and tumorigenesis through Wnt/β-catenin pathway in human colorectal adenocarcinoma DLD1 cells. *Toxicol Appl Pharmacol* 2011; 256: 114-121.
12. Cheng X, Xu X, Chen D, Zhao F, Wang W. Therapeutic potential of targeting the Wnt/β-catenin signaling pathway in colorectal cancer. *Biomed Pharmacother* 2019; 110: 473-481.
13. Taciak B, Pruszynska I, Kiraga L, Bialasek M, Krol M. Wnt signaling pathway in development and cancer. *J Physiol Pharmacol* 2018; 69: 185-196.
14. Zhou YN, Xu CP, Han B, *et al.* Expression of E-cadherin and β-catenin in gastric carcinoma and its correlation with the clinicopathological features and patient survival. *World J Gastroenterol* 2002; 8: 987-993.
15. Katoh M. Multi-layered prevention and treatment of chronic inflammation, organ fibrosis and cancer associated with canonical WNT/β catenin signaling activation. *Int J Mol Med* 2018; 42: 713-725.
16. Munemitsu S, Albert I, Souza B, *et al.* Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Biochemistry* 1995; 92: 3046-3050.
17. Cha PH, Cho YH, Lee SK, *et al.* Small-molecule binding of the axin RGS domain promotes β-catenin and Ras degradation. *Nat Chem Biol* 2016; 12: 593-600.
18. Stamos JL, Weis WI. The β-catenin destruction complex. *Cold Spring Harb Perspect Biol* 2013; 5: a007898. doi: 10.1101/cshperspect.a007898
19. Plakis P, Hart M, Rubinfeld B. Defects in the regulation of β-catenin in colorectal cancer. *Adv Exp Med Biol* 1999; 470: 23-32.
20. Bondi J, Bukholm G, Nesland JM, Bukholm IR. Expression of non-membranous β-catenin and γ-catenin, c-Myc and cyclin D1 in relation to patient outcome in human colon adenocarcinomas. *APMIS* 2004; 112: 49-56.
21. Zielinska-Gorska M, Gorski K, Biernacka K, Sawosy E, Kaminska T, Gajewska A. Gonadotrpin-releasing hormone and kisspeptin-10 regulate nuclear receptor subfamily 5 group A member/catenin-beta/nuclear receptor subfamily group B member activity in female rat anterior pituitary gland. *J Physiol Pharmacol* 2018; 69: 463-474.

22. Rybicka A, Eyileten C, Taciak B, *et al.* Tumour-associated macrophages influence canine mammary cancer stem-like cells enhancing their pro-angiogenic properties. *J Physiol Pharmacol* 2016; 67: 491-500.
23. Olson JA, Krinsky NI. Introduction: the colorful, fascinating world of the carotenoids: important physiologic modulators. *FASEB J* 1995; 9: 1547-1550.
24. Lu R, Dan H, Wu R, *et al.* Lycopene: features and potential significance in the oral cancer and precancerous lesions. *J Oral Pathol Med* 2011; 40: 361-368.
25. van Breemen RB, Pajkovic N. Multitargeted therapy of cancer by lycopene. *Cancer Lett* 2008; 269: 339-351.
26. Salman H, Bergman M, Djaldetti M, Bessler H. Lycopene affects proliferation and apoptosis of four malignant cell lines. *Biomed Pharmacother* 2007; 61: 366-369.
27. Teodoro AJ, Oliveira FL, Martins NB, Maia Gde A, Martucci RB, Borojevic R. Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. *Cancer Cell Int* 2012; 12: 36. doi: 10.1186/1475-2867-12-36
28. Assar EA, Vidalle MC, Chopra M, Hafizi S. Lycopene acts through inhibition of IκB kinase to suppress NF-κB signaling in human prostate and breast cancer cells. *Tumour Biol* 2016; 37: 9375-9385.
29. Shanbhag VK. Lycopene in cancer therapy. *J Pharm Bioallied Sci* 2016; 8: 170-171.
30. Russo M, Spagnuolo C, Tedesco I, Russo GL. Phytochemicals in cancer prevention and therapy: truth or dare? *Toxins* (*Basel*) 2010; 2: 517-551.
31. Wertz K. Lycopene effects contributing to prostate health. *Nutr Cancer* 2009; 61: 775-783.
32. Tang FY, Shih CJ, Cheng LH, Ho HJ, Chen HJ. Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. *Mol Nutr Food Res* 2008; 52: 646-654.
33. Velmurugan B, Bhuvaneswari V, Nagini S. Antiperoxidative effects of lycopene during N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis. *Fitoterapia* 2002; 73: 604-611.
34. Luo C, Wu XG. Lycopene enhances antioxidant enzyme activities and immunity function in N-methyl-N'-nitro-N-nitrosoguanidine-enduced gastric cancer rats. *Int J Mol Sci* 2011; 12: 3340-3351.
35. Zhou L, Chen X, Lu M, *et al.* Wnt/β-catenin links oxidative stress to podocyte injury and proteinuria. *Kidney Int* 2019; 95: 830-845.
36. Zhang S, Huang Q, Wang Q, *et al.* Enhanced renal afferent arteriolar reactive oxygen species and contractility to endothelin-1 are associated with canonical Wnt signaling in diabetic mice. *Kidney Blood Press Res* 2018; 43: 860-871.
37. Park B, Lim JW, Kim H. Lycopene treatment inhibits activation of Jak1/Stat3 and Wnt/β-catenin signaling and attenuates hyperproliferation in gastric epithelial cells. *Nutr Res* 2018; Jul 21: S0271-S5317. doi: 10.1016/j.nutres.2018.07.010
38. Vallee A, Lecarpentier Y. Crosstalk between peroxisome proliferator-activated receptor gamma and the canonical WNT/β-catenin pathway in chronic inflammation and oxidative stress during carcinogenesis. *Front Immunol* 2018, 9: 745. doi: 10.3389/fimmu.2018.00745
39. Pagliara P, Scarano A, Barca A, *et al.* Ostreopsis cf. ovata induces cytoskeletal disorganization, apoptosis, and gene expression disregulation on HeLa cells. *J Appl Phycol* 2015; 27: 2321-2332.
40. Park M, Lim JW, Kim H. Docoxahexaenoic acid induces apoptosis of pancreatic cancer cells by suppressing activation of STAT3 and NF-κB. *Nutrients* 2018; 10: E1621. doi: 10.3390/nu10111621
41. Lim S, Hwang S, Yu JH, Lim JW, Kim H. Lycopene inhibits regulator of calcineurin 1-mediated apoptosis by reducing oxidative stress and down-regulating Nucling in neuronal cells. *Mol Nutr Food Res* 2017; 61: doi: 10.1002/mnfr.201600530
42. Maruyama K, Ochiai A, Akimoto S, *et al.* Cytoplasmic beta-catenin accumulation as a predictor of hematogenous metastasis in human colorectal cancer. *Oncology* 2000; 59: 302-309.
43. Zheng R, Deng Q, Liu Y, Zhao P. Curcumin inhibits gastric carcinoma cell growth and induces apoptosis by suppressing the Wnt/β-catenin signaling pathway. *Med Sci Monit* 2017; 23: 163-171.
44. Dai H, Deng HB, Wang YH, Guo JJ. Resveratrol inhibits the growth of gastric cancer via the Wnt/β-catenin pathway. *Oncol Lett* 2018; 16: 1579-1583.
45. Polakis P. Wnt signaling and cancer. *Genes Dev* 2000; 14: 1837-1851.
46. Nunez F, Bravo S, Cruzat F, Montecino M, De Ferrari GV. Wnt/β-catenin signaling enhances cyclooxygenase-2 (COX2) transcriptional activity in gastric cancer cells. *PloS One* 2011; 6: 18562. doi: 10.1371/journal.pone.0018562
47. Su Y, Fu C, Ishikawa S, *et al.* APC is essential for targeting phosphorylated beta-catenin to the SCFbeta-TrCP ubiquitin ligase. *Mol Cell* 2008; 32: 652-661.
48. Gao X, Schoettker B. Reduction-oxidation pathways involved in cancer development: a systematic review of literature reviews. *Oncotarget* 2017; 8: 51888-51906.
49. Jeong WJ, Yoon J, Park JC, *et al.* Ras stabilization through aberrant activation of Wnt/β-catenin signaling promotes intestinal tumorigenesis. *Sci Signal* 2012; 5: ra30. doi: 10.1126/scisignal.2002242
50. Mao J, Fan S, Ma W, *et al.* Roles of Wnt/β-catenin signaling in the gastric cancer stem cells proliferation and salinomycin treatment. *Cell Death Dis* 2014; 5: e1039. doi: 10.1038/cddis.2013.515
51. Palozza P, Sheriff A, Serini S, *et al.* Lycopene induces apoptosis in immortalized fibroblasts exposed to tobacco smoke condensate through arresting cell cycle and downregulating cyclin D1, pAKT and pBad. *Apoptosis* 2005, 10: 1445-1456.
52. McHugh ML. Multiple comparison analysis testing in ANOVA. *Biochem Med* (*Zagreb*) 2011; 21: 203-209.

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