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Cancer Chemopreventive Effects of Lycopene: Suppression of MMP-7 Expression and Cell Invasion in Human Colon Cancer Cells

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ABSTRACT: Clinical studies indicate that high blood levels of leptin or matrix metalloproteinase-7 (MMP-7; matrilysin) proteins are associated with tumor progression of human colorectal cancer (CRC). Leptin could play an important role in cell migration and invasion of cancer cells. Our previous study indicated that lycopene could inhibit the proliferation of human colon cancer cells *in vitro*. However, the inhibitory effects of lycopene on the progression of human colon cancer cells have not been demonstrated yet. In this study, we investigated the inhibitory effects of lycopene on tumor progression including cell invasion and MMP-7 expression in leptin-stimulated human colon cancer cells *in vitro*. Our results demonstrated that lycopene significantly inhibited leptin-mediated cell invasion and MMP-7 expression in human colon cancer HT-29 cells. Lycopene could augment the expression and stability of E-cadherin proteins. Our results showed that MAPK/ERK and PI3K/Akt signaling pathways played important roles in leptin-mediated MMP-7 expression and cell invasion. Lycopene could effectively inhibit the phosphorylation of Akt, glycogen synthase kinase-3β (GSK-3β) and ERK 1/2 proteins. The molecular mechanisms of lycopene were in part through decreases in nuclear levels of AP-1 and β-catenin proteins. These novel findings suggested that lycopene could act as a chemopreventive agent to suppress MMP-7 expression and leptin-mediated cell invasion in human colon cancer HT-29 cells.

KEYWORDS: lycopene, leptin, MMP-7, invasion, AP-1, β-catenin, human colon cancer cells

1. INTRODUCTION

Colorectal cancer (CRC) is one of the most important causes of cancer mortality in many countries. In the United States, at least thousands of cancer deaths are attributed to this cancer every year.[1] Colon carcinogenesis is featured with aberrant regulation of tumor growth and progression. Acquisition of several tumor-associated mutations including aberrant activation of k-*ras* and Phosphatidylinositol 3-kinase (PI3K) could lead to genomic instability.[2,3] It is well-known that PI3K protein and its downstream target molecules such as Akt protein could regulate cell-cycle progression, migration, invasion and proliferation of cancer cells.[4] The mitogen activated protein kinase (MAPK)/ERK signaling cascades activate AP-1 (c-fos and c-jun proteins) transcription factors and regulate expression of several important genes involved in cell proliferation, migration and invasion. Therefore, oncogenic pathways such as PI3K/Akt and MAPK/ERK signaling cascades play important roles in the development of CRC.[3]

Previous study suggests that MMP-7 proteins are mainly expressed in colon cancer cells and strongly associated with tumor progression.[5,6] A high plasma level of leptin was correlated with tumor progression in colon cancer patients.[7−9] Studies suggest that leptin could play crucial roles in different clinical stages of CRC.[8,10] Leptin is a bioactive polypeptide that is secreted from mature adipocytes and epithelial cells, circulates as a 16 kDa protein partially interacting with plasma proteins, and contributes its actions through specific receptors (ObR) by different mechanisms.[11,12] In physiological status, leptin controls the balance of normal body weight through the regulation of food intake and other biological activities.[13] In pathological circumstances, leptin is identified as a mitogen and associated with the development of intestinal malignancy.[7] Leptin could also induce tumor angiogenesis and tumor progression of several types of cancer.[14,15] Overexpression of leptin and ObR has been demonstrated in several types of human cancer including colorectal cancer.[8,10,16] Previous studies indicated that leptin significantly activated the PI3K/Akt signaling pathway in several types of human cancer cells.[17−20] Leptin also mediated cell migration and invasion through activation of PI3K and Src kinase pathways in metastatic colon cancer LS174T and HM7 cells.[21] Invasion of colon cancer cells into the surrounding microenvironment occurs through the activation of several matrix metalloproteinases (MMPs).[22] MMPs degrade extracellular matrix and create a microenvironment supporting maintenance of tumor angiogenesis, growth and metastasis.[22] Studies demonstrated that expression of MMP genes including MMP-2, MMP-7 and MMP-9 is modulated by extracellular growth factors and proinflammatory cytokines in cancer cells.[22−24] Clinical studies suggested that increase in expression of MMP-7 is associated with cancer recurrence, poor prognosis and low survival rate in colorectal cancer patients.[25] Studies demonstrated that increases in nuclear levels of AP-1 and β-catenin transcription factors could modulate the expression of *MMP-7*, *c-Myc*, and *cyclin D1* genes.[4,22] A correlation between β-catenin and MMP-7 overexpression was also observed in several types of cancer.[4,26] Moreover, activation of the PI3K signaling pathways could augment nuclear levels of β-catenin proteins through inactivation of GSK-3β activity and reduction of ubiquitin-dependent degradation of β-catenin.[27] Therefore, activation of PI3K/Akt cascades could lead to the translocalization of β-catenin from cytoplasm to nucleus where β-catenin functions as a coactivator

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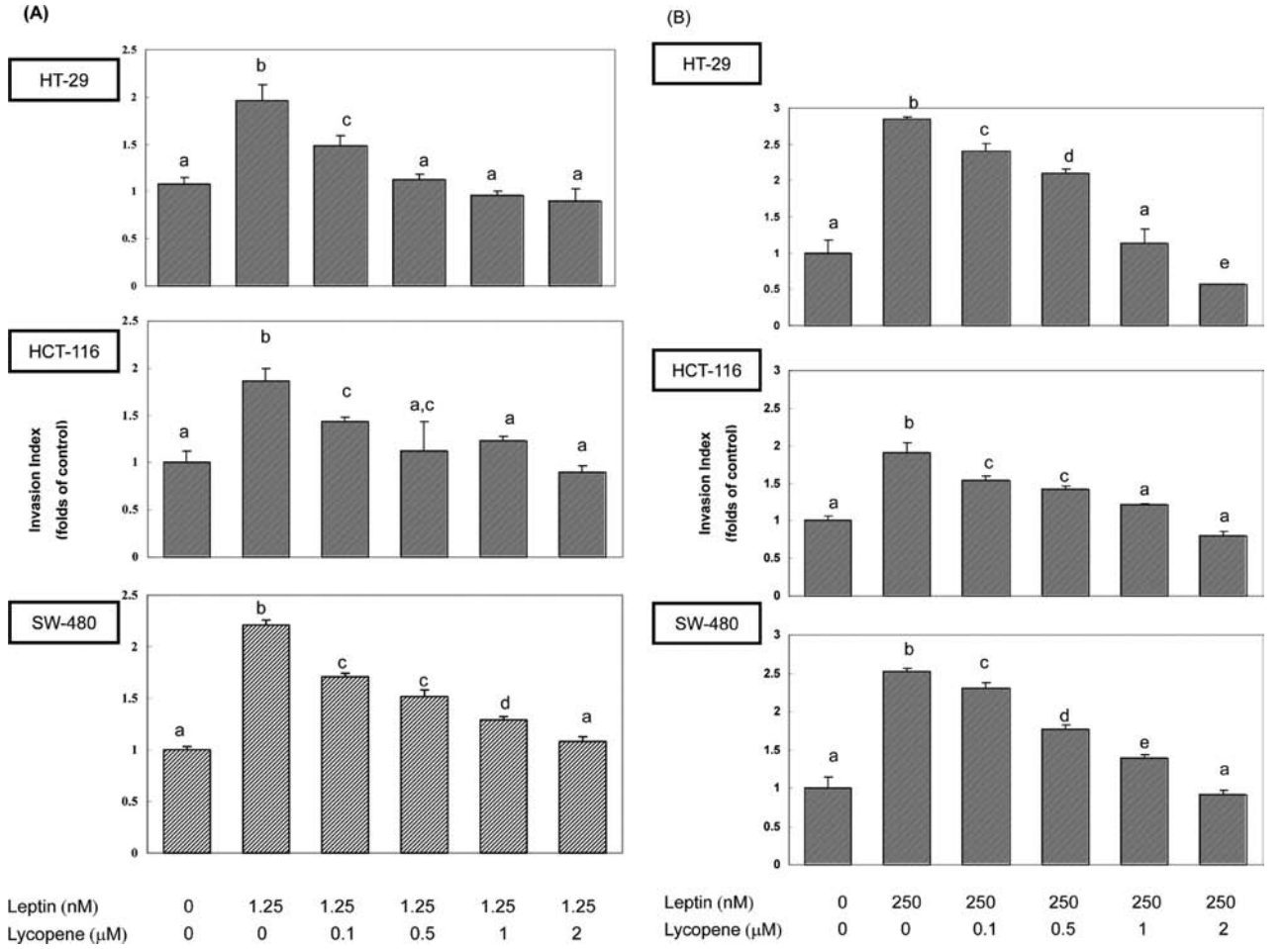


Figure 1. Lycopene inhibited leptin-mediated cell invasion in human colon cancer cells. The invasion of tumor cells was analyzed in Transwell Boyden chambers with a polyvinyl/pyrrolidone-free polycarbonate filter of 8 μm pore size. Each filter was coated with 50 μL of a 1:5 diluted Matrigel in cold McCoy's medium to form a thin continuous layer on the top of the filter. Confluent human colon cancer cells (HT-29, SW-480 and HCT-116) were cultured in McCoy's medium with 10% fetal bovine serum at 37 °C. After washing out the medium, colon cancer cells were trypsinized, prestained with calcein AM and transferred to Matrigel coated Transwell Boyden chambers. Human colon cancer cells (5,000 cells/well) were added to each of triplicate wells in McCoy's medium containing leptin at concentrations of either 1.25 nM (A) or 250 nM (B) in the presence or absence of various concentrations of lycopene (0, 0.1, 0.5, 1, and 2 μM). After incubation for 18 h, cells were counted as described above, and the number of cells invading the lower side of the filter was measured as invasive activity. Figures represent the level of invasion index. Different letters represent statistically significant difference, $p < 0.05$. The data shown are representative of three independent experiments.

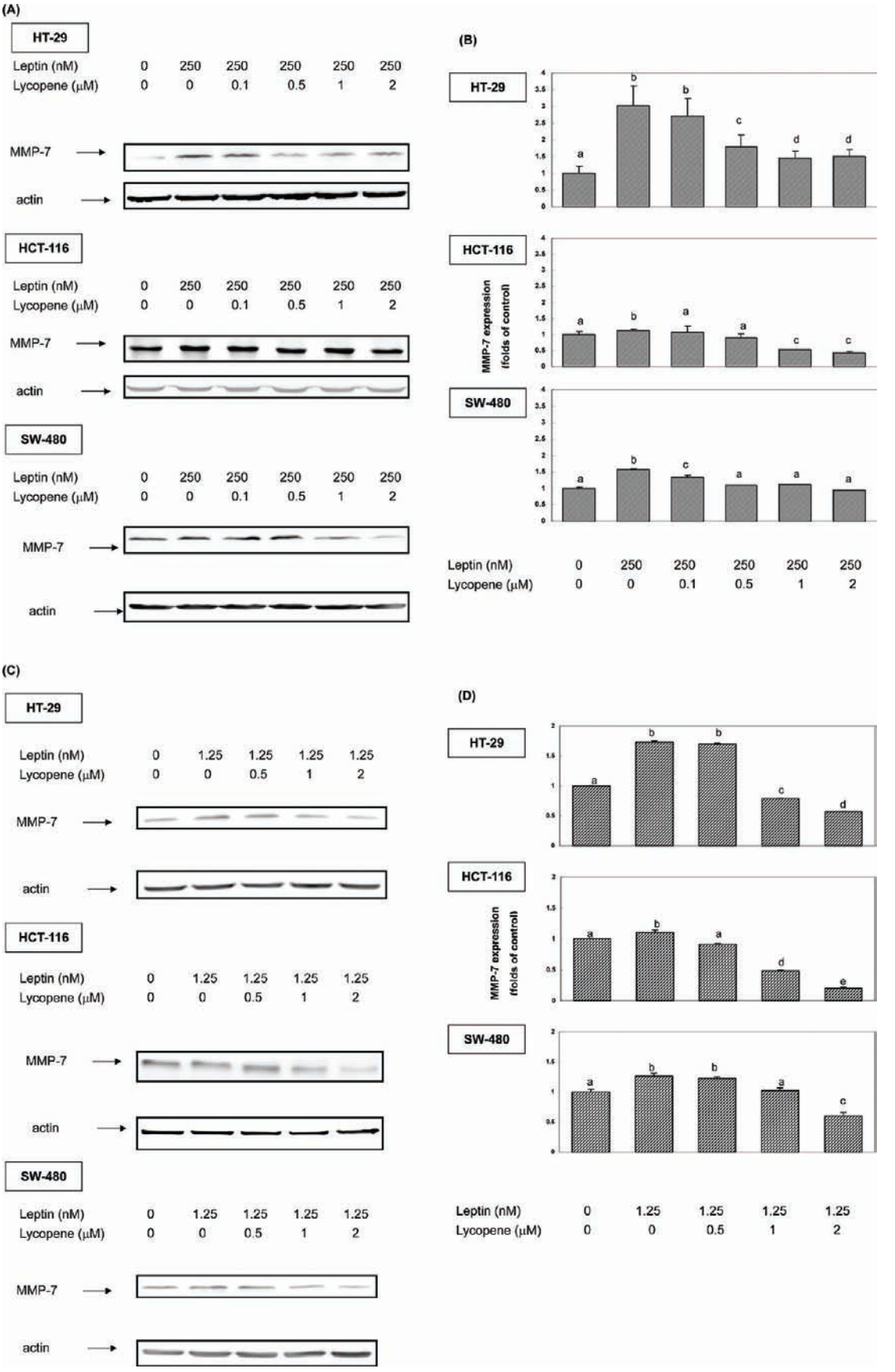
for transcription factors such as T-cell factor/lymphoid enhancing factor (TCF/LEF) family.[27]

Epidemiologic studies reported significant inverse correlations between tomato consumption and colon cancer risk for men and women.[28−32] Lycopene, a major component in tomato, exhibited potential anticarcinogenesis activity in many types of cancer.[33−35] Our previous study demonstrated that lycopene could inhibit PI3K/Akt signaling pathways and have anticancer effects on human colon cancer HT-29 cells.[36,37] However, the chemopreventive effects of lycopene on MMP-7 expression and leptin-mediated cell invasion in human colon cancer cells have not been demonstrated yet. In this study, we examined the molecular mechanisms of lycopene during tumor progression including increased expression of MMP-7 and cell invasion in human colon cancer cells.

2. MATERIALS AND METHODS

Reagents and Antibodies. Antiphosphorylation GSK-3β, antiphosphorylation β-catenin, antiphosphorylation ERK 1/2, antiphosphorylation Akt, antiphosphorylation p38, antiphosphorylation c-Jun, anti-MMP-7, anti-β-catenin, anti-c-Fos, anti-c-Myc and anti-lamin A/C antibodies were purchased from Cell signaling Technology Inc. (Danvers, MA). Anti-ERK1/2, anti-p38, anti-Akt and anti-GSK-3β antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). McCoy's medium and calcein AM were purchased from Invitrogen Inc. (Carlsbad, CA). Anti-β-actin antibody, AG 490 (JAK inhibitor), SP600125 (JNK inhibitor), PD098059 (MEK inhibitor), Wortmannin (PI3K inhibitor), SB 203580 (p38 inhibitor) and Ipegal CA-630 were purchased from Sigma (St. Louis, MO). Human leptin recombinant protein was purchased from R & D Systems, Inc. (Minneapolis, MN). Human colon cancer HT-29, SW-480 and HCT-116 cell lines were purchased from American Type Culture Collection (Walkersville, MD). NE-PER Nuclear and Cytoplasmic Extract reagent Kit was purchased from Pierce Biotechnology (Rockford, IL).

Cell Culture. Briefly, human 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 media. Medium was supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine and 1.5 g/L sodium bicarbonate. Cells used in different experiments have similar passage numbers.



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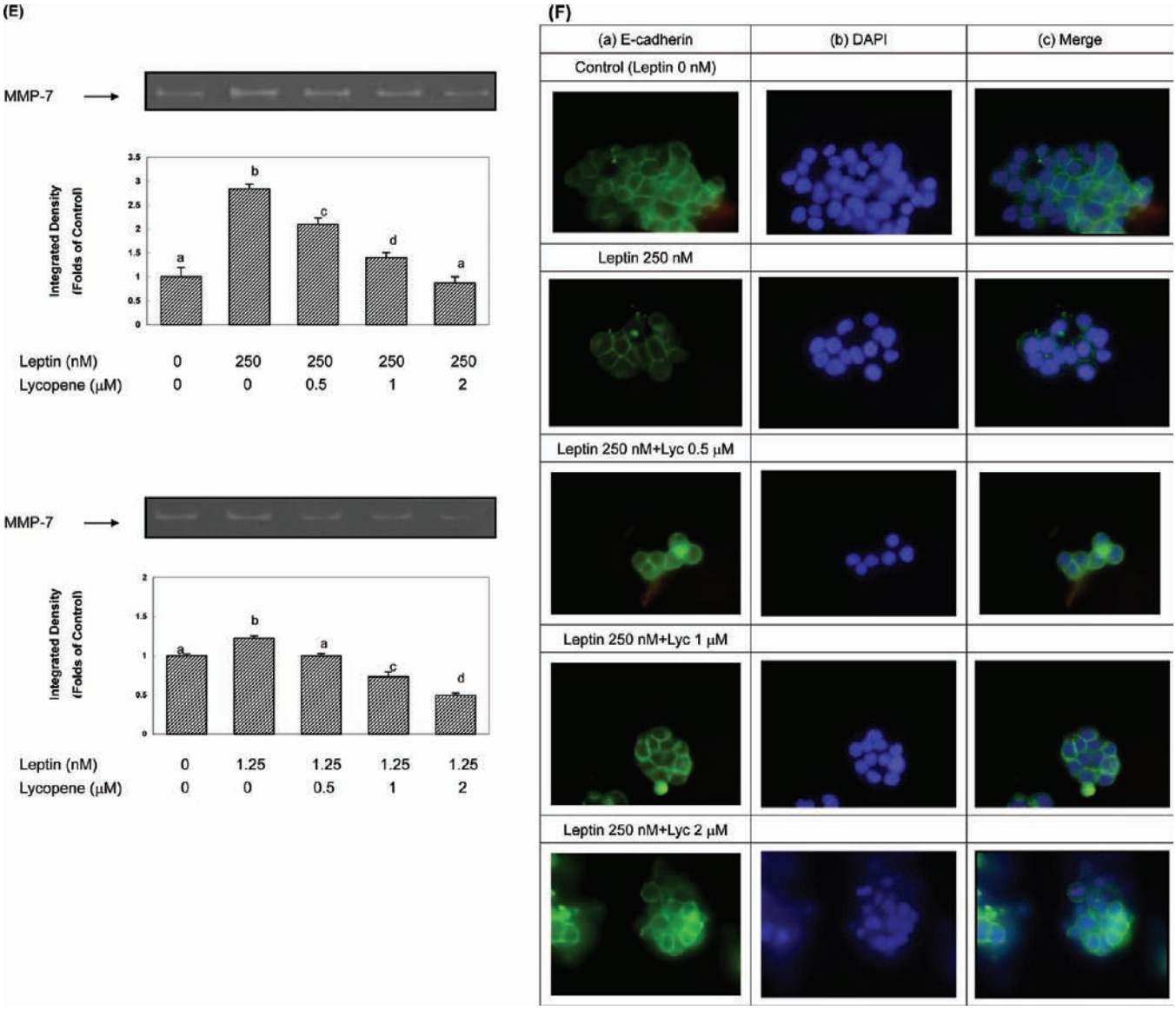

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Preparation of Cell Lysates. Human colon cancer cells were cultured in cell culture media in the presence or absence of lycopene at different concentrations. At the end of experiment, human colon cancer cells were washed with phosphate buffered saline (PBS) twice and then lysed in ice-cold lysis buffer. Lysis buffer contains 1× PBS, 1% Ipegal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS) with 100 μM of phenylmethylsulfonyl fluoride (PMSF), aprotinin and specific phosphatase inhibitors, sodium orthovanadate. The cell lysates were sonicated and then centrifuged at 10000*g* for 15 min at 4 °C to remove cell debris, and the supernatants were referred to as whole cell lysates.

Nuclear and cytoplasmic fractions from cells were prepared by using NE-PER nuclear and cytoplasmic extract reagent kit containing protease inhibitor and phosphatase inhibitors. Cross contamination between nuclear and cytoplasmic fractions were barely found (data not shown).

Western Blotting Analysis. Cytoplasm proteins were fractionated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE), transferred to nitrocellulose membrane, and blotted with anti-MMP-7 monoclonal antibody, according to the manufacturer's instructions. The blots were stripped and reprobed with β-actin antibody as loading control. Protein levels of phosphorylation ERK 1/2, phosphorylation p38, phosphorylation GSK-3 and phosphorylation β-catenin were measured by using the same procedure described above.

Nuclear proteins were also fractionated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE), transferred to nitrocellulose membrane, and blotted with anti-β-catenin monoclonal antibody, according to the manufacturer's instructions. The blots were stripped and reprobed with Lamin A/C antibody as loading control. Protein levels of c-Fos, phosphorylation c-Jun and c-Myc were measured by using the same procedure described above.

Casein Zymography. Casein zymography was performed for control, leptin and lycopene treatment groups as follows. Gel were polymerized with 0.1% casein. For each sample, an equal amount of protein (100 μg) was loaded. Electrophoresis was carried out using the minigel slab apparatus Mini Protean 2 (Biorad) at a constant voltage of 150 V, until the dye reached the bottom of the gel. Following electrophoresis, gels were washed in renaturation buffer (2.5% Triton X-100 in 50 mM Tris−HCl (pH 7.5)) for 1 h in an orbital shaker. The zymograms were incubated for 24 h at 37 °C in incubation buffer (0.15 M NaCl, 10 mM $CaCl_2$, 0.02% NaN_3 in 50 mM Tris−HCl (pH 7.5)). Gels were

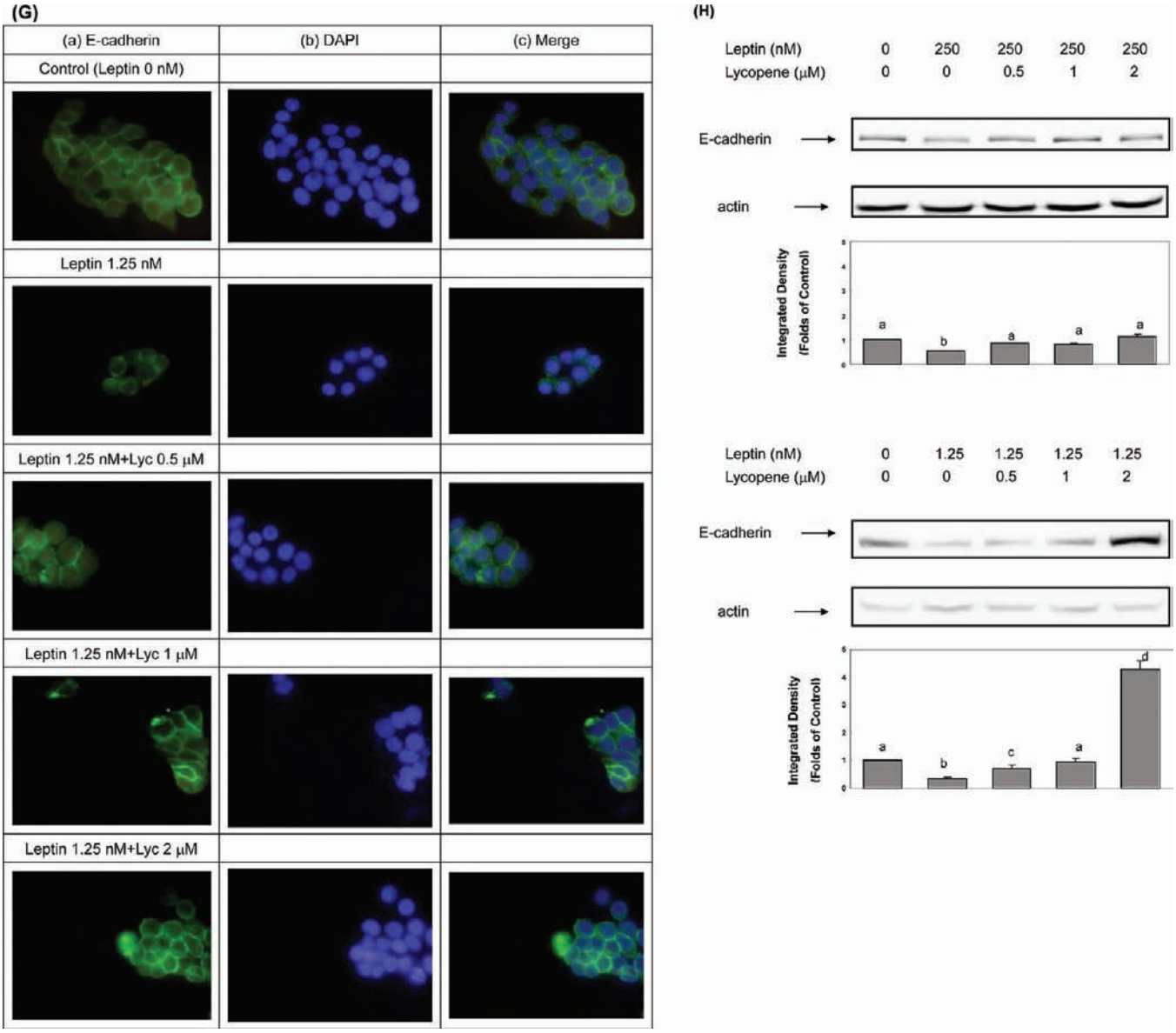


Figure 2. Lycopene differentially inhibited leptin-mediated MMP-7 expression in human colon cancer cells. Human colon cancer cells, cultured in McCoy's medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation, and resuspended in the same medium. After washing out the medium, human colon cancer cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with leptin at concentrations of either 250 nM (A, B) or 1.25 nM (C, D) for 18 h in the presence or absence of various concentrations of lycopene (0, 0.5, 1, and 2 μM). Total cell lysates were blotted with anti-MMP-7 antibody as described in Materials and Methods. The levels of detection in cell lysates represent the amount of MMP-7 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 (A, C). The densitometric analyses are shown in B, D. Different letters represent statistically significant difference among different groups, $p < 0.05$. The data shown are representative of three independent experiments. Conditioned medium was collected and loaded into casein-containing zymogram gel. The gel was stained with coomassie blue stains as described in Materials and Methods. The levels of detection represent the zymogen expression of MMP-7 in human colon cancer HT-29 cells. The zymogen bands are noted with arrows (E). The densitometric analyses are shown at the bottom panels (E). Different letters represent statistically significant difference among different groups, $p < 0.05$. Post confluent colon cancer cells cultured on Tek chambers were incubated in McCoy's medium with 10% FBS at 37 °C. After washing out the media, colon cancer HT-29 cells were incubated in McCoy's medium in the presence of leptin at concentrations of either 250 nM (F) or 1.25 nM (G) at 37 °C for 18 h in the presence or absence of various concentrations of lycopene (0, 0.5, 1, and 2 μM). Imaging was documented at 1000× magnification. Green fluorescence area represents distribution of E-cadherin adherent junction molecule in human colon cancer HT-29 cells stained with monoclonal antibody. Blue fluorescence area represents the location of cell nuclei stained with 4,6-diamidino-2-phenyindole (DAPI) (F, G). Human colon cancer HT-29 cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with leptin at either concentrations of 250 nM or 1.25 nM (H) for 18 h in the presence or absence of various concentrations of lycopene (0, 0.5, 1, and 2 μM). Total cell lysates were blotted with anti-E-cadherin antibody as described in Materials and Methods. The levels of detection in cell lysates represent the amount of E-cadherin in human colon cancer HT-29 cells. The blots were stripped and reprobed with anti-actin antibody as loading control. The immunoreactive bands are noted with arrows (H). The densitometric analyses are shown at the bottom panels (H). Different letters represent statistically significant difference among different groups, $p < 0.05$. The data shown are representative of three independent experiments.

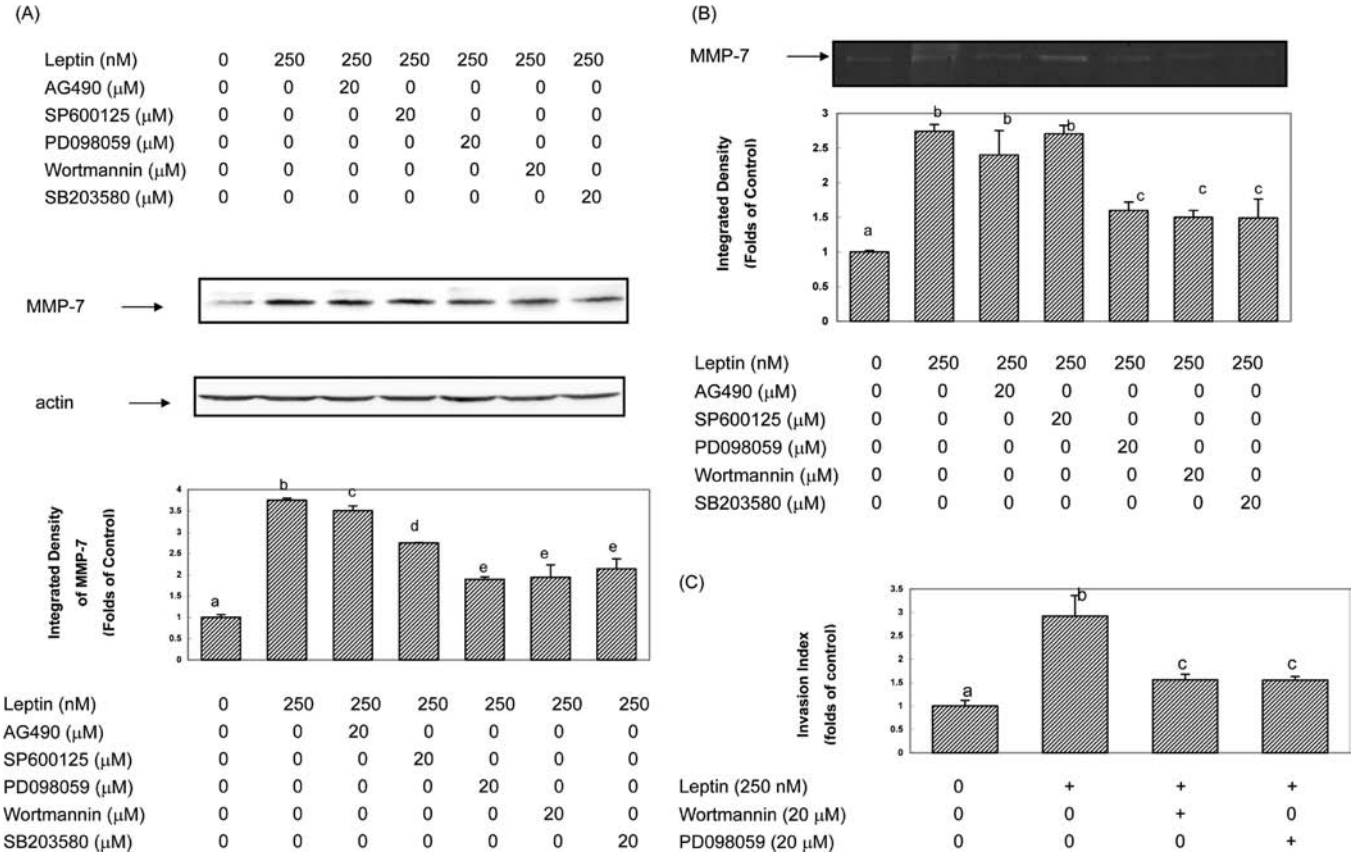


Figure 3. Leptin-mediated expression of MMP-7 and cell invasion in human colon cancer HT-29 cells was in part through MAPK/ERK and PI3K/Akt signaling pathways. Human colon cancer cells, cultured in McCoy's medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation, and resuspended in the same medium. After washing out the medium, human colon cancer cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with different concentration of leptin (0, 250 nM) for 18 h in the presence of different inhibitors (AG490, SP600125, PD098059, wortmannin, SB203580). Total cell lysates were blotted with anti-MMP-7 antibody as described in Materials and Methods. The levels of detection in cell lysates represent the amount of MMP-7 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 (A). The densitometric analysis is shown at the bottom panels (A). Statistical results represent the level of MMP-7. Different letters represent statistically significant difference among different groups, $p < 0.05$. The data shown are representative of three independent experiments. Conditioned medium was collected and loaded into casein-containing zymogram gel. The gel was stained with coomassie blue stains as described in Materials and Methods. The levels of detection represent the zymogen expression of MMP-7 in human colon cancer HT-29 cells. The zymogen bands are noted with arrows (B). The densitometric analysis is shown at the bottom panels (B). Different letters represent statistically significant difference among different groups, $p < 0.05$. The data shown are representative of three independent experiments. The invasion of tumor cells was analyzed in Transwell Boyden chambers with a polyvinyl/pyrrolidone-free polycarbonate filter of 8 μm pore size. Each filter was coated with 50 μL of a 1:5 diluted Matrigel in cold McCoy's medium to form a thin continuous layer on the top of the filter. Confluent human colon cancer HT-29 cells were cultured in McCoy's medium with 10% fetal bovine serum at 37 °C. After washing out the medium, colon cancer cells were trypsinized, prestained with calcein AM and transferred to Matrigel coated Transwell Boyden chambers. Human colon cancer cells (5,000 cells/well) were added to each of triplicate wells in McCoy's medium containing leptin at concentrations of 250 nM in the presence of wortmannin or PD098059 (20 μM). After incubation for 18 h, cells were counted as described above, and the number of cells invading the lower side of the filter was measured as invasive activity. Figures represent the level of invasion index (C). Different letters represent statistically significant difference among different groups, $p < 0.05$. The data shown are representative of three independent experiments.

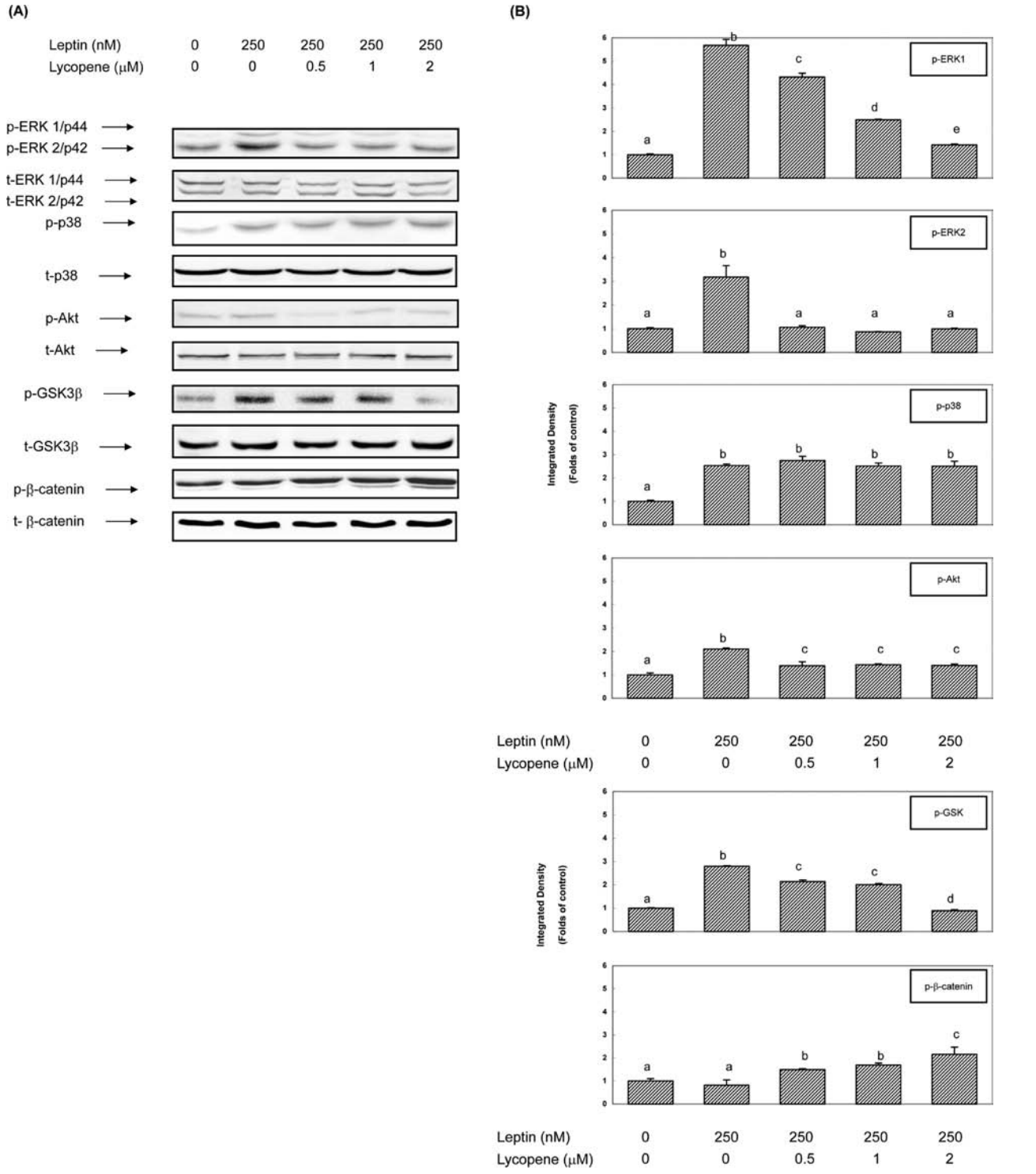
then stained with Coomassie blue and destained with 7% methanol and 5% acetic acid. Areas of catalytic activity appeared as clear bands over the dark background.

Quantum Dot Based Immunofluorescence. 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 8-well Tek Chamber and stimulated with leptin at either 1.25 or 250 nM. Leptin-stimulated colon cancer cells were treated with various concentrations of lycopene (0, 0.5, 1, and 2 μM) for 24 h. At the end of the 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-E-cadherin primary antibody at 1:50 in blocking solution. At the end of incubation, cells were washed with PBS and incubated with quantum dot (Qdot) 525 secondary antibody for another 1 h in 1.5% BSA/PBS. Images (1000×) were acquired on an Olympus BX-51 microscope using the Olympus DP-71 digital camera and imaging system.

Assessment of Cell Invasion. The invasion of tumor cells was analyzed in Transwell Boyden chambers with a polyvinyl/pyrrolidone-free polycarbonate filter of 8 μm pore size. Each filter was coated with 50 μL of a 1:5 diluted Matrigel in cold McCoy medium to form a thin continuous film on the top of the filter. Human colon

cancer cells prestained with calcein AM and treated with lycopene were added to each of triplicate wells in McCoy medium (5,000 cells/well). To examine the molecular mechanisms, cells were treated with wortmannin or PD098059 at concentrations of 20 μM, respectively. After incubation for 18 h, cells in ten randomly selective fields were counted on an Olympus IX-71 inverted fluorescence microscope. The number of cells invading the lower side of the filter was measured as invasion activity (invasion index).

Electrophoresis Mobility Shift Assay (EMSA). Human colon cancer HT-29 cells were treated with leptin (1.25 and 250 nM) in the presence of lycopene (0.5, 1, and 2 μM). The cell lysates were sonicated and then centrifuged at 10000*g* for 15 min at 4 °C to remove cell debris, and the supernatants were referred to as whole cell lysates. Nuclear and cytoplasmic fractions from cells were prepared by using NE-PER nuclear and cytoplasmic extract reagent kit containing protease inhibitor and phosphatase inhibitors. Cross contamination between nuclear and



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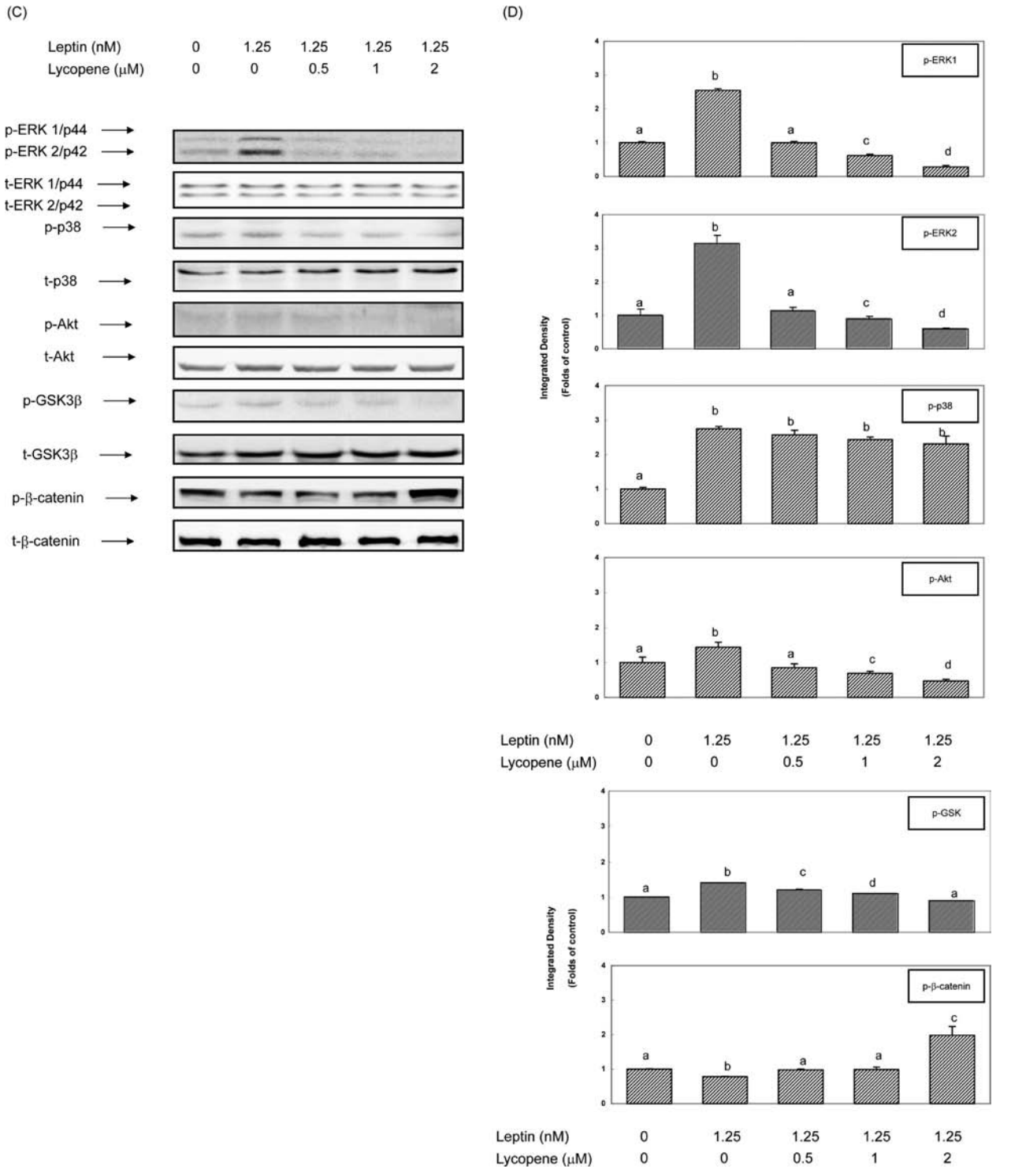
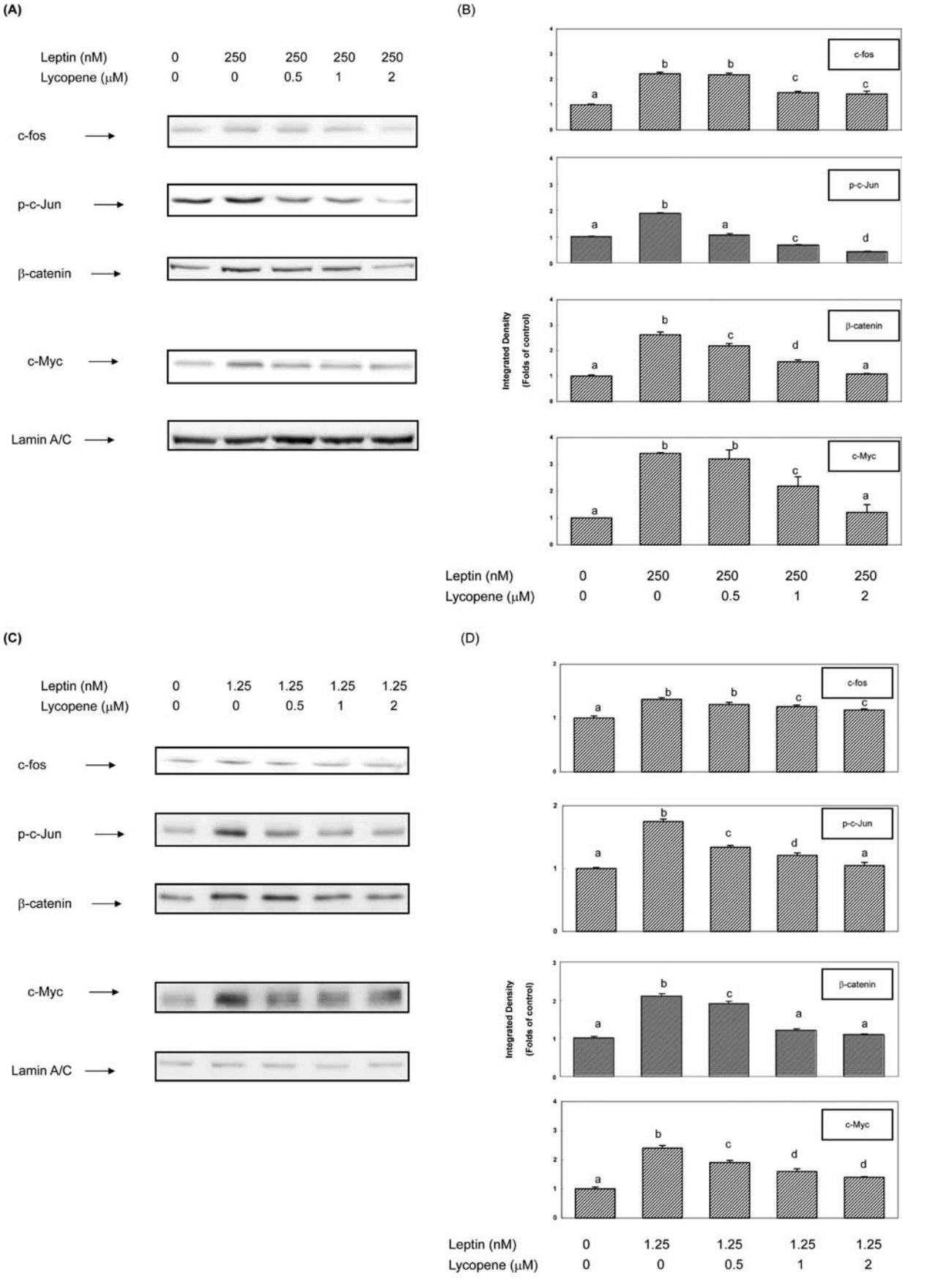


Figure 4. Lycopene modulated phosphorylation levels of ERK 1/2, Akt, GSK-3β and β-catenin in leptin-stimulated human colon cancer HT-29 cells. Human colon cancer cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with leptin at either concentrations of 250 nM (A, B) or 1.25 nM (C, D) for 1 h in the presence or absence of various concentrations of lycopene (0, 0.5, 1, and 2 μM). Total cell extracts were blotted with anti-p-ERK 1/2 antibody as described in Materials and Methods. The levels of detection in total cellular extracts represent the amount of phosphorylated ERK 1/2 in human colon cancer cells. Protein levels of p-p38, p-Akt, p-GSK-3β and p-β-catenin were measured by using the same procedure described above. The Western blotting analyses were performed by using individual total protein (ERK1/2, p38, Akt, GSK-3β, and β-catenin) antibody as loading control. The immunoreactive bands are noted with arrows (A, C). The densitometric analyses are shown in B, D. Statistical results represent the level of phosphorylation proteins under the treatment of leptin at either 250 nM (B) or 1.25 nM (D). Different letters represent statistically significant difference among different groups, $p < 0.05$. The data shown are representative of three independent experiments.



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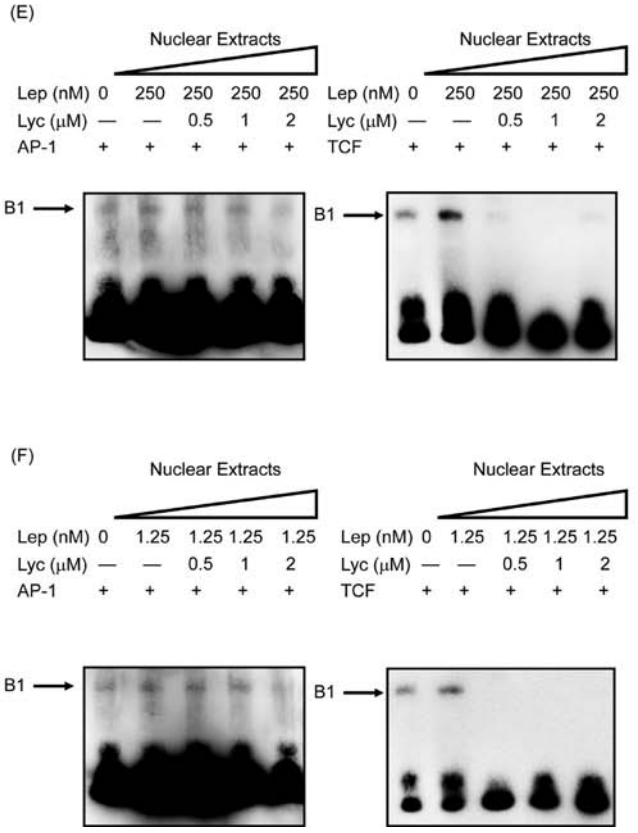

Figure 5. Lycopene reduced nuclear levels of AP-1, β-catenin and c-Myc proteins in leptin-stimulated human colon cancer HT-29 cells. Human colon cancer cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with leptin at either concentrations of 250 nM (A, B) or 1.25 nM (C, D) for 1 h in the presence or absence of various concentrations of lycopene (0, 0.5, 1, and 2 μM). Total nuclear extracts were blotted with anti-β-catenin antibody as described in Materials and Methods. The levels of detection in nuclear extracts represent the amount of β-catenin in human colon cancer cells. Protein levels of c-Fos, p-c-Jun and c-Myc were measured by using the same procedure described above. The blots were stripped and reprobed with anti-lamin A/C antibody as loading control. The immunoreactive bands are noted with arrows (A, C). Statistical results represent the level of phosphorylation proteins under the treatment of leptin at either 250 nM (B) or 1.25 nM (D). Different letters represent statistically significant difference among different groups, $p < 0.05$. The data shown are representative of three independent experiments. Human colon cancer cells were incubated in McCoy's medium with 10% FBS in a tissue culture dish with leptin at either concentrations of 250 nM (E) or 1.25 nM (F) for 1 h in the presence or absence of various concentrations of lycopene (0, 0.5, 1, and 2 μM). EMSA were performed using HT-29 nuclear extracts and dsDNA harboring a consensus site for TCF or AP-1 binding sequences. B1 indicates the transcription factor protein complex with biotinylated DNA probes.

cytoplasmic fractions were not found (data not shown). EMSA was performed using 5 μg of nuclear extracts and mixed with biotin-labeled dsDNA oligonucleotide probes (labeled TCF sequences or labeled AP-1 sequences). Labeled TCF sequences (5′ → 3′): GCAAAATCCTTTGAAAGACAAATCCCTCTCCTT. Labeled AP-1 sequences (5′ → 3′): CGCTTGATGACTCAGCCGGAA. The reaction mixtures were resolved on a 6% nondenaturing polyacrylamide gel in Tris borate−EDTA buffer at 250 V for 30 min. At the end of gel electrophoresis, gels were transferred to membrane to visualize the shifts bands that correspond to the protein/DNA complexes.

Statistical Analysis. The biostatistic methodology was used to determine the difference in the cell invasion among experimental sets of human colon cancer cells. In brief, statistical analyses of the differences in invasive capability among triplicate sets of experimental conditions were performed using SYSTAT software. Confirmation of difference in invasion index as being statistically significant requires rejection of the null hypothesis of no difference between mean invasion indices obtained from replicate sets at the $P = 0.05$ level with the one way ANOVA model. Post hoc test was used to determine differences among different groups.

3. RESULTS

Lycopene Inhibited Leptin-Mediated Cell Invasion in Human Colon Cancer Cells. Previous studies indicated that cell invasion played an important role in the metastasis of human CRC. High plasma levels of leptin were associated with tumor progression of CRC. Therefore, we examined the effect of leptin on cell invasion of human colon cancer cells (HT-29, HCT-116 and SW-480 cells) in this study. To investigate the molecular mechanisms of leptin, CRC cell lines were treated with either low (1.25 nM) or high (250 nM) concentration of leptin in this study. These human colon cancer cells have different subtypes of oncogenic mutations. Human colon cancer HT-29 cells had a PI3K mutation. SW-480 cells were mutated at k-*ras* oncogene. HCT-116 cells had a double mutation of k-*ras* and PI3K oncogenes. Leptin significantly induced cell invasion of human colon cancer cells (HT-29, HCT-116 and SW-480 cells) at concentrations of 1.25 nM (Figure 1A) and 250 nM (Figure 1B). The results demonstrated that these CRC cells had differential responses to leptin-mediated invasion (HT-29 > SW-480 > HCT-116). Leptin could significantly induce cell invasion of HT-29 cells up to almost

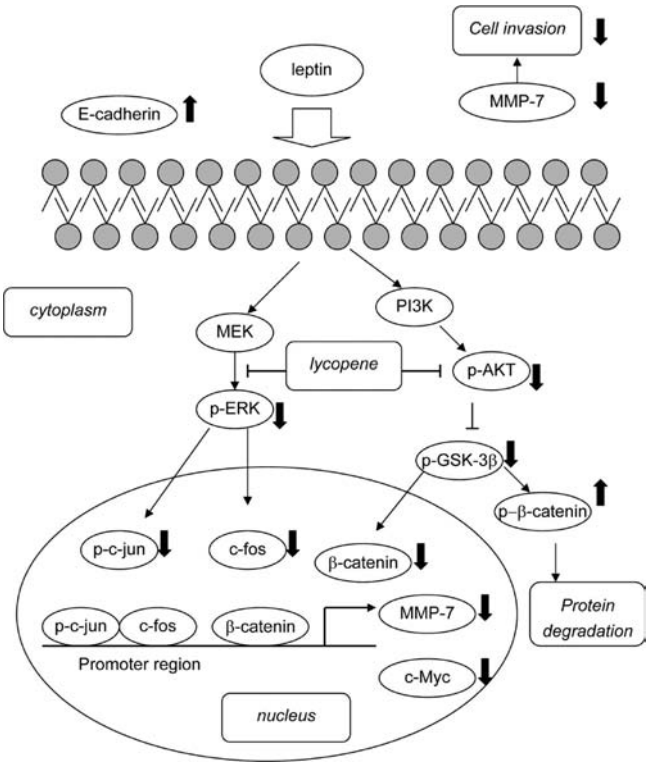

Figure 6. Proposed mechanisms of signaling pathways associated with lycopene-mediated suppression of MMP-7 expression and cell invasion in human colon cancer HT-29 cells. Black closed arrows indicated effects mediated by lycopene in human colon cancer HT-29 cells. Induction is indicated by lightface arrows. Suppression is indicated by a horizontal line with a small vertical line at one end.

3-fold at either low or high concentrations. Moreover, treatment of lycopene (at concentrations of 0.1, 0.5, 1, and 2 μM) could significantly inhibit leptin-mediated cell invasion of human colon cancer cells in a dose-dependent manner (Figure 1). Our results also indicated that the inhibitory effects of lycopene on cell invasion were not through its cytotoxic effects (data not shown). These findings suggest that leptin could effectively induce cell invasion, whereas lycopene could act as a chemopreventive agent to suppress these actions even at physiological concentration (0.5−2 μM).

Lycopene Differentially Inhibited Leptin-Mediated MMP-7 Expression in Human Colon Cancer Cells. Since upregulation of leptin and MMP-7 was highly associated with tumor progression of CRC, we further investigated whether leptin could induce the expression of MMP-7 proteins in human colon cancer cells. As shown in Figure 2A,B, leptin could differentially induce the expression of MMP-7 protein in human colon cancer cells. With normal k-*ras* activity, HT-29 cells were sensitive to leptin stimulation even at concentration of 250 nM. However, SW-480 and HCT-116 cells were resistant to leptin treatment. The results suggested that leptin significantly induced MMP-7 expression in human colon cancer HT-29 cells (Figure 2A−D). However, CRC cells with k-*ras* mutation (SW-480, HCT-116 cells) were resistant to leptin-mediated stimulation at either low dose (1.25 nM) or high dose (250 nM). Therefore, we further examined the chemopreventive effects of lycopene on tumor progression including MMP-7 expression and functional loss of E-cadherin in human colon cancer cells. As shown in Figure 2, lycopene could dose-dependently inhibit leptin-mediated expression of MMP-7 proteins in human colon cancer HT-29 cells. Even without the exogenous effects of leptin, lycopene was still able to inhibit the expression of MMP-7 in SW-480 and HCT-116 cells. Moreover, our results also indicated that endogenous levels of MMP-7 were differentially expressed in different human CRC cell lines (HCT-116 > SW-480 > HT-29). These results suggested that leptin could significantly induce MMP-7 expression only in human colon cancer HT-29 cell, which has normal k-*ras* activity and low endogenous level of MMP-7 protein. However, lycopene could significantly suppress leptin-mediated expression of MMP-7 protein in human colon HT-29 cancer cells. Lycopene could also inhibit expression of MMP-7 in SW-480 and HCT-116 cancer cells, which were sensitive to leptin stimulation and had endogenous levels of MMP-7 proteins. These results suggested that lycopene effectively suppressed endogenous or exogenous (leptin-mediated) levels of MMP-7 proteins in these CRC cell lines. Since the catalytic activity of MMP is associated with cell invasion, we further investigated the effects of lycopene on leptin-mediated catalytic activities of MMP-7 by using casein zymogram analysis. As shown in Figure 2E, leptin at either low dose (1.25 nM) or high dose (250 nM) significantly stimulated the catalytic activities of MMP-7 in human colon cancer HT-29 cells. Since previous study indicated that MMP-7 could cleave the E-cadherin adherent molecule in cells,[38] we further investigated the effects of lycopene on expression and distribution of E-cadherin proteins in leptin-sensitive HT-29 cancer cells. Interestingly, our results demonstrated that lycopene could dose-dependently stabilize the assembly (Figure 2F,G) and expression (Figure 2H) of E-cadherin proteins in human colon cancer HT-29 cells. It is probable that cell invasion was strongly associated with MMP-7 expression in leptin-sensitive HT-29 cells. Our results further suggested that lycopene-mediated stabilization and upregulation of E-cadherin adherent complexes were associated with suppression of MMP-7 proteins in human colon

cancer HT-29 cells. These findings suggested that lycopene could act as a novel chemopreventive agent to inhibit cell invasion in part through the suppression of MMP-7 and stabilization of E-cadherin proteins in human colon cancer HT-29 cells.

Leptin-Mediated Expression of MMP-7 and Cell Invasion in Human Colon Cancer HT-29 Cells Was in Part through MAPK/ERK and PI3K/Akt Signaling Pathways. To further investigate the molecular mechanisms of leptin-mediated MMP-7 expression and cell invasion, we applied specific kinase inhibitors toward human colon cancer HT-29 cells, which were adopted as a major cell model in this study. In the current study, our results demonstrated that treatment of PD098059 (MEK specific inhibitor) and wortmannin (PI3K specific inhibitor) could significantly suppress leptin-mediated MMP-7 expression in human colon cancer HT-29 cells (Figure 3A). These results suggested that PI3K/Akt and MAPK/ERK signaling pathways played crucial roles in the expression of MMP-7 proteins in human colon cancer HT-29 cells. We further investigated whether catalytic activities of MMP-7 would be regulated by different inhibitors. As shown in Figure 3B, the results indicated that treatment of PD098059 (MEK specific inhibitor) and wortmannin (PI3K specific inhibitor) also significantly suppressed the catalytic activities of MMP-7 in human colon cancer HT-29 cells. To validate our important findings, we further examined the effects of different specific kinase inhibitors on cell invasion. As shown in Figure 3C, PD098059 and wortmannin could effectively inhibit cell invasion of human colon cancer HT-29 cells. These results suggested that a leptin-mediated catalytic activity of MMP-7 was associated with cell invasion. The molecular mechanism was, in part, through the activation of PI3K/Akt and MAPK/ERK signaling pathways. It is plausible that lycopene might suppress leptin-mediated MMP-7 expression, activities and cell invasion in part through the modulation of at least different signaling cascades such as PI3K and MAPK/ERK signaling molecules.

Lycopene Modulated Phosphorylation Levels of ERK 1/2, Akt, GSK-3β and β-Catenin in Leptin-Stimulated Human Colon Cancer HT-29 Cells. To further examine the molecular actions of lycopene, we investigated the inhibitory effects of lycopene on the downstream molecules of PI3K/Akt and MAPK/ERK signaling pathways in human colon cancer HT-29 cells. As shown in Figure 4A,B, leptin at high concentration (250 nM) could significantly induce phosphorylation of ERK1/2, Akt, GSK-3β and p38 molecules. However, lycopene could only significantly suppress leptin (250 nM)-mediated phosphorylation of ERK 1/2 proteins. There was no significantly inhibitory effect of lycopene on leptin-mediated phosphorylation of p38 molecules. Since β-catenin could act as an important regulator for the expression of *MMP-7* gene, we further investigated the effects of lycopene on the downstream signaling molecules of PI3K/Akt signaling pathways. Our results showed that treatment of lycopene could suppress the leptin (250 nM)-mediated phosphorylated level of Akt and GSK-3β proteins. Moreover, treatment of lycopene could increase the phosphorylation levels of β-catenin proteins. Similar effects of lycopene were observed in low concentration of leptin-treated group at 1.25 nM, although the stimulatory effects of leptin were not so robust as high dose of leptin (250 nM)-treated group (Figure 4C,D). The results suggested that lycopene could still inhibit the endogenous expression of MMP-7 proteins through the suppression of PI3K/Akt and MAPK/ERK signaling pathways even at low concentration of leptin (1.25 nM) in human colon cancer HT-29 cells. These results suggested that lycopene might be able to induce ubiquitin-dependent degradation of β-catenin molecule through the increased activity of GSK-3β protein and phosphorylated level of β-catenin protein in human colon cancer HT-29 cells.

Lycopene Reduced Nuclear Levels of AP-1, β-Catenin and c-Myc Proteins in Leptin-Stimulated Human Colon Cancer HT-29 Cells. As described above, lycopene could inhibit the expression of MMP-7 in part through the MAPK/ERK and PI3K/Akt/GSK3β signaling cascades. We further investigated the inhibitory effects of lycopene on these downstream transcription factors in human colon cancer HT-29 cells. As shown in Figure 5A,B, leptin at a concentration of 250 nM significantly induced nuclear accumulation of c-Fos, p-c-Jun and β-catenin proteins in human colon cancer HT-29 cells. Treatment of lycopene could significantly suppress nuclear accumulation of c-Fos, p-c-Jun and β-catenin proteins in a dose-dependent manner. Lycopene could even inhibit nuclear levels of c-Fos, p-c-Jun and β-catenin proteins even under the stimulation of leptin at a dose of 1.25 nM (Figure 5C,D). Previous studies already demonstrated that β-catenin could play an important role in the regulation of *c-Myc* expression. As shown in Figure 5A−D, our results demonstrated leptin could significantly induce the expression of c-Myc protein. However, lycopene significantly inhibited the leptin-mediated c-Myc expression (Figure 5A−D). These results suggested that lycopene might be able to inhibit MMP-7 expression in part through the blockade of nuclear accumulation of c-Fos, p-c-Jun and β-catenin proteins in leptin-stimulated human colon cancer HT-29 cells. To validate the molecular mechanisms of lycopene, we further investigated the effects of lycopene on the interactive bindings between transcription factors and response elements in promoter regions of MMP-7 gene. As shown in Figure 5E,F, lycopene could effectively suppress the interactive bindings between these transcription factors (AP-1 and β-catenin proteins) and the corresponding binding sequences (AP-1 and TCF probes) in the promoter regions of *MMP-7* gene. These results suggested that leptin at either high concentration of 250 nM (Figure 5E) or low concentration of 1.25 nM (Figure 5F) induced the nuclear accumulation of AP-1 and β-catenin proteins in human colon cancer HT-29 cells. Lycopene might act as a chemopreventive agent to suppress the expression of MMP-7 in part through suppression of transcription factors/binding sequences complexes in the promoter regions (Figure 5E,F).

4. DISCUSSION

Previous studies suggest that leptin could contribute to cell transformation and tumorigeneisis of human CRC.[9] High plasma levels of leptin are associated with the malignancy and poor prognosis of CRC patients.[7] Many studies suggest that overexpression of MMP-7 could induce the tumorgenicity of CRC and become an important biomarker of colorectal carcinoma. Our previous studies demonstrated that lycopene could significantly inhibit cell proliferation at concentrations of 2, 5, and 10 μM, respectively.[36] However, the inhibitory effects of lycopene on MMP-7 expression and tumor progression of human CRC cells have not been demonstrated yet. Therefore, we would investigate the molecular mechanisms of lycopene during the leptin-mediated MMP-7 expression and tumor progression including cell invasion in human colon cancer cells in this study.

We adapted three CRC cell lines (HT-29, SW-480, and HCT-116 cells) with different mutation characteristics to investigate the molecular mechanisms of leptin. In contrast to HT-29 cells with normal *ras* proto-oncogene activity, SW-480 and HCT-116 have oncogenic k-*ras* mutations.[2,39] Moreover, HCT-116 and HT-29 cells have PI3K oncogenic mutation, which was normal in SW-480 cells.[2] The results from this study demonstrated that leptin differentially induced cell invasion of human colon cancer cells (leptin sensitivity: HT-29 > SW-480 > HCT-116 cells) (Figure 1). Interestingly, our results showed that the basal levels of cell invasion (HCT-116 > SW-480 > HT-29) were correlated with the status of k-*ras* and PI3K mutation (data not shown). It is plausible that the stimulatory effects of leptin on cell invasion were inversely correlated with the status of k-*ras* and PI3K mutation. HCT-116 cells (k-*ras* mutation; PI3K mutation) had high levels of endogenous MMP-7 and were highly insensitive to leptin stimulation. Moreover, SW-480 cells (wild type PI3K; k-*ras* mutation) had low sensitivity to leptin treatment. However, HT-29 cells (wild type k-*ras*; PI3K mutation) were highly sensitive to leptin stimulation. It is probable that leptin-mediated MMP-7 expression and cell invasion of human colon cancer cells were associated with the status of k-*ras*/ PI3K mutations and various aspects of downstream signaling cascades. In the leptin-sensitive and -resistant cancer cells, lycopene even at low concentrations could effectively inhibit leptin-mediated MMP-7 expression and cell invasion in a dose-dependent manner (Figures 1 and 2). These results suggested that lycopene could work as a chemopreventive agent with anti-invasion capability. Our results also demonstrated that leptin upregulated the expression of MMP-7 in human colon cancer HT-29 cells. Treatment of lycopene could effectively suppress leptin-mediated expression and catalytic activities of MMP-7 even at physiological levels (0.5, 1, and 2 μM) (Figure 2). Lycopene could also inhibit the expression of MMP-7 in leptin-resistant CRC cells (SW-480, HCT-116) even with high levels of endogenous expression of MMP-7 (Figure 2). These findings suggested that leptin at low concentration of 1.25 nM could effectively induce MMP-7 expression in HT-29 cells (normal *ras*; mutant *PI3K* genes). However, leptin could significantly augment MMP-7 expression even at high concentration of 250 nM. Our results support the hypothesis that increases in leptin levels could contribute to the risk of colon cancer especially in normal *ras* and mutant *PI3K* colon cancer HT-29 cells which is featured with upregulated expression of MMP-7. These results also suggested that lycopene could effectively inhibit the expression of MMP-7 in those CRC cells containing mutant ras and PI3K genes even under low or high levels of leptin. Therefore, consumption of lycopene could be a chemopreventive agent for protection of most colon cancer patients to prevent the carcinogenic effects of leptin. Overexpression of MMP-7 protein is strongly correlated with the malignant progression of human colon cancer. Previous studies suggested that MMP-7 could cleave the E-cadherin adherent junction molecule. Our results suggested that leptin could induce the expression of MMP-7 protein and instability of E-cadherin molecule in human colon cancer HT-29 cells. It is probable that leptin could augment tumor progression through upregulation of MMP-7 molecules and loss of E-cadherin in human colon cancer HT-29 cells. The results further demonstrated that lycopene upregulated the expression of E-cadherin even in the presence of leptin in human colon cancer HT-29 cells (Figure 2). Our results showed that immunofluorescence staining results were slightly different from the protein levels detected by Western blotting analysis (Figure 2F,G vs Figure 2H). The difference could be explained by the abundant expression of E-cadherin protein on the cell surface.

To investigate the molecular mechanisms involved in the MMP-7 expression in human colon cancer cells, we adopted several inhibitors specifically targeted to different signaling pathways. The results demonstrated that PI3K/Akt and MAPK/ERK signaling pathways could play important roles in leptin-mediated expression and catalytic activities of MMP-7 (Figure 3). Our preliminary finding suggested that these two PI3K/Akt and MAPK/ERK signaling pathways worked independently to regulate the expression of MMP-7 in human colon cancer HT-29 cells (data not shown). Furthermore, our results suggested that catalytic activity of MMP-7 is associated with cell invasion. To further examine the molecular mechanisms of lycopene in the suppression of MMP-7 expression, we investigated the inhibitory effects of lycopene on these downstream signaling molecules. As shown in Figure 4, lycopene could significantly inhibit the leptin (at even concentration 250 nM)-mediated phosphorylated levels of ERK 1/2, Akt and GSK-3β molecules in human colon cancer HT-29 cells. As stated above, suppression of PI3K/Akt and MAPK/ERK signaling pathways and subsequent signaling molecules could inhibit the expression of MMP-7. The results suggested that lycopene significantly inhibited both PI3K/Akt and MAPK/ERK signaling pathways individually. It is probable that lycopene could effectively inhibit the expression of MMP-7 in part through the suppression of ERK 1/2 and Akt molecules. Furthermore, lycopene could enhance the phosphorylated level of β-catenin protein. It is suggested that lycopene might be able to induce the degradation of β-catenin protein through the induction of GSK-3β activity and increased phosphorylated levels of β-catenin protein. However, no inhibitory effects of lycopene on the phosphorylated level of p38 were observed in this study. The results suggested that lycopene might specifically target to the Akt, GSK-3β and ERK1/2 signaling molecules and suppress the downstream transcription factors such as β-catenin and AP-1 proteins. Since previous studies already indicated that β-catenin and AP-1 could modulate the expression of MMP-7 in several types of cells, we further examine the inhibitory effects of lycopene on expression of β-catenin and AP-1 transcription factors in leptin-stimulated human colon cancer cells. As shown in Figure 5, we demonstrated that lycopene could significantly inhibit the nuclear accumulation of AP-1 and β-catenin transcription factors. We also demonstrated that lycopene could significantly inhibit the expression of c-Myc protein in human colon cancer cells. These results suggested that lycopene might be able to suppress the expression of MMP-7 and c-Myc proteins in part through the modulation of β-catenin and AP-1 transcription factors.

Previous studies indicated that strong immunohistochemical staining of MMP-7 was observed in various types of colonic adenomas.[5] Several studies also indicated that the expression of MMP-7 is significantly correlated with the presence of nodal or distant metastases.[40,41] Our results are consistent with previous findings and provide the mechanism of leptin in tumor progression of human colon cancer cells. As shown in Figure 6, the proposed mechanisms suggested that leptin would modulate the

cell invasion and MMP-7 expression through the modulation of Akt, GSK-3β and ERK1/2 signaling pathways in human colon cancer HT-29 cells (normal *ras*; mutant *PI3K* genes). The results indicated that leptin induced nuclear accumulation of AP-1 and β-catenin proteins in human colon cancer HT-29 cells. Lycopene, an anticancer agent, could effectively suppress leptin-mediated nuclear expression of AP-1, β-catenin, c-Myc, and MMP-7 proteins. The molecular actions of lycopene were also in part through the activation of GSK-3β molecule and destabilization of β-catenin in human colon cancer HT-29 cells. Lycopene could effectively inhibit MMP-7 expression and cell invasion in either *ras* or *PI3K* mutation CRC cells under the stimulation of leptin at low concentration of 1.25 nM. Our results indicated that leptin at high concentration of 250 nM could induce MMP-7 expression and cell invasion in HT-29 cells (normal *ras* gene). With the increasing levels of leptin up 250 nM, lycopene could still suppress MMP-7 expression and cell invasion in all three CRC cells featured with either *ras* or *PI3K* gene mutation. In conclusion, lycopene could exert its novel anticancer effects in part through suppression of MMP-7 expression as needed for cell invasion during development of colorectal tumor. These findings provide a novel insight into the anticancer effects of lycopene on tumor progression of human colorectal cancer. Although we only test the effects of lycopene on human colon cancer HT-29 cell lines *in vitro*, our results provide the first findings that lycopene could significantly inhibit leptin-mediated MMP-7 expression and cell invasion which are associated with malignancy as evident from population studies and animal experiments showing enhancement of tumor progression and cancer development.

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REFERENCES

(1) Jemal, A.; Siegel, R.; Xu, J.; Ward, E. Cancer statistics, 2010. *CA—Cancer J.Clin.* **2010**, *60*, 277–300.

(2) Yeh, J. J.; Routh, E. D.; Rubinas, T.; Peacock, J.; Martin, T. D.; Shen, X. J.; Sandler, R. S.; Kim, H. J.; Keku, T. O.; Der, C. J. KRAS/BRAF mutation status and ERK1/2 activation as biomarkers for MEK1/2 inhibitor therapy in colorectal cancer. *Mol. Cancer Ther.* **2009**, *8*, 834–843.

(3) Velho, S.; Oliveira, C.; Ferreira, A.; Ferreira, A. C.; Suriano, G.; Schwartz, S, Jr; Duval, A.; Carneiro, F.; Machado, J. C.; Hamelin, R.; Seruca, R. The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur. J. Cancer* **2005**, *41*, 1649–1654.

(4) Brabletz, T.; Jung, A.; Dag, S.; Hlubek, F.; Kirchner, T. beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. *Am. J. Pathol.* **1999**, *155*, 1033–1038.

(5) Kirimlioglu, H.; Kirimlioglu, V.; Yilmaz, S.; Sagir, V.; Coban, S.; Turkmen, E.; Hilmioglu, F. Role of matrix metalloproteinase-7 in colorectal adenomas. *Dig. Dis. Sci.* **2006**, *51*, 2068–2072.

(6) Oshima, T.; Akaike, M.; Yoshihara, K.; Shiozawa, M.; Yamamoto, N.; Sato, T.; Yamada, R.; Fujii, S.; Rino, Y.; Kunisaki, C.; Tanaka, K.; Masuda, M.; Imada, T. Clinicopathological significance of the gene expression of matrix metalloproteinase-7, insulin-like growth factor-1, insulin-like growth factor-2 and insulin-like growth factor-1 receptor in patients with colorectal cancer: insulin-like growth factor-1 receptor gene expression is a useful predictor of liver metastasis from colorectal cancer. *Oncol. Rep.* **2008**, *20*, 359–364.

(7) Howard, J. M.; Pidgeon, G. P.; Reynolds, J. V. Leptin and gastrointestinal malignancies. *Obes. Rev.* **2010**, *11* (12), 863–874.

(8) Stattin, P.; Palmqvist, R.; Soderberg, S.; Biessy, C.; Ardnor, B.; Hallmans, G.; Kaaks, R.; Olsson, T. Plasma leptin and colorectal cancer risk: a prospective study in Northern Sweden. *Oncol. Rep.* **2003**, *10*, 2015–2021.

(9) Garofalo, C.; Surmacz, E. Leptin and cancer. *J. Cell. Physiol.* **2006**, *207*, 12–22.

(10) Uddin, S.; Bavi, P. P.; Hussain, A. R.; Alsbeih, G.; Al Sanea, N.; Abduljabbar, A.; Ashari, L. H.; Alhomoud, S.; Al Dayel, F.; Ahmed, M.; Al Kuraya, K. S. Leptin receptor expression in Middle Eastern colorectal cancer and its potential clinical implication. *Carcinogenesis* **2009**, *30*, 1832–1840.

(11) Fruhbeck, G.; Sopena, M.; Martinez, J. A.; Salvador, J. [Nutrition, energy balance, and obesity]. *Rev. Med. Univ. Navarra* **1997**, *41*, 185–192.

(12) Muoio, D. M.; Dohm, G. L.; Fiedorek, F. T., Jr.; Tapscott, E. B.; Coleman, R. A. Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* **1997**, *46*, 1360–1363.

(13) Muoio, D. M.; Lynis, D. G. Peripheral metabolic actions of leptin. *Best Pract. Res., Clin. Endocrinol. Metab* **2002**, *16*, 653–666.

(14) Fruhbeck, G.; Jebb, S. A.; Prentice, A. M. Leptin: physiology and pathophysiology. *Clin. Physiol.* **1998**, *18*, 399–419.

(15) Saxena, N. K.; Sharma, D.; Ding, X.; Lin, S.; Marra, F.; Merlin, D.; Anania, F. A. Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. *Cancer Res.* **2007**, *67*, 2497–2507.

(16) Wolf, I.; Sadetzki, S.; Kanety, H.; Kundel, Y.; Pariente, C.; Epstein, N.; Oberman, B.; Catane, R.; Kaufman, B.; Shimon, I. Adiponectin, ghrelin, and leptin in cancer cachexia in breast and colon cancer patients. *Cancer* **2006**, *106*, 966–973.

(17) Uddin, S.; Bu, R.; Ahmed, M.; Hussain, A. R.; Ajarim, D.; Al Dayel, F.; Bavi, P.; Al kuraya, K. S. Leptin receptor expression and its association with PI3K/AKT signaling pathway in diffuse large B-cell lymphoma. *Leuk. Lymphoma* **2010**, *51*, 1305–1314.

(18) Uddin, S.; Bavi, P.; Siraj, A. K.; Ahmed, M.; Al Rasheed, M.; Hussain, A. R.; Ahmed, M.; Amin, T.; Alzahrani, A.; Al Dayel, F.; Abubaker, J.; Bu, R.; Al Kuraya, K. S. Leptin-R and its association with PI3K/AKT signaling pathway in papillary thyroid carcinoma. *Endocr.-Relat. Cancer* **2010**, *17*, 191–202.

(19) Frankenberry, K. A.; Skinner, H.; Somasundar, P.; McFadden, D. W.; Vona-Davis, L. C. Leptin receptor expression and cell signaling in breast cancer. *Int. J. Oncol.* **2006**, *28*, 985–993.

(20) Somasundar, P.; Frankenberry, K. A.; Skinner, H.; Vedula, G.; McFadden, D. W.; Riggs, D.; Jackson, B.; Vangilder, R.; Hileman, S. M.; Vona-Davis, L. C. Prostate cancer cell proliferation is influenced by leptin. *J. Surg. Res.* **2004**, *118*, 71–82.

(21) Jaffe, T.; Schwartz, B. Leptin promotes motility and invasiveness in human colon cancer cells by activating multiple signal-transduction pathways. *Int. J. Cancer* **2008**, *123*, 2543–2556.

(22) Westermarck, J.; Kahari, V. M. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J.* **1999**, *13*, 781–792.

(23) Benbow, U.; Brinckerhoff, C. E. The AP-1 site and MMP gene regulation: what is all the fuss about? *Matrix Biol.* **1997**, *15*, 519–526.

(24) Vincenti, M. P.; White, L. A.; Schroen, D. J.; Benbow, U.; Brinckerhoff, C. E. Regulating expression of the gene for matrix metalloproteinase-1 (collagenase): mechanisms that control enzyme activity, transcription, and mRNA stability. *Crit. Rev. Eukaryotic Gene Expression* **1996**, *6*, 391–411.

(25) Fang, Y. J.; Lu, Z. H.; Wang, G. Q.; Pan, Z. Z.; Zhou, Z. W.; Yun, J. P.; Zhang, M. F.; Wan, D. S. Elevated expressions of MMP7, TROP2, and survivin are associated with survival, disease recurrence, and liver metastasis of colon cancer. *Int. J. Colorectal Dis.* **2009**, *24*, 875–884.

(26) Matono, H.; Oda, Y.; Nakamori, M.; Tamiya, S.; Yamamoto, H.; Yokoyama, R.; Saito, T.; Iwamoto, Y.; Tsuneyoshi, M. Correlation between beta-catenin widespread nuclear expression and matrix metalloproteinase-7 overexpression in sporadic desmoid tumors. *Hum. Pathol.* **2008**, *39*, 1802–1808.

(27) Giles, R. H.; van Es, J. H.; Clevers, H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim. Biophys. Acta* **2003**, *1653*, 1–24.

(28) Franceschi, S.; Bidoli, E.; La Vecchia, C.; Talamini, R.; D'Avanzo, B.; Negri, E. Tomatoes and risk of digestive-tract cancers. *Int. J. Cancer* **1994**, *59*, 181–184.

(29) Franceschi, S.; Favero, A.; La Vecchia, C.; Negri, E.; Conti, E.; Montella, M.; Giacosa, A.; Nanni, O.; Decarli, A. Food groups and risk of colorectal cancer in Italy. *Int. J. Cancer* **1997**, *72*, 56–61.

(30) Terry, P.; Jain, M.; Miller, A. B.; Howe, G. R.; Rohan, T. E. Dietary carotenoid intake and colorectal cancer risk. *Nutr. Cancer* **2002**, *42*, 167–172.

(31) Erhardt, J. G.; Meisner, C.; Bode, J. C.; Bode, C. Lycopene, beta-carotene, and colorectal adenomas. *Am. J. Clin. Nutr.* **2003**, *78*, 1219–1224.

(32) Murtaugh, M. A.; Ma, K. N.; Benson, J.; Curtin, K.; Caan, B.; Slattery, M. L. Antioxidants, carotenoids, and risk of rectal cancer. *Am. J. Epidemiol.* **2004**, *159*, 32–41.

(33) Nahum, A.; Hirsch, K.; Danilenko, M.; Watts, C. K.; Prall, O. W.; Levy, J.; Sharoni, Y. Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27(Kip1) in the cyclin E-cdk2 complexes. *Oncogene* **2001**, *20*, 3428–3436.

(34) Amir, H.; Karas, M.; Giat, J.; Danilenko, M.; Levy, R.; Yermiahu, T.; Levy, J.; Sharoni, Y. Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutr. Cancer* **1999**, *33*, 105–112.

(35) Karas, M.; Amir, H.; Fishman, D.; Danilenko, M.; Segal, S.; Nahum, A.; Koifmann, A.; Giat, Y.; Levy, J.; Sharoni, Y. Lycopene interferes with cell cycle progression and insulin-like growth factor I signaling in mammary cancer cells. *Nutr. Cancer* **2000**, *36*, 101–111.

(36) Tang, F. Y.; Shih, C. J.; Cheng, L. H.; Ho, H. J.; Chen, H. J. Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. *Mol. Nutr. Food Res.* **2008**, *52*, 646–654.

(37) Tang, F. Y.; Cho, H. J.; Pai, M. H.; Chen, Y. H. Concomitant supplementation of lycopene and eicosapentaenoic acid inhibits the proliferation of human colon cancer cells. *J. Nutr. Biochem.* **2009**, *20*, 426–434.

(38) Lynch, C. C.; Vargo-Gogola, T.; Matrisian, L. M.; Fingleton, B. Cleavage of E-Cadherin by Matrix Metalloproteinase-7 Promotes Cellular Proliferation in Nontransformed Cell Lines via Activation of RhoA. *J. Oncol.* **2010**, *2010*, 530745.

(39) Agarwal, B.; Bhendwal, S.; Halmos, B.; Moss, S. F.; Ramey, W. G.; Holt, P. R. Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. *Clin. Cancer Res.* **1999**, *5*, 2223–2229.

(40) Adachi, Y.; Yamamoto, H.; Itoh, F.; Arimura, Y.; Nishi, M.; Endo, T.; Imai, K. Clinicopathologic and prognostic significance of matrilysin expression at the invasive front in human colorectal cancers. *Int. J. Cancer* **2001**, *95*, 290–294.

(41) Adachi, Y.; Yamamoto, H.; Itoh, F.; Hinoda, Y.; Okada, Y.; Imai, K. Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. *Gut* **1999**, *45*, 252–258.