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Lycopene inhibits matrix metalloproteinase-9 expression and down-regulates the binding activity of nuclear factor-kappa B and stimulatory protein-1

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Abstract

The carotenoid lycopene has been associated with decreased risks of several types of cancer, such as hepatoma. Although lycopene has been shown to inhibit metastasis, its mechanism of action is poorly understood. Here, we used SK-Hep-1 cells (from a human hepatoma) to test whether lycopene exerts its anti-invasion activity via down-regulation of the expression of matrix metalloproteinase (MMP)-9, an important enzyme in the degradation of basement membrane in cancer invasion. The activity and expressions of MMP-9 protein and mRNA were detected by gelatin zymography, Western blotting and RT-PCR, respectively. The binding abilities of nuclear factor-kappa B (NF-κB), activator protein-1 and stimulatory protein-1 (Sp1) to the binding sites in the MMP-9 promoter were measured by the electrophoretic mobility shift assay. We showed that lycopene (1–10 μM) significantly inhibited SK-Hep-1 invasion (P<.05) and that this effect correlated with the inhibition of MMP-9 at the levels of enzyme activity (r^2=.94, P<.001), protein expression (r^2=.80, P=.007) and mRNA expression (r^2=.94, P<.001). Lycopene also significantly inhibited the binding abilities of NF-κB and Sp1 and decreased, to some extent, the expression of insulin-like growth factor-1 receptor (IGF-1R) and the intracellular level of reactive oxygen species (P<.05). The antioxidant effect of lycopene appeared to play a minor role in its inhibition of MMP-9 and invasion activity of SK-Hep-1 cells because coincubation of cells with lycopene plus hydrogen peroxide abolished the antioxidant effect but did not significantly affect the anti-invasion ability of lycopene. Thus, lycopene decreases the invasive ability of SK-Hep-1 cells by inhibiting MMP-9 expression and suppressing the binding activity of NF-κB and Sp1. These effects of lycopene may be related to the down-regulation of IGF-1R, while the antioxidant activity of lycopene appears to play a minor role.
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Keywords: Lycopene; MMP-9; NF-κB; Sp1; IGF-1R

1. Introduction

Metastasis is a characteristic of highly malignant cancers with poor clinical outcome. Malignant tumor progression depends upon the capacity to invade, metastasize and promote the angiogenic host response. One critical characteristic that metastasis cancer cells have acquired is the ability to dissolve basement membranes and the extracellular matrix (ECM). This degradative process is mediated largely by matrix metalloproteinases (MMPs) [1], which are a large family of at least 20 zinc-dependent neutral endopeptidases that together can degrade all known components of ECM [2]. MMP-9 is abundantly expressed in various malignant tumors and is postulated to play a critical role in tumor invasion and angiogenesis [3,4]. Thus, the inhibition of MMP activity, including MMP-9, is important for the prevention of cell invasion [5,6]. Interestingly, MMP-9 expression levels are especially high in hepatoma cells, such as SK-Hep-1 cells, and the enzyme has been studied in diverse malignant tumor cells because of its inducible character [7]. The 5′ flanking region of the *MMP-9* gene contains several functional regulatory motifs that interact with well-characterized transcription factors [8], including nuclear factor-kappa B (NF-κB), activator protein-1 (AP-1) and stimulatory protein-1 (Sp1) [8,9]. MMP-9 is regulated at a posttranslational level through interaction with tissue inhibitor of metalloproteinase-1 (TIMP-1) [6].

Lycopene is an antioxidative pigment found in vegetables and fruit. Previous studies have suggested that higher intakes of lycopene are associated with a reduced risk of several types of cancer, such as prostate cancer and

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hepatoma [10–12]. The anticancer effects of carotenoids such as lycopene have been related to their antioxidant actions such as singlet oxygen quenching and free radical scavenging [13–16]. Kozuki et al. [17] have suggested that the antioxidative property of lycopene may partly explain its anti-invasive action. By contrast, Collins [18] indicated that the antioxidant property of carotenoids is not related to their anticancer abilities. Recently, several studies have shown that carotenoids affect the transcription of various genes such as *connexin 43* [18–20]. We have recently shown that lycopene inhibits the migration and invasion of SK-Hep-1 cells and that these actions are associated with up-regulation of *nm23-H1*, a metastasis suppressor gene [21]. Using the same cell line (SK-Hep-1), Hwang and Lee [22] recently reported that lycopene inhibits its migration, invasion and adhesion and decreases the secretion of MMP-2 and MMP-9. However, the mechanism underlying the inhibition of MMPs by lycopene is not clear.

Because liver cancer is the most endemic cancer in Taiwan and in a large region of the world, we employed SK-Hep-1 cells to study the antimetastatic mechanisms of lycopene. We examined the effects of lycopene on MMP-9, insulin-like growth factor-1 receptor (IGF-1R), TIMP-1 and the transcription factors NF-κB, Sp1 and AP-1 in order to provide insights into the nuclear events that control the ability of cell invasion in response to lycopene.

2. Materials and methods

2.1. Chemicals

The cell line SK-Hep-1 was a generous gift from Dr. Liu TZ (Graduate Institute of Clinical Medicine, Chang Gung University, Taoyuan, Taiwan). All chemicals used are of reagent or higher grade. Lycopene (Wako, Japan) was delivered to the cell using tetrahydrofuran (THF; Merck, Germany) solvent, containing 0.025% butylated hydroxytoluene to avoid formation of peroxides. Dulbecco's minimal essential medium (DMEM), fetal bovine serum (FBS), trypsin, penicillin, streptomycin, sodium pyruvate, nonessential amino acid and Giemsa stain were from Gibco/BRL (Bethesda, MD, USA). Transwells were from Costar (Cambridge, MA). Matrigel, anti-IGF-1R-phycoerythrin-conjugated mouse IgG1 antibody and fluorescein isothiocyanate (FITC)-conjugated anti-IGFR antibody were from BD Biosciences (USA). TIMP-1, insulin-like growth factor-1 (IGF-1) and the electrophoretic mobility shift assay (EMSA) kit were purchased from Amersham and Pierce Biotechnology (Rockford, IL). Anti-MMP-9 mouse monoclonal antibody and antimouse IgG-HRP antibody were purchased from US Biological Co. (USA) and Santa Cruz Biotechnology Co. (USA), respectively.

2.2. Cell culture and lycopene incorporation

SK-Hep-1 cells were grown in DMEM medium containing 10% (v/v) FBS, 0.37% (w/v) $NaHCO_3$, penicillin (100 U/ml) and streptomycin (100 U/ml) in a humidified incubator under 5% CO_2 and 95% air at 37°C. The cells were harvested at approximately 90% confluence (10^6 cells/dish). The survival rate of cells was always higher than 95% by trypan blue assay [23]. A stock THF–lycopene solution (10 mM) was prepared freshly before each experiment. The purity of commercial lycopene was approximately 97%, which compares well with the 98% purity claimed by the supplier (Wako), as determined using HPLC in the laboratory. By passing the stock solution in THF through a 0.22-μm filter, the concentration of lycopene stock solution was >93% (i.e., >9.3 mM). THF–lycopene was added to the culture medium at a calculated final concentration of 1, 2.5, 5 or 10 μM. THF at 0.1% (v/v; or 1.2 μM) served as the control for lycopene, which did not significantly affect the assays described below. SK-Hep-1 cells (ca. 10^6 cells/dish) were incubated with THF–lycopene at 37°C in the dark for 2 h, as described in other cell lines [21,24]. The cells were then washed three times in phosphate-buffered saline (PBS; pH 7.4). The cellular uptake of lycopene was determined by HPLC, with detection at 470 nm following extraction with ethanol and hexane [24]. In order to calculate the actual uptake of lycopene, the cellular uptake obtained from 37°C was subtracted from the adherent lycopene, which was measured by incubating lycopene with the cells at 4°C for 2 h.

2.3. Cell viability and morphology

Cells were cultured in 24-well plates at 1×10^4 cells/well in media for 24 h. Each well was washed with PBS, filled with 1 ml of DMEM containing various doses of lycopene and preincubated for 2, 12 and 24 h at 37°C, respectively, followed by 24 h incubation. Each well was then incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol terazolium bromide for 1 h at 37°C. The liquid was removed, and DMSO was added to dissolve the solid residue. The optical density at 570 nm of each well was then determined using a microplate reader (FLUOstar OPTIMA, BMG Labtechnologies GmbH, Germany). In addition, cell morphology was photographed under ×100 magnification microscopy just before each assay.

2.4. Cell invasion assay

Tumor cell invasion was assayed in transwell chambers (Costar) according to the methods reported by Repesh [25] with some modifications. Briefly, transwell chambers (Costar) with 6.5-mm polycarbonate filters that have an 8-μm pore size were used, and each filter was coated with 100 μl of a 1:20 diluted matrigel in cold DMEM to form a thin continuous film on the top of the filter. The number of cells was adjusted to 5×10^5/ml, and a 100-μl aliquot containing 5×10^4 cells was added to each of the triplicate wells in DMEM containing 10% FBS. After incubation for 24 h, cells were stained and counted as described above, and the number of cells invading the lower side of the filter was measured as the invasive activity. For each replicate, the tumor cells in 10 randomly selected fields were determined,

and the counts were averaged. The percentage inhibition of invasion was calculated by the following formula: [1−(treatment/control)]×100%.

2.5. Gelatin zymography

MMP-9 activity was assayed using gelatin zymography according to the methods reported by Huang et al. [26] with some modifications. The cells (5×10^4 cells/ml) were pretreated with lycopene for 2 h in DMEM medium containing 10% (v/v) FBS and incubated for 24 h at 37°C in serum-free DMEM; then, the culture medium was harvested and stored at −20°C until use. For the assay of gelatin zymography, the culture medium was electrophoresed in a 10% sodium dodecyl sulfate (SDS)-PAGE gel containing 0.1% (w/v) gelatin. The gel (MMP gel) was washed for 30 min at room temperature in a solution containing 2.5% (v/v) Triton X-100 with two changes and subsequently transferred to a reaction buffer for enzymatic reaction containing 1% NaN_3, 10 mM $CaCl_2$ and 40 mM Tris–HCl, pH 8.0, at 37°C with shaking overnight (for 12 and 15 h). Finally, the MMP gel was stained for 30 min with 0.25% (w/v) Coomassie blue in 10% acetic acid (v/v) and 50% methanol (v/v) and destained in 10% acetic acid (v/v) and 50% methanol (v/v). The relative MMP-9 activities were quantitated by Matrox Inspector 2.1 software.

2.6. Western blotting

MMP-9 and IκBα protein levels were assayed by Western blotting as described previously [27]. Total cellular proteins were prepared using lysis buffer containing 20% SDS and 1 mM phenylmethyl sulfonyl fluoride. The lysate was sonicated for 30 s on ice, followed by centrifugation for 30 min at 4°C. Cytosolic fractions and nuclear fractions were prepared according to previous studies [28]. The protein concentrations of extracts were determined by Bio-Rad assay as outlined by the manufacturer (Bio-Rad, Hercules, CA). The relative MMP-9 levels were quantitated by Matrox Inspector 2.1 software.

2.7. RT-PCR (RNA isolation and sequencing)

Total cellular RNA was isolated from cell culture (RNAzol kit) and reverse transcribed into cDNA (MMLV-Reverse Transcriptase, Gibco/BRL) using oligo $(dT)_{15}$ as primers and then coamplified with four primer bases on MMP-9 and β-actin (internal control) sequences. The primers for amplifying MMP-9 cDNA were 5′-AGGCCTC-TACAGAGTCTTTG-3′, located in the 5′-untranslated region, and 5′-CAGTCCAACAAGAAAGGACG-3′, located in the 3′-untranslated region. The primers for amplifying β-actin cDNA were 5′ -GTGGGGCGCCCCAGGCACCA-3′ and 5′-CTCCTTAATGTCACGCACGATTTC-3′. PCR amplification was performed with a thermal cycler as follows: denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 90 s (29 cycle), followed by a final incubation at 72°C for 7 min. The sizes of the amplification products of MMP-9 and β-actin were 825 and 541 bp, respectively. The PCR products were subjected to 1% agarose gel electrophoresis and stained with ethidium bromide. The relative *MMP-9* levels were quantitated by Matrox Inspector 2.1 software.

2.8. Enzyme-linked immunosorbent assay (ELISA)

After incubation with different concentrations of lycopene, the culture media from SK-Hep-1 cells were collected and processed for IGF-1, and TIMP-1 was quantified by an ELISA system using human IGF-1 and TIMP-1 sandwich ELISA kit (Amersham). The assays were performed in triplicate by following the instructions of the manufacturer.

2.9. Preparation of nuclear extracts and EMSA

Nuclear protein extracts (5 μg) were prepared according to the modified method of a previous study [29]. Binding activities of transcription factors including NF-κB, Sp1 and AP-1 were analyzed by gel mobility shift assays (EMSA). EMSA was performed in the nuclear extracts of SK-Hep-1 cells using LightShift Chemiluminescent EMSA Kit (Pierce Biotechnology), as described previously [30], using double-stranded oligonucleotides based on sequences of NF-κB (5′ -AGTTGAGGGGACTTTCCCAGGC-3′) and Sp1 (5′-ATTCGATCGGGGCGGGGCGAG-3′) sites in the human MMP-9 promoter or a consensus AP-1 (5′-CGCTTGAT-GACTCAGCCGGAA-3′) site. The relative NF-κB and Sp1 levels were quantitated by Matrox Inspector 2.1 software.

2.10. Surface IGF-1R expression

Cell surface IGF-1R levels in SK-Hep-1 cells were determined by flow cytometry. The cells were trypsinized and washed twice with PBS, centrifuged, resuspended and counted. The cells (10^6 cells/sample in FACS buffer) were incubated for 30 min on ice with IGF-1R-phycoerythrin-conjugated mouse IgG1 antibody (2 μg/ml), washed and stained with FITC-conjugated anti-IGFR antibody. Cells

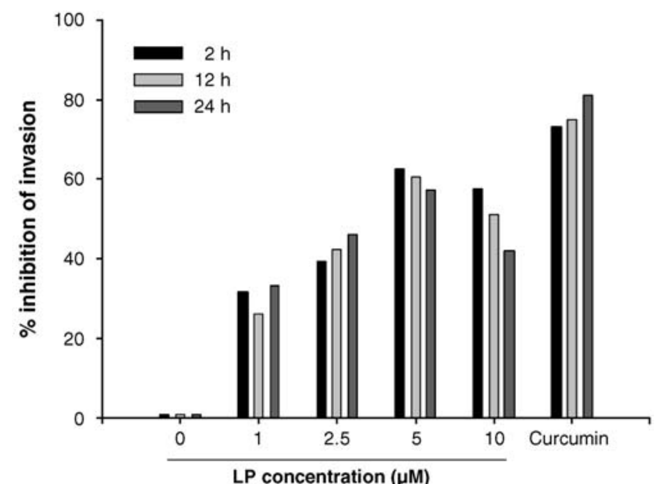

Fig. 1. Effects of lycopene (LP) concentration on invasion of SK-Hep-1 cells at preincubation times of 2, 12 and 24 h. THF (0.1%) is the solvent control for lycopene; curcumin (20 μM) is the positive control.

were washed, resuspended and analyzed for fluorescence intensity using CellQuest software (BD Biosciences).

2.11. Intracellular reactive oxygen species (ROS) assay

Intracellular ROS were assayed using 2′,7′-dichlorofluorescin-diacetate (DCFH-DA) as reported previously [31,32], which diffuses readily through cell membranes and is oxidized by peroxide to form a highly fluorescent DCF that can be detected by flow cytometry. The cells were preincubated with lycopene (1–10 μM) for 2 h. After washing with PBS for three times, the cells were incubated with 10 μM DCFH-DA for 15 min, and the intracellular fluorescence was measured using flow cytometry (FACS-Calibur, BD Biosciences).

2.12. Statistical analysis

Values are expressed as means±S.D. and analyzed using one-way ANOVA followed by Duncan's Multiple Range Test for comparisons of group means. When only two groups are compared, we used unpaired Student's t test for statistical analysis. The statistical analysis was performed using SPSS for Windows, version 10 (SPSS, Inc.); $P<.05$ was considered statistically significant.

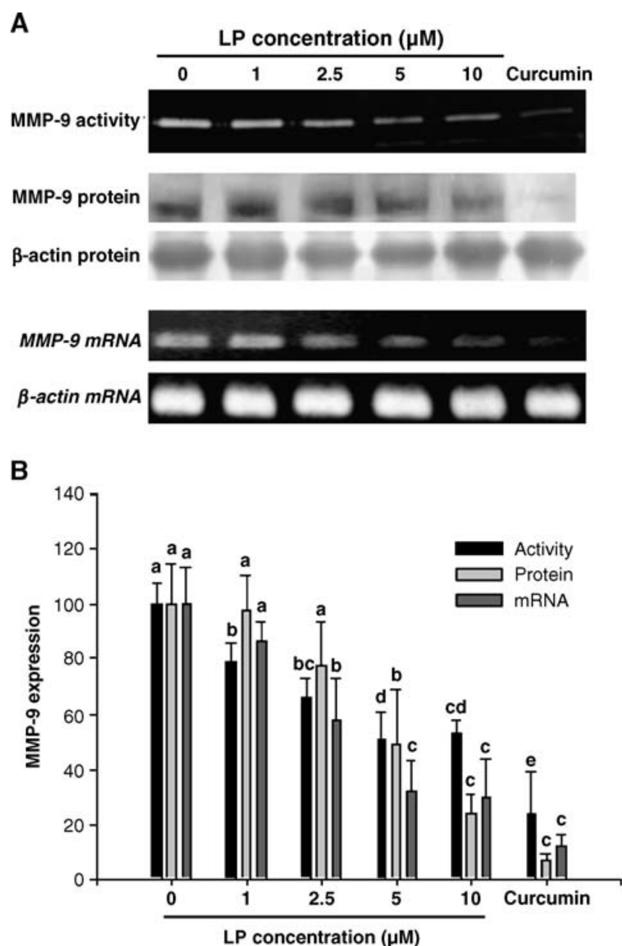

Fig. 2. Effects of lycopene (LP) concentration on MMP-9 production in SK-Hep-1 cells. Cells were incubated with lycopene for 2 h and washed twice in PBS before incubation with DMEM for 24 h. THF (0.1%) is the solvent control for lycopene; curcumin (20 μM) is the positive control. For loading control, expression of β-actin was analyzed using the same lysate. Values are means±S.D., $n \geq 3$; means without a common letter differ ($P<.05$).

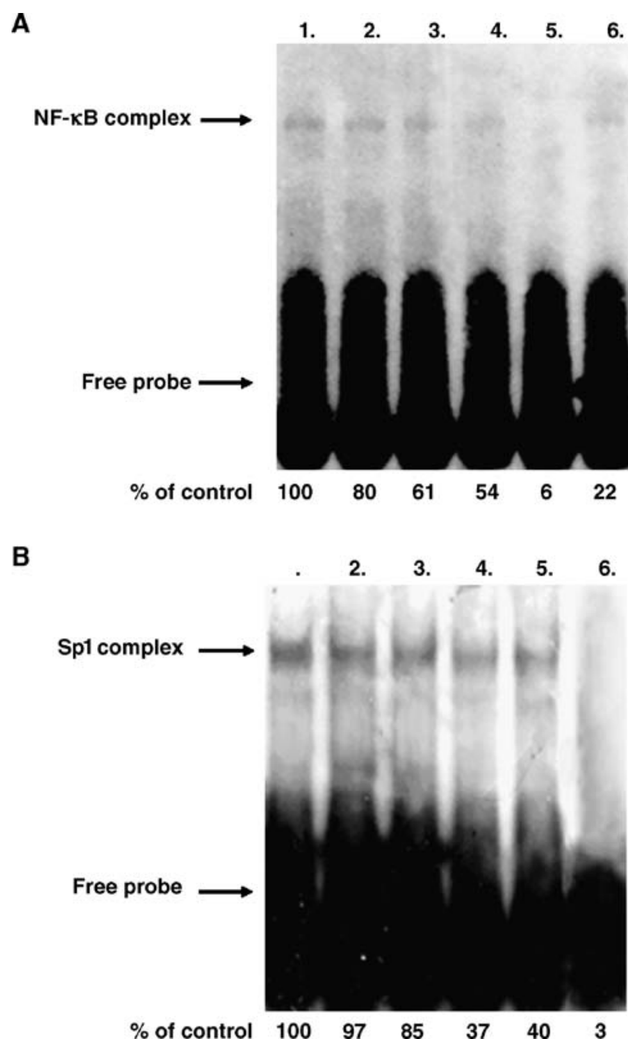

Fig. 3. Inhibition of lycopene on the binding activity of NF-κB (A) and Sp1 (B). Nuclear extracts were prepared from SK-Hep-1 cells pretreated with indicated concentrations of lycopene for 2 h and washed; then, incubation was continued for 24 h. EMSA was carried out as described in Section 2. Lane 1, solvent control (THF, 0.1%); lane 2, lycopene (1 μM); lane 3, lycopene (2.5 μM); lane 4, lycopene (5 μM); lane 5, lycopene (10 μM); lane 6, positive control (curcumin, 20 μM).

3. Results

3.1. Lycopene inhibits cell invasion in vitro

Treatment of SK-Hep-1 cells with lycopene up to 10 μM neither reduced cell viability nor induced cell morphological changes (data not shown). By contrast, lycopene treatment led to a dose-dependent inhibition of cell invasion at preincubation times of 2, 12 and 24 h (Fig. 1). The extent of inhibition by lycopene was similar among these time points and was the highest at 5 μM lycopene (57–63%, $P<.001$) at each time point. Therefore, we chose a preincubation time of 2 h for the following studies.

3.2. Lycopene uptake

After incubation with 1, 2.5, 5 and 10 μM lycopene (i.e., 10, 25, 50 and 100 nmol, respectively) for 2 h at 37°C in the dark, the lycopene uptake of SK-Hep-1 cells was 0.16±0.04, 0.49±0.10, 0.78±0.02 and 1.62±0.11 nmol, respectively (data not shown), which is equivalent to 1.6–2.0% uptake of lycopene (calculated as [(nmol lycopene/10^6)/(nmol/dish)]×100%).

3.3. Lycopene inhibits MMP-9 at both the protein and mRNA levels

Lycopene dose-dependently inhibited the MMP-9 activity between 1 and 5 μM ($P<.05$), whereas the levels of MMP-9 of 10 μM lycopene (53±5%) were not significantly different from those of 5 μM (51±10%; Fig. 2). We then analyzed the level of MMP-9 protein and mRNA in the cells, and we found that the expression of MMP-9 protein and mRNA was also suppressed by lycopene in a similar manner.

To determine whether lycopene directly inhibits MMP-9 activity, we incubated lycopene (10 μM) with the reaction buffer containing MMP gel in the absence of SK-Hep-1 cells in zymographic analysis [33], and we found no inhibition of MMP-9 by lycopene. We also determined the level of TIMP-1, the endogenous inhibitor of MMP-9 [6], and we found that lycopene at 1–10 μM did not significantly affect the level of TIMP-1 (data not shown).

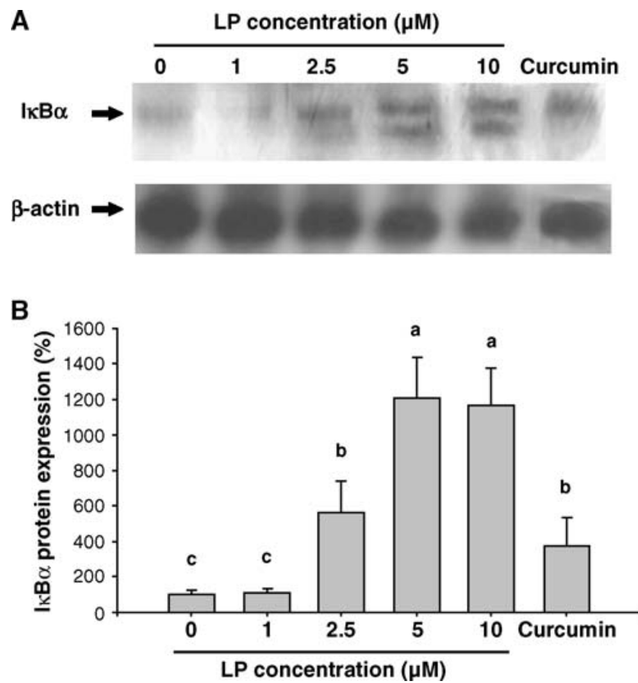

Fig. 4. Effects of lycopene on IκBα protein expression in SK-Hep-1 cells. Cells were incubated with lycopene for 2 h and washed twice in PBS before incubation in DMEM for 24 h. The protein extracts were subjected to Western blot analysis and compared with control. THF (0.1%) is the solvent control for lycopene; curcumin (20 μM) is the positive control. Values are means±S.D., $n \geq 3$; means without a common letter differ ($P<.05$).

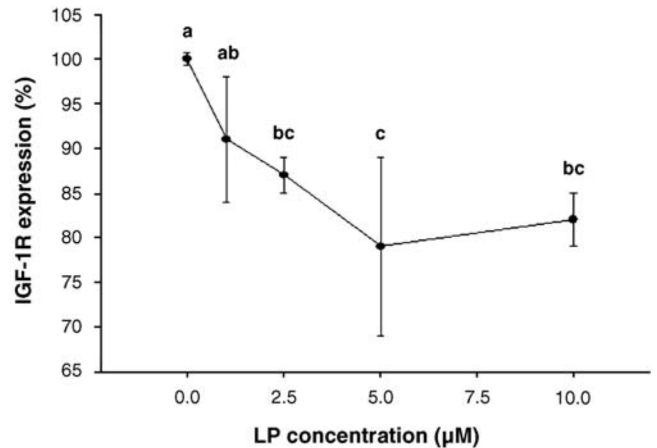

Fig. 5. Effects of lycopene (LP) on surface expression levels of IGF-1R in SK-Hep-1 cells. Cells were incubated with lycopene for 2 h and washed twice in PBS before incubation with DMEM for 24 h, after which cells were used for examining surface expression levels of IGF-1R by flow cytometry. THF (0.1%) is the solvent control for lycopene. Values are means±S.D., $n>3$; means without a common letter differ significantly ($P<.05$).

3.4. Lycopene inhibits the binding activity of NF-κB and Sp1

The MMP-9 promoter contains several transcription-factor-binding motifs, including NF-κB, AP-1 and Sp1 [34], and activation of binding activities of these transcription factors in tumor cells may contribute to MMP-9 transcription and cell invasion. We found that lycopene inhibited the binding activities of NF-κB and Sp1 in a dose-dependent manner (Fig. 3) and that these effects of lycopene were similar to those on MMP-9 protein and mRNA expression. Lycopene at 1–10 μM slightly inhibited AP-1 binding activity and the expression of c-Jun nuclear proteins (data not shown).

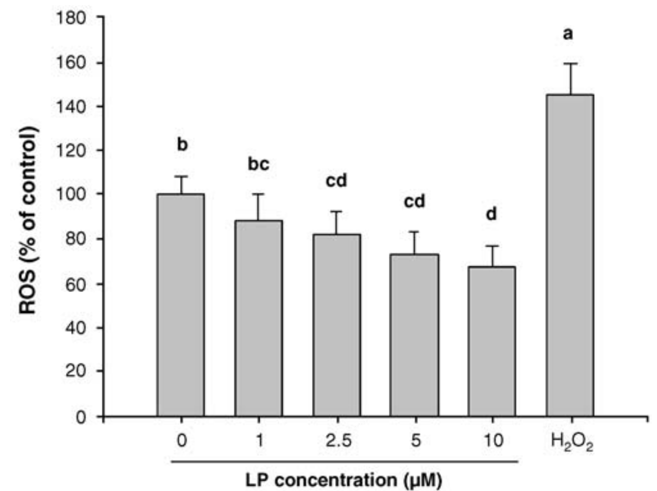

Fig. 6. Effects of lycopene (LP) on intracellular ROS of SK-Hep-1 cells. Cells were incubated with lycopene for 2 h and washed twice in PBS before incubation with DMEM for 24 h. THF (0.1%) is the solvent control for lycopene; H_2O_2 (1 mM) is the positive control. Values are means±S.D., $n>3$; means not sharing a common letter differ significantly ($P<.05$).

Table 1
Effects of lycopene, β-carotene and lycopene plus hydrogen peroxide (H_2O_2) on intracellular ROS, MMP-9 activity and invasion of SK-Hep-1 cells

Treatments	ROS (%)	MMP-9 (%)	Inhibition of invasion (%)
Control	100±6[b] (As 100)	100±8[a] (As 100)	100±13[a] (As 100)
Lycopene (10 μM)	67±16[c] (33↓)	53±5[c] (47↓)	59±6[b] (41↓)
β-carotene (10 μM)	71±4[c] (29↓)	95±6[ab] (5↓)	87±6[a] (13↓)
H_2O_2 (1 mM)	139±14[a] (39↑)	107±11[a] (7↑)	102±11[a] (2↑)
Lycopene+H_2O_2	98±14[b] (2↓)	81±14[b] (19↓)	66±4[b] (34↓)

The cells were incubated with lycopene, β-carotene or lycopene plus hydrogen peroxide at 37°C for 2 h. Values are means±S.D., $n \geq 3$; means in a column without a common letter are significantly different (P<.05). Data in parentheses are percentage changes, which are calculated as [1−(treatment/control)]×100%.

3.5. Lycopene enhances IκBα protein levels

Western blot analyses showed that lycopene enhanced IκBα protein expression and that the effects at 5 μM and 10 μM were similar (Fig. 4).

3.6. Lycopene reduces surface expression of IGF-1R

Lycopene significantly reduced the expression of IGF-1R in a dose-dependent manner in SK-Hep-1 cells (P<.05), with the highest inhibition found at 5 μM (ca. 21%, P<.001; Fig. 5).

3.7. Effects of lycopene, β-carotene and concomitant addition of hydrogen peroxide on ROS, MMP-9 and cell invasion

Lycopene significantly decreased the intracellular ROS in a dose-dependent manner (1–10 μM), with a reduction of 33% (P<.01) at 10 μM (Fig. 6). To examine whether the reduction of ROS by lycopene contributes to its reduction of the expression of MMP-9 and invasion activity of SK-Hep-1 cells, we compared the effects of lycopene with those of β-carotene. We found that β-carotene at 10 μM significantly decreased the intracellular ROS (by 29%, P<.01), an effect that was essentially the same as that of lycopene at the same concentration (10 μM). However, β-carotene at 10 μM only slightly inhibited MMP-9 protein expression (5%, P=.355) and cell invasion (13%, P=.192; Table 1). We also added hydrogen peroxide concomitantly with lycopene in order to minimize or abolish the antioxidant effect of lycopene in the cells, and we found that a coincubation of 1 mM hydrogen peroxide with lycopene was sufficient to raise the level of ROS in the lycopene treatment group (67±16%) to 98±14%, which is essentially the same as the control group (100±6%, i.e., without lycopene or hydrogen peroxide treatment). This treatment (i.e., hydrogen peroxide plus lycopene) significantly increased MMP-9 activity (from 53±5% for lycopene alone to 81±14%, P<.05), which was still below the control (100%), and the difference (19%) is attributable to the non-antioxidant effect of lycopene. However, this treatment did not significantly change the anti-invasion effect of lycopene (from 59±6% to 66±4%, P=.137).

4. Discussion

Lycopene has been reported to inhibit the invasion of SK-Hep-1 cells [21,22], but the mechanism underlying this action is not clear. In this study, we first confirmed our previous finding [21] that lycopene significantly inhibited cell invasion in a dose-dependent manner up to 5 μM. We then showed that lycopene significantly and dose dependently down-regulated the expression of MMP-9. We further showed that lycopene inhibited MMP-9 activity at the transcriptional level rather than inhibited the enzyme directly. The anti-invasive effect of lycopene correlated (r^2=.94, P<.001) with the down-regulation of MMP-9 secretion by lycopene, suggesting that the anti-invasive effect of lycopene is associated with the inhibition of enzymatically degradative processes of tumor invasion.

The mechanism by which lycopene inhibits MMP-9 appears to be complex (Fig. 7). Carotenoids including β-carotene and lycopene have been shown to regulate pathways converging at the AP-1, Sp1 and NF-κB binding sites [35–40]. Here, we showed that lycopene inhibited the binding activity of NF-κB and Sp1 in SK-Hep-1 cells, although it only slightly inhibited AP-1. The decrease in Sp1 and NF-κB binding ability affected by lycopene may be mediated in part by its suppression of IGF-1 signal cascades. It has been suggested that the IGF-1 signal pathway activates Sp1 and NF-κB [41,42], which then enhances the expression of MMPs [43,44]. Here, we showed that lycopene significantly decreased the surface expression of IGF-1R but only slightly decreased IGF-1 in SK-Hep-1 cells and that these actions of lycopene may lead

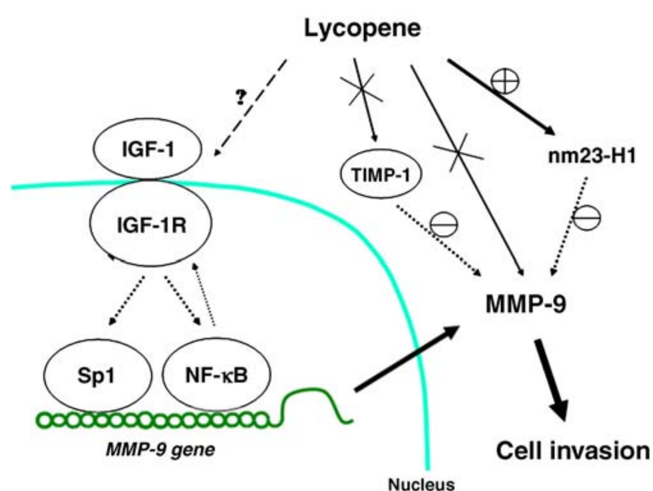

Fig. 7. Proposed mechanisms by which lycopene inhibits the invasion of SK-Hep-1 cells. Solid arrows indicate the evidence obtained from the present study and a prior study of ours [21]; dotted arrows are suggestive or based on evidence from the literature. ⊕, promotional; ⊖, inhibitory; ×, no effect; ?, suggestive.

to partial reduction of the MMP-9 expression. However, it should be noted that decreased NF-κB expression may also decrease IGF-1R signaling by feedback inhibition [45]. Several studies have demonstrated that lycopene possesses anticancer activity through the IGF-1 signal cascade [46–49]. For instance, Liu et al. [46] showed that lycopene supplementation (4.3 mg/kg BW/day, for 9 weeks) significantly increased the plasma level of IGF-binding protein-3 (165% of control) in ferrets. In rats, lycopene supplementation (200 ppm) for 8 weeks reduces IGF-1 expression in normal rat prostate [47] and in prostate cancer [48]. Further studies are needed to prove that lycopene may affect NF-κB and Sp1 through the suppression of the IGF-1 cascade.

Another possible mechanism by which lycopene inhibits MMP-9 activities is through increased expression of *nm23-H1*, a tumor metastasis suppressor gene [50]. The expression of MMPs, including MMP-9 and MMP-2, has been shown to be down-regulated by nm23-H1 protein [51,52]. We recently reported that lycopene inhibits the invasion of SK-Hep-1 cells and that this effect of lycopene is related to its up-regulation of nm23-H1 [21]. It has been reported that MMP-9 can be inhibited by forming a 1:1 complex with TIMP-1 [53,54]. However, we found that TIMP-1 expression in SK-Hep-1 cells was not significantly affected by lycopene treatment, suggesting that the inhibition of MMP-9 activity by lycopene is not related to TIMP-1.

Intracellular ROS have been shown to activate the NF-κB signal cascades, and antioxidants including carotenoids have been shown to block these cascades [35–40,55]. Indeed, the antioxidant activity of lycopene has been suggested to be related to its anti-invasion activity [17]. However, several lines of evidence obtained from the present study indicate that the antioxidant activity of lycopene does not play a major role in its anti-invasion activity. First, we found that the decrease of intracellular ROS by lycopene was dose dependent up to 10 μM, rather than 5 μM, as we found in other effects of lycopene including those of NF-κB and MMP-9. Second, β-carotene, a carotenoid similar to lycopene, inhibited intracellular ROS to the same extent as lycopene (both at 10 μM) but did not significantly affect cell invasion. Third, concomitant incubation of cells with lycopene and hydrogen peroxide (1 mM) abolished the antioxidant activity of lycopene but did not affect the anti-invasion ability of lycopene.

In summary, we demonstrate that lycopene strongly inhibits the invasion of SK-Hep-1 cells and that this effect is likely mediated by inhibition of NF-κB and Sp1 binding activity, leading to decreased secretion of MMP-9. By contrast, the antioxidant activity of lycopene appears to play a minor role, if any, in the anti-invasion ability of lycopene.

Acknowledgments

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