Lycopene Inhibits Cell Migration and Invasion and Upregulates Nm23-H1 in a Highly Invasive Hepatocarcinoma, SK-Hep-1 Cells

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ABSTRACT The carotenoid lycopene has been associated with decreased risks of several types of cancer, such as prostate cancer and hepatoma. Tumor metastasis is the most important cause of cancer death. Although lycopene was shown to inhibit metastasis, the mechanism underlying this action is not well understood. Here, we tested the possibility that lycopene may inhibit cancer cell metastasis by upregulating the expression of nm23-H1, a metastasis suppressor gene, in SK-Hep-1 cells, a highly invasive hepatoma cell line, and we determined migration and invasion activities and the expression of nm23-H1 protein and mRNA. We showed that lycopene inhibited SK-Hep-1 migration and invasion in a bell-shaped manner, with the highest effect at 5 μmol/L (91 and 63% inhibition for migration and invasion, respectively; \( P < 0.05 \)). At the same test level (10 μmol/L), lycopene was much more effective than β-carotene in reducing cell invasion (by ~87%). In contrast to the effects on migration and invasion, lycopene enhanced nm23-H1 expression at both the protein and mRNA levels; the effects were also bell shaped, and at 5 μmol/L, lycopene enhanced nm23-H1 protein and mRNA expressions by 220 and 153 ± 22% \( (P < 0.01) \), respectively. These bell-shaped effects of lycopene may be related to autooxidation of lycopene at elevated concentrations (≥10 μmol/L). Significant correlations existed between nm23-H1 protein expression and migration \( (r^2 = 0.78, P < 0.001) \) and between nm23-H1 protein expression and invasion \( (r^2 = 0.84, P < 0.001) \) in lycopene-treated SK-Hep-1 cells. We conclude that lycopene has significant antimigration and anti-invasion activity, and that this effect is associated with its induction of nm23-H1 expression. J. Nutr. 135: 2119–2123, 2005.

KEY WORDS: • lycopene • migration • invasion • nm23-H1 • hepatocellular carcinoma cell

Tumor metastasis, the process by which tumor cells leave a primary tumor to colonize other sites of the body, is a major cause of death for cancer patients. Metastasizing cells must first disseminate from the primary tumor, invade the surrounding tissue, intravasate and extravasate the circulatory system, arrest, initiate angiogenesis, and colonize distant sites, while evading the immune system (1,2). The nm23 gene was first identified as a gene whose expression was reduced in highly metastatic rodent tumors relative to poorly metastatic tumor cells (3). It is located on chromosome 17q 21 and codes for an 18.5-kDa protein containing 166 amino acids with nucleoside diphosphate kinase and protein-histidine kinase activities, as well as serine auto phosphorylation activity (4,5). The transcription of nm23 cDNA into various cancer cell lines results in the suppression of metastatic potential of motility, invasion, or colonization (6–12), indicating that nm23 is a potential metastasis suppressor gene that could function on the invasion and migration steps of the metastatic pathway. Eight human nm23 genes have been characterized to date, of which the H1 gene is most closely correlated with the metastatic phenotype in human breast carcinoma, colorectal carcinoma, ovarian carcinoma, and hepatocarcinoma (13–19).

Carotenoids including β-carotene and lycopene possess several common biological functions such as photoprotection, antioxidant effects, and immunomodulatory and anticancer activity (20,21). Elevated intakes of lycopene have been associated with lowered risk of several types of cancer (22–24). Studies suggested that the anticancer effects of carotenoids such as lycopene are related to their effectiveness as antioxidants, singlet oxygen quenchers, and free radical scavengers (25–30). However, the mechanisms by which lycopene decreases the risk of cancer are not well understood.

Kozuki et al. (31) suggested that the antioxidant property of lycopene may partly explain its anti-invasive action. By contrast, Collins (32) indicated that the antioxidant property of carotenoids is not related to their anticancer ability. Recently, several studies showed that carotenoids affect the transcription of various genes, such as connexin 43 (32–34). In the present study, we hypothesized that lycopene may exert its antimetastatic effects through upregulation of an antimetastatic gene, nm23-H1. Because liver cancer is the most endemic cancer in Taiwan and in much of the world, we employed a highly invasive hepatocarcinoma, SK-Hep-1 cells, to examine the effects of lycopene on cell migration and invasion and the possible mechanisms underlying such actions.
MATERIALS AND METHODS

Chemicals. The cell line SK-Hep-1 was a generous gift from Dr. T. Z. Liu (Graduate Institute of Clinical Medicine, Chang Gung College of Medicine and Technology, Taiwan). All chemicals used were of reagent or higher grade. Lycopene was delivered to the cell using tetrahydrofuran (THF, Merck) solvent, containing 0.25% BHT to avoid formation of peroxides. DMEM, fetal bovine serum (FBS), trypsin, penicillin, streptomycin, sodium pyruvate, nonessential amino acid, and G418 media were from Gibco/BRL. Transwells were from Costar. Anti-nm23 mouse monoclonal antibody (mAb) and anti-mouse IgG-HRP antibody were purchased from BD and Stressgen, respectively.

Cell culture and lycopene incorporation. SK-Hep-1 cells were grown in DMEM medium containing 10% (v/v) FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml sodium pyruvate, 0.37% sodium bicarbonate, penicillin (100 U/ml), streptomycin (100 μg/ml) in a humidified incubator under 5% CO2 and 95% air at 37°C. The cells were harvested at ~90% confluence (106 cells/dish). The survival rate of cells was always >95% by Trypan-blue assay (35). A stock THF-lycopene solution (20 mmol/L) was freshly prepared before each experiment, and the concentration of the stock solution was always ≥19 mmol/L, as determined using an extinction coefficient of 1.85 × 104 (mol/L)−1 cm−1 at 472 nm after 1.104 dilution in THF. The purity of commercial lycopene was ~97%, which compares well with the 98% purity claimed by the supplier (Wako). THF-lycopene was added to the culture medium at a final concentration of 1, 2.5, 5, 10, or 20 μmol/L. The final concentration of THF in the culture medium was 0.1% (v/v, ~1.2 μmol/L), which did not affect the assays described below. SK-Hep-1 cells (~105 cells/dish) were incubated with THF-lycopene at 37°C in the dark for 2 h, as described in other cell lines (36,37). The cells were then washed 3 times in PBS (pH7.4).

MTT assay. The effect of lycopene on cell viability was estimated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide (MTT) assay, as described previously (38). Cells were cultured in 24-well plates at 105 cells/well in DMEM for 24 h, and each well was washed and then filled with 1 mL of DMEM containing various concentrations of lycopene and incubated for 2 h at 37°C, washed twice in PBS, and then incubated in DMEM for 24 h to observe cytotoxicity, if any (the longest incubation time for various assays employed in this study was 24 h). Each well was then incubated with MTT for 1 h, after which the liquid was removed, and dimethyl sulfoxide 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).

Cell proliferation assay. SK-Hep-1 cells were seeded at ~105 cells/well in DMEM containing 10% FBS, 0.1% (v/v, NaHCO3 free), placed in the upper transwell chamber, and then incubated for 5 h at 37°C. Then, the cells on the upper surface of the filter were completely wiped away with a cotton swab. The cells on the lower surface of the filter were fixed in methanol, stained with G418, and counted under a microscope. For each replicate, the tumor cells in 10 randomly selected fields were determined, and the counts were averaged.

Cell invasion assay. The procedure reported by Repesh (39) for the cell invasion assay was similar to that for cell migration. The invasion of tumor cells was assessed in transwell chambers with a 6.5-mm-diameter polyvinyl/pyrrolidone-free polycarbonate filter of 8-μm pore size. 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 × 105/L and a 100 μL aliquot containing 5 × 104 cells was added to each of 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 invasive activity.

Western blotting. Expression levels of endogenous nm23-H1 protein were determined by immunoblotting. Briefly, the medium was removed and cells were lysed with 20% SDS containing 1 mmol/L phenylmethylsulfonyl fluoride. The lystate was sonicated for 30 s on ice, followed by centrifugation at 12,000 × g for 30 min at 4°C. An amount of protein (40 μg) from the supernatant was resolved by SDS-PAGE and transferred onto a nitrocellulose membrane. After blocking with TBS buffer (20 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.4) containing 5% nonfat milk, the membrane was incubated with anti-nm23-H1 monoclonal antibody (BD Biosciences) followed by horseradish peroxidase-conjugated anti-mouse IgG, and then visualized using an ECL chemiluminescent detection kit (Amersham).

RT-PCR (RNA isolation and sequencing). Total cellular RNA was isolated from cell culture (RNAzol-kit), reverse-transcribed into cDNA (MMLV-Reverse Transcriptase, Gibco/BRL) using oligo (dT)15 as primers, and then coamplified with 4 primer pairs on nm23-H1 and β-actin (internal control) sequences. The primers for amplifying nm23-H1 cDNA were 5′-CTGGAACCAGCCTGGG-3′, located in the 5′-untranslated region, and 5′-TCAGGGATGTAAACACTGTA-3′, located in the 3′-untranslated region. The primers for amplifying β-actin cDNA were 5′-GTTGGGCGCGCCTCACCAAC-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, followed by a final incubation at 72°C for 7 min. The optimal number of cycles was 29. The sizes of the amplification products of nm23-H1 and β-actin were 702 and 541 bp, respectively. The PCR products were subjected to 1% agarose gel electrophoresis and stained with ethidium bromide. The relative nm23-H1 levels were quantitated by Matrox Inspector 2.1 software.

Statistical analysis. Values are expressed as means ± SD and analyzed using 1-way ANOVA followed by Duncan’s multiple range test for comparisons of group means. Differences were considered significant at P < 0.05.

RESULTS

Effects of lycopene and β-carotene on in vitro migration and invasion of SK-Hep-1 cells. Neither lycopene (1 to 20 μmol/L) nor β-carotene (10 μmol/L) induced morphological changes or cytotoxicity of SK-Hep-1 cells (data not shown). However, lycopene inhibited SK-Hep-1 cell migration in a bell-shaped manner at preincubation times of 2, 6, 12, and 24 h. LP1, LP2.5, LP5, LP10, and LP20 represent 1, 2.5, 5, 10, and 20 μmol/L, respectively. THF is the solvent for lycopene. Values are means ± SD, n = 3; means without a common letter differ, P < 0.05.

FIGURE 1 Effects of lycopene (LP) and β-carotene (BC, 10 μmol/L) on migration of SK-Hep-1 cells at preincubation times of 2, 6, 12, and 24 h. LP1, LP2.5, LP5, LP10, and LP20 represent 1, 2.5, 5, 10, and 20 μmol/L, respectively; THF is the solvent for lycopene. Values are means ± SD, n = 3; means without a common letter differ, P < 0.05.
and 870%, respectively, compared with a common letter differ, and LP20 represent 1, 2.5, 5, 10, and 20 μmol/L protein (220 mRNA (153/H11006 lycopene (174 nm23-H1 Lycopene treatment moderately upregulated the expression of nm23-H1 protein (220). The expression of nm23-H1 protein was affected by lycopene in a bell-shaped manner (Fig. 3). The solvent control (THF, 0.1%) did not affect the expression of nm23-H1 protein. At 5 μmol/L, lycopene induced the highest expression of nm23-H1 protein (220 ± 33%, P < 0.01); at 10 and 20 μmol/L lycopene, nm23-H1 protein levels were 174 ± 17 and 125 ± 8% of the control, respectively. β-Carotene at 10 μmol/L also enhanced nm23-H1 protein expression, but the level (149 ± 14%) was lower (P < 0.01) than that of 10 μmol/L lycopene (174 ± 17%).

Upregulation of nm23-H1 by lycopene at the protein level. Lycopene treatment moderately upregulated the expression of nm23-H1 in a dose-dependent manner (Fig. 4). The optimal concentration of lycopene was 5 μmol/L, which induced the highest expression of nm23-H1 mRNA (153 ± 22% of the control, P = 0.006). In contrast, lycopene was somewhat more effective in inducing nm23-H1 mRNA than β-carotene (10 μmol/L; 133 ± 14 vs. 121 ± 12%).

Correlation of nm23-H1 with cell migration and cell invasion. Nm23-H1 protein expression was negatively correlated with migration (r² = 0.78, P < 0.001) and invasion (r² = 0.84, P < 0.001) in SK-Hep-1 cells (Fig. 5).

Effects of autoxidized lycopene and concomitant addition of α-tocopherol. Because the antimigration and anti-invasion effects of 10 μmol/L lycopene were lower than those of 5 μmol/L lycopene, we suspected that autoxidation of lycopene might have occurred at elevated concentrations of lycopene (≥10 μmol/L). Indeed, we found that a combination of 10 μmol/L lycopene with 10 μmol/L α-tocopherol during the 2-h incubation with SK-Hep-1 cells reduced the oxidation of lycopene from 35 to 12% (data not shown) and that the combined incubation significantly increased the antimigration and anti-invasion ability and the expression of nm23-H1 induced by 10 μmol/L lycopene, whereas α-tocopherol itself at 10 μmol/L had no effect (Table 1).

FIGURE 3 Effects of lycopene (LP) concentration and β-carotene (BC, 10 μmol/L) on nm23-H1 protein expression in SK-Hep-1 cells. Cells were incubated with lycopene for 2 h and then washed twice in PBS before incubation with DMEM for 5 h. LP1, LP2.5, LP5, LP10, and LP20 represent 1, 2.5, 5, 10, and 20 μmol/L, respectively; THF is the solvent for lycopene. (A) Western blots of nm23-H1 and β-actin. (B) Densitometric analysis of Panel A. For loading control, expression levels of β-actin were analyzed using the same lysate. Values are means ± SD, n = 3; means without a common letter differ, P < 0.05.

DISCUSSION

Both lycopene and β-carotene were reported to possess antimitastatic activity (31,40), but the mechanisms underlying such actions are not clear. In this study, we showed that lycopene inhibited the metastasis of the human hepatoma cell line, SK-Hep-1 cells, as evidenced by decreased cell migration and invasion in a dose-dependent manner up to 5 μmol/L. At the same concentration, lycopene was more effective than β-carotene in reducing cell invasion (by ~870%). These findings indicate that lycopene is a potent antimitastatic nutrient. Our results agree with those of Kozuki et al. (31), who showed that lycopene inhibits the invasion of rat ascites hep-
ataoma AH109A cells in a dose-dependent manner up to 5 μmol/L. Rooprai et al. (41) also showed that lycopene is able to reduce the motility of brain tumor cells. Although Okajima et al. (29) found no significant effects of lycopene on the invasion of rat urinary bladder transitional cell carcinomas, it should be noted that these authors used tomato juice rather than pure lycopene.

The decrease in the metastasis-associated phenotypes, such as cell migration and cell invasion, induced by lycopene treatment may be mediated in part by its upregulation of nm23-H1 expression because these effects are highly correlated. Moreover, the expression of nm23-H1 protein was similar to that of nm23-H1 mRNA, indicating that induction of nm23-H1 protein in response to lycopene may rely entirely on upregulation at the transcriptional level.

An interesting observation of the present study is that the effects of lycopene on cell migration, invasion, and the expression of nm23-H1 (at both the protein and mRNA levels) were all bell-shaped; i.e., the effects were all lower at 10 μmol/L lycopene than at 5 μmol/L. In agreement with the present finding, carotenoids including β-carotene and lycopene were shown to have lowered effectiveness as antioxidants and antcarcinogens in vitro at concentrations > 10 μmol/L (42,43). A possible explanation for the bell-shaped effects is that the antioxidant activity of β-carotene may shift into a prooxidant activity, depending on carotenoid concentration inside the cells and on the oxygen tension of the biological environment as well as on cell redox status (44). Here, our results suggest that lycopene at concentrations ≥ 10 μmol/L may undergo more rapid autoxidation and lead to decreased effects because concomitant incubation of SK-Hep-1 cells with α-tocopherol and lycopene (both at 10 μmol/L) protected against lycopene autoxidation and increased the anti-invasion, antimigration and nm23-H1-enhancing effects of lycopene. These results appear to support the notion that the antimetastatic effects are also related to the antioxidant properties of lycopene (29–31,45). However, this issue is unsettled because β-carotene, a carotenoid similar to lycopene, inhibits only cell migration, not cell invasion.

![FIGURE 4](image)

**FIGURE 4**  Effects of lycopene (LP) concentration and β-carotene (BC, 10 μmol/L) on nm23-H1 mRNA expression in SK-Hep-1 cells. Cells were incubated with lycopene for 2 h and then washed twice in PBS before incubation with DMEM for 24 h. LP1, LP2.5, LP5, LP10, and LP20 represent 1, 2.5, 5, 10, and 20 μmol/L, respectively; THF is the solvent for lycopene. (A) RT-PCR of nm23-H1 and β-actin. (B) Densitometric analysis of Panel A. Values are means ± SD, n = 3; means without a common letter differ, P < 0.05.

![FIGURE 5](image)

**FIGURE 5**  Correlation of nm23-H1 protein expression with numbers of migration cells (A) and with numbers of invasion cells (B). Data are from Figures 2 and 4 (A) and from Figures 3 and 4 (B).

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<th>TABLE 1</th>
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<tr>
<th>Treatment</th>
<th>Migrating cells</th>
<th>Invading cells</th>
<th>Relative nm23-H1 protein expression</th>
<th>Relative nm23-H1 mRNA expression</th>
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<tr>
<td>Control</td>
<td>910 ± 61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>280 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100 ± 11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>α-T</td>
<td>980 ± 68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>319 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108 ± 13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>98 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>LP</td>
<td>227 ± 22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>172 ± 18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>174 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>133 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>LP + α-T</td>
<td>168 ± 37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>213 ± 24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>1</sup> Values are means ± SD, n = 3; means in a column without a common letter differ, P < 0.05.

<sup>2</sup> SK-Hep-1 cells were incubated with lycopene (10 μmol/L), α-tocopherol (10 μmol/L), or their combination at 37°C for 2 h.
The concentrations of lycopene (1–5 μmol/L) used in the present study are relatively high compared with plasma lycopene concentrations in humans (50–900 nmol/L) (22,23,46). However, plasma levels of lycopene can increase markedly with lycopene supplementation. For instance, Edwards et al. (47) reported an increase in plasma lycopene from 428 to 960 nmol/L over 3 wk using 18.4 mg/d lycopene from 240 g (~1 cup/d) of tomato juice. In addition, pure lycopene was reported to be ~3 times as bioavailable to humans as lycopene from steamed and pureed tomatoes (48). Thus, it appears that the concentrations of lycopene used in our study are not exceedingly high and may be reached in vivo by lycopene supplementation.

In summary, we demonstrated that lycopene has significant antimigration and anti-invasion activities against SK-Hep-1 cells, and that this effect is associated with its induction of nm23-H1 expression. Further studies are warranted to verify the in vivo relevance of these findings.

LITERATURE CITED