Lycopene Inhibits Proliferation, Invasion and Migration of Human Breast Cancer Cells

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Abstract – Breast cancer has been estimated as one of the most common causes of cancer death among women. The major cause of death from breast cancer is the metastatic spread of the disease from the primary tumor to distant sites in the body. Lycopene is one of the major carotenoids in fruits and vegetables including tomatoes. Epidemiological studies have shown that the dietary intake of lycopene is associated with decreased risk of cancer. Although mounting evidence shows the chemopreventive effect of lycopene, the role of lycopene in the prevention of metastatic potential of breast cancer has not been determined yet. In the present study, we investigated the inhibitory effect of lycopene on invasive and migratory phenotypes of two highly aggressive breast cancer cell lines, H-Ras-transformed MCF10A human breast epithelial cells (H-Ras MCF10A) and MDA-MB-231 human breast cancer cells. Here, we report that lycopene significantly inhibits invasion and migration as well as proliferation of H-Ras MCF10A and MDA-MB-231 cells. This study suggested an *in vitro* anti-cancer and anti-metastatic potential of lycopene. We also showed that activations of ERKs and Akt were inhibited by lycopene in H-Ras MCF10A cells, suggesting that the ERKs and Akt signaling pathways may be involved in lycopene-induced anti-proliferative and/or anti-invasive/migratory effects in these cells. Taken in conjunction with the fact that breast cancer metastasis is one of the most lethal malignancies in women, our findings may provide useful information for the application of lycopene in establishing strategy to prevent the metastatic breast cancer.

Keywords: Lycopene, H-Ras MCF10A, MDA-MB-231, Invasion, Breast cancer

INTRODUCTION

Breast cancer has been estimated as the most commonly diagnosed type of cancer and the second most common cause of cancer death among women (Jemal *et al.*, 2007). Metastasis, a characteristic of highly malignant cancers with poor clinical outcome, has been one of the major causes of mortality in breast cancer patients. Thus, inhibition of the invasion and metastasis of cancer cells is of great significance in cancer treatment (Hwang and Lee, 2006). Since metastasis represents the most important cause of cancer death, anti-tumor agents that may inhibit this process have been extensively pursued. Metastasis of malignantly transformed cells is a multi-step process, which involves detachment of cells from the primary tumor,

attachment to the extracellular matrix (ECM), degradation of the ECM components (invasion) and migration of cells through degraded matrix (Stetler-Stevenson *et al.*, 1993). A role for members of matrix metalloproteinase (MMP) family on tumor invasion and metastasis has been suggested, especially, MMP-2 and MMP-9 (Liotta *et al.*, 1979; DeClerck *et al.*, 1992).

Elevated levels of the Ras protein are detected in 60-70% of human primary breast carcinomas (Clair *et al.*, 1987) and Ras has been suggested as a marker of tumor aggressiveness in breast cancer. We previously showed that H-Ras, but not N-Ras, induced invasive and migratory phenotypes in MCF10A human breast epithelial cells (Moon *et al.*, 2000). We also showed that H-Ras-induced invasiveness was associated with the activation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERKs), resulting in induction of MMP-9 and MMP-2 (Kim *et al.*, 2003; Shin *et al.*, 2005; Song *et al.*, 2006).

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Chemoprevention is arguably one of the major weapons in the anticancer arsenal (Ferguson, 1994; Stavric, 1994). Efforts have been made to develop a chemoprevention strategy that can inhibit proliferation of malignant cancer cells. There has been a growing body of evidence that phytochemicals in our diet can reduce the risk of cancers. Cancer chemoprevention has emerged as a new strategy against cancer, using of pharmacologic or natural agents that inhibit the development of invasive cancer (Kim and Moon, 2004).

Carotenoids are important phytochemicals that are known for the protective effects of fruits and vegetables (Rao and Agarwal, 1999). Epidemiological studies reported that dietary intake of a carotenoid lycopene is associated with decreased risk of cancer (Franceschi *et al.*, 1994; Giovannucci, 1999; Agarwal and Rao, 2000). Lycopene is the pigment principally responsible for the bright red color found in ripe tomatoes (*Lycopersicon esculentum*) and tomato products (Omoni and Aluko, 2005). The structure of lycopene is a lipophilic, 40-carbon atom, highly unsaturated, straight chain hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds as depicted in Fig. 1 (Boileau *et al.*, 1999; Rao and Agarwal, 2000; Shi, 2000). The 11 conjugated double bonds of lycopene make it a potentially powerful anti-oxidant and lycopene has most anti-oxidant effect among carotenoids (Stahl and Sies, 1996; Arab and Steck, 2000). Epidemiological studies have demonstrated that high consumption of lycopene reduces the risk of breast and other types of human cancers (Rao and Agarwal, 1999; Weisburger, 2002; Wu *et al.*, 2003). Giovannucci (1999) reported that intake of tomatoes and tomato-based products and plasma levels of lycopene have been relatively consistently associated with a lower risk of a variety of cancers. Several *in vitro* studies have demonstrated the anti-proliferation property of lycopene in endometrial, mammary, lung, and prostate human cancer cells (Levy *et al.*, 1995; Kim *et al.*, 2002). In an *in vivo* study, lycopene-fed mice had delayed onset and reduced spontaneous mammary tumor growth and development (Nagasawa *et al.*, 1995). Recently, it has been demonstrated that lycopene reduced experimental tumor metastasis and such an action was associated with

attenuation of proliferation, invasion and angiogenesis of hepatoma cells (Huang *et al.*, 2008).

In the present study, we attempted to investigate the *in vitro* chemopreventive effect of lycopene and the possible mechanism of action on two highly aggressive breast cancer cell lines, H-Ras-transformed MCF10A human breast epithelial cells (H-Ras MCF10A) and MDA-MB-231 human breast cancer cells. Here, we report that lycopene inhibits proliferation of H-Ras MCF10A and MDA-MB-231 cells. We also provide evidence that lycopene reduces invasive and migratory properties of breast cancer cells, suggesting a possible application of lycopene for prevention of metastatic breast cancer.

MATERIALS AND METHODS

Cell lines and culture condition

H-Ras MCF10A cells were established as previously described (Moon *et al.*, 2000). H-Ras MCF10A cells were maintained at 37°C in a humidified atmosphere of 5% CO₂/95% air in DMEM/F12 medium supplemented with 5% heat-inactivated horse serum, 10 µg/ml insulin, 100 ng/ml cholera toxin, 0.5 µg/ml hydrocortisone, 20 ng/ml recombinant EGF, 2 mM L-glutamine, and 100 µg/ml penicillin-streptomycin and 0.5 µg/ml fungizone. MDA-MB-231 cells were kindly provided by Dr. Dong Young Noh (Seoul National University, Seoul, Korea) and cultured in DMEM supplemented with 10% FBS and 100 µg/ml penicillin-streptomycin. The cells were maintained in humidified atmosphere with 95% air and 5% CO₂ at 37°C.

Preparation of lycopene

Lycopene (99%) was purchased from Sigma Chemical (St. Louis, MI). Lycopene was dissolved in tetrahydrofuran (THF) containing 0.25% butylated hydroxytoluene (BHT). Stock solution was prepared under the minimum of exposure to air and light and stored at -70°C. Immediately before the experiment, aliquots from the stock solution were added to the cell culture medium, and the final concentration of lycopene was ranged from 0.1 µM to 20 µM. All procedures including cell treatment were carried out under dim light and on the ice. The concentration of THF in the media was 0.25%, which did not cause cytotoxicity (data not shown).

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay

Cells (5×10^3) cultured in a 96-well plate were treated with lycopene for 24 hr. After 24 hr of incubation, 25 µl of 0.5 mg/ml of MTT was added and incubated for 4 hr.

Fig. 1. Structure of all-trans lycopene which is the most prominent isomer found in fresh tomatoes (Cunningham *et al.*, 1994).

Conversion of MTT into purple formazan by metabolically active cells indicates the extent of cell viability. The crystals of produced formazan were dissolved with 100 μ l of DMSO and the optical density was measured at 540 nm using a micro-ELISA reader (Molecular Devices, Sunnyvale, CA) for quantification of cell viability. Assays were performed triplicate.

In vitro invasion assay

In vitro invasion assay was performed using 24-well transwell unit with polycarbonate filters (Corning Costar, Cambridge, MA) as previously described (Kim *et al.*, 2003). The lower side of the filter was coated with type I collagen, and the upper side was coated with Matrigel (Collaborative Research, Lexington, KY). Lower compartment was filled with serum-free media containing 0.1% BSA. Cells were placed in the upper part of the Transwell plate, incubated for 17 hr, fixed with methanol and stained with hematoxylin for 10 min followed briefly by eosin. The cells on the upper surface of the filter were removed using a cotton swab. The invasive phenotypes were determined by counting the cells that invaded to the lower side of the filter with microscopy at $\times 400$. Thirteen fields were counted for each filter and each sample was assayed in triplicate.

Transwell migration assay

In vitro migration assay was performed using a 24 well transwell unit with polycarbonate filters as previously described (Kim *et al.*, 2003). Experimental procedures were the same as the *in vitro* invasion assay described above except that the filter was not coated with Matrigel for the migration assay.

Western blot analysis

Western blot analysis was performed as previously described (Shin *et al.*, 2005). Protein extracts in lysis buffer (0.5% Triton X-100, 0.15M NaCl, 50 mM Tris-HCl, pH7.4, 25 mM NaF, 20 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4)

containing protease inhibitor cocktail (Roche, Mannheim, Germany) were subjected to 12% SDS-PAGE analysis and transferred to nitrocellulose membrane. The levels of Bcl-2 and Bax were detected using anti-Bcl-2 (DAKO, Denmark) and anti-Bax (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) antibodies. Anti-ERK, anti-phosphorylated ERK, anti-p38, anti-phosphorylated p38, anti-Akt, anti-phosphorylated Akt, anti-SAPK/JNK, anti-phosphorylated-SAPK/JNK were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Enhanced chemiluminescence (ECL, Amersham-Pharmacia) system was used for detection. Relative band intensities were determined by quantitation of each band with an Image Analyzer (Vilber Lourmet).

RESULTS AND DISCUSSION

A dose response study was conducted to examine the effect of lycopene on the growth of breast cancer cell lines. Treatment with lycopene for 24 hr inhibited the growth of H-Ras MCF10A cells in a dose-dependent manner with the IC_{50} value of 1.3 μ M (Fig. 2, open circle). Following 24 hr of treatment with lycopene, the proliferation of MDA-MB-231 cells was also decreased with the IC_{50} value of 13.4 μ M (Fig. 2, closed circle). Lycopene was more effective in inhibiting proliferation of H-Ras MCF10A cells compared to that of MDA-MB-231 cells. Consistent with our results, it has previously been reported that lycopene inhibited the growth of MCF-7 and MDA-MB-231 breast cancer cells (Prakash *et al.*, 2001).

Numerous natural products have been shown to exert growth-inhibitory effects on malignant cells through induction of apoptosis. It has been shown that lycopene exerts anti-proliferative effect on human colon carcinoma cells, human prostate carcinoma cells and a prototype of Burkitt lymphoma cells by induction of apoptosis (Hwang and Bowen, 2004; Salman *et al.*, 2007). We then investigated if the anti-proliferative effect of lycopene involves

Fig. 2. Effects of lycopene on the growth of H-Ras MCF10A cells and MDA-MB-231 cells. Cells were treated with various concentrations of lycopene for 24 hr and subjected to MTT assay. The results represent means $\pm$ SE of triplicates. *, **Statistically different from control at $p < 0.05$ and $p < 0.01$, respectively.

Fig. 3. Effects of lycopene on the expression of Bcl-2 and Bax. H-Ras MCF10A cells and MDA-MB-231 cells were treated with various concentrations of lycopene for 24 hr. Western blot analysis was performed on cell lysates using anti-Bax antibody (A) and anti-Bcl-2 antibody (B). β -actin was used as a loading control.

Fig. 4. Effects of lycopene on the invasion and migration of H-Ras MCF10A cells and MDA-MB-231 cells. The abilities of the invasion and migration of H-Ras MCF10A cells (A) and MDA-MB-231 cells (B) were measured using Transwell. The number of invaded or migrated cells per field was counted ($\times 400$) in thirteen arbitrary visual fields. *, **Statistically different from control at $p < 0.05$ and $p < 0.01$, respectively.

apoptosis in H-Ras MCF10A and MDA-MB-231 cells. Bcl-2 is the first identified member of a large family of apoptosis-regulating proteins, consisting of blockers (such as Bcl-2) and promoters (such as Bax) of cell death (Yang and Korsmeyer, 1996). In order to examine the mode of growth inhibition by lycopene, we determined the protein levels of two key apoptosis-linked gene products, Bcl-2

and Bax. As shown in Fig. 3A, the expression level of the death-promoting Bax was not affected by lycopene treatment in H-Ras MCF10A cells and MDA-MB-231 cells. Lycopene did not alter the expression of the anti-apoptotic oncoprotein Bcl-2 in both cell lines (Fig. 3B). These data suggest that the inhibitory effect of lycopene on proliferation of H-Ras MCF10A cells and MDA-MB-231 cells

may not be due to induction of Bax or Bcl-2-related apoptotic cell death.

Invasive and migratory properties of cells are considered crucial in metastatic process of cancer. While many studies support the anti-proliferative effect of lycopene in a variety of cancers, there has been a limited amount of information on the anti-invasive and anti-metastatic effects of lycopene. A recent report showed that lycopene inhibited migration of platelet derived growth factor (PDGF)-BB-induced human Hs68 skin fibroblast (Chiang *et al.*, 2007). Lycopene significantly inhibited the invasive ability of hepatoma cells (Huang *et al.*, 2007). Lycopene supplementation *in vivo* markedly inhibited the growth of metastatic tumors with attenuation of proliferation, invasion and angiogenesis in hepatoma cells (Huang *et al.*, 2008). In this study, we investigated the effects of lycopene on the invasive and migratory phenotypes of human breast cancer cells. Treatment with 10 μ M of lycopene for 17 hr significantly inhibited invasion and migration of H-Ras MCF10A cells by 50% and 48%, respectively (Fig. 4A). Invasive and migratory properties of MDA-MB-231 cells were also inhibited by 10 μ M of lycopene by 24% and 37%, respectively (Fig. 4B). MMP-2 and/or MMP-9 have been

shown to be responsible for the induction of invasive phenotype in many cell systems including breast cells (Kim *et al.*, 2003; Song *et al.*, 2006; Kim *et al.*, 2010). We next examined if lycopene down-regulated MMP-2/-9 in H-Ras MCF10A and MDA-MB-231 cells by gelatin zymogram assay. Neither MMP-2 nor MMP-9 was affected upon lycopene treatment (data not shown).

In order to investigate the association of signaling molecules in lycopene-induced inhibition of proliferation and/or invasiveness of breast cancer cell lines, we examined the effect of lycopene on the activation of signaling molecules; ERK1/2, p38, c-Jun N-terminal kinase (JNK) and Akt. A kinetic study was conducted on H-Ras MCF10A and MDA-MB-231 cells to examine the activations of these signaling molecules upon treatment with 20 μ M lycopene. As shown in Fig. 5, phosphorylated forms of ERK1/2 and Akt were significantly reduced after exposure of lycopene in H-Ras MCF10A cells while activation of p38 or JNK was not altered. These data suggest that activation of ERKs and Akt signaling pathways may be involved in lycopene-induced anti-proliferative and/or anti-invasive/migratory effects in H-Ras MCF10A cells. Consistent with our results, a recent study showed that lycopene inhibited

Fig. 5. Effects of lycopene on the phosphorylation of ERK1/2, p38, JNK and Akt in H-Ras MCF10A cells. Cells were treated with 20 μ M lycopene for indicated time points. Western blot analysis was performed using antibodies against phosphorylated or total forms of ERK1/2, p38, JNK and Akt. Band intensities were quantitated by densitometric measurements and the phosphorylated forms of ERK1/2, p38, JNK and Akt were plotted.

PDGF-BB-induced migration of retinal pigment epithelial cell by suppression of PI3K/Akt and ERK1/2 pathways (Chan *et al.*, 2009). In MDA-MB-231 cells, however, neither ERK1/2 nor Akt was significantly inhibited by lycopene (data not shown). Since lycopene is one of the members of vitamin A family compounds (Cunningham *et al.*, 1994; Freemantle *et al.*, 2003), a possible mechanism for the anti-proliferative, anti-invasive, and anti-migratory effects of lycopene may involve the retinoid receptor signaling. Further studies would be required to elucidate the molecular mechanism for the inhibitory effect of lycopene in proliferation and invasion/migration of breast cancer cells.

Taken together, the present study demonstrated that lycopene inhibited proliferation, invasion and migration of highly invasive breast cancer cell lines, H-Ras MCF10A and MDA-MB-231 cells. Given that breast cancer metastasis is one of the most lethal malignancies in women, our findings may provide useful information for the application of lycopene in establishing strategy to prevent the metastatic breast cancer.

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