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Journal of Nutritional Biochemistry 23 (2012) 8–17

Journal of Nutritional Biochemistry

Lycopene inhibits the proliferation of androgen-dependent human prostate tumor cells through activation of PPARγ-LXRα-ABCA1 pathway

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Received 21 April 2010; received in revised form 24 September 2010; accepted 11 October 2010

Abstract

The activation of nuclear receptors, peroxisome proliferator-activated receptor gamma (PPARγ) and liver X receptor alpha (LXRα), has been shown to inhibit the growth of prostate cancer cells. This study examined whether the anti-proliferative effect of lycopene on androgen-dependent human prostate cancer (LNCaP) cells involves the up-regulation of the expression of PPARγ and LXRα. As expected, lycopene treatment (2.5–10 μM) significantly inhibited the proliferation of LNCaP cells during incubation for 96 h. Lycopene significantly increased the protein and mRNA expression of PPARγ and LXRα at 24 and 48 h, while the increased in the expression of ATP-binding cassette transporter 1 (ABCA1) was only evident 96 h. In addition, lycopene significantly decreased cellular total cholesterol levels and increased apoA1 protein expression at 96 h. Incubation of LNCaP cells with lycopene (10 μM) in the presence (20 μM) of a specific antagonist of PPARγ (GW9662) and LXRα (GGPP) restored the proliferation of LNCaP cells to the control levels and significantly suppressed protein expression of PPARγ and LXRα as well as increased cellular total cholesterol levels. LXRα knockdown by siRNA against LXRα significantly enhanced the proliferation of LNCaP cells, whereas si-LXRα knockdown followed by incubation with lycopene (10 μM) restored the proliferation to the control level. The present study is the first to demonstrate that the anti-proliferative effect of lycopene on LNCaP cells involves the activation of the PPARγ-LXRα-ABCA1 pathway, leading to reduced cellular total cholesterol levels.

Keywords: ABCA1; Cholesterol efflux; Lycopene; Nuclear receptors; Prostate cancer

1. Introduction

Prostate cancer is the most common diagnosed malignancy and is the second cause of cancer death in American men, among to 192,280 new cases and 27,360 deaths during 2009 [1]. The well-known risk factors for prostate cancer are age, race and family history of the disease. Androgen ablation therapy may be used to treat early stage of prostate cancer, whereas hormonal, chemotherapy or radiation is used to treat advanced stage [1].

Lycopene, one of more than 600 carotenoids synthesized by plants and photosynthetic microorganisms, is a tetraterpene hydrocarbon containing 40 carbon atoms and 56 hydrogen atoms [2]. Lycopene contains 11 conjugated and two non-conjugated double bonds, which make it highly reactive towards oxygen and free radicals [3]. Epidemiological studies have suggested that higher intakes of lycopene are associated with a reduced risk of several types of cancer such as prostate cancer [4-6]. Acyclic carotenoids, such as lycopene, β-carotene and phytofluene, all of which are present in tomatoes, inhibit proliferation of prostate cancer [6]. Previous cell culture studies have indicated that lycopene possesses multi-functions in cancer chemoprevention such as antioxidant activity (quenching singlet oxygen and free radicals) [7], induced apoptosis and cell cycle arrest of prostate cancer cells [8-11], inhibits the metastasis of human hepatoma SK-Hep-1 cells both in vitro [12-14] and in vivo in nude mice [15]. Lycopene also inhibits growth rates of prostate tumor cell lines DU145 and PC-3 [16,17] and reduces the development of spontaneous mammary tumors in animal studies [18].

Peroxisome proliferator-activated receptors (PPARs) belong to the members of the nuclear hormone receptor superfamily and are ligand-activated intracellular transcription factors [19]. The PPAR family comprises three subtypes-PPARα, PPARβ/δ and PPARγ [20], and PPARγ is the most extensively studied subtype of the PPARs. PPARγ transcriptional activity depends on the binding of ligands. Ligand activation of PPARγ inhibits the growth and differentiation of several types of cancer cells, such as prostate cancer, breast cancer and lung cancer [21-23]. Liver X receptors (LXRs) α and β, which are nuclear receptors activated by oxysterols such as 22(*R*)- and 24(*S*)-hydroxycholesterol, are involved in a central transcriptional regulator for lipid homeostasis [24]. LXRα is expressed at high levels in liver, intestine, adipose tissue and macrophages, whereas LXRβ is expressed ubiquitously [25]. T0901317, a synthetic LXR agonist, has been shown to inhibit the proliferation of androgen-dependent prostate cancer cells [26] and has been developed as antiatherogenic drugs [27].

Several studies have demonstrated that the activation of nuclear receptors, such as PPARγ and LXRα, is related to reduced growth of

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doi:10.1016/j.jnutbio.2010.10.006

prostate cancer [21,26]. However, little attention has been paid on the relation between lycopene and nuclear receptor in prostate cancer progression. In the present study, we employed the LNCaP cell line, a cell model for early androgen-dependent prostate cancer [28], to examine the effects of lycopene on cell proliferation and the possible mechanisms underlying such an action. We hypothesized that lycopene may exert its anti-proliferative effects through up-regulation of the PPARγ-LXRα-ABCA1 pathway.

2. Material and methods

2.1. Chemicals and lycopene preparation

All chemicals used are of the highest grade. tetrahydrofuran (THF) and THF containing 0.0025% butylhydroxytoluene (BHT) were obtained from Merck (Darmstadt, Germany). RPMI1640, nonessential amino acid, penicillin/streptomycin, sodium pyruvate, fetal bovine serum (FBS) and trypsin were from Gibco/BRL (Grand Island, NY, USA). Lycopene were purchased from Wako (Japan). Lycopene was soluble in THF/BHT to form a stock solution of 10 mM lycopene, which was diluted with THF at indicated ratios (1:1, 1:3, 1:7), and then diluted with FBS at indicated ratio (1:9). THF/BHT-FBS-lycopene was added to the culture medium at a calculated final concentration of 2.5, 5, 10 or 20 μM. THF at 0.2% (v/v) and FBS at 1.8% (v/v) served as the solvent control for lycopene, which did not significantly affect the assays described below.

2.2. Cell culture and cell proliferation assay

The human androgen-dependent prostate cancer cell lines LNCaP cells (BCRC 60088) were purchased from the Food Industry Research and Development Institute, Hsin Chu, Taiwan. LNCaP cells were grown in RPMI1640 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. Cells were cultured in six-well plates at 6×10^4 cells/well in RPMI1640 for 48 h, and each well was washed with phosphate-buffered saline (PBS) and then filled with 1 ml of RPMI1640 containing various concentrations of lycopene and incubated for 24, 48, 96 h at 37°C. After treatment, the cells were trypsinized and the cell number was measured by trypan-blue exclusion assay.

2.3. Annexin V/PI double staining

Cell apoptosis was determined by flow cytometric assay using double staining with FITC-conjugated Annexin V and propidium iodide (PI) (BD Pharmingen). After incubation with lycopene, the cells were washed twice in PBS, harvested by trypsinization, suspended in 3 ml of PBS, and centrifuged at 300×*g* for 5 min. After removing the supernatant, the cells were incubated in a binding buffer containing

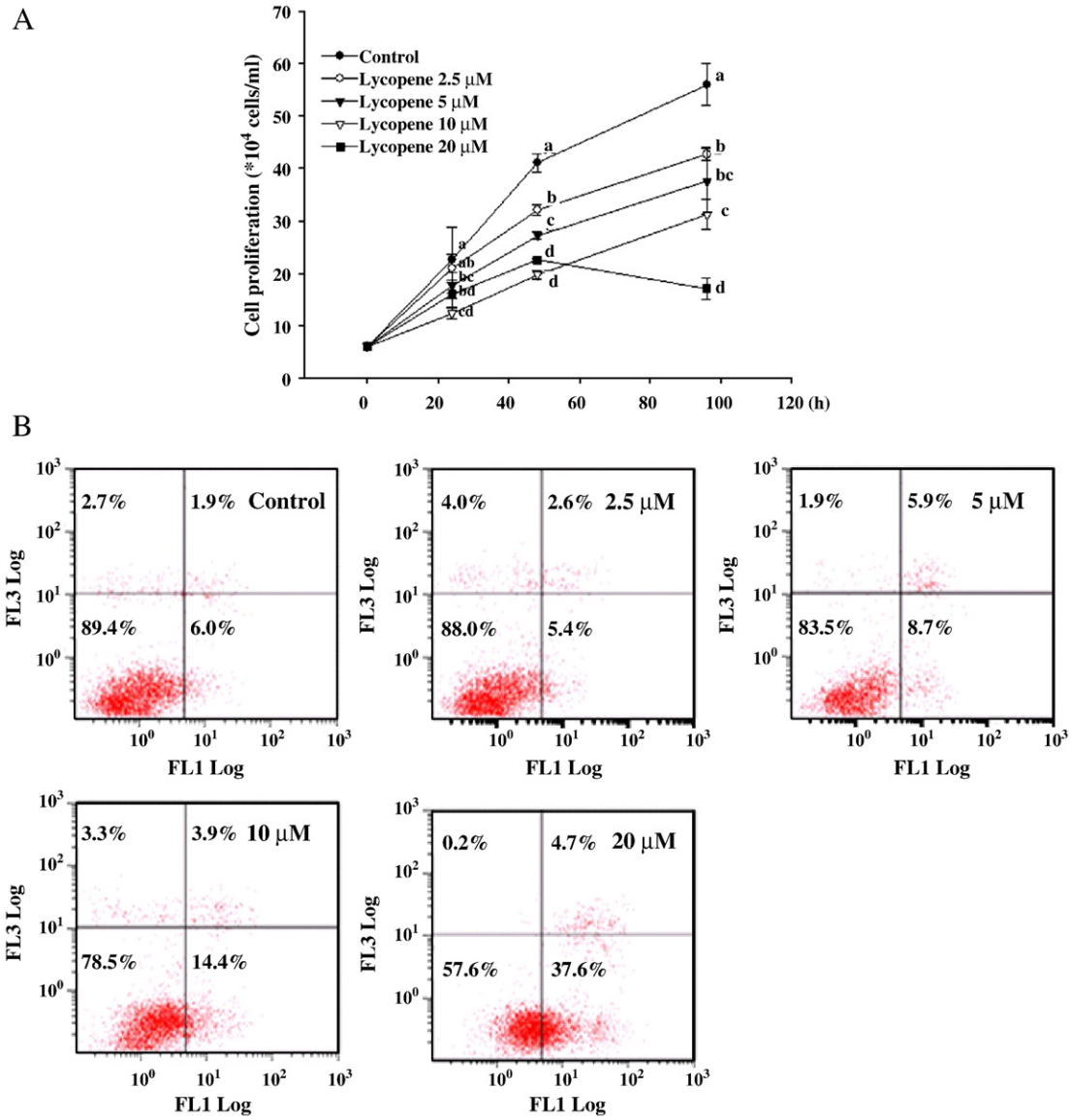

Fig. 1. Effects of lycopene on cell proliferation and apoptosis of LNCaP cells. Cell proliferation of LNCaP cells treated with lycopene (0–20 μM) for 24, 48 and 96 h (A). Apoptosis (%) of LNCaP cells treated with lycopene (0–20 μM) for 96 h (B). Values are means±S.D., $n=3$; means from the same incubation time not sharing an alphabetic letter differ significantly ($P<.05$).

Annexin V-FITC and PI at room temperature for 15 min. Fluorescence analysis was performed using a flow cytometer (Beckman Coulter FC500).

2.4. Western blotting

LNCaP cells were incubated with lycopene at 37°C for 24, 48 or 96 h. The medium was removed and cells were lysed with RIPA buffer containing proteinase inhibitors. The cell lysate were scraped, followed by centrifugation for 30 min at 4°C. The proteins (50 μg) from the supernatant were resolved by sodium docecyl sulfate polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride (PVDF) membrane. After blocking with Tris-buffered saline buffer (20 mM Tris–HCl, 150 mM NaCl, pH 7.4) containing 5% nonfat milk, the membrane was incubated with mouse anti-human PPARγ, LXRα, ABCA1 and apoA1 antibody (Santa Cruz Biotechnology) and then incubated with horseradish peroxidase-conjugated antirabbit IgG

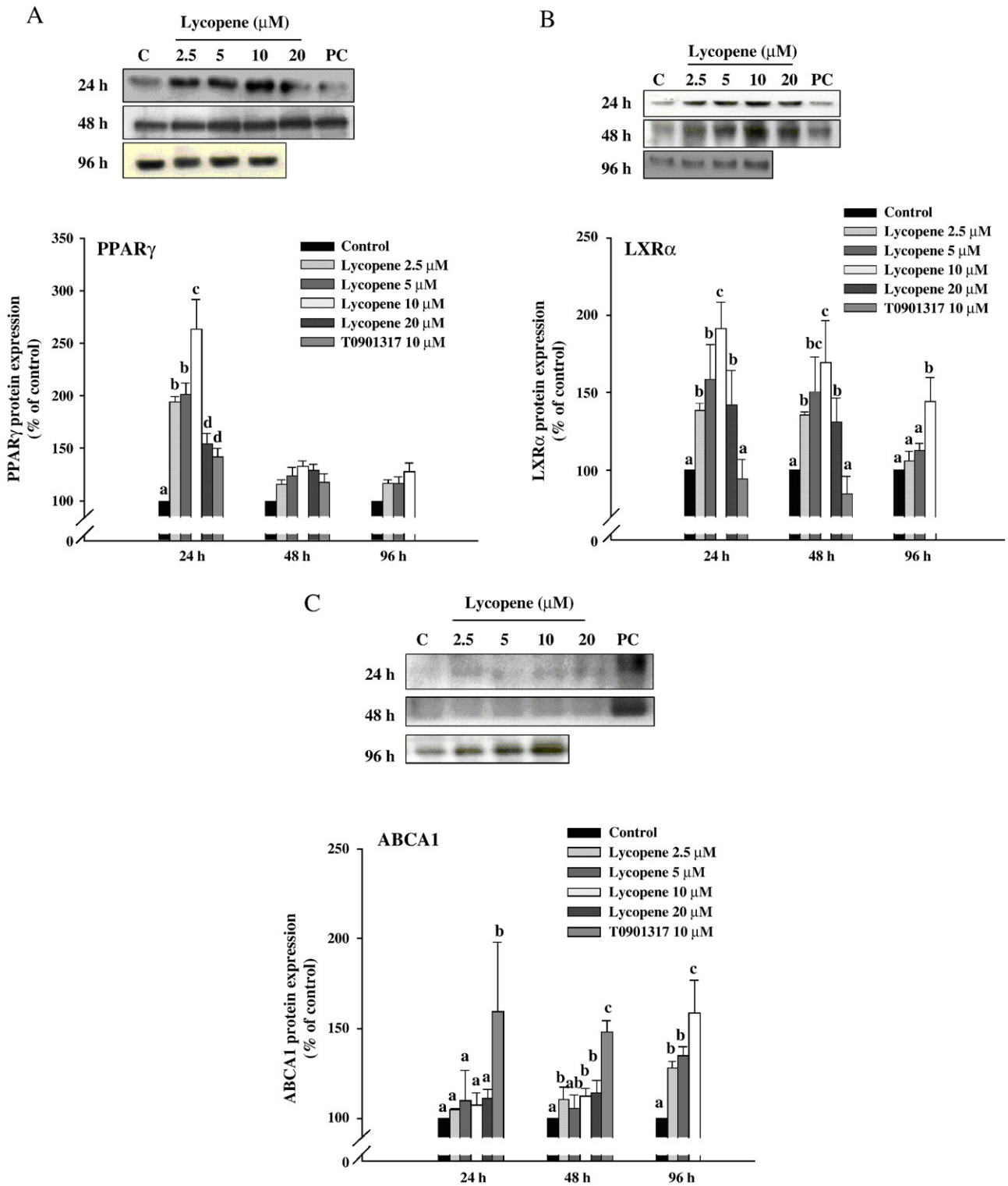

Fig. 2. The protein expression PPARγ (A); LXRα (B) and ABCA1 (C) of LNCaP cells treated with lycopene (0–20 μM) for 24, 48 and 96 h. PC represents T0901317 (10 μM) as positive control. No assays were performed at 96 h for 20 μM lycopene and PC owing to cytotoxicity. Values are means±S.D., $n=3$; means from the same incubation time not sharing an alphabetic letter differ significantly ($P<.05$).

for 1 h, follow by visualization using an ECL chemiluminescent detection kit (Amersham Bucks, UK). The relative density of the protein expression was quantitated by densitometry (Matrox Inspector 2.1 software.).

2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR) (RNA isolation and sequencing)

Total cellular RNA was isolated from cell culture by RNAzol kit and reverse transcribed into cDNA (MMLVReverse Transcriptase, Gibco/BRL) using oligo (dT) as primers and then amplified with ten primer bases on PPARγ, LXRα, ABCA1, apoA1 and β-actin (internal control) sequences. The primers for amplifying PPARγ cDNA were 5′-TCTCCAGCATTTCTACTCCAC-3′, located in the 5′-untranslated region, and 5′-GCC-AACAGCTTCTCCTTCTCG-3′, located in the 3′-untranslated region. The primers for amplifying LXRα cDNA were 5′- TCAGCCGGGAGGACCAGATTG-3′ and 5′-CCGGAGGC-TCACCAGTTTCATTAG-3′. The primers for amplifying ABCA1 cDNA were 5′-ACAAC-CAAACCTCACACTACTG-3′ and 5′-ATAGATCCCATTACAGACAGCG-3′. The primers for amplifying apoA1 cDNA were 5′-CTTGGCCGTGCTCTTCCTGA-3′ and 5′-CGCAGCCTTG-CTGAAGGTGGA-3′. The primers for amplifying β-actin were 5′-GAGCGGGAAAT-CGTGCGTGAC-3′ and 5′-GCCTAGAAGCATTTGCGGTGGAC-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 (35 cycle), followed by a final incubation at 72°C for 7 min. The sizes of the amplification products of PPARγ, LXRα, ABCA1, apoA1 and β-actin were 460, 404, 439, 215 and 518 bp, respectively. The PCR products were subjected to 1% agarose gel electrophoresis and stained with ethidium bromide. The relative PPARγ, LXRα, ABCA1 and apoA1 levels were quantitated by Matrox Inspector 2.1 software.

2.6. Cholesterol loading and efflux Assay

After incubation with different concentrations of lycopene, the cell lysate and the culture medium were collected and processed for total cholesterol was quantified by cholesterol/cholesterol ester quantitation kit (BioVision). The assay was performed in triplicate by following the instructions of the manufacturer.

2.7. Transient transfection of siRNA against LXRα

The plasmid containing siRNA against LXRα (si-LXRα; ON-TARGETplus SMART pool), non-targeting siRNA (si-control) and the DharmaFECT transfection reagent were

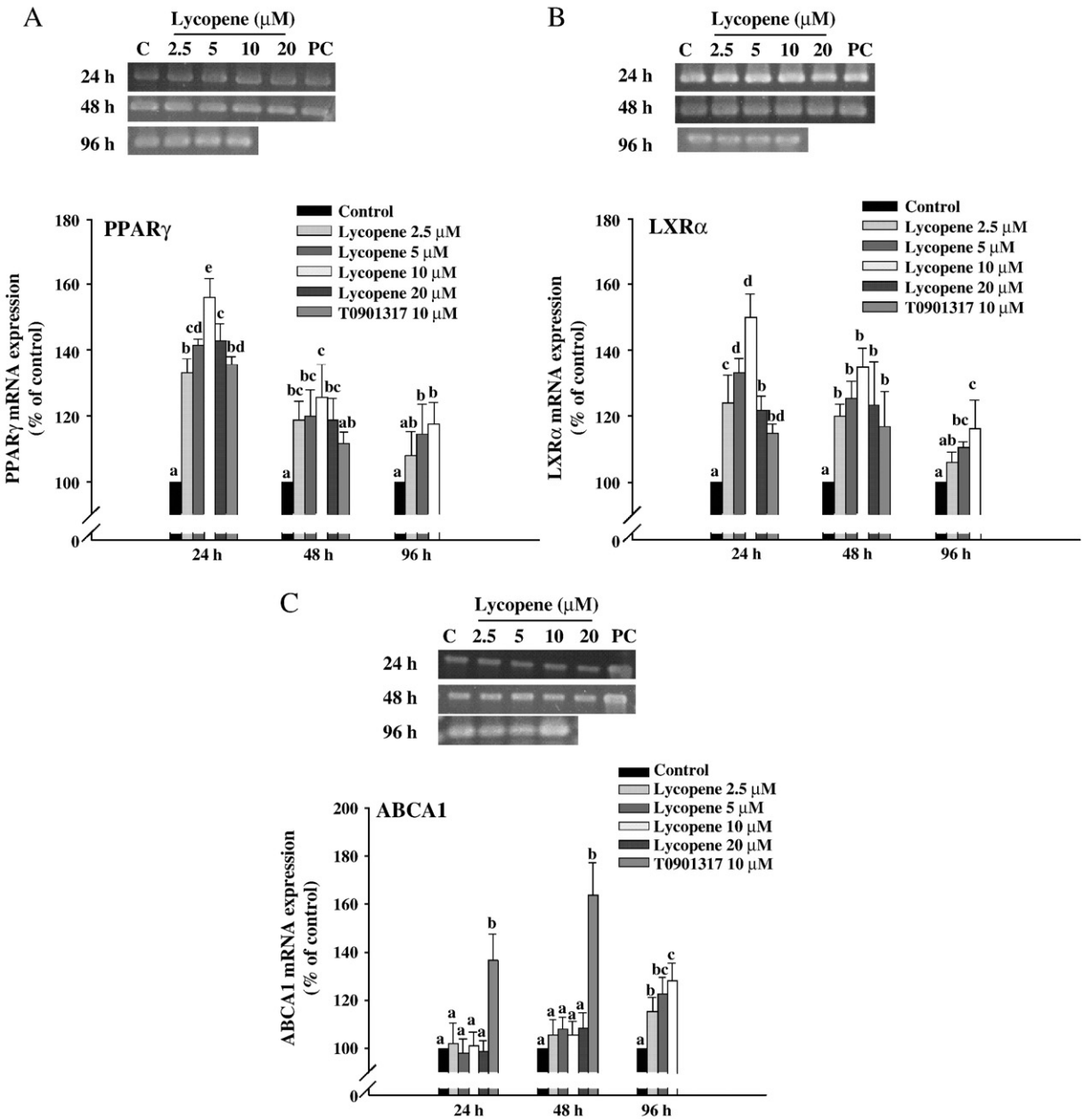

Fig. 3. The mRNA expression of PPARγ (A); LXRα (B) and ABCA1 (C) in LNCaP cells incubated with lycopene (0-20 μM) for 24, 48 and 96 h. PC represents positive control (10 μM T0901317). No assays were performed at 96 h for 20 μM lycopene and PC owing to cytotoxicity. Values are means±S.D., $n=3$; means from the same incubation time not sharing an alphabetic letter differ significantly ($P<.05$).

purchased from Thermo Scientific Dharmacon (USA). Si-LXRα and si-control stock solutions (20 μM) were diluted with diethyl pyrocarbonate (DEPC) water to form 5-μM solutions. The DharmaFECT transfection reagent was mixed with 5 μM si-LXRα or si-control, incubated for 20 min and then added to the culture medium at a final concentration of 25 nM. The cells were incubated with si-LXRα and si-control for 48 h.

2.8. Statistical analysis

Values are expressed as means and standard deviations and analysed by one-way analysis of variance followed by least significant difference (LSD) test for comparisons of group means. $P<.05$ was considered significant.

3. Results

3.1. Antiproliferative effect of lycopene in LNCaP cells

As expected, in LNCaP cells incubated for 24, 48 and 96 h, lycopene markedly decreased the cell proliferation, and the inhibitory effect of lycopene increased with increasing concentration up to 10 μM but was weakened at 20 μM (Fig. 1). Treatment of LNCaP cells with 20 μM lycopene for 96 h resulted in cytotoxicity, as evidenced by lower cell numbers at 96 h than at 48 h. However, only the LNCaP cells incubated with 20 μM lycopene for 96 h produced significant cell apoptosis (Fig. 1B). For these reasons, all following experiments were conducted only up to 48 h of incubation, when 20 μM lycopene was used.

3.2. Lycopene up-regulates the protein expression of PPARγ, LXRα and ABCA1 in LNCaP cells

Incubation of LNCaP cells with lycopene (0–20 μM) significantly increased the protein expression of PPARγ (Fig. 2A) and LXRα (Fig. 2B), and the effect was strongest at 24 h of incubation (by 91% for PPARγ and by 70% for LXRα, $P<.05$). The protein expression of ABCA1 was not affected by lycopene at 24 and 48 h of incubation but was significantly increased by 10 μM lycopene at 96 h of incubation (59%, $P<.05$) (Fig. 2C). In these experiments, T0901317, a LXR agonist, was used as positive control, and it did not significantly affect the LXRα expression but markedly increased the expression of ABCA1 in LNCaP cells. Because 10 μM T0901317 caused cytotoxicity in LNCaP cells after incubation for 48 h (data not shown), no further tests on the effect of T0901317 were conducted at 96 h.

3.3. Lycopene up-regulates the mRNA expression of PPARγ, LXRα and ABCA1 in LNCaP cells

Incubation of LNCaP cells with lycopene (0–20 μM) significantly increased the mRNA expression of PPARγ (Fig. 3A) and LXRα (Fig. 3B), and the effect was strongest at 24 h of incubation. The mRNA expression of ABCA1 was not affected by lycopene at 24 h and 48 h of incubation but was significantly increased by 10 μM lycopene at 96 h of incubation (28%, $P<.05$) (Fig. 3C). Lycopene at 10 μM resulted in the highest increment in mRNA expression for PPARγ, LXRα and ABCA1. As in protein expression, the LXR agonist T0901317 did not significantly affect the LXRα mRNA expression but markedly increased the expression of ABCA1 in LNCaP cells.

3.4. Effect of lycopene on cholesterol efflux and apoA1 expression in LNCaP cells

As shown in Fig. 4A, incubation of LNCaP cells with lycopene for 96 h markedly decreased the intracellular total cholesterol level, and the effect increased with increasing concentrations, with an inhibition of 62% ($P<.05$) at 10 μM lycopene. In contrast, incubation of LNCaP cells with lycopene for 96 h markedly increased the protein and mRNA expression of apoA1 (Fig. 4B) in a concentration-dependent

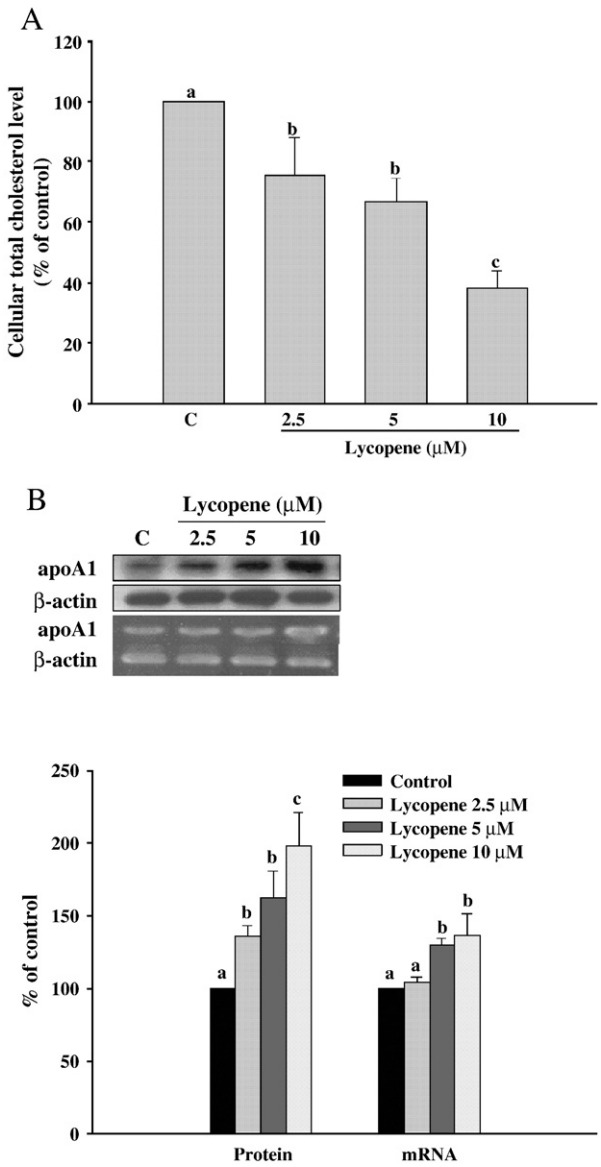

Fig. 4. Levels of cellular total cholesterol and the expression of apoA1 protein and mRNA in LNCaP cells treated with lycopene (0–10 μM) for 96 h. (A) Cellular total cholesterol; (B) Western blot and RT-PCR of apoA1; (C) Densitometric analysis of (B). Values are means±S.D., $n=3$; means without a common letter differ significantly ($P<.05$).

manner, with a 98% increase in protein expression ($P<.05$) and a 37% increase ($P<.05$) in mRNA expression at 10 μM lycopene (Fig. 4C).

3.5. Effect of lycopene in combination with a specific antagonist of PPARγ (GW9662) and LXRα (GGPP) on proliferation, PPARγ and LXRα protein expression as well as cellular total cholesterol levels in LNCaP cells

To confirm the involvement of the PPARγ-LXRα-ABCA1 pathway, we incubated LNCaP cells with lycopene (10 μM) in the presence or absence of a specific antagonist of PPARγ (GW9662, 10 and 20 μM) or LXRα (GGPP, 10 and 20 μM) for 24 h. As shown in Fig. 5A, GW9662 alone markedly increased the proliferation of LNCaP cells in a concentration-dependent manner. GW9662 in combination with lycopene effectively restored the anti-proliferative effect of lycopene to the control levels. Similarly, GGPP alone significantly increased the proliferation of LNCaP cells in a concentration-dependent manner. GGPP in combination with lycopene prevented the anti-proliferative

effect of lycopene, with 20 μM GGPP completely restoring the anti-proliferative effect of lycopene to the control levels (Fig. 5B).

GW9662 significantly inhibited the protein expression of PPARγ and LXRα to a level that was lower than that of the control, whereas GGPP inhibited LXRα protein expression without affecting PPARγ protein expression (Fig. 5C). The combination of GW9662 and lycopene (10 μM) significantly attenuated the protein expression of PPARγ and LXRα, with 20 μM GW9662+lycopene reaching the

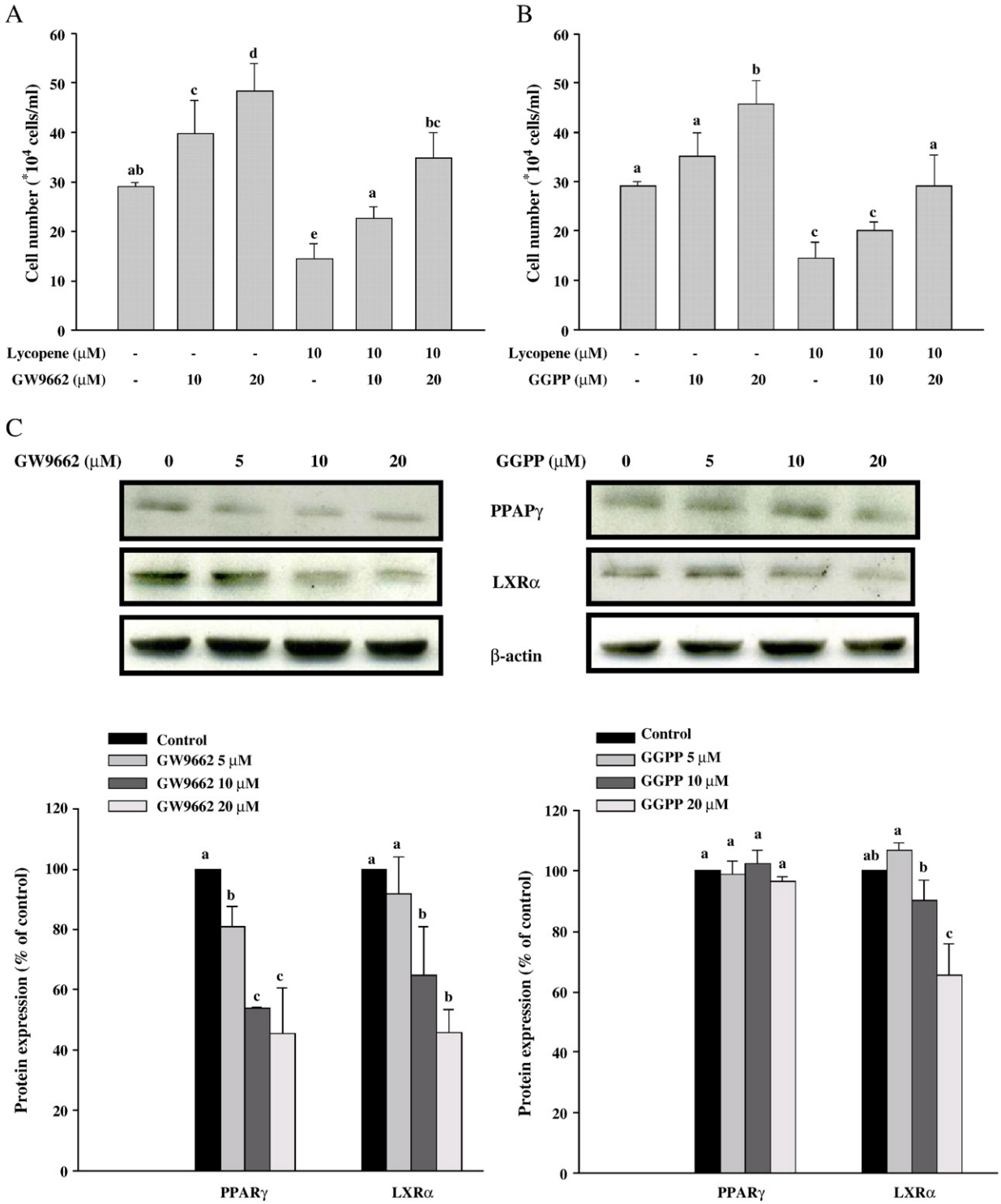

Fig. 5. Cell proliferation and PPARγ and LXRα protein expression in LNCaP cells (6×10^4 cells/ml) incubated 24 h with lycopene (10 μM) in the presence or absence of an antagonist of PPARγ (GW9662, 10 and 20 μM) and of LXRα (GGPP, 10 and 20 μM). (A) Effect of GW9662 and lycopene on cell proliferation. (B) Effect of GGPP and lycopene on cell proliferation. (C) The upper panels are Western blots of PPARγ and LXRα protein expression of GW9662 (left) and GGPP (right), respectively. The lower panels are the densitometric analyses of the upper panels. (D) The upper panels are western blots of PPARγ and LXRα protein expression of cells incubated with lycopene (10 μM) in the presence or absence of GW9662 (left) and GGPP (right), respectively. The lower panels are the densitometric analyses of the upper panels. (E) Cellular total cholesterol in LNCaP cells incubated with lycopene (10 μM) in the presence or absence of GW9662 (10 and 20 μM) and GGPP (10 and 20 μM). Values (means±S.D., $n=3$) without a common letter differ significantly ($P<.05$).

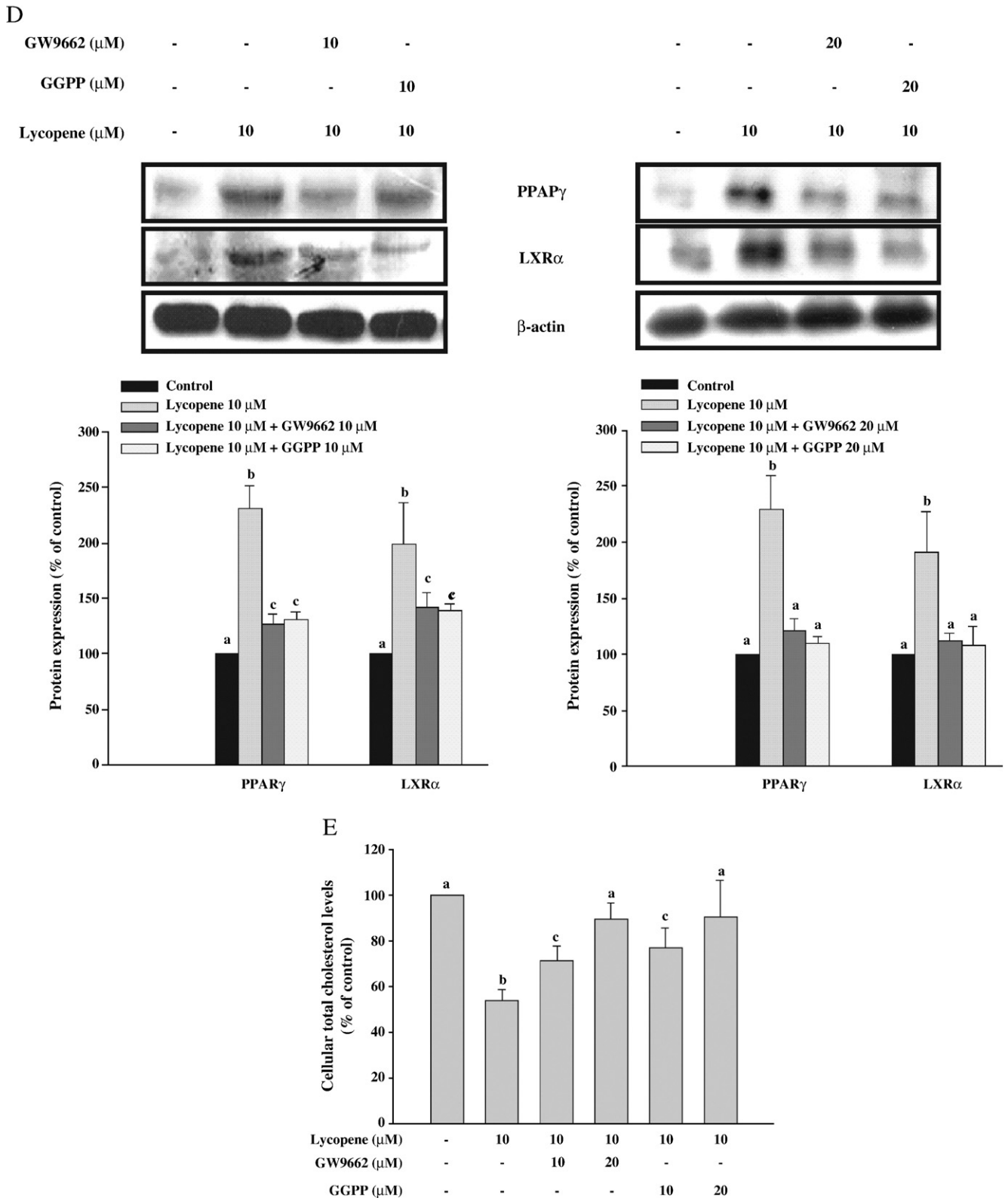

Fig. 5 (*continued*).

control level (Fig. 5D). The effects of GGPP in combination with lycopene (10 μM) were similar to those of GW9662+lycopene on the protein expression of PPARγ and LXRα in the LNCaP cells (Fig. 5D). In addition, GW9662 (10 and 20 μM)+lycopene (10 μM) and GGPP (10 and 20 μM)+ lycopene (10 μM) significantly restored lycopene-reduced cellular total cholesterol levels, with 20 μM GW9662+lycopene or 20 μM GGPP+lycopene reaching the control levels (Fig. 5E).

3.6. PPARγ-LXRα-ABCA1 pathway involves the anti-proliferative effects of lycopene in LNCaP cells

Si-RNA techniques were used to further confirm the involvement of PPARγ-LXRα–ABCA1 pathway in the anti-proliferative action of lycopene. The introduction of si-LXRα to LNCaP cells decreased the expression of LXRα and ABCA1 without affecting PPARγ expression (Fig. 6A). Pretreatment of LNCaP cells with si-LXRα or si-control alone for 48 h revealed that LXRα knockdown significantly increased cell proliferation (131% of the control level) (Fig. 6B), and this result supports the critical role of LXRα in the proliferation of LNCaP cells, as indicated by Fukuchi et al [26]. In addition, si-LXRα+lycopene restored the cell proliferation to the control level (Fig. 6B), indicating the involvement of LXRα in the antiproliferative action of lycopene in LNCaP cells.

4. Discussion

PPARγ and LXRα are members of nuclear receptors which bind a ligand in the cytoplasm and are then translocated to the nucleus [29]. PPARγ and LXRα exist as a heterodimer with retinoid X receptor (RXR) partners [19], and LXRα is a direct target gene for the PPARγ/RXR heterodimer [30]. It has recently been shown that the activation of PPARγ/RXR or LXRα/RXR expression by ligands inhibits the growth of prostate cancer cells [23,26]. However, little is known whether lycopene or other carotenoids can activate the nuclear X receptors expression, except that short-term lycopene supplementation was shown to increase the PPARγ mRNA expression in prostate tissue of F344 rats, especially in castrated rats [31]. In the present study, we demonstrated that the antiproliferative effect of lycopene on LNCaP cells was associated with the PPARγ-LXRα-ABCA1 pathway, as lycopene not only increased the expression of PPARγ, LXRα and ABCA1 at both protein and mRNA levels but also decreased intracellular cholesterol levels in LNCaP cells. These findings suggest that lycopene is an exogenous agonist of PPARγ and LXRα. Receptors can be activated or inactivated either by endogenous (such as hormones and neurotransmitters) or by exogenous (such as drugs) agonists and antagonists, resulting in stimulating or inhibiting a biological response. An agonist is a ligand that binds to a receptor, alters the function of the receptor and triggers a physiological response [32]. To the best of our knowledge, this is the first report to indicate that lycopene inhibits cell proliferation through up-regulating PPARγ-LXRα-ABCA1 pathway in human androgen-dependent prostate cancer cells.

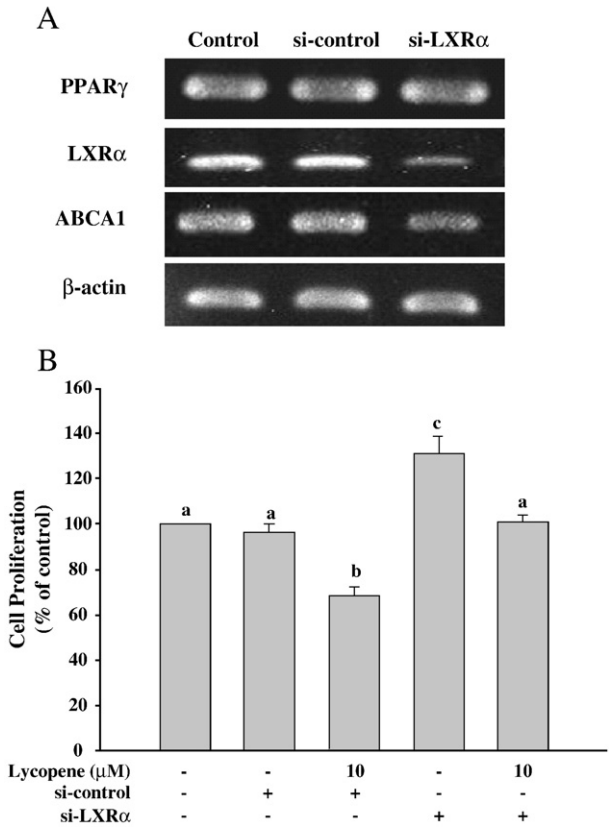

Fig. 6. mRNA expression levels of PPARγ, LXRα and ABCA1 (A) and cell proliferation (B) after knockdown of LXRα in LNCaP cells. Cells were transfected with si-RNA against LXRα for 48 h, and mRNA expression was determined using RT-PCR. After transfection, the cells were incubated with lycopene (10 μM) for 24 h, and the cell proliferation was determined using the MTT assay. Values (means±S.D., $n=3$) not sharing an alphabetic letter differ significantly ($P<.05$).

Epidemiological and preclinical studies suggest that cholesterol accumulation promotes prostate cancer development and progression [33]. As early as in 1942, Swyer reported a higher cholesterol content in malignant tumors than that in normal tissues [34]. Thus, modulation of intracellular cholesterol transport and metabolic related-gene is important. LXRs regulate intracellular cholesterol levels through mediating the expression of ABCA1, which modulates cholesterol efflux and reverse cholesterol transport (RCT) from peripheral tissues [35]. RCT, a multi-step process, is mediated by high-density lipoprotein (HDL) resulting in the net movement of cholesterol from peripheral tissues back to the liver [36]. The cholesterol efflux by apoA1, a major apolipoprotein of HDL, is the first step in RCT [37]. ABCA1 is a member of the ATP-binding cassette-transporter family [38]. PPARγ activators mediate ABCA1 expression, probably by the inductive effects on LXRα expression [39,40]. In addition, the ligand of LXR/RXR heterodimer regulates the ABCA1 expression and the apoA1-mediated cholesterol efflux [41]. In macrophages, the PPARγ-LXR-ABCA1 pathway is involved in cholesterol efflux and atherogenesis [30]. It has been shown that ABCA1 overexpression inhibits the growth of LNCaP cells, whereas LNCaP cells grow faster when the expression of the ABCA1 gene is down-regulated [42]. The reason may be that a decrease in ABCA1 expression may modulate cholesterol levels in lipid raft domains, which function as membrane platforms for cellular signal transduction [43]. Our findings that lycopene increased ABCA1 and apoA1 expression and decreased total intracellular cholesterol levels in LNCaP cells further demonstrate that lycopene is involved in PPARγ-LXR-ABCA1 pathway by mediating the cholesterol efflux in LNCaP cells.

To further confirm the involvement of the PPARγ-LXRα-ABCA1 pathway in the anti-proliferative effect of lycopene on LNCaP cells, we used the antagonists, GW9662 and GGPP, for PPARγ and LXRα, respectively. We showed that these antagonists not only attenuated lycopene-induced PPARγ and LXRα expression in LNCaP cells but also reversed the anti-proliferative effect of lycopene. Using the si-RNA techniques, we found that si-LXRα inhibited the expression of LXRα and ABCA1 without affecting the expression of PPARγ in LNCaP cells, confirming that LXRα is a target gene for the PPARγ, as reported previously [30]. It has been shown that LXRα activation inhibits the proliferation of prostate cancer cells [26]. In contrast, knockdown of ABCA1 expression by RNA interference increases the proliferation of androgen-dependent prostate cancer cells [42]. Thus, the inactivation of LXRα and ABCA1 may increase cell proliferation. Indeed, we show here that si-LXRα treatment increased the proliferation of LNCaP cells, whereas subsequent incubation of LNCaP cells with lycopene restored si-LXRα-induced cell proliferation to the level of control. The present results obtained from the use of antagonists (GW9662 and GGPP) and LXRα knockdown support the critical role of PPARγ-LXRα-ABCA1 pathway in the anti-proliferative effects of lycopene.

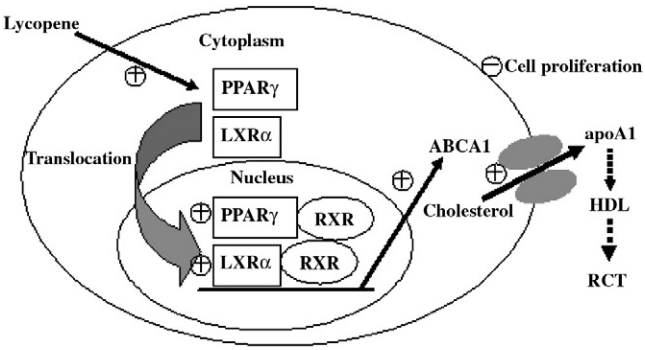

Fig. 7. Proposed mechanisms by which lycopene inhibits the proliferation of LNCaP cells. Solid arrows indicate the evidence obtained from the present study; dotted arrows are suggestive or based on evidence from the literature. ⊕, promotional; ⊖, inhibitory.

Lycopene has been shown to cause cell cycle arrest and apoptosis of LNCaP cells [44], but we did not find significant cell apoptosis in the present study, except the incubation for 96 h with the highest concentration of lycopene (20 μM). The discrepancy may be attributed to the forms of lycopene, i.e., we used lipid-soluble lycopene as compared to the water-soluble lycopene (0.5–5 μM) used by Hwang et al. [44]. Interestingly, it has been shown that PPARγ activation inhibits the growth of prostate cancer cells and that this inhibition is accompanied by morphological changes but not by signs of apoptosis [45].

Although the present study has furnished new knowledge of the molecular mechanisms for the anti-proliferative effect of lycopene, some limitations exist in the present study that may undermine the significance of our findings. One limitation of this study is that only the in vitro cell culture system was used. Indeed, the growth of prostate cancer cells is much more complex in vivo, and many factors such as androgens, estrogens and growth factors may affect the cell growth. Another limitation is that only the androgen-dependent prostate cancer cells were studied in this study, although work involving the androgen-independent prostate cancer cells (DU-145 and PC-3 cells) is currently underway in this laboratory. Still another limitation of this study is that it is unclear whether lycopene itself or its bioactive metabolites, such as apo-8′-lycopenal and apo-12′-lycopenal [46], is involved in the antiproliferative action. These lycopenoids have been suggested to be more bioactive and more central to the health outcomes seen in vivo than the parent compound [47]. These lycopenoids are not commercially available at present, but such metabolites need to be investigated in the future.

In conclusion, the results of the present study have clearly demonstrated that the anti-proliferative effect of lycopene on androgen-dependent prostate cancer cells is associated with up-regulation of the PPARγ-LXRα-ABCA1 pathway (Fig. 7). The results suggest that lycopene, and other agents that can enhance this particular pathway, could be used as adjuvant in the chemoprevention or chemotherapy of androgen-dependent prostate cancer cells.

Acknowledgment

This research was supported by grants (NSC-97-2320-B-005-003-my3) from the National Science Council (Republic of China).

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