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Lycopene modulates growth and survival associated genes in prostate cancer☆

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Abstract

Lycopene is a fat soluble red-orange carotenoid pigment present in tomato that reduces the risk for prostate cancer, a common malignancy among men. However, the mechanism by which lycopene attenuates prostate cancer is not fully defined. In this study we examined the effect of lycopene on proliferation, survival, and biomarker gene expression in prostate cancer (PC-3) cells in culture. WST-1 assay showed that lycopene induces a biphasic effect on PC-3 cells with a modest increase in proliferation at 1–5 μM, no change at 10–25 μM and a decrease at 50–100 μM doses in culture. Interestingly, combination treatment with lycopene induced anti-proliferative effect of Temozolomide on PC-3 cells. Lycopene also augmented the anti-proliferative effect of peroxisome proliferator-activated receptor gamma (PPARγ) agonists, but not Doxorubicin or Taxol, in prostate cancer. Flow cytometry analyses showed that lycopene, in combination with chemotherapeutic agents and PPARγ agonists, induced modest cell cycle arrest with significant increase in cell death by apoptosis and necrosis on prostate cancer. Gene array and quantitative reverse transcription polymerase chain reaction analyses showed that lycopene alters the expression of growth and apoptosis associated biomarkers in PC-3 cells. These findings highlight that lycopene attenuates prostate cancer by modulating the expression of growth and survival associated genes.

Keywords: Prostate cancer; PC-3 cells; Carotenoids; Lycopene; Cancer prevention

1. Introduction

Plant-derived bioactive dietary compounds play important roles in the regulation of health and diseases. Carotenoids are a family of over 600 fat-soluble plant pigments and about 40 of them are commonly present in human diet [1,2]. Several studies have shown that carotenoids modify diseases such as cancer, cardiovascular disease, cataracts and age-related macular degeneration [3,4]. Carotenoids also improve immune responses, pro-vitamin A activity, gap junction communication, antioxidant function, and metabolism of drugs and xenobiotics [2]. Lycopene is a fat soluble red-orange carotenoid pigment present in tomato, watermelon, papaya, guava and pink grapefruit [1,2]. Structure-function analyses revealed that lycopene ($C_{40}H_{56}$) is an acyclic isomer of β-carotene with a 40 carbon polyisoprenoid chain and 13 conjugated double bond structure [5]. Tomato and tomato products are rich source of lycopene that have been studied for their benefits in health and disease. Lycopene exhibits antioxidant, anti-inflammatory, and anti-cancer properties [6–8]. The dietary consumption of fruits and vegetables rich in lycopene associates with a reduced risk for different types of human cancers [9,10].

Prostate cancer is a commonly diagnosed cancer and second leading cause of cancer-related death among men in the United States [11]. The National Cancer Institute estimates about 241,740 new cases and 28,170 deaths due to prostate cancer among American men in 2012 [11]. While the etiology is not known, genetic makeup, age, food habits and environment are considered risk factors for prostate cancer [12]. Epidemiological and clinical studies suggest that high lycopene consumption through fruits and vegetables and high levels of serum lycopene associates with 30–40% reduction in the risk for prostate cancer [12–14]. Lycopene supplements (8–30 mg/day) augment serum and tissue lycopene and reduce prostate specific antigen (PSA) levels in men with high-grade prostate cancer [14–16]. Laboratory studies have also demonstrated that lycopene inhibits the growth and survival of prostate cancer cells in culture and in animal models, suggesting its significance in the treatment of prostate cancer [17,18].

Current treatment for prostate cancer includes surgery, radiation, hormone and chemotherapy, which yield poor prognosis and toxic side effects. Doxorubicin and Taxol are two chemotherapeutic agents

Abbreviations: TGFβ-2, Transforming growth factor beta-2; CDK-9, cyclin dependent kinase–9; EGFR, epidermal growth factor receptor; BCL-2, B-cell lymphoma–2; BCL2L1, B-cell lymphoma-2 like 1; IGF1R, insulin-like growth factor 1 receptor; CDK-7, cyclin dependent kinase-7; BRCA1, breast cancer 1; Real-Time PCR, Real-Time polymerase chain reaction; FBS, fetal bovine serum; PBS, phosphate-buffered saline.

☆ Competing interests: The authors declare no competing interests.

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commonly used in treating prostate cancer. Doxorubicin is an anthracycline compound that intercalates with DNA, causing topoisomerase II-mediated DNA cleavage and apoptosis in prostate cancer cells. Taxol is an alkaloid isolated from Japanese and Pacific yew that induces abnormal bundling of microtubules thereby blocking mitotic division in cancer cells [19,20]. Despite its in vitro effects on prostate cancer cells, a clinical trial using Taxol showed only modest benefits in patients with hormone refractory prostate cancer [21]. Whereas, combination treatment with Docetaxel improves the activity of Doxorubicin in PC-3 and DU-145, two hormone resistant prostate cancer cells [22]. Moreover, PectaSol, a modified citrus pectin, potentiates the effect of Doxorubicin in DU-145 and LNCaP prostate cancer cells in culture [23]. However, the potentiating effect of lycopene on these chemotherapeutic agents to inhibit prostate cancer is not well defined.

Temozolomide is an alkylating agent that has been commonly used to treat patients with malignant glioma and other types of tumors, but not prostate cancer. Temozolomide induces O^6-methylguanine formation that pairs with thymine during DNA replication leading to the activation of apoptotic pathways [24]. Although the exact mechanism is not known, earlier studies have shown that Temozolomide inhibits brain tumor by modulating p53/MGMT signaling pathways [25,26]. However, the influence of lycopene on the therapeutic efficacy of TMZ on prostate cancer is not known.

Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor transcription factor that regulates cell growth, differentiation and homeostasis [27]. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ2) is a natural ligand for PPARγ, while thiazolidinediones including Ciglitazone and Pioglitazone serve as synthetic agonists for PPARγ. PPARγ agonists also modulate lipid metabolism and glucose homeostasis and have been used as prescription drugs for type 2 diabetes. We and others have shown earlier that PPARγ agonists induce growth-arrest and apoptosis in different types of tumors [28–30]. Other studies have also demonstrated that PPARγ agonists induce growth-arrest by modulating the expression and activation of PPARγ in prostate cancer [30,31]. Troglitazone induces G0/G1 cell cycle arrest and apoptosis by blocking GSK3/nuclear factor κB pathways in prostate cancer [32]. Another study showed that lycopene up-regulates PPARγ-LXRα-ABCA1 pathway, suggesting its significance in the treatment of prostate cancer [33]. However, the potentiating effect of lycopene on PPARγ agonists to inhibit prostate cancer is not known.

In this study we examined the anti-tumor effect of lycopene alone or in combination with chemotherapeutic agents and PPARγ agonists in prostate cancer. We found that lycopene augments growth-arrest and apoptosis by modulating growth and survival associated genes in prostate cancer cells, suggesting its significance in the treatment of prostate cancer.

2. Materials and methods

2.1. Cells and reagents

RPMI 1640 medium, fetal bovine serum (FBS), penicillin, streptomycin, propidium iodide and RNase A were purchased from Life Technologies (Grand Island, NY, USA). Lycopene ($C_{40}H_{56}$, ≥90% pure isolated from tomato, Fig. 1) was purchased from Sigma Chemicals (St. Louis, MO) and diluted in phosphate buffered saline (PBS) or RPMI medium. Doxorubicin, paclitaxel (Taxol), temozolomide, pioglitazone and 15d-PGJ2 were obtained from Sigma (St. Louis, MO, USA). Ciglitazone was purchased from EMD Biosciences (Billerica, MA, USA). The human prostate cancer biomarker array kit was obtained from Superarray/ SA Bioscience (Valencia, CA, USA). The reagents and primer sets specific to TGFβ-2, IGF1R, EGFR, CDK-9, CDK-7, BRCA1, BCL-2 and GAPDH for quantitative reverse transcription polymerase chain reaction (qRT-PCR) were obtained from Applied Biosystems (Foster City, CA, USA). WST-1 reagent (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) and Annexin-V-Fluos staining kit were purchased from Roche (Indianapolis, IN).

2.2. Cell culture

Hormone-refractory human prostate cancer (PC-3) cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). PC-3 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml of penicillin and 100 μg/ml of streptomycin in 5% CO_2 incubator at 37°C. Cells were passaged in T-75 tissue culture flasks as and when they were ~85% confluent. Cells from passage 2 and 3 were used in the experiments.

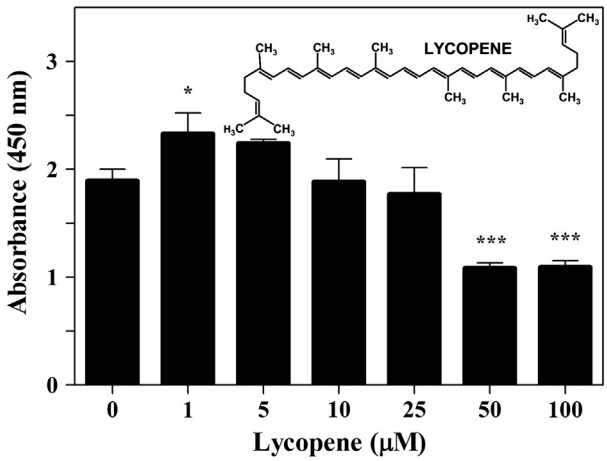

Fig. 1. Influence of lycopene on the proliferation of prostate cancer cells. PC-3 cells were cultured in RPMI medium in 96 well plates with increasing doses of lycopene. After 48 hours WST-1 reagent was added and the absorbance measured at 450 nm. The experiment was repeated three times and values are mean±S.D. *P<.05, **P<.01, ***P<.001. The chemical structure of lycopene is shown as an insert.

2.3. Proliferation assay

The effect of lycopene on prostate cancer cell proliferation was measured by WST-1 assay. PC-3 cells were cultured in RPMI medium (1×10^4/200 μl/well) in 96-well tissue culture plates in 5% CO_2 incubator at 37°C. Increasing doses of lycopene alone or in combination with chemotherapeutic agents and PPARγ agonists were added at the initiation of culture. After 48 h, 10 μl of WST-1 reagent was added to each well and the absorbance measured at 450 nm using a titer-plate reader (Alpha Diagnostics, San Antonio, TX, USA).

2.4. Cell cycle analysis

The effect of lycopene on cell cycle progression of prostate cancer cells was determined by propidium iodide staining and flow cytometry. PC-3 cells were cultured in RPMI medium (5×10^5/ml) in the absence or presence of 25 μM lycopene alone and in combination with 1 mM Doxorubicin, 100 μM Taxol, and 25 μM of Temezolomide, 15d-PGJ2, Ciglitazone or Piogliatzone in 12 well plates. Cells treated with medium, vehicle or 25 μM lycopene were used as controls. Cells were harvested after 48 h of culture and fixed in 70% ethanol overnight. Cells were then incubated in PBS containing 100 μg/ml of propidium iodide, 0.6% NP-40 and 20 μg/ml RNase (Sigma Chemicals, St Louis, MO, USA) at 4°C for 1 h. The percentage of cells at different phases of cell cycle (G0/G1, G2/M and S phases) and Debris (subG0/G1) were determined based on DNA content by flow cytometry using FACS Calibur Flow Cytometer (BD Biosciences). The data were analyzed using ModFit LT2.0 software (Verity software house, Topsham, ME, USA).

2.5. Apoptosis assay

The effect of lycopene on apoptosis of prostate cancer cells was determined by Annexin V-FITC staining and flow cytometry. PC-3 cells were cultured in RPMI medium (5×10^5/ml) in the absence or presence of 25 μM lycopene alone and in combination with 1 mM Doxorubicin, 100 μM Taxol, and 25 μM of Temezolomide, 15d-PGJ2, Ciglitazone or Piogliatzone in 12 well plates. Cells treated with medium, vehicle or 25 μM lycopene were used as controls. Cells were harvested after 48 h of culture and washed 3 times in PBS containing 0.1% BSA. Cells were then stained with Annexin V-FITC (Roche, Indianapolis, IN, USA) in binding buffer (0.1M Hepes/NaOH, pH 7.4, 1.4 M NaCl, 0.2 mM) containing 100 μg/ml propidium iodide according to the manufacturer's instruction (Roche). The cells were incubated at room temperature for 30 min in dark, acquired using FACS Calibur Flow Cytometer (BD Biosciences) and the data analyzed using FlowJo 8.2.6 software (Tree Star, Ashland, OR, USA).

2.6. DNA microarray

The effect of lycopene on gene expression profile of PC-3 cells was determined by Super array containing 263 biomarker genes associated with the diagnosis and prognosis of prostate cancer (Superarray/Sa Bioscience, Valencia, CA, USA). In brief, PC-3 cells were cultured in RPMI medium in the absence or presence of 25 μM lycopene. The cells were

harvested after 18 hrs and total RNA was extracted using Tri-Reagent according to manufacturer's instruction (Sigma, St. Louis, MO, USA). Equal quantities of RNA samples were reverse transcribed into cDNA and then converted to cRNA using the SuperArray TrueLabeling-AMP 2.0 kit (Superarray Bioscience, Frederick, MD, USA). The cRNA samples were labeled with Biotin (Roche, Indianapolis, IN) and hybridized with Superarray for 24 h. The arrays were then developed using a Chemiluminescence detection system and visualized by exposing to X-ray films. The images were uploaded to GEArray Expression Analysis Suite 2.0 computer software that reports gene expression profile normalized to GAPDH as internal control. Microarray data were also analyzed and quantified by using FluorChem HD2 software (Alpha Innotech/Quansys Biosciences, West Logan, UT, USA). The microarray experiments were conducted in triplicates and a mean percent change in gene expression between untreated and lycopene treated PC-3 cells was calculated after normalizing to GAPDH. Heat map was constructed using Excel software (Mirosoft, Redmond, WA, USA). Box plot and scatter plot were generated using GraphPad Prizm 5.0 software (GraphPad, La Jolla, CA, USA).

2.7. qRT-PCR

The effect of lycopene on the expression of selected genes in PC-3 cells was quantified using qRT-PCR technique. In brief, PC-3 cells were cultured in RPMI medium in the absence or presence of 25 μM lycopene. The cells were harvested after 18 hrs and total RNA was extracted using Tri-Reagent. Equal quantities of RNA samples were reverse transcribed into cDNA using TaqMan reverse transcription kit (Applied Biosystems, Foster City, CA, USA). qRT-PCR was performed using primer sets and probes (Taqman) for eight selected genes (EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, BCL-2, and GAPDH) obtained from Applied Biosystems (Foster City, CA, USA) in 96-well format with iCycler MYIQ Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA). The data were analyzed using the MYIQ Real-Time PCR quantification (delta-delta-Ct) study software and the gene expression levels were normalized to GAPDH and presented as relative fold change (RQ) compared to control. The qRT-PCR experiments were conducted in triplicates and an average percent change in gene expression between untreated and lycopene-treated PC-3 cells was also calculated.

2.8. Statistical analysis

The experiments were repeated three or more times and the values are expressed as mean±S.D./S.E.M. The differences between groups were analyzed by one way ANOVA using GraphPad Prism 5.0 software (La Jolla, CA, USA) and the values with $P<0.05^{*,\#}$, $P<0.01^{**,\#\#}$ and $P<0.001^{***,\#\#\#}$ were considered significant.

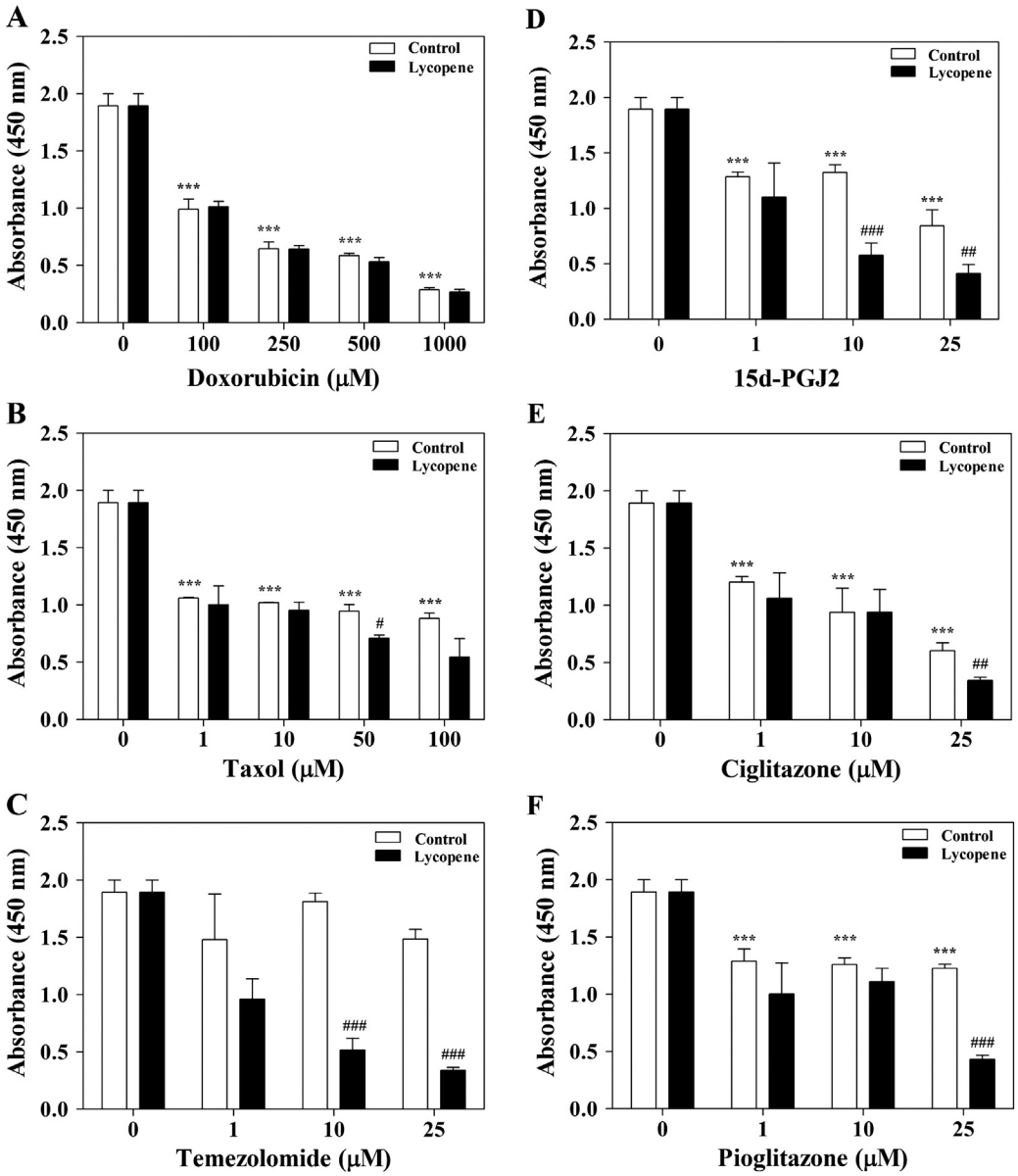

Fig. 2. Potentiating effect of lycopene on chemotherapeutic agents and PPARγ agonists in prostate cancer. PC-3 cells were cultured in RPMI medium in 96-well plates with doxorubicin (A) and paclitaxel (B), temezolomide (C), 15d-PGJ2 (D), ciglitazone (E) or pioglitazone (F) in the absence or presence of 25 μM lycopene. WST-1 reagent was added after 48 hrs and the absorbance measured at 450 nm. The experiment was repeated three times, values are mean±S.D., $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ compared to medium control and $^{\#}P<.05$, $^{\#\#}P<.01$, and $^{\#\#\#}P<.001$ compared to corresponding dose without lycopene.

3. Results

3.1. Lycopene influences the proliferation of prostate cancer cells

Earlier studies have presented conflicting results on the anti-tumor effect of lycopene in prostate cancer. In this study we found that the hormone refractory PC-3 cells grow and expand significantly in RPMI medium in culture. Interestingly, PC-3 cells cultured with 1 and 5 μM lycopene resulted in 23% and 18% increase in proliferation ($P<.05$), respectively that reversed to control levels at 10 and 25 μM doses. However, addition of 50 and 100 μM lycopene in culture induced 43% and 42% decrease in proliferation ($P<.001$), respectively (Fig. 1). These findings suggest that lycopene exerts a biphasic effect on the proliferation of PC-3 cells, with a modest increase at low dose and a significant decrease at high dose.

3.2. Lycopene augments the anti-proliferative effect of chemotherapeutic agents in prostate cancer

To further understand the beneficial effect of lycopene, we examined its potentiating effect on Doxorubicin and Taxol, two clinically available chemotherapeutic agents for prostate cancer. As shown in Fig. 2, in vitro treatment of PC-3 cells with Doxorubicin resulted in a dose-dependent decrease in proliferation, reaching 85% inhibition ($P<.001$) at 1 mM dose. Treatment with Taxol also induced a significant decrease in proliferation, reaching 54% inhibition ($P<.001$) at 100 μM dose in culture. However, combination treatment with lycopene failed to improve the anti-proliferative effects of Doxorubicin or Taxol on PC-3 cells in culture (Fig. 2 A and B). On the other hand, treatment with Temezolomide, a drug of choice for glioma, showed no

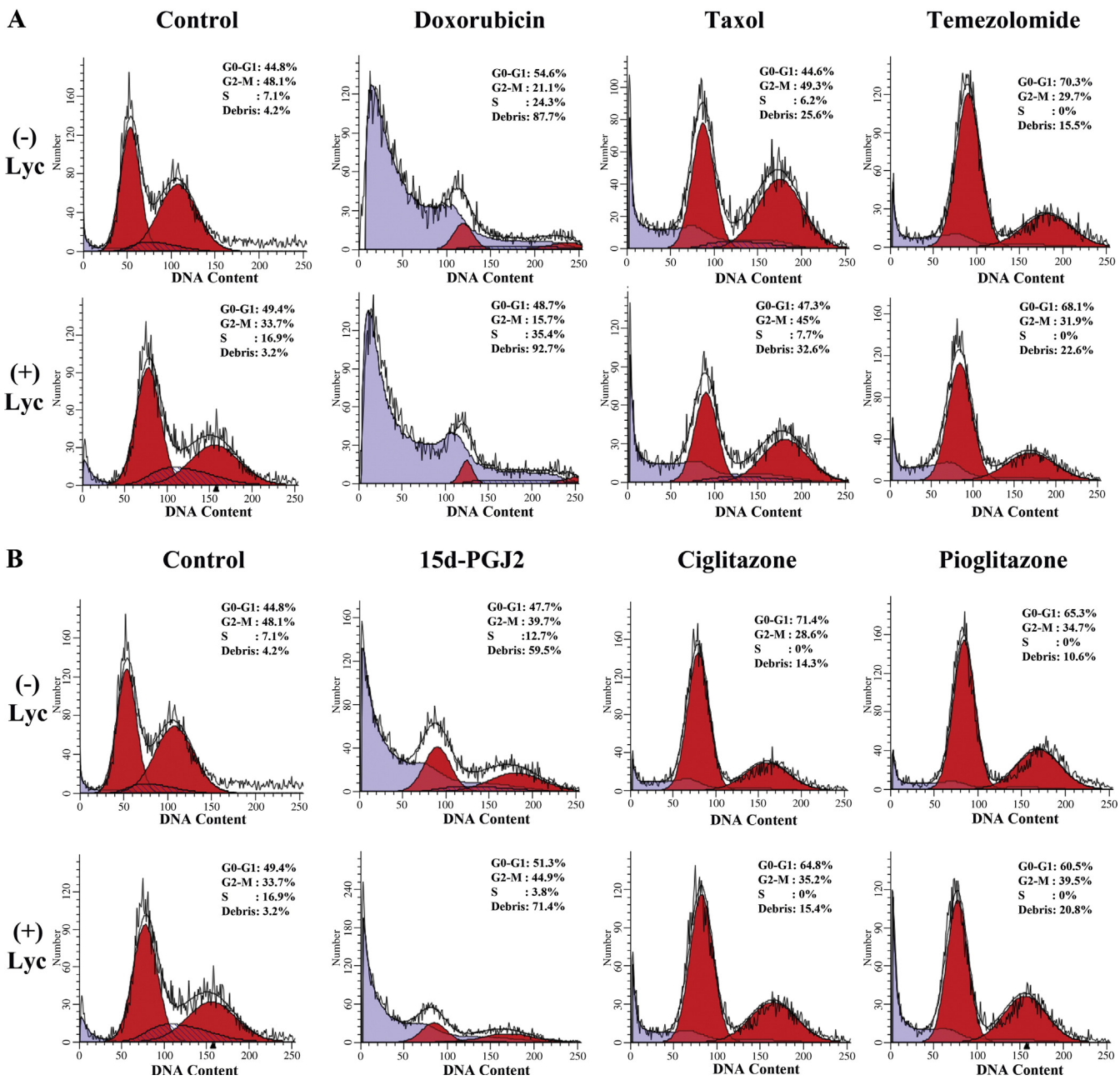

Fig. 3. Effect of lycopene on chemotherapeutic agent and PPARγ agonist induced cell cycle arrest. PC-3 cells were cultured with different chemotherapeutic agents (A) or PPARγ agonists (B) in the absence or presence of lycopene. Cells were collected after 48 h and fixed in ethanol and stained with propidium iodide. The cell cycle progression was determined by acquiring the samples through a flow cytometer and analyzing the results with ModFit software. The experiment was repeated three times.

significant antiproliferative effect on PC-3 cells. Interestingly, combination treatment with 25 μM lycopene resulted in a significant and dose-dependent decrease in PC-3 cell proliferation, reaching 72% and 77% inhibition ($P<.001$) at 10 μm and 25 μM Temezolomide, respectively (Fig. 2 C). These results suggest that lycopene induces anti-tumor effect of Temezolomide on prostate cancer.

3.3. Lycopene augments anti-proliferative effect of PPARγ agonists in prostate cancer

We have shown earlier that PPARγ agonists inhibit the growth and survival of brain tumor stem cells, while other studies demonstrated inhibition of prostate cancer cells [28,29,32]. In this study we examined the potentiating effect of lycopene on PPARγ agonists in PC-3 cells. We found that in vitro treatment of PC-3 cells with 15d-PGJ2, a natural ligand for PPARγ, resulted in a dose-dependent decrease in proliferation reaching 56% inhibition at 25 μM dose and that further increased to 78% inhibition ($P<.01$) in combination with 25 μM lycopene (Fig. 2D). Similarly, PC-3 cells treated with Ciglitazone, a synthetic agonist for PPARγ, showed a dose-dependent decrease in proliferation reaching 68% inhibition at 25 μM dose that increased to 82% inhibition ($P<.01$) in combination with 25 μM lycopene (Fig. 2E). Moreover, in vitro treatment of PC-3 cells with Pioglitazone, a clinically available synthetic agonist for PPARγ, also induced a significant decrease in proliferation, reaching 35% inhibition at 25 μM dose and that further increased to 77% inhibition ($P<.001$) in combination with 25 μM lycopene (Fig. 2F). These results suggest that lycopene potentiates the anti-proliferative effects of PPARγ agonists in prostate cancer.

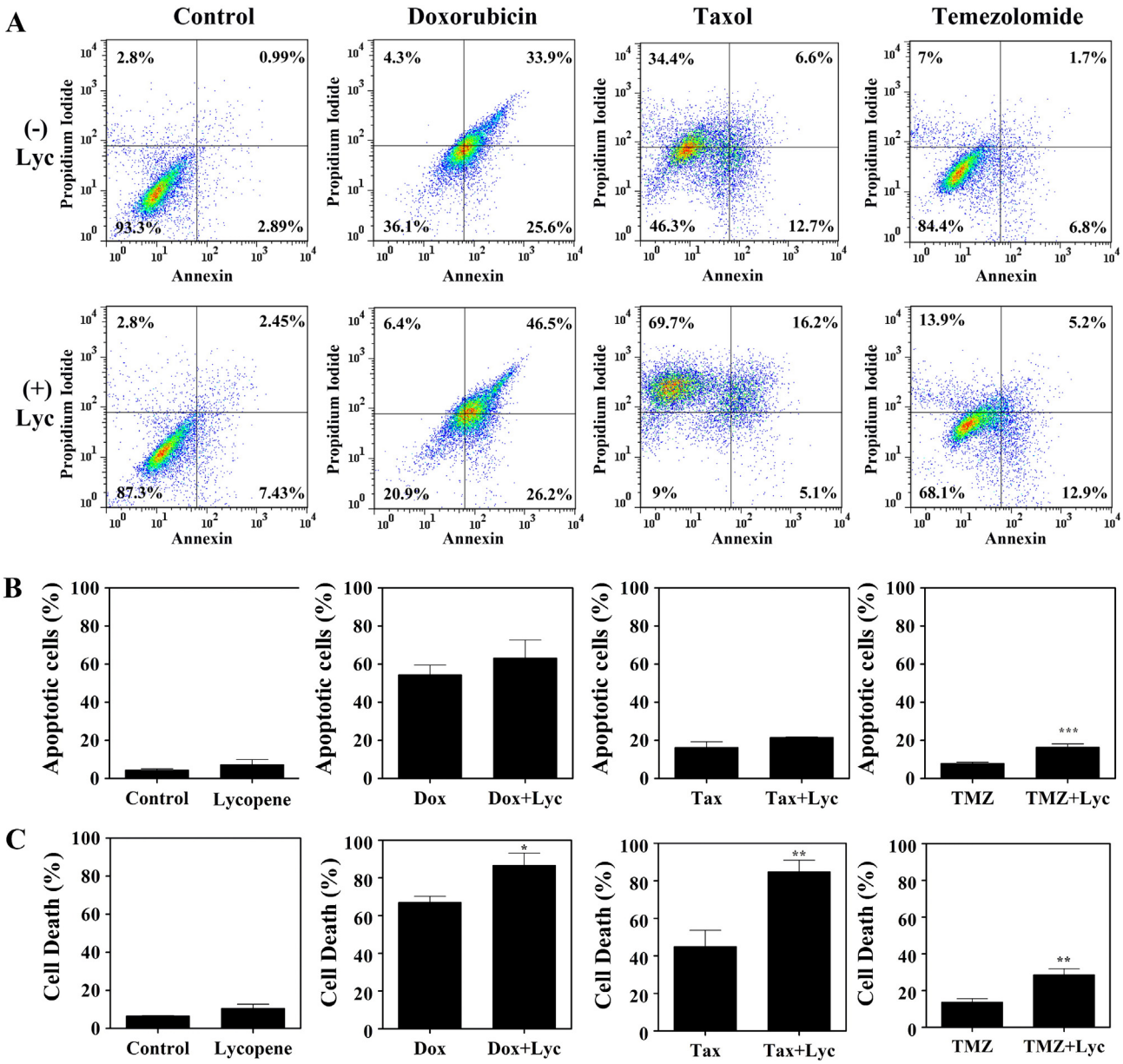

Fig. 4. Effect of Lycopene on chemotherapeutic agent induced apoptosis in PC-3 cells. PC-3 cells were cultured in RPMI medium in 12 well plates with different chemotherapeutic agents in the absence (A) or presence of lycopene (B). Cells were collected after 48 h and stained with Annexin V Fluos and propidium iodide after washing. Samples were acquired using a flow cytometer and analyzed using FlowJo 8.4.2 software. Total dead cells (C) and apoptotic cells (D) were determined. The experiment was repeated three times, values are mean±S.D., *$P<.05$, **$P<.01$, ***$P<.001$ compared to drug alone.

3.4. Lycopene modulates cell cycle progression in prostate cancer

In order to determine the mechanism by which lycopene inhibits the proliferation of prostate cancer cells we studied its influence on cell cycle progression. As shown in Fig. 3, treatment of PC-3 cells with 25 μM lycopene induced significant changes in the distribution of G0/G1, S and G2/M phase of cell cycle in the absence or presence of chemotherapeutic agents. We found that PC-3 cells cultured in the presence of lycopene showed a marked decrease in G2/M phase cells in association with an increase in G0/G1 and S phase without any change in debris compared to control (Fig. 3A). On the other hand, PC-3 cells cultured with Doxorubicin showed 88% debris that increased to 93% in the presence of lycopene. Moreover, PC-3 cells cultured with Taxol showed 26% debris that increased to 33% in combination with lycopene. However, neither Doxorubicin nor Taxol induced any significant changes in the cell cycle progression of PC-3 cells in the absence or presence of lycopene. On the other hand, Temezolomide showed a marked increase in G0/G1 arrest (70%) compared to control (45%) (Fig. 3A). In addition, PC-3 cells cultured with 25 μM PPARγ agonists showed an increase in G0/G1 and a decrease in G2/M phase (Fig. 3B). Interestingly, combination treatment with 25 μM lycopene led to a dramatic increase in debris in combination with 15d-PGJ2 and Pioglitazone, but not Ciglitazone. These results suggest that the chemotherapeutic agents and PPARγ agonists induce marked changes in cell cycle progression, but the induction of cell cycle arrest is not the mechanism by which lycopene exerts anti-cancer effect in prostate cancer.

3.5. Lycopene augments chemotherapeutic agent induced apoptosis in prostate cancer

To further understand the anti-cancer property of lycopene, we studied its effect on apoptosis in combination with chemotherapeutic

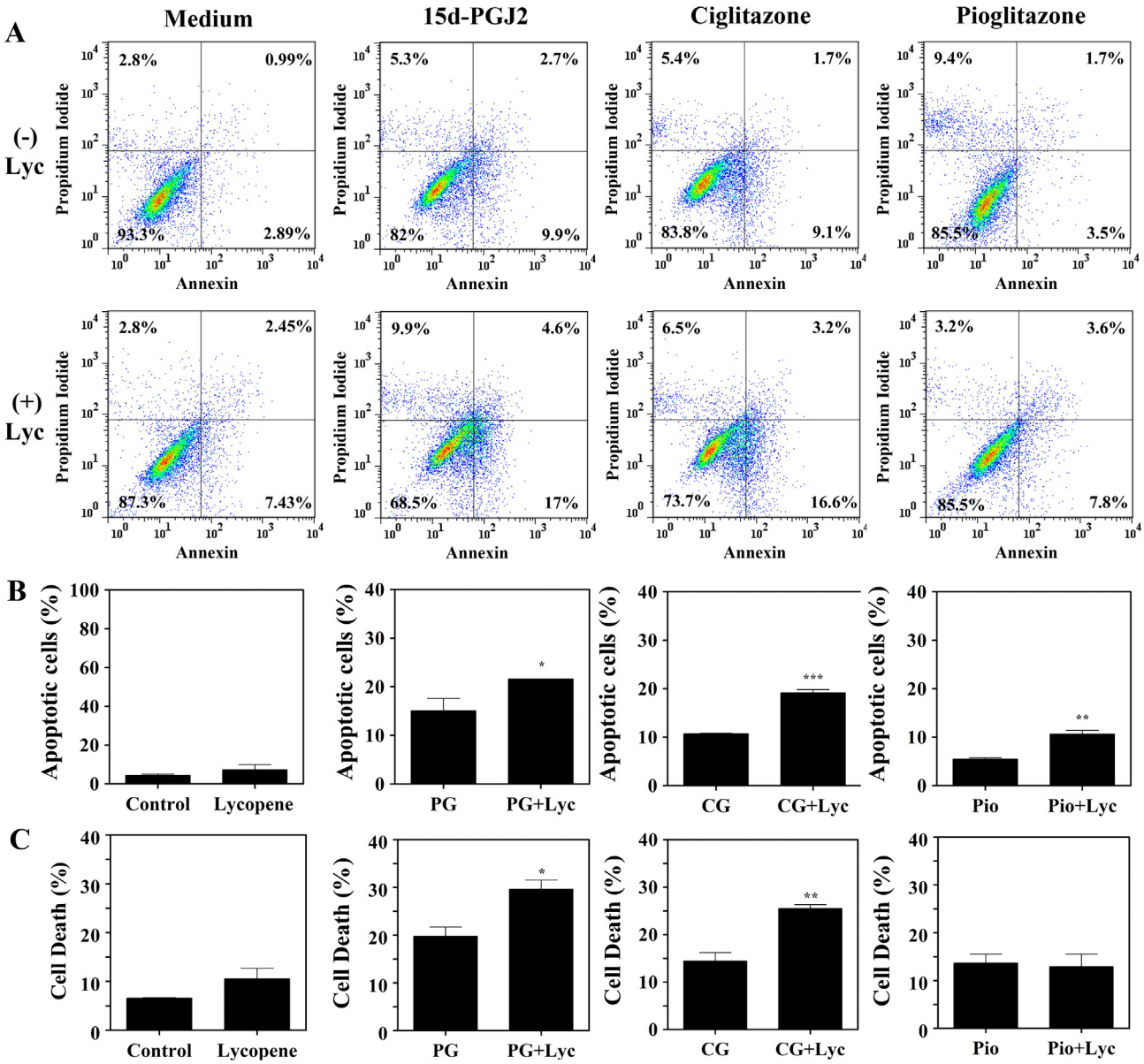

Fig. 5. Effect of lycopene on chemotherapeutic agent induced apoptosis in PC-3 cells. PC-3 cells were cultured in RPMI medium in 12-well plates with different PPARγ agonists in the absence (A) or presence of Lycopene (B). Cells were collected after 48 h and stained with Annexin V Fluos and propidium iodide after washing. Samples were acquired using a flow cytometer and analyzed using FlowJo 8.4.2 software. Total dead cells (C) and apoptotic cells (D) were determined. The experiment was repeated three times, values are mean±S.D., $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ compared to drug alone.

agents in prostate cancer. As shown in Fig. 4, PC-3 cells cultured with 25 μM lycopene showed no significant increase in apoptosis compared to control. Interestingly, treatment with Doxorubicin, Taxol and Temezolomide induced 54%, 16%, and 8% Annexin positive apoptotic cells that increased to 63%, 22% and 16% ($P<.001$) in combination with lycopene, respectively (Fig. 4A and B). In addition, PC-3 cells treated with Doxorubicin, Taxol and Temezolomide showed 67%, 45% and 14% dead cells (apoptosis+necrosis) that increased to 87% ($P<.05$), 85% ($P<.01$) and 29% ($P<.01$) in combination with lycopene, respectively (Fig. 4C). These results indicate that lycopene modulates drug-induced apoptosis in prostate cancer.

3.6. Lycopene modulates PPARγ induced apoptosis in prostate cancer

To further determine the anti-cancer property of lycopene, we studied its effect on apoptosis in combination with PPARγ agonists in prostate cancer. As shown in Fig. 5, we found that the PC-3 cells cultured with 25 μM 15d-PGJ2, ciglitazone and pioglitazone showed 15%, 11% and 5% apoptosis that increased to 22% ($P<.05$), 19% ($P<.001$) and 11% ($P<.01$) in combination with lycopene (Fig. 5 A and B). Moreover, PC-3 cells treated with 25 μM 15d-PGJ2, Ciglitazone and Pioglitazone also showed 20%, 14% and 14% Annexin and PI positive dead cells that changed to 30% ($P<.05$), 26% ($P<.01$) and 13% in combination with lycopene (Fig. 5C). These results indicate that lycopene augments PPARγ agonist-induced apoptosis in prostate cancer.

3.7. Lycopene alters the expression of biomarker genes in prostate cancer

To further understand the molecular mechanism by which lycopene attenuates prostate cancer, we used Superarray to detect the expression of 263 prostate cancer biomarker genes in PC-3 cells. This array consists of 288 spots that also include other housekeeping genes (Table 1). As shown in Fig. 6, microarray analysis demonstrated that in vitro treatment with 25 μM lycopene resulted in significant alterations in the expression of several biomarker genes in PC-3 cells compared to control. Among the 288 spots examined, we found detectable expression of only 182 with the remaining 106 were undetected in either medium or lycopene treated PC-3 cells (Fig. 6,

Table 1
Prostate cancer biomarker gene array map

RPS27A	RPS27A	AGR2	AGTR2	AIG1	AKAP1	AKT1	APC	APOC1	GAPDH	GAPDH	GAPDH
1.6	1.3	0.9	0	D-ND	0.3	0.4	0.08	0.6	1.0	1.0	1.0
RPS27A	AR	BAK1	BAX	BCL2	BCL2L1	BMP6	BRCA1	CANT1	CASP1	CASP3	CASP7
1.8	D-ND	1.6	0.8	0.65	0.6	0	0.74	0.9	D-ND	0	0.35
CAV1	CCND1	CD164	CD44	CDH1	CDH10	CDH12	CDH13	CDH18	CDH19	CDH20	CDH7
1.65	2.0	0.92	0.7	0.33	D-ND	D-ND	0	D-ND	0.4	0	D-ND
CDH8	CDH9	CDK2	CDK3	CDK4	CDK5	CDK6	CDK7	CDK8	CDK9	CDKN1A	CDKN1B
0	0	0.9	0	1.06	0.6	0	0.71	D-ND	D-ND	0.84	0.3
CDKN1C	CDKN2A	CDKN2B	CDKN2C	CDKN3	CHGA	CHGB	CLDN3	CLN3	CLU	COL1A1	COL6A1
1.05	0.94	1.0	1.04	1.01	0	0	0.34	0.81	0.9	D-ND	0.8
CYB5	CYC1	DAB2IP	DAPK1	DES	DNCL1	E2F1	EGF	EGFR	EGR3	ELAC2	ELL
0.91	1.43	0.85	0	0	1.1	1.1	0.31	0.4	0	0.9	0.41
ENO1	ENO2	ENO3	ERBB2	ERK8	ESR1	ESR2	EZH1	EZH2	FASN	FGF1	FGF10
1.53	1.2	1.22	1.3	0	0.83	0	0	0.6	0.54	0	0
FGF11	FGF12	FGF13	FGF14	FGF16	FGF17	FGF18	FGF19	FGF2	FGF20	FGF21	FGF22
0	0	0	D-ND	0	D-ND	0	0	D-ND	0	0	0
FGF23	FGF3	FGF4	FGF5	FGF6	FGF7	FGF8	FGF9	FHIT	FLJ12584	FLJ25530	FOLH1
0	D-ND	0	0	D-ND	0	0	0	0	0.9	0	0
GAGEB1	GAGEC1	GGT1	GNRH1	GRP	GSTP1	HIF1A	HIP1	HK2	HK3	HRAS	HUMCYT2A
0	0	0	0	0	0.94	0.007	D-ND	D-ND	0	0.65	0
IGF1	IGF1R	IGF2	IGFBP3	IGFBP6	IL12A	IL1A	IL1B	IL2	IL24	IL29	ILK
0	0.52	1.05	1.07	0.94	1.2	0.4	0.94	0	0	0	1.42
INHA	INSL3	INSL4	ITGA1	JUN	K6HF	KAI1	KLK1	KLK10	KLK11	KLK12	KLK13
0.3	0.9	0.83	0	1.5	1.05	0.71	0.91	0.92	0	0	D-ND
KLK14	KLK15	KLK2	KLK3	KLK4	KLK5	KLK6	KLK7	KLK8	KLK9	KRT1	KRT2A
0.9	0	1.11	0	0	0.72	D-ND	D-ND	D-ND	0.11	0	0.71
MAP2K4	MAP3K1	MAPK1	MAPK10	MAPK11	MAPK12	MAPK13	MAPK14	MAPK3	MAPK4	MAPK6	MAPK7
1.2	0	1.02	0	0	D-ND	0.7	D-ND	1.6	0	0.72	0
MAPK8	MAPK9	MIB1	MMP2	MMP9	MSMB	MTSS1	MYC	NCOA4	NFKB1	NFKB1A	NKX3-1
1.23	1.42	D-ND	1.8	D-ND	0	D-ND	0.8	0.91	1.13	1.8	D-ND
NOX5	NROB1	NROB2	NR1D1	NR1D2	NR1H2	NR1H3	NR1H4	NR1I2	NR1I3	NR2C1	NR2C2
0.8	0	0	D-ND	0	0.44	1.0	0	1.1	0.2	0.3	1.01
NR2E1	NR2E3	NR2F1	NR2F2	NR2F6	NR3C1	NR3C2	NR4A1	NR4A2	NR4A3	NR5A1	NR5A2
0	1.2	1.6	0	1.2	0.7	0	D-ND	0.84	0	0	0
NR6A1	NTN4	ODZ1	PALM2	PAP	PART1	PATE	PAWR	PCA3	PCNA	PGR	PIAS1
0	0.8	0	D-ND	D-ND	0	0	0.46	0	1.11	1.23	0.8
PIAS2	PIK3CG	PLAU	PLG	PPID	TMEM37	PRKCA	PRKCB1	PRKCD	PRKCE	PRKCG	PRKCH
0.41	1.6	1.6	0	0.83	1.1	0	0	0.75	0	1.0	1.0
PRKCI	PRKD3	PRKCQ	PRKCZ	PRKD1	PRKD2	PRL	PSAP	PSCA	PTEN	RARB	RASSF1
D-ND	1.44	0.6	0.6	0	D-ND	0	0	0.6	0	0	1.22
RB1	RNASEL	RNF14	ROBO2	SERPINA3	SHBG	SLC2A2	SLC33A1	SLC43A1	SOX2	SRC	SRD5A2
1.6	0.9	0.55	0	0	D-ND	0	D-ND	0	0	D-ND	D-ND
HSPCB	STEAP	STEAP2	TGFA	TGFB1	TGFB1I1	TGFB2	TGFB3	TIMP3	TNF	POL1	PUC18
1.9	1.75	1.3	1.23	1.23	0.09	0.17	D-ND	0	0	0	0
B2M	BLANK	BLANK	TNFSF10	TP53	TPM1	TMP2	18SrRNA	AS1R3	AS1R2	AS1R1	AS1
1.6	0	0	0	D-ND	0.2	0.94	D-ND	D-ND	0	0	0
B2M	B2M	ACTB	TRPC6	TRPS1	TYK2	VEGF	BAS2C	BAS2C	BAS2C	BAS2C	BAS2C
1.74	2.62	2.5	0	0	0	0	0	0	0.71	1.6	1.75

Table depicts the symbols of prostate cancer biomarker genes included in the Superarray. Numbers represent fold change in gene expression of lycopene treated PC-3 cells compared to control, as measured by densitometry (0, not detected, 1, no change; D-ND, detected to not detected).

Table 1). After normalizing the 182 data points to GAPDH as an internal control, we found an increase in the expression of 56 genes (↑), decrease in 78 genes (↓), decrease to undetectable level in 41 genes (D-ND) and 7 unchanged genes in lycopene treated PC-3 cells compared to medium control. Among the 56 up-regulated genes, 3 showed ≥2-fold increase, 19 showed 1.5–1.9-fold increase and 34 genes showed 1–1.4 fold increase following treatment with lycopene. Moreover, among the 78 down-regulated genes, three showed ≤0.09-fold decrease, 20 showed 0.1–0.49-fold decrease and 55 genes showed 0.5–0.99-fold decrease in lycopene treated cells compared to control (Table 1, Fig. 8A).

As shown in Fig. 7, further analyses showed significant alterations in the expression of many prostate cancer biomarker genes by lycopene in culture. Spot densitometry analysis of the microarray data plotted as heat map (Fig. 7A) demonstrates alterations in gene expression in lycopene treated cells compared to untreated control. Similarly, scatter plot analysis (Fig. 7B) shows the fold changes in lycopene treated PC-3 cells, compared to control with dots above 1 indicating increase in expression, while dots below 1 represent inhibition. Moreover, the scatter plot of gene expression level between control and lycopene treated cells shows an r^2value of 0.7834 (Fig. 7C). The genes in the microarray are clustered under several groups of which we further analyzed apoptosis, cell cycle and proliferation associated genes in detail. As shown in Fig. 7D, we found that the median gene expression of inducers of apoptosis and caspases were decreased in lycopene treated cells compared to control, whereas the inhibitors and other regulators of apoptosis were increased in lycopene treated cells. Among the cell cycle family of genes the checkpoint, inhibitor and regulator genes showed increased expression following lycopene treatment (Fig. 7E). Interestingly, of the proliferation associated genes the inhibitors of proliferation showed a marked increase in expression in lycopene treated cells, with no significant difference in cytokines, growth factors and inducers of proliferation when compared to control (Fig. 7F). These results suggest that lycopene alters the expression of growth and survival associated biomarker genes in prostate cancer.

3.8. Lycopene inhibits the expression of growth and survival-associated genes in prostate cancer

Further analyses of microarray data revealed that in vitro treatment with lycopene resulted in significant decreased in the expression of eight selected growth and survival associated genes in PC-3 cells. These genes are BCL-2, BCL2L1, BRCA1, CDK-7, CDK-9, EGFR, IGF1R, TGFβ-2, which showed 35%, 40%, 26%, 29%, 100%, 60%, 48% and 83% inhibition, respectively, after treatment of PC-3 cells with lycopene compared to control (Fig. 8B). Further analyses by qRT-PCR showed 30%, 50%, 34%, 56%, 72%, 61% and 44% decrease in BCL-2, BCL2L1, BRCA1, CDK-7, CDK-9, EGFR, IGF1R, TGFβ-2 expression, respectively, in PC-3 cells treated with lycopene compared to control (Fig. 8C). These results further confirm that lycopene inhibits the expression of growth and survival-associated biomarker genes in prostate cancer.

4. Discussion

Plant-derived dietary carotenoids are known to exert beneficial effects on metabolism, cancer and cardiovascular diseases [2]. Lycopene is a carotenoid pigment not synthesized in the body but primarily consumed through food containing tomatoes and tomato products such as tomato sauce; tomato paste and ketchup; and relatively smaller amounts from dried apricots, watermelon and pink grapefruit [1,2,18]. Clinical studies have observed that the dietary consumption of lycopene from fruits and vegetables reduces the risk

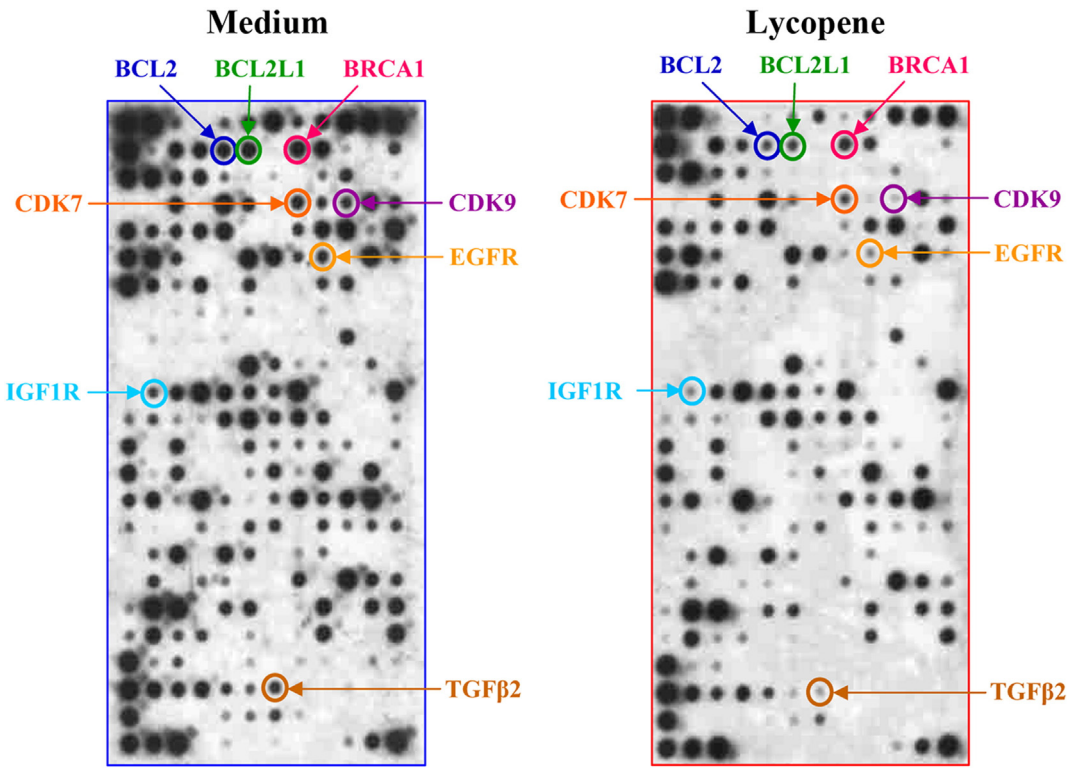

Fig. 6. Effect of lycopene on prostate cancer biomarker genes. PC-3 cells were cultured in the absence or presence 25 μM lycopene for 18 hrs and gene expression was analyzed using Superarray with 288 spots containing 263 prostate cancer biomarkers and other housekeeping genes. The arrays were developed using a chemiluminescence detection system and visualized by exposing to X-ray films. Arrows indicate selected biomarker genes. The figure is a representative of three independent experiments.

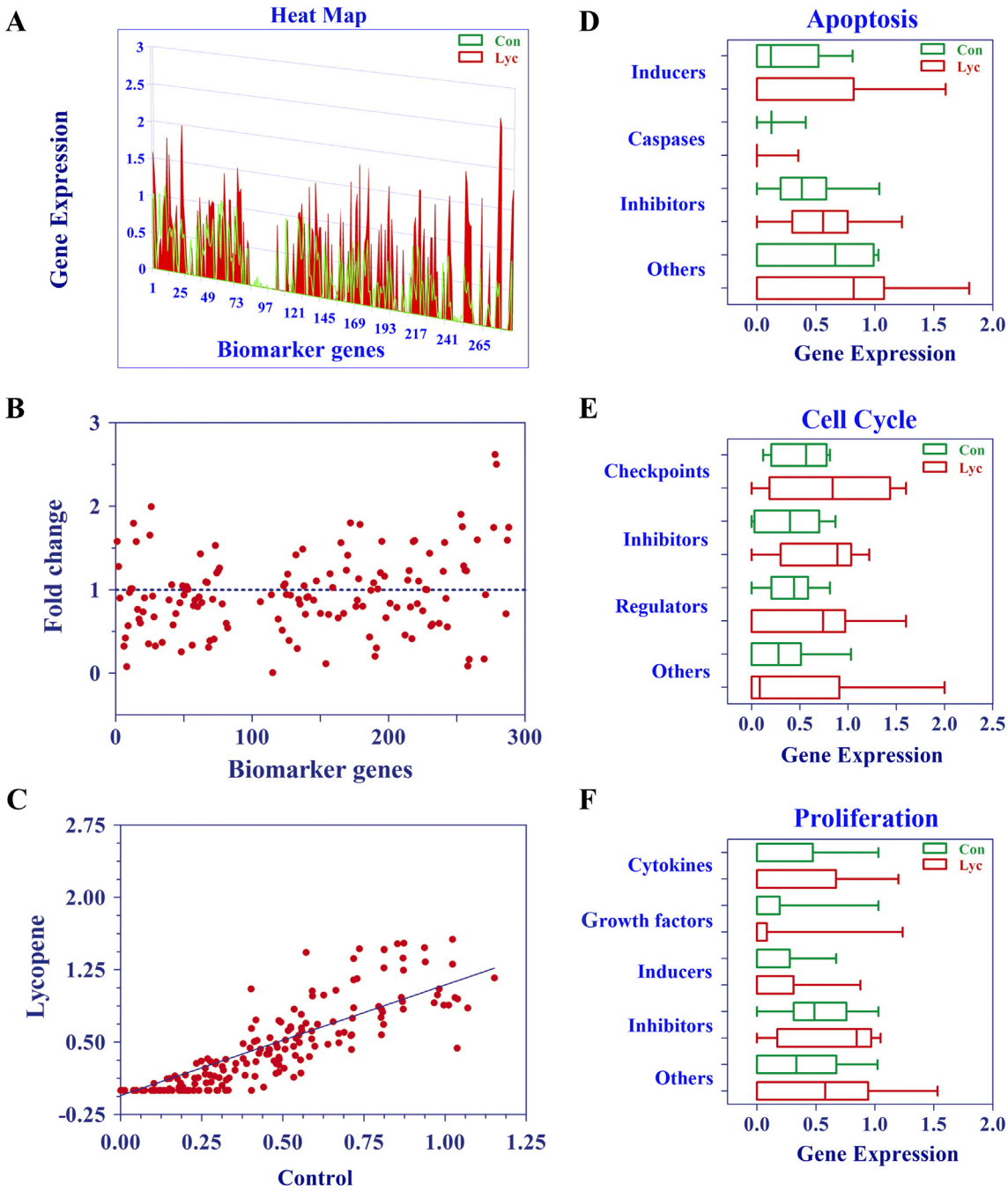

Fig. 7. Analysis of lycopene regulated prostate cancer biomarker genes. The gene expression profiles of super array blots were quantified using FluorChem HD2 software. Heat map was constructed using Excel (A), scatter plots (B, C) and box plots (D, E, F) were generated using GraphPad Prizm 5.0 software. Legends show medium control (Con) and 25 μM Lycopene (Lyc) in the figure.

of prostate cancer [12–14]. In addition laboratory experiments showed significant changes in the growth pattern of lycopene treated prostate cancer cells in culture and in animal models [17,18]. While some studies showed significant and dose-dependent inhibition of proliferation by lycopene, others demonstrated partial decrease, partial increase or no effect on prostate cancer [6,34–36]. Moreover, the mechanism by which lycopene attenuates prostate cancer is not fully defined.

In this study we found that lycopene induces a biphasic effect on prostate cancer cells with an initial increase in proliferation at lower dose followed by a moderate decrease at high dose. Doxorubicin and Taxol are two important clinically available chemotherapeutic agents in the treatment of prostate cancer, but they produce only modest beneficial effects with many side effects [22,37]. Therefore we examined the potentiating effect of lycopene on these drugs for the treatment of prostate cancer. We found that both Doxorubicin and Taxol induced a dose-dependent decrease in the proliferation of PC-3 cells in culture. Interestingly, combination treatment with lycopene resulted in a significant improvement in the anti-tumor effect of Doxorubicin and Taxol at lower doses. Further analyses revealed that lycopene also augments drug-induced apoptosis without affecting cell cycle. These findings indicate the use of lycopene to potentiate the effect of chemotherapeutic agents on prostate cancer.

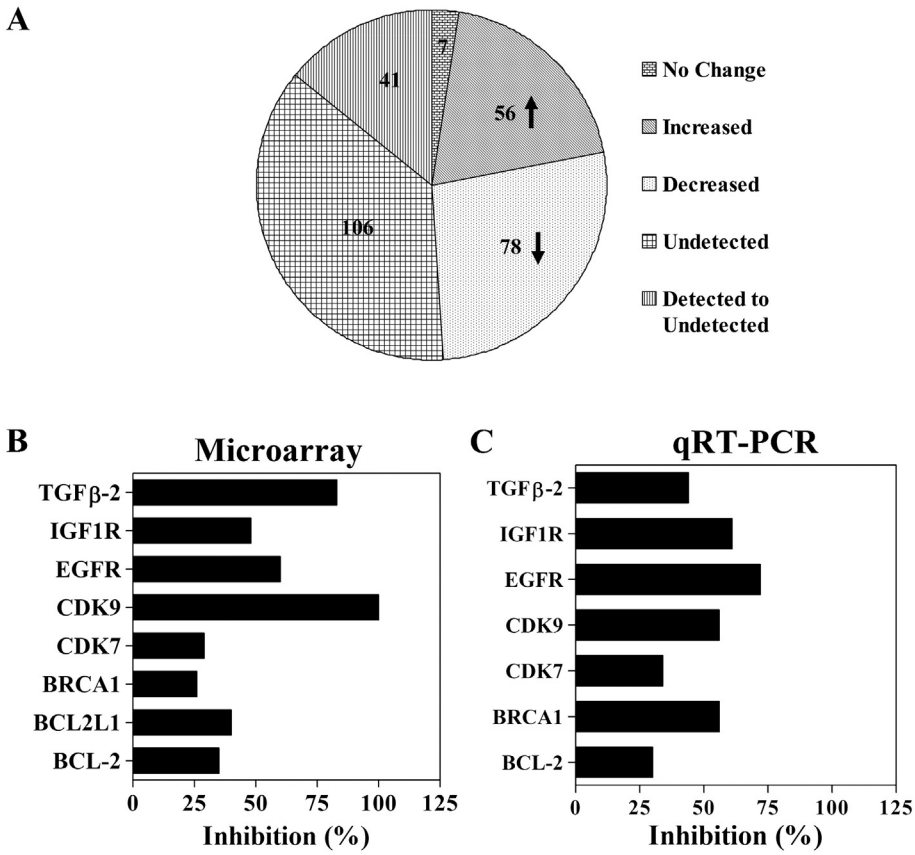

Fig. 8. Effect of lycopene on growth and survival associated genes in prostate cancer. (A) Pie chart analysis showing differential regulation of biomarker genes by lycopene in prostate cancer. Inhibition of growth and survival-associated genes by lycopene in prostate cancer, as determined by microarray (B) and qRT-PCR (C) analysis are shown.

Temozolomide is a DNA alkylating agent that has been used to treat glioma, either alone or in conjunction with irradiation, but not for prostate cancer. We found that Temozolomide has no anti-proliferative effect on PC-3 cells in culture. Interestingly in the presence of lycopene, Temozolomide induced a dose-dependent decrease in the proliferation in prostate cancer. Lycopene in combination with Temozolomide also induced cell cycle-arrest and apoptosis in prostate cancer cells. Previous studies have reported that PI3K/AKT and ERK1/2 survival pathways are constitutively active in glioma [38]. Although the exact mechanism is not know, earlier reports on the regulation of p53/MDMT pathway by lycopene and Temozolomide in glioma suggest that lycopene could regulate this pathway to sensitize prostate cancer cells to Temozolomide [25].

PPARγ is a nuclear receptor transcription factor that regulates growth and differentiation of many cell types. PPARγ modulates lipid metabolism and glucose homeostasis and selective PPARγ agonists have been used as prescription drugs for type 2 diabetes. Earlier studies have also shown that PPARγ agonists exert anti-tumor effects in many tumors including prostate cancer [28–32]. We demonstrated earlier that PPARγ agonists inhibit the growth and expansion of brain tumor stem cells by modulating stemness and differentiation genes in glioma [28,29]. In this study we found that PPARγ agonists 15d-PGJ2, Ciglitazone and Pioglitazone induced a dose-dependent decrease in proliferation of PC-3 cells. Interestingly, lycopene improved the anti-proliferative effect of PPARγ agonists on prostate cancer cells. Moreover, in vitro treatment with lycopene also increased cell cycle-arrest and apoptosis induced by PPARγ agonists on prostate cancer cells. Thus our findings suggest that lycopene could be useful in potentiating the therapeutic effect of PPARγ agonists for prostate cancer. Although the mechanisms are not known, recent reports on the ability of lycopene to up-regulate and interact with PPARγ suggest an association between lycopene and PPARγ pathway in modulating prostate cancer [33].

Earlier studies have used microarray to identify new therapeutic targets in the treatment of prostate cancer [39]. In order to determine the molecular mechanisms in the regulation of prostate cancer by lycopene in this study we use a prostate cancer biomarker gene array approach. Our results showed that in vitro treatment with lycopene induced considerable changes in the expression of many biomarker genes in PC-3 cells. Interestingly there are different functional gene clusters that are increased, decreased, unchanged or not expressed, suggesting complex mechanisms in the regulation of prostate cancer by lycopene. In particular, we found a marked increase in the inhibitors of proliferation, cell cycle regulators and check point genes as well as regulators of apoptosis after treatment with lycopene, suggesting their significance to the attenuation of prostate cancer.

Further analyses by qRT-PCR confirmed our microarray data that 8 growth and survival associated biomarker genes were consistently down-regulated by lycopene in prostate cancer. In particular, lycopene inhibits the expression of growth-associated genes such as EGFR, IGF1R and TGFβ-2 that are known to promote the survival, progression and metastasis of androgen-independent prostate cancer [40–44]. Similarly, lycopene inhibits the expression of CDK-7 and CDK-9 that can affect prostate cancer cell growth and expansion [45]. Moreover, lycopene inhibits the expression of BCL-2 that can influence the survival and apoptosis of prostate cancer [46,47]. Taken together, in this study we have demonstrated that lycopene attenuates prostate cancer by modulating the expression of growth and survival associated genes.

References

[1] Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Mol Aspects Med 2005;26:459–516.
[2] Rao AV, Rao LG. Carotenoids and human health. Pharmacol Res 2007;55:207–16.
[3] Eid SY, El-Readi MZ, Wink M. Carotenoids reverse multidrug resistance in cancer cells by interfering with ABC-transporters. Phytomedicine 2012;19:977–87.
[4] Kong KW, Khoo HE, Prasad KN, Ismail A, Tan CP, Rajab NF. Revealing the power of the natural red pigment lycopene. Molecules 2010;15:959–87.
[5] Bhuvaneswari V, Nagini S. Lycopene: a review of its potential as an anticancer agent. Curr Med Chem Anticancer Agents 2005;5:627–35.
[6] Ilic D, Misso M. Lycopene for the prevention and treatment of benign prostatic hyperplasia and prostate cancer: a systematic review. Maturitas 2012;72:269–76.
[7] McEneny J, Wade L, Young IS, Masson L, Duthie G, et al. Lycopene intervention reduces inflammation and improves HDL functionality in moderately overweight middle-aged individuals. J Nutr Biochem [e-pub 2012].
[8] Nagal S, Kaur C, Choudhary H, Singh J, Bhushan Singh B, et al. Lycopene content, antioxidant capacity and colour attributes of selected watermelon (Citrullus lanatus (Thunb.) Mansfeld) cultivars grown in India. Int J Food Sci Nutr [epub 2012].
[9] La Vecchia C, Negri E, Pelucchi C, Franceschi S. Dietary folate and colorectal cancer. Int J Cancer 2002;102:545–7.
[10] Holick CN, Michaud DS, Stolzenberg-Solomon R, Mayne ST, Pietinen P, et al. Dietary carotenoids, serum beta-carotene, and retinol and risk of lung cancer in the alpha-tocopherol, beta-carotene cohort study. Am J Epidemiol 2002;156: 536–47.
[11] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62: 10–29.
[12] Pal RP, Maitra NU, Mellon JK, Khan MA. Defining prostate cancer risk before prostate biopsy. Urol Oncol [epub 2012].
[13] Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC. A prospective study of tomato products, lycopene, and prostate cancer risk. J Natl Cancer Inst 2002;94: 391–8.
[14] Mohanty NK, Saxena S, Singh UP, Goyal NK, Arora RP. Lycopene as a chemopreventive agent in the treatment of high-grade prostate intraepithelial neoplasia. Urol Oncol 2005;23:383–5.
[15] Chakrabarti R, Robles LD, Gibson J, Muroski M. Profiling of differential expression of messenger RNA in normal, benign, and metastatic prostate cell lines. Cancer Genet Cytogenet 2002;139:115–25.
[16] Chen L, Stacewicz-Sapuntzakis M, Duncan C, Sharifi R, Ghosh L, et al. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. J Natl Cancer Inst 2001;93:1872–9.
[17] Tang L, Jin T, Zeng X, Wang JS. Lycopene inhibits the growth of human androgen-independent prostate cancer cells in vitro and in BALB/c nude mice. J Nutr 2005;135:287–90.
[18] Rafi MM, Yadav PN, Reyes M. Lycopene inhibits LPS-induced proinflammatory mediator inducible nitric oxide synthase in mouse macrophage cells. J Food Sci 2007;72:S069–74.
[19] Horwitz SB, Parness J, Schiff PB, Manfredi JJ. Taxol: a new probe for studying the structure and function of microtubules. Cold Spring Harb Symp Quant Biol 1982;46(Pt 1):219–26.
[20] De Brabander M, Geuens G, Nuydens R, Willebrords R, De Mey J. Microtubule stability and assembly in living cells: the influence of metabolic inhibitors, taxol and pH. Cold Spring Harb Symp Quant Biol 1982;46(Pt 1):227–40.
[21] Roth BJ, Yeap BY, Wilding G, Kasimis B, McLeod D, et al. Taxol in advanced, hormone-refractory carcinoma of the prostate. A phase II trial of the Eastern Cooperative Oncology Group. Cancer 1993;72:2457–60.
[22] Tsakalozou E, Eckman AM, Bae Y. Combination effects of docetaxel and doxorubicin in hormone-refractory prostate cancer cells. Biochem Res Int 2012;2012:832059.
[23] Tehranian N, Sepehri H, Mehdipour P, Biramijamal F, Hossein-Nezhad A, et al. Combination effect of PectaSol and Doxorubicin on viability, cell cycle arrest and apoptosis in DU-145 and LNCaP prostate cancer cell lines. Cell Biol Int 2012;36: 601–10.
[24] Taverna P, Catapano CV, Citti L, Bonfanti M, D'Incalci M. Influence of O6-methylguanine on DNA damage and cytotoxicity of temozolomide in L1210 mouse leukemia sensitive and resistant to chloroethylnitrosoureas. Anticancer Drugs 1992;3:401–5.
[25] Zhang WB, Wang Z, Shu F, Jin YH, Liu HY, et al. Activation of AMP-activated protein kinase by temozolomide contributes to apoptosis in glioblastoma cells via p53 activation and mTORC1 inhibition. J Biol Chem 2010;285:40461–71.
[26] Sato A, Sunayama J, Matsuda K, Seino S, Suzuki K, et al. MEK-ERK signaling dictates DNA-repair gene MGMT expression and temozolomide resistance of stem-like glioblastoma cells via the MDM2-p53 axis. Stem Cells 2011;29:1942–51.
[27] Rosen ED, Spiegelman BM. PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem 2001;276:37731–4.
[28] Pestereva E, Kanakasabai S, Bright JJ. PPARgamma agonists regulate the expression of stemness and differentiation genes in brain tumour stem cells. Br J Cancer 2012;106:1702–12.
[29] Chearwae W, Bright JJ. PPARgamma agonists inhibit growth and expansion of CD133+ brain tumour stem cells. Br J Cancer 2008;99:2044–53.
[30] Mansour M, Schwartz D, Judd R, Akingbemi B, Braden T, et al. Thiazolidinediones/PPARgamma agonists and fatty acid synthase inhibitors as an experimental combination therapy for prostate cancer. Int J Oncol 2011;38:537–46.
[31] Xu Y, Iyengar S, Roberts RL, Shappell SB, Peehl DM. Primary culture model of peroxisome proliferator-activated receptor gamma activity in prostate cancer cells. J Cell Physiol 2003;196:131–43.
[32] Ban JO, Oh JH, Son SM, Won D, Song HS, et al. Troglitazone, a PPAR agonist, inhibits human prostate cancer cell growth through inactivation of NFkappaB via suppression of GSK-3beta expression. Cancer Biol Ther 2011;12:288–96.
[33] Yang CM, Lu YL, Chen HY, Hu ML. Lycopene and the LXRalpha agonist T0901317 synergistically inhibit the proliferation of androgen-independent prostate cancer cells via the PPARgamma-LXRalpha-ABCA1 pathway. J Nutr Biochem 2012;23: 1155–62.
[34] Yang CM, Lu IH, Chen HY, Hu ML. Lycopene inhibits the proliferation of androgen-dependent human prostate tumor cells through activation of PPARgamma-LXRalpha-ABCA1 pathway. J Nutr Biochem 2012;23:8–17.
[35] Yang CM, Yen YT, Huang CS, Hu ML. Growth inhibitory efficacy of lycopene and beta-carotene against androgen-independent prostate tumor cells xenografted in nude mice. Mol Nutr Food Res 2011;55:606–12.
[36] Burgess LC, Rice E, Fischer T, Seekins JR, Burgess TP, et al. Lycopene has limited effect on cell proliferation in only two of seven human cell lines (both cancerous and noncancerous) in an in vitro system with doses across the physiological range. Toxicol In Vitro 2008;22:1297–300.
[37] Stearns ME, Wang M. Taxol blocks processes essential for prostate tumor cell (PC-3 ML) invasion and metastases. Cancer Res 1992;52:3776–81.
[38] Tian Y, Nan Y, Han L, Zhang A, Wang G, et al. MicroRNA miR-451 downregulates the PI3K/AKT pathway through CAB39 in human glioma. Int J Oncol 2012;40: 1105–12.
[39] Calvo A, Gonzalez-Moreno O, Yoon CY, Huh JI, Desai K, et al. Prostate cancer and the genomic revolution: advances using microarray analyses. Mutat Res 2005;576:66–79.
[40] Hernes E, Fossa SD, Berner A, Otnes B, Nesland JM. Expression of the epidermal growth factor receptor family in prostate carcinoma before and during androgen-independence. Br J Cancer 2004;90:449–54.
[41] Shah RB, Ghosh D, Elder JT. Epidermal growth factor receptor (ErbB1) expression in prostate cancer progression: correlation with androgen independence. Prostate 2006;66:1437–44.
[42] Blanchere M, Saunier E, Mestayer C, Broshuis M, Mowszowicz I. Alterations of expression and regulation of transforming growth factor beta in human cancer prostate cell lines. J Steroid Biochem Mol Biol 2002;82:297–304.
[43] Hellawell GO, Turner GD, Davies DR, Poulsom R, Brewster SF, et al. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. Cancer Res 2002;62: 2942–50.
[44] Loughran G, Huigsloot M, Kiely PA, Smith LM, Floyd S, et al. Gene expression profiles in cells transformed by overexpression of the IGF-I receptor. Oncogene 2005;24:6185–93.
[45] Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol 2006;24:1770–83.
[46] Yoshino T, Shiina H, Urakami S, Kikuno N, Yoneda T, et al. Bcl-2 expression as a predictive marker of hormone-refractory prostate cancer treated with taxane-based chemotherapy. Clin Cancer Res 2006;12:6116–24.
[47] Yamanaka K, Rocchi P, Miyake H, Fazli L, Vessella B, et al. A novel antisense oligonucleotide inhibiting several antiapoptotic Bcl-2 family members induces apoptosis and enhances chemosensitivity in androgen-independent human prostate cancer PC3 cells. Mol Cancer Ther 2005;4:1689–98.