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An appraisal of the therapeutic value of lycopene for the chemoprevention of prostate cancer: A nutrigenomic approach

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ABSTRACT

Lycopene is the predominant and bioactive carotenoid present in the tomato (*Solanum lycopersicum*), a widely appreciated healthful vegetable. *In vivo*, *in vitro* and clinical studies conducted in recent years have revealed an inverse association between the dietary intakes of lycopene with the risk of prostate cancer (PCa). Lycopene has been proposed to protect against PCa by the reduction of lipid oxidation, inhibition of cancer cell proliferation, and its antioxidative properties. Attention has been paid towards the interactive impact of lycopene and its genetic predisposition with the prostate carcinogenesis. In particular, lycopene is believed to play a key role in regulating the gene expressions and modulating the development of PCa. This paper presents a state of art review of the lycopene × gene interaction for the protective treatment of PCa. The key pathway for deoxyribonucleic acid (DNA) repair, insulin-like growth factor (IGF), cell cycle, androgen receptor, steroid signaling, inflammation and inflammatory cytokines, gap junction communications, carotenoid cleavage enzymes, and endogenous antioxidant enzymes are concisely elucidated. Furthermore, the evidence underlying its potent impacts on the integrin, nitric oxide synthase (NOS), prostate specific antigen (PSA) and urokinase plasminogen activator receptor (uPAR) expressions is outlined.

1. Introduction

The growing knowledge about food functionality, bioactivity and its implication are among the topics of interest in the scientific community, with the ultimate objective of improving the human health. Marked advances were seen in deciphering the molecular mechanisms on how nutritional factors can impact on the regulation of genes, and subsequently their function without modification of the genetic code (Ruemmele & Garnier-Lengliné, 2012). The term 'nutrigenomic' is introduced to explain the complex interaction of nutritional factors as an exogenous determinant on gene regulation and expression. To date, nutrigenomic as a fast developing field considers the contribution of individual genotypes to wellness and the risk of chronic diseases, and how such genetic predisposition can be modified by appropriate diets

Abbreviations: ABC, Adenosine triphosphate binding cassette; ABCA1, Adenosine triphosphate binding cassette transporter A1; AF-1, Ligand-independent transcriptional activation domain; AF-2, Ligand-binding protein; AKT, Protein kinase B; ApoA-I, Apolipoprotein A-I; AS, Active surveillance; BER, Base excision repair; CDK, Cyclin-dependent kinase; cDNA, Complementary DNA; CI, Confidence interval; CMO-I, β,β-carotene 15,15′-monooxygenase 1; CMO-II, carotene-9′,10′-monooxygenase; COX-2, Cyclooxygenase-2; CS, Control diet; Cx43, Connexin43; CyP7B1, 25-Hydroxycholesterol 7-alpha-hydroxylase; DHEA, Dehydroepiandrosterone; DHT, Dihydrotestosterone; DNA, Deoxyribonucleic acid; eNOS, Endothelial nitric oxide synthase; ERβ, Estrogen receptor β; FABP3, Fatty acid binding protein 3; GSTP1, Glutathione S-transferase P1; hOGG1, 8-Oxoguanine DNA glycosylase; HDL, High-density lipoprotein; HPLC, High performance liquid chromatography; IGF, Insulin-like growth factor; IGF-1R, Type 1 insulin-like growth factor receptor; IGFBP2, Insulin-like growth factor binding protein 2; IL, Interleukine; iNOS, Inducible nitric oxide synthase; LBS, Lycopene beadlets; LOX-12, Lipooxygenase-12; LXR, Liver X receptor; LYC, Lycopene-containing; MIC-1, Macrophage-inhibitory cytokine-1; MnSOD, Manganese superoxide dismutase; MPC, Markers of prostate cancer; MRI, Magnetic resonance imaging; mRNA, Messenger ribonucleic acid; MTT, 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; NO, Nitrite oxide; NOS, Nitric oxide synthase; NR, Nuclear receptor; Nrf-2, NF-E2-related factor; OR, Odds ratio; PAI-1, Plasminogen activator inhibitor 1; PCa, Prostate cancer; PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; PHS, Physicians' Health Study; PI, Propidium iodide; PI3K, Phosphatidylinositol 3-kinase; PPARγ, Peroxisome proliferator-activated receptor gamma; PSA, Prostate specific antigen; Rb, Retinoblastoma protein; RCT, Reverse cholesterol transport; RNA, Ribonucleic acid; ROS, Reactive oxygen species; RR, Relative risk; RT, Red tomato paste; RT-PCR, Real time-polymerase chain reaction; RTS, Red tomato; RXR, Retinoid X receptor; SNPs, Single nucleotide polymorphisms; TMD, Transmembrane spanning domain; TNF-α, Tumor necrosis factor-α; TP, Tomato powder; uPA, Urokinase plasminogen activator; uPAR, Urokinase plasminogen activator receptor; VEGF, Vascular endothelial growth factor; WCRF, World cancer research foundation; XMRV, Xenotropic murine leukemia virus-related virus; XRCC1, X-ray repair cross-complementing group 1; YTS, Yellow tomato; 5α-DHT, 5α-Dihydrotestosterone; 17β-HSD, 17β-Hydroxysteroid-dehydrogenases; 3β-Adiol, 5α-Androstane-3β,17β-diol.

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(Ferguson & Schlothauer, 2012; García-Rodríguez, Romero-Segura, Sanz, Sánchez-Ortiz, & Pérez, 2011; Romero-Segura, García-Rodríguez, Sánchez-Ortiz, Sanz, & Pérez, 2012).

Tomato (*Solanum lycopersicum*), originated from South America, is a horticultural plant species that belongs to the family *Solanaceae* and genus of *Solanum* (Feudo, Macchione, Naccarato, Sindona, & Tagarelli, 2011; Navarro-González, García-Valverde, García-Alonso, & Periago, 2011). It is an important vegetable crop, with an annual production of 150 million tons (FAO, 2009). Lycopene (ψ,ψ-carotene), the contributor of red pigment in tomatoes, is a tetraterpene hydrocarbon containing 11 conjugated and two non-conjugated double bonds (Bravo et al., 2012; Colle, Van Buggenhout, Lemmens, Van Loey, & Hendrickx, 2012; Fernández-García et al., 2012; van Breemen & Pajkovic, 2008). Epidemiological studies have suggested the health beneficial implications of lycopene to protect against prostate cancer (PCa) (Wei & Giovannucci, 2012). Scientific evidences have suggested that a higher dietary intake of lycopene is related to a lower risk of developing PCa (Illic & Misso, 2012; Thapa & Ghosh, 2012).

PCa is the most common diagnosed malignancy, and is the second cause of cancer death in American men, with 217,730 of new cases, and 32,050 of deaths in 2010 (ACS, 2010). Epidemiological studies have shown remarkably variations of PCa incidence and mortality across the geographic regions (Bassett et al., 2012; Brawley, 2012), with the highest rates in Western countries and lowest in the Asian countries. The most important recognized risk factors of PCa are age, race and family history. Despite the dramatic impact of PCa, the underlying etiology is still relatively unknown, with both genetic predisposition and environmental factors are likely contributing to the risk (Schleutker, 2012). Today, androgen ablation therapy is a common method for the treatment of PCa, where chemotherapy, hormonal or radiation is used to treat the advanced stage (ACS, 2010). Nevertheless, these conventional androgen deprivation therapy, radiotherapy and chemotherapy have been evident to provide only a transient benefit (Eichholz, Ferraldeschi, Attard, & de Bono, 2012). Importantly, these treatment methods are prohibited when PCa turns to the metastatic stage. This has prompted to a potpourri of herbal preparations and natural phytochemicals for the preventive treatment of PCa.

Of major interest, lycopene has been proposed to present antiproliferative properties on PCa (Hwang & Bowen, 2004; Tang, Jin, Zeng, & Wang, 2005). Although the biological function of lycopene has not been fully delineated, its beneficial impact in modulating the gene expressions of PCa has gained popularity worldwide. In view with the matter, this paper attempts to postulate an initial platform to arise the unique implications of lycopene for the preventive treatment of PCa. The present work is aimed at providing an up to date picture of the prominent role of lycopene for the regulation of cell cycle, gap junction communication, insulin-like growth factor (IGF) signaling, integrin modulation, deoxyribonucleic acid (DNA) methylation and DNA repair pathway. The comprehensive literature has been summarized to familiarize the readers with pertinent information regarding the potential of lycopene as a viable chemopreventive agent of PCa.

2. Lycopene, gene expressions and PCa

2.1. Lycopene, XRCC1 gene polymorphisms and PCa

X-ray repair cross-complementing group 1 (*XRCC1*) gene is one of the key players in the base excision repair (BER) system mapped to the human chromosome 19q13.2–13.3 (Wang et al., 2012). This special gene functions as the central scaffolding protein for DNA polymerase β, and DNA ligase III (Caldecott, Aoufouchi, Johnson, & Shall, 1996), and interacts with poly(ADP-ribose) polymerase and polynucleotide kinase (Dantzer et al., 1999; Masson et al., 1998). Three common single nucleotide polymorphisms (SNPs) have been described for the *XRCC1* gene: one at codon 194 (*Arg*3*Trp*), one at codon 280 (*Arg*3*His*), and one at codon 399 (*Arg*3*Gln*). These SNPs were reported to demonstrate a functional impact on BER capacity (Savas, Kim, Ahmad, Shariff, & Ozcelik, 2004).

In the perspective, van Gils, Bostick, Stern, and Taylor (2002) have initiated the first markers of prostate cancer (MPC) study to evaluate the PCa risk among men with polymorphisms in the *XRCC1* gene across different strata of antioxidant intake. Seventy seven PCa patients and 183 community controls from the Piedmont Triad metropolitan area were recruited, and the Block-National Cancer Institute Health Habits and History Questionnaire (Block et al., 1986) was used to estimate the individual's dietary intake in the previous year (as a proxy for usual dietary intake), and the daily intakes of vitamin C, vitamin E, vitamin A, β-carotene, lycopene, and calories were estimated. DNA was extracted from peripheral blood lymphocytes and the *XRCC1* genotypes were determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Results revealed that the PCa risk with lycopene intake was significantly lower than the control group ($p < 0.05$); and men with one or two copies of variant alleles at the *XRCC1* codons 194 and 399 depicted a lower PCa risk than those who were homozygous for the common allele [codon 194: odds ratio (OR) = 0.80; 95% confidence interval (CI), 0.40–1.80 and codon 399: OR = 0.80; 95% CI, 0.50–1.30]. Whereas, the variant at codon 280 was related to a slightly increase of PCa risk (OR = 1.50; 95% CI, 0.70–3.60). The combined effect of *XRCC1* codon 399 genotype and dietary intake showed that *Arg*/*Arg* genotype in combination with low lycopene (OR = 2.0; 95% CI, 0.8–4.9) and vitamin E (OR = 2.4; 95% CI, 1.0–5.6) intake gave a higher PCa risk than expected on the basis of the independent effects. The study indicated *XRCC1* genotype polymorphisms could modulate a higher PCa risk associated with a lower intake of lycopene and vitamin E.

Four years later, an expanded analysis of the original MPC study was conducted in the North Carolina to address the intake of lycopene across the levels of both plasma antioxidants and *XRCC1* genotypes (Goodman et al., 2006). Plasma α-tocopherol and β-carotene levels were measured using a high performance liquid chromatography (HPLC), and the *XRCC1* genotypes were detected using PCR-RFLP. Stratification according to the *XRCC1* 399 genotype showed that among men with two *Arg* alleles, lycopene intake was related to a more pronounced decrease of PCa risk with ORs of 0.59 (0.23–1.50) and 0.21 (0.06–0.71) for medium (732–1529 μg/day) and high (>1529 μg/day) exposure tertiles, respectively ($p < 0.01$). Similarly, potential four-way interaction using two hierarchically logistic regression showed that the combination of above median (1048 μg/day) lycopene and above median levels of α-tocopherol and β-carotene was associated with an OR of 0.11 (0.02–0.65) among men with the *Arg*/*Arg* genotype but not for those with at least one *Gln* allele ($p = 0.01$). The study emphasized that high lycopene intake was inversely associated with PCa risk for men with a common variant of *XRCC1* gene (codon 399 *Arg*/*Arg*). In addition, the relation between lycopene and PCa risk might be modified by other antioxidants and *XRCC1* genotype.

Meanwhile, the modulation effects of plasma antioxidants with polymorphisms in the *XRCC1* and 8-oxoguanine DNA glycosylase (*hOGG1*) genotypes on the risk of PCa were examined by Zhang, Dhakal, Greene, Lang, and Kadlubar (2010a) in Central Arkansas. For 193 cases and 197 controls, the lymphocytes DNA were extracted and genotyped by high throughput chip-based matrix assisted laser desorption time-of-flight mass spectrometry, and the plasma concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, and α-tocopherol were measured using a HPLC. After the adjustment for age, race, body mass index, education and smoking status, unconditional logistic regression analysis reported a significant increase of risk for individuals who carried one or two copies of the variant allele (399*Gln*) (OR = 1.56; 95% CI, 1.01–2.45) as compared with those who harbored only the wild-type allele (299*Arg*). Specifically, the promoting effect of the *XRCC1* 399*Gln* allele was

more striking among individuals who were heterozygous or homozygous of the 399*Gln* allele, but a statistically significant trend was observed only for lutein/zeaxanthin ($p = 0.015$), β-cryptoxanthin ($p = 0.003$), and lycopene ($p = 0.032$). Similarly, individuals who were heterozygous or homozygous for the variant allele (326*Cys*) of *hOGG1* appeared to have a lower risk of PCa than those who were homozygous for the wild-type allele (326*Ser*) (OR = 0.72; 95% CI, 0.46–1.10). Importantly, a reduction of 50%, 54% and 62% of PCa risk were pronounced for individuals who have one or two copies of the 326*Cys* allele and low plasma levels of β-cryptoxanthin, lycopene and α-tocopherol, respectively. The study highlighted the variant of *XRCC1* and *hOGG1* genes for the repair of DNA oxidative damage and modulation of PCa risk, and the beneficial effects are more pronounced among the subjects with low plasma levels of antioxidants.

The role of antioxidants for cancer prevention remains the focus of debate in the research world. The biological mechanisms to support the protective effect of antioxidants in the presence of *XRCC1 Arg/Arg* genotypes, are still unclear. Similarly, the phenotypic significance of *hOGG1* genetic polymorphisms, and its interaction with antioxidants intake on PCa risk is still under investigation. Previous *in vitro* assay has suggested that *XRCC1 Gln → Arg* substitution at codon 399 may be related to the reduction of BER capacity, however, controversial results on the risk for different cancer cites have been reported (Savas et al., 2004; Wang et al., 2003). It is biologically plausible that the *XRCC1 Arg/Arg* genotypes and *hOGG1* 326*Cys* allele might interact with antioxidants to modulate PCa risk with the presence of oxidative stress challenge (Zhang et al., 2010a).

2.2. Lycopene, carotenoid cleavage enzyme pathway and testosterone production

Provitamin A carotenoids such as β-carotene are oxidatively cleaved by the enzyme β,β-carotene 15,15′-monooxygenase 1 (*CMO-I*) through central chain cleavage, with the formation of retinoic acid and other retinoids. Whereas, carotene-9′,10′-monooxygenase (*CMO-II*) is responsible for eccentric cleavage of acyclic carotenoids, including lycopene, to form aldehyde metabolites, such as β-apo-10′-carotenal and β-ionone (Ford, Moran, Smith, Clinton, & Erdman, 2012; Lan, Hanh, Osorio-Puentes, & Waché, 2011). Both enzymes are located at different sites within the cell, with *CMO-I* being a protein in the cytosol, and *CMO-II* is located in the mitochondria (Lietz, Oxley, Boesch-Saadatmandi, & Kobayashi, 2012). The expression of *CMO-I*$^{-/-}$ in mice fed with lycopene showed a significantly less hepatic lycopene, but more lycopene in prostate, seminal vesicles and testes as compared to the wild-type mice (Lindshield et al., 2008).

A preliminary *in vivo* study investigating the impact of *CMO-I* genotype (*CMO-I*$^{-/-}$ or wild type) in combination with the dietary intake of tomato powder (TP) or lycopene on the testosterone production has been examined by Ford et al. (2012). Nine to twelve weeks old mice without the expression of *CMO-I*$^{-/-}$ or wild-type mice were randomly assigned to either a 10% TP (204 nmol lycopene/g), lycopene-containing (LYC) (248 nmol/g) or their respective control diets for four days. Testicular testosterone was extracted and quantified together with serum testosterone using the testosterone coated-tube radioimmunoassay kit. Testicular gene expression was determined by isolating testicular ribonucleic acid (RNA), while determination of the concentrations and quality of messenger ribonucleic acid (mRNA) was quantified using spectrophotometry and agarose gel electrophoresis, respectively. Superscript II reverse transcriptase and random hexamers were used to synthesize the complementary DNA (cDNA). *CMO-II* was measured using primer pairs, and real-time PCR (RT-PCR) was performed with a RT-PCR detection system and SYBR green fluorescence dye.

Factorial analysis reported a significant interaction between the diet and genotype where TP reduced the serum testosterone concentrations ($p = 0.02$) and testicular testosterone ($p = 0.01$) in the *CMO-I*$^{-/-}$ mice but not in the wild-type mice. The result implied that testosterone synthesis may be inhibited in this group. Similarly, the lycopene diet significantly reduced serum testosterone concentrations ($p = 0.03$), and the expression of *CMO-I* and lycopene were significantly interacted to alter testicular testosterone ($p = 0.03$). In term of tissue carotenoid concentration, β-carotene was found significantly accumulated in the testicular tissue of *CMO-I*$^{-/-}$ mice as compared to the wild-type mice ($p < 0.0001$). The expression of the gene encoding *CMO-II* enzyme responsible for eccentric oxidative carotenoid cleavage in the testes was significantly upregulated in the testicular tissues of *CMO-I*$^{-/-}$ mice. However, the lycopene or TP diets did not impact directly to the expression of *CMO-II* in the mouse testes. The study proposed that genetic polymorphisms impacting *CMO-I* expression, and its interaction with *CMO-II* coupled with the serum testosterone concentrations and testicular testosterone synthesis, may be modulated by the variations of tomato products and dietary lycopene status. Hence, the alterations in the function of *CMO-I* and *CMO-II* enzymes from SNP may explain the effects of tomato carotenoids on endocrine processes related to the PCa risk.

Testosterone is the critical hormone that impacts prostate development and prostate carcinogenesis (Erdman, Ford, & Lindshield, 2009). Previous studies have consistently suggested a higher intake of tomato phytochemicals and serum lycopene to reduce the serum testosterone (Campbell, Stroud, Nakamura, Lila, & Erdman, 2006; Kumar et al., 2008). These observations proposed that tomato or its active compounds (lycopene) could impact testosterone signaling, and may in part illustrate the inverse association between tomato consumption and the risk of PCa. In the study, the increase of *CMO-II* gene expression may in turn result in the production of lycopene metabolites, which indicated that *CMO-II* action is a key step for the degradation and excretion of lycopene. However, future studies are needed to unravel the complex interrelationship between tomato carotenoids, endocrine process and the PCa risk.

2.3. Lycopene, inflammation and inflammatory cytokine and PCa risk

Investigation of the prostatectomy specimens and prostate biopsy evidenced that inflammation plays a key role in the PCa etiology (Gueron, De Siervi, & Vazquez, 2012). Xenotropic murine leukemia virus-related virus (XMRV) has been identified in the malignant prostate epithelium, and found to be integrated into the human genome DNA extracted from the prostate tumor tissue (Schlaberg, Choe, Brown, Thaker, & Singh, 2009). Epidemiological research studies have revealed that chronic inflammation is associated with DNA damages through radical oxygen and nitrogen species, leading to the prostate tumorigenesis (McArdle et al., 2006).

Cyclooxygenase-2 (COX-2), that involves in the synthesis of prostaglandins and thromboxanes, is located in the chromosome 1q25.2–q25.3 (Tan et al., 2011). Several research findings have suggested that COX-2 is overexpressed in the PCa tissue compared to benign tissue from the same patient (Denkert et al., 2007; Uotila et al., 2001). Some pro-inflammatory and anti-inflammatory cytokine genes have been proven to influent the expression of encoding proteins and affect the host predispose to PCa. The most common gene polymorphisms of COX-2 are rs5277, rs689466, rs2206593, rs689470, and rs2745557. The possibility of genetic polymorphisms in different inflammatory cytokines [interleukin-1β (IL-1β), IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and macrophage-inhibitory cytokine-1 (MIC-1)] and PCa risk have been examined (McCarron et al., 2002; Michaud et al., 2006; Sun et al., 2004).

In particular, Zhang, Dhakal, Lang, and Kadlubar (2010b) have conducted a case–control study to evaluate the possibility of the sequence variants in the inflammatory genes, and its interaction with plasma antioxidants to influent PCa risk. A total of 193 PCa patients and 197 healthy controls (aged 40–80) were matched, and their lymphocytes and plasma were obtained. Polymorphisms genotyping for

COX-2 (rs689466), lipoxygenase-12 (LOX12) (rs1126667), TNF-α (rs1800629), IL-1β (rs16944), IL-6 (rs1800795), IL-8 (rs4073), and IL-10 (rs1800871) were conducted. Plasma concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin and α-tocopherol were determined by HPLC, while selenium level was measured by inductively coupled plasma-mass spectrometry. From the study, polymorphisms in the COX-2 (rs689466) and IL-8 (rs4073) were found not significantly associated with the PCa risk after adjustment for confounders. Nevertheless, the protecting effect of the mutant allele of COX-2 was more remarkable among the AG/GG genotype subjects who had a higher plasma concentrations of lycopene (OR = 0.41, p = 0.09), β-cryptoxanthin (OR = 0.37, p = 0.12), β-carotene (OR = 0.48, p = 0.25), and selenium (OR = 0.45). Conversely, the promoting effect of the variant allele of IL-8 (AT/TT *vs.* AA) was more pronounced among the subjects with a lower plasma level of lutein/zeaxanthin (OR = 2.13), β-carotene (OR = 2.44), and β-cryptoxanthin (OR = 2.15). This observation showed that the interactions between the genetic variability in the inflammation pathway and plasma antioxidants could play a role in the PCa etiology. Exogenous antioxidant, such as lycopene, has been shown to lower the biomarkers of oxidative stress (Basu & Imrhan, 2007). Therefore, lowering the PCa risk by increasing the dietary intake of antioxidants appears to be particularly relevant and important for individuals who carry the risk alleles of inflammatory genes.

2.4. Lycopene, gap junction communications and PCa

Connexin43 (Cx43) is a membrane phosphoprotein that mediates direct intercellular communication by forming gap junctions (Yeganeh et al., 2012). As such, Cx43 plays an important role in gene expression, tissue homeostasis, cell growth and differentiation (Laird, 2006). Accumulating evidences have indicated that lycopene exposure could increase Cx43 expression, stabilize Cx43 mRNA, restore cell-to-cell communication and modulate cell cycle progression (Chalabi et al., 2007; Fornelli, Leone, Verdesca, Minervini, & Zacheo, 2007; Livny et al., 2003).

In the perspective, the effects of red tomato, yellow tomato and lycopene on Cx43 expression have been examined by Gitenay et al. (2007). Forty male Wistar rats were randomized into four groups: control diet (CS), control diet supplemented with lyophilized red tomato (RTS), control diet supplemented with lyophilized yellow tomato (YTS), and control diet supplemented with lycopene beadlets (LBS). Human PCa cells (PC3AR) were incubated with serum from rats. RNA extraction was conducted using PC3AR, and intracellular lycopene concentration was determined by mass spectrometry. Western blot analysis was carried out to determine the Cx43 protein levels, and the quantification was performed using the ImageJ Software. The expression of Cx43 was significantly ($p < 0.05$) increased after exposure to RTS or YTS for 48 h as compared to cells exposed to CS. In contrast, serum from rats fed with lycopene-supplemented diet did not exhibit a significant effect on the Cx43 expression. Cells incubated with RTS and LBS showed similar lycopene levels, but those incubated with YTS contained no lycopene. Therefore, it was speculated that the effect of RTS in up-regulating Cx43 expression is not likely due entirely to lycopene but may be attributed to a combination of other micronutrients of tomatoes. Cx43 expression in the RTS supplemented group could probably linked to the restoration of cell-to-cell communication typically lost in the cancer cells (Bertram, 2004), suggesting the cancer-preventive mechanism of tomato. Conclusively, the study suggested that tomato supplementation, rather than lycopene alone could induce metabolic changes in the rat serum and up-regulate Cx43 expression. The changes are independent of the tomato lycopene content, and are more pronounced than the lycopene feeding alone.

Relatively, the proliferation, apoptosis, cell cycle progression, and gap junction communication activities of androgen-independent DU145 cells were tested using lycopene, apo-8′-lycopenal or apo-12′-lycopenal (lycopene metabolites) (Ford, Elsen, Zuniga, Linshield & Erdman, 2011). Results showed that the supra-physiological levels of lycopene (15 μM and 25 μM) and apo-12′-lycopenal significantly ($p < 0.05$) reduced DU145 cells proliferation through the alteration of normal cell cycle. These physiological changes also induced a significant reduction ($p < 0.05$) in the total number of cells in the S phase. However, the Cx43 expression was not altered by either lycopene or apo-lycopenal treatments. The study provided an evidence that lycopene and at least one of its metabolite (apo-12′-lycopenal) might contribute to the reduction of PCa risk. No changes in the protein levels of Cx43 revealed that DU 145 cells may require treatment other than lycopene to regain proper gap junction communication. However, King and Bertram (2005) have demonstrated an opposite opinion that only forced expression of connexins, gene transfer, or hypermethylation could overcome the loss of expression of the connexin genes. Hence, further research in this area is warranted to explain the role of lycopene and its metabolites in the regulation of Cx43 expression for PCa cells.

2.5. Lycopene, PSA mRNA expression and PCa

PSA is a routinely used biomarker for the early detection of PCa. PSA exists in two forms, free (unbound) and bound to alpha-1-antichymotrypsin, with a lower percentage of free PSA is associated with a higher risk for aggressive PCa (Stephan et al., 2009). A growing body of evidence has raised the concern that potential agents used for the prevention of PCa may modulate PSA mRNA expression or protein secretion independently from the alterations in tumor growth (Peternac et al., 2008). Identification of the potential chemopreventive agent is important not only to decrease the morbidity and mortality, but also to minimize health care costs in the high-risk males.

The inhibitory growth effects of lycopene *in vitro* were evaluated to determine their influence on PSA mRNA and protein expressions (Peternac et al., 2008). In the study, two cell lines, LNCaP (androgen-dependent and nonmetastatic) and C4-2 (androgen-independent and metastatic state) were cultured and incubated with lycopene (0.04, 0.4 and 4.0 μg/ml). The antiproliferative activity was evaluated using the (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT) cell proliferation assay, the PSA mRNA expression was assessed by quantitative RT-PCR, while the secreted total PSA protein levels were quantified using the microparticle enzyme immunoassay. All lycopene concentrations were found capable of inhibiting LNCaP cell growth ($p < 0.05$), while a concentration of 0.04 and 4.0 μg/ml lycopene induced a significant proliferation inhibition of the C4-2 cells ($p < 0.05$). However, lycopene did not affect the PSA mRNA and protein expressions in both cell lines. This supported the findings of an epidemiological study that lycopene inhibited the growth of several androgen-dependent PCa cells (DU145, PC-3 and LNCaP) (Tang et al., 2005). The study highlighted the feasibility of lycopene in suppressing the proliferation of LNCaP and C4-2 cell lines. Since PSA transcript and protein were not affected by lycopene treatment in both cell lines, lycopene could be eventually used as a surrogate endpoint marker in the nutritional intervention trials.

2.6. Lycopene, nitric oxide synthase gene polymorphism and PCa

Nitric oxide synthase (NOS) is a family of enzymes that generates nitrite oxide (NO) from L-arginine. It can be further classified into three isoenzymes, neuronal NOS (*nNOS*/*NOS1*), calcium-independent inducible NOS (*iNOS*/*NOS2*), and calcium-dependent endothelial NOS (*eNOS*/*NOS3*) (Stuehr, 1999). The expression of inducible NOS (*NOS2A*) has been reported to elevate prostate carcinoma (Uotila et al., 2001), however, *eNOS* was shown capable of protecting PCa cells from apoptosis (Tong & Li, 2004). An examination in a nested case–control Caucasian population illustrated that the occurrence of PCa was related to *eNOS* polymorphisms (T-786C and intron 4a/b), while

neither the PCa incidence nor PCa clinical pathologic features were associated with *eNOS G894T* polymorphism (Safarinejad, Safarinejad, Shafiei, & Safarinejad, 2012). Spurring interest has been devoted to establish the relationship between genetic variants of NOS, together with the environmental exposure in affecting PCa risk.

In Korea, the first study investigating the association between NOS gene polymorphisms, antioxidant intake and PCa risk has been undertaken by Lee et al. (2009). 1320 PCa and 1842 control cases were selected using risk-set sampling, a 137 item food frequency questionnaire was used to assess the usual dietary intake, and total antioxidant score (3–7 indicated a lower antioxidant intake and 8–12 revealed a higher antioxidant intake) was created for both groups by summing the quartile levels of β-carotene, lycopene and vitamin E. *NOS2A* [*−2892T>C* (rs9282799), *Ex16 + 14C>T* (S608L) (rs2297518), *IVS16 + 88T>G* (rs9282801), *IVS20 + 524G>A* (rs944722)] and *NOS3* [(*IVS1 − 762C>T* (rs2853796), *Ex7 − 43C>T* (D258D) (rs1549758), *IVS7 − 26A>G* (rs1007311), *Ex8 − 63G>T* (E298D) (rs1799983) and *IVS15 − 62G>T* (rs2853796)] genotyping were performed. *NOS2A* (*NOS2A IVS16 + 88GT/TT*) and *NOS3* (*IVS7 − 26GG*) genetic polymorphisms were associated with the aggressive PCa risk (OR = 1.26, 95% CI, 1.03–1.55; OR = 1.44, 95% CI, 1.07–1.95) in both Caucasians and African-Americans population. Stratified and multiplicative interaction analysis by comparison of log-likelihood statistics revealed that *NOS2A IVS16 + 88GT/TT* was significantly ($p = 0.01$) associated with aggressive PCa among the highly antioxidant intakers (OR = 1.61, 95% CI, 1.18–2.19). A possible explanation could be due to the higher antioxidant intake level that inhibits reactive oxygen species (ROS)-induced apoptosis or increase angiogenesis in the PCa cells (Wartenberg, Schallenberg, Hescheler, & Sauer, 2003). The study indicated that NOS gene polymorphisms are genetic susceptibility factors for aggressive PCa, particularly among the highly antioxidants intakers.

2.7. Lycopene, PPARγ–LXRα–ABCA1 pathway and PCa

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated intracellular transcription factor belongs to the superfamily of nuclear hormone receptors (Olefsky, 2001). Three known isoforms of PPARs: PPARα, PPARβ/δ and PPARγ share common molecular structure and functional domains [distinct N-terminal ligand-independent transcriptional activation domain (AF-1), DNA binding domain, the hinge region, and the ligand-binding domain, (AF-2)] (Penumetcha & Santanam, 2012). PPARγ is the extensively studied subtype of PPARs, and its transcriptional activity depends on the binding of ligands. In recent years, a flurry of research has revealed the biological effects of PPARγ ligand-activation in the inhibition of growth and differentiation of PCa cells (Matsuyama & Yoshimura, 2008; Mueller et al., 2000).

Oxysterol-activated receptors Liver X receptor (LXR), LXRα and LXRβ are the members of the nuclear receptor (NR) family, responsible for the regulation of transcription factors. LXRs function as the physiological master regulators of lipid and cholesterol metabolism, and present anti-inflammatory activities (Jakobsson, Treuter, Gustafsson, & Steffensen, 2012). LXRα expression is restricted to metabolically active tissues, such as intestine, liver, macrophages and adipose tissue, where LXRβ is expressed at a moderate level in most of the physiological systems (Jakobsson et al., 2012). A widely used synthetic LXR agonist, T0901317, has been shown to inhibit the proliferation of androgen-dependent PCa cells (Fukuchi, Kokontis, Hiipakka, Chuu, & Liao, 2004).

Adenosine triphosphate binding cassette (ABC) transporters are one of the largest protein families ubiquitously conserved from bacteria to man (Holland, Cole, Kuchler, & Higgins, 2003). ABC transporter A1 (ABCA1) is a full-sized ABC transporter, consists of a transmembrane spanning domain (TMD). The lipid sensitive ABCA1 is a lipid translocator and the modulator of lipid architecture (Zarubica, Trompier, & Chimini, 2007). It is localized at the plasma membrane and intracellular sites to facilitate the transportation of lipid to the external acceptors, or internalized apolipoprotein A-I (ApoA-I) (Schmitz & Langmann, 2005). Previous study has reported the involvement of PPARα and PPARγ in the up-regulation of ABCA1 expression, and reverse cholesterol transport by the transcription of LXRα (Chawla et al., 2001; Chinetti et al., 2001; Chinetti-Gbaguidi et al., 2005). Moreover, the activation of PPARγ and LXRα was found associated with a reduced growth of prostate tumor xenografts (Fukuchi et al., 2004; Tsubouchi et al., 2000).

The antiproliferative effect of lycopene by PPARγ–LXRα–ABCA1 pathway activation on androgen-dependent human PCa (LNCaP) and androgen-independent PCa cells (DU145 and PC-3 cells) has been examined by Yang, Lu, Chen, and Hu (2012a, 2012b). Lycopene was found significantly increased the protein and mRNA expressions of PPARγ, LXRα, ABCA1, and cholesterol efflux. Incubation with lycopene (10 μM) was noted capable to restore the proliferation to the control levels in the presence of specific antagonist of PPARγ (GW9662) and LXRα (GGPP). The result indicated the antiproliferative action of lycopene for the activation of PPARγ–LXRα–ABCA1 pathway, leading to an enhanced cholesterol efflux in both androgen-independent and androgen-dependent PCa cells. The findings supported the use of lycopene as an adjuvant for the chemoprevention or chemotherapy of PCa cells.

2.8. Lycopene, cell cycle regulation and PCa

Continuous cell division is a hallmark of cancer and cell cycle regulators (von Bergwelt-Baildon, Kondo, Klein-González, & Wendtner, 2011). They represent the targeted molecule for tumor therapy and have been widely used as biomarkers to indicate the development of cancer. Among these targets, cyclins and consequent cyclin-dependent kinase (CDK) have received much attention due to their unique functions at specific cell cycle in coordinating the sequential completion of DNA replication and cell division (Besson, Dowdy, & Roberts, 2008). For instance, the best understood biological function of cyclin D is to promote cell proliferation as a regulatory partner for CDK4 or CDK6 (Musgrove, Caldon, Barraclough, Stone, & Sutherland, 2011).

In the perspective, Obermüller-Jevic et al. (2003) have conducted an examination of the effects of lycopene on the normal human prostate epithelial cells (PrEC) by treating them with synthetic all-E-lycopene (up to 5 μmol/L). Cell proliferation was determined using the [^{3}H]thymidine incorporation assay, and the effects of lycopene on cell cycle was assessed using the flow cytometry analysis. Results revealed that a dosage of 2 μmol/L lycopene has significantly inhibited the growth of PrEC cells (~80%), while treatment with 5 μmol/L of lycopene has led to a significant accumulation of cells in the G0/G1 phase (71% *vs.* 56%; $p < 0.05$), and fewer cells in the S phase (22% *vs.* 31%; $p < 0.05$) as compared with the vehicle-treated cells. Western blot analyses showed that no cyclin D1 expression was detected, while cyclin E remain unaffected throughout the experiments. The findings demonstrated that lycopene could effectively inhibit the growth of PrEc *in vitro* by antiproliferation, regulated the cell cycle, and may have an impact on the prostate development.

Ivanova et al. (2007) have investigated the molecular antiproliferative action, mitotic index, phosphorylation states of cell cycle regulatory, and changes in the absolute levels and phosphorylation states of signaling proteins in LNCaP and PC-3 cells using two lycopene-based agents, each contained 3% and 38% lycopene by weight. Both lycopene extracts showed antiproliferative activity in both LNCaP and PC-3 cells with the reduction of cyclins D1, cyclin E and CDK4, which are critical for the suppression of phosphorylation of retinoblastoma protein (Rb). These findings indicated lycopene could induce cell cycle arrest by altering the expression and activation of the same key G1/S phase cell cycle regulatory factors in both LNCaP and PC-3 cells. The responses were found correlated with a down-regulation of IGF-1R expression, increase of IGF binding protein 2 (IGFBP2) expressions and decrease of protein kinase B (AKT) activation. The study

suggested the potential of lycopene exposure to suppress phosphatidylinositol 3-kinase (PI3K)-dependent proliferative and survival signaling of LNCaP and PC-3 cells, and outlined the cytotoxic actions of lycopene for the induction of G0/G1 cell cycle arrest. Similarly, Hwang and Bowen (2004) have reported that 1 μM of lycopene was able to inhibit 31% of the PCa cell growth, while 5 mM of lycopene could increase the number of cells in the G2/M phase from 13% to 28%, and decrease the S-phase cells from 45% to 29%.

2.9. Lycopene, insulin-like growth factor (IGF) system and PCa

The IGF axis, which consists of two ligands (IGF-I and IGF-II) that principally signal *via* IGF-IR and a family of six high-affinity IGFBPs plays a vital role in the development and progression of many epithelial cancers, including PCa (Meinbach & Lokeshwar, 2006). Prospective epidemiology study has consistently shown strong associations between the circulating IGF-I levels and the subsequent risk of developing PCa (Roddam et al., 2008).

The mechanism action of lycopene on the IGF system and apoptosis was examined (Kanagaraj et al., 2007) by treating the PC-3 human prostate cells with lycopene in different concentrations (20, 40 and 60 μM) for 24, 48, 72 and 96 h. PC-3 cell proliferation was performed using MTT assay, and the IGF-1, IGFBP-3 and IGF-IR levels in the lycopene-treated cells were measured. The induction of apoptosis was studied using flow cytometric analysis, together with the annexin V and propidium iodide (PI) binding studies. Lycopene treatment showed antiproliferative activity by increasing the number of PC-3 dead cells. A dosage of 40 μM lycopene significantly increased the level of IGFBP-3. A dramatic decrease in the IGF-IR expression ($p < 0.05$) was observed in the PC-3 cells when the cells were treated with both lycopene and IGF-I. The annexin binding studies of lycopene treated cells also revealed a significant increase in the number of apoptotic cells. These results suggested that lycopene could inhibit the expression of IGF-IR, increase the levels of IGFBP-3 and induce apoptosis in the PC-3 cells. The promising lycopene-induced biological effects in regulating the IGF system supported therapeutic value of lycopene for PCa treatment.

2.10. Lycopene, DNA methylation and PCa

Recent emerging molecular biology knowledge has identified the deregulation of cellular epigenetic pattern as an early event in the human carcinogenesis, and DNA methylation as an epigenetic mechanism for the development of PCa (Cooper & Foster, 2009). Previous study has shown that the global and gene-specific DNA methylation was greatly affected by the environmental and dietary factors (Majumdar, Buckles, Estrada, & Koochekpour, 2011). In particular, the role of glutathione S-transferase P1 (GSTP1) as a specific biomarker of PCa has been investigated extensively (Woodson et al., 2008). Generally, GSTP1 encodes the pi-class gluthathione S-transferase, an enzyme responsible for the detoxification of electrophilic and oxidant carcinogens (Costello & Plass, 2001), making it an attractive early detection biomarker for PCa (Nakayama et al., 2004).

The effects of lycopene and apo-10′-lycopeneal on DNA methylation of GSTP1 have been observed by Liu and Erdman (2011). LNCaP cell lines were dosed daily with lycopene at 1, 2, or 4 μM for seven days, and in the subsequent experiments, apo-10′-lycopenal of 0.01, 0.1, or 1 μM were dosed. Placebo bedlets were used as the vehicle, and the cellular uptake of carotenoids was determined using the reverse-phase HPLC-PDA system. GSTP1 expression was determined using RT-PCR, while methylated GSTP1 was quantified by MethPrimer. Result showed that lycopene was taken up by LNCaP cells in a dose-dependent manner ($p = 0.008$). In contrast, the average content of apo-10′-lycopenal was only 2.5 and 2.3 pmol per million cells for 1 μM and 0.1 μM dose levels, respectively. Lycopene or apo-10′-lycopenal treatment did not alter the expression of GSTP1 and DNA methylation of the GSTP1 promoter. The results suggested that both lycopene and apo-10′-lycopenal were not an effective demethylating agent in the human LNCaP cell lines. Additional research is required to explore the epigenetic effects of lycopene and its metabolites.

2.11. Lycopene, integrin expression and PCa

Integrins are a family of heterodimeric transmembrane proteins that contain α- and β-subunits. They function as the cell surface receptors for extracellular matrix proteins and cell cytoskeleton (Goel, Alam, Johnson, & Languino, 2009), and modulate cell growth, survival, invasion, migration and differentiation (Baust et al., 2010). In terms of cancer etiology, the expression of integrins $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ have been found to facilitate the migration and metastatic spread of cancer cells, and correlate with the increased tumor invasiveness (Hall, Dai, van Golen, Keller, & Long, 2006; McCabe, De, Vasanji, Brainard, & Byzova, 2007). Therefore, integrin is identified as a key biomarker for cancer progression, which enables the assessment of influence of bioactive nutrients on cancer growth and development.

The efficacy of the physiologically relevant concentrations of nutrients lycopene, vitamin E and fish oil on a range of normal prostate epithelial cells (RWPE-1) and highly invasive PCa cells (22Rv1, LNCaP, and PC-3) was investigated (Bureyko, Hurdle, Metcalfe, Clandinin, & Mazurak, 2009). The cells were cultured with or without lycopene (10 nM), vitamin E (5 mM) or fish oil (100 mM) for 48 h. The expression levels of integrins and monoclonal antibodies ($\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$) were determined for each prostate cell lines. All lycopene-containing culture media (10 nM) resulted in no reductions of cell growth in these prostate cell lines tested. The findings showed that the physiologically relevant concentrations of lycopene did not show significant effect on the growth of either normal or malignant prostate cells. $\alpha_2\beta_1$ supplementation of the culture medium with lycopene produced a significant increase ($p < 0.0001$) in the integrin $\alpha_2\beta_1$ expression. In contrast, the addition of lycopene reduced $\alpha_2\beta_1$ in the malignant 22Rv1, LNCaP and PC-3 cell lines and, likewise, decreased $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in the PC-3 cell line. Therefore, lycopene may impact the PCa progression by altering the invasive and migratory potential. Extensive work is essential to determine the functional changes related to the differences in integrin expressions due to lycopene in PCa.

2.12. Lycopene, endogenous manganese superoxide dismutase (MnSOD) polymorphisms and PCa

Manganese superoxide dismutase (*MnSOD* or *SOD2*), a single-copy gene with five exons and four introns, is among the primary endogenous antioxidant enzyme in the mitochondria of human body. The fragment patterns specific for the three *MnSOD* genotypes are *VV* (*GTT*; 351 bp, 87 bp), *VA* (*GTT*/*GCT*; 438 bp, 351 bp, 87 bp), and *AA* (*GCT*; 438 bp) (Li et al., 2005). *MnSOD* plays a prominent role to reduce the oxidative damages by converting ROS to oxygen and hydrogen peroxide, and further catalyzed into water by catalase and glutathione peroxidase (Ambrosone et al., 1999). A cytosine to thymine (*C*/*T*) SNP in the *MnSOD* gene at nucleotide 47 would result in a substitution of alanine (*Ala*) to valine (*Val*) at the 9th position (16th codon from the beginning of the signal sequence), which represents a disruption of the α-helix structure of the *MnSOD* enzyme (rs4880) (Rosenblum, Gilula, & Lerner, 1996). Laboratory evidence has demonstrated that *MnSOD* might function as a tumor suppressor agent, possibly by modulating the *in situ* apoptotic and proliferation pathways (Li, Oberley, Oberley, & Zhong, 1998). Specifically, *MnSOD* was shown to modulate the proliferation of PC-3 cells by retarding G1 to S transition in the cell cycle and regulate the cell survival (Venkataraman et al., 2004, 2005).

In 2005, Li and his co-workers have examined the relationship between the clinical significant of PCa and *MnSOD* polymorphism, its interactions with the baseline plasma antioxidant levels (selenium, lycopene, and α-tocopherol) and β-carotene treatment of 567 cases,

and 764 controls nested in the prospective Physicians' Health Study (PHS). The *MnSOD* (*Val* → *Ala*) polymorphism was genotyped through a series of DNA extraction, amplification, digestion with *BsaWI*, and visualized on a 2% agarose gel stained with ethidium bromide. The plasma antioxidant levels were measured using the instrumental neutron activation analysis and HPLC. High plasma selenium level was found associated with a relative risk (RR) of 0.3 (95% CI, 0.2–0.7) and 0.2 (95% CI, 0.1–0.5) for total PCa and clinically aggressive PCa, respectively among men with the *Ala*/*Ala* genotype. In contrast, those with *Val* allele were much less sensitive to the variation of selenium. Similar interactions have been found between the *MnSOD* polymorphisms and plasma levels of lycopene or α-tocopherol. The high (*versus* low) total antioxidant score was associated with a significant reduction of the risk of total (5-fold; RR, 0.20; 95% CI, 0.09–0.49) and aggressive PCa (10-fold; RR, 0.10; 95% CI, 0.03–0.29) among men homozygous for the *Ala* allele. Meanwhile, the *AA* genotype men who were randomly assigned to β-carotene treatment showed a RR of 0.6 (95% CI, 0.2–0.9). The study provided a strong evidence that the association between antioxidant status and PCa risk is partially attributed to the *MnSOD* genotype, and both endogenous and exogenous antioxidants are important for the clinical significant of PCa.

Whereas, a nested case–control study (612 subjects each) was undertaken to investigate the role of *MnSOD* gene *Ala16Val* polymorphism, and its joint association with plasma carotenoid concentrations in relation to the risk of total PCa and aggressive PCa (advanced stage or Gleason sum ≥7) (Mikhak et al., 2008). The plasma and dietary lycopene levels were summed to reflect the long term lycopene status. No statistically significant interaction ($p > 0.05$) was found between the *MnSOD* genotypes, and plasma carotenoid (or plasma total carotenoids) in relation to the risk of total PCa or aggressive PCa. However, at lycopene levels below the median, men with *Ala*/*Ala* genotype showed a nearly 2-fold elevated aggressive PCa risk compared with those with *Val*/*Val* genotype. For aggressive PCa, men with *Ala*/*Ala* genotype were found to have a 3-fold greater risk (RR = 3.10, 95% CI, 1.37–7.02, $p = 0.02$) compared with those with *Ala*/*Val* and *Val*/*Val* genotypes. This study suggested that men with *MnSOD Ala*/*Ala* genotype and lower lycopene status were found to have a higher risk of aggressive PCa compared with individuals with other *MnSOD* genotypes.

The potential mechanism for the interaction between *MnSOD*, antioxidant status and PCa risk is still under investigation. In mitochondria, the ROS and superoxide anion are dismutated by *MnSOD* into oxygen and hydrogen peroxide, which is further detoxified by mitochondrial glutathione peroxidase, or catalased into water. However, over-expression of *MnSOD* might lead to enzyme imbalance and induce toxicity if the glutathione peroxidase activity is low, and the risk of PCa would increase (Li et al., 2005). Previous study has shown that the *Ala*-containing *MnSOD* is transported through the mitochondrial matrix more efficiently compared with *Val*-variant, suggesting that the *Ala*/*Ala* homozygous subjects might have a higher *MnSOD* activity than the *Val*/*Val* individuals (Sutton et al., 2003).

2.13. Lycopene, urokinase plasminogen activator receptor (uPAR) expression and PCa

Urokinase plasminogen activator (uPA) is a 411 residues serine protease, originally identified for its ability to activate plasminogen and generate plasmin, a broad-spectrum matrix- and fibrin-degrading enzyme (Carriero et al., 2011). The uPA system, which consists of uPA, uPA receptor (uPAR), and the inhibitor plasminogen activator inhibitor 1 (PAI-1), is recognized as the crucial player in the migration and invasion of tumor (Sidenius & Blasi, 2003). Meanwhile, uPAR is a glycosylphosphatidylinositol membrane anchored 55–60 kDa glycoprotein with three homologous domains, denoted as I, II, and III (Almasi et al., 2011). During the last few years, accumulating evidence from animal models and clinical studies have shown an association between the high levels of uPA expression in tumors with the poor patient prognosis (Carriero & Stoppelli, 2011).

The inhibitory potential of lycopene against the advanced stage of PCa has been tested on PCa bone metastatic cell line (PC-2MM2) by Forbes, Gillette, and Sehgal (2003). In the study, 1 μM of lycopene was added into the cell cultures, and the lysates were assessed for the uPAR expression. Surprisingly, lycopene treatment showed a higher expression of uPAR, indicating its contribution for constitutive cleavage and release. To examine the invasiveness property of uPAR, a standard *in vitro* invasion assay was conducted. Results illustrated that the lycopene-treated cells were almost twice invasive as compared to the untreated cells ($p < 0.05$). Additionally, the lycopene treated cells were found unable to inhibit the proliferation, or to induce a detectable level of Cx43 expression. The overall results suggested that lycopene was not effective to inhibit the metastatic PCa cell lines. The use of lycopene as a chemopreventive agent in advanced stage PCa needs to be further justified before definite recommendations can be proposed.

3. Efficacy of tomato and lycopene supplementation, gene expressions and PCa

The effects of tomato (or lycopene intake) and its relation with the PCa development remains a global debate. The World Cancer Research Foundation (WCRF) has proposed a strong correlation between the consumption of tomato with the risk of PCa (Kavanaugh, Trumbo, & Ellwood, 2007; WCRF, 2007). A number of *in vivo* and human clinical trials have been undertaken to explore the prominent role of tomato or lycopene interventions for modulating the targeted gene expressions, directed to the progression of PCa.

3.1. In vivo study

In 2004, an early study investigating the effects of lycopene and vitamin E supplementation on the autocrine/paracrine loops in the Dunning PCa model has been conducted by Siler et al. (2004). Thirty Copenhagen rats (8–10 weeks of age) were randomly assigned to receive either lycopene (200 μg/g diet), vitamin E (540 μg/g diet), lycopene + vitamin E (200 μg lycopene + 540 μg vitamin E/g diet), vehicle (40 μg vitamin E/g diet) or control diet (<5 μg vitamin E/g diet) supplementations for four weeks. Subsequently, 1×10^5 MatLyLu Dunning PCa cells were injected into the ventral prostate, and the supplementation was continued for 18 days of tumor growth. Results showed that lycopene and vitamin E were accumulated in the tumor tissue, and macroscopic evaluations depicted a significant increase of necrotic area in the lycopene ($p = 0.022$) and vitamin E ($p = 0.006$) treatment groups. The steroid 5-α-reductase 1 expression was inhibited in the lycopene (0.36-fold) and co-treated (0.48-fold) groups, while steroid target genes expression (cystatin-related proteins 1 and 2, prostatic spermine binding protein, prostatic steroid binding proteins C1, C2 and C3 chain, probasin) was reduced in both lycopene-treated groups. The prostatic expression of IGF-1 was significantly downregulated by lycopene treatment and in combination with vitamin E, whereas IL-6 expression was inhibited by lycopene treatment alone. The results suggested that lycopene and vitamin E contributed to the reduction of PCa risk by interfering with internal autocrine or paracrine loops of sex steroid hormone, growth factor activation, and signaling in the prostate.

To investigate the effect of lycopene on normal prostate tissue, 42 young and normal Copenhagen rats were randomly supplemented with either 200 μg lycopene/g diet or placebo (40 μg vitamin E/g diet) for eight weeks (Herzog et al., 2005). Thereafter, all rats were killed under deep isoflurance anesthesia by bleeding, and the four pairs of prostate lobes (anterior, dorsal, lateral and ventral) were isolated. Lycopene was extracted from the prostate lobes, and its isomers were determined using HPLC. All-trans lycopene was found as the predominant lycopene isoform (59.8 ± 5.7%) of total lycopene for all

time points tested. Transcriptomic analysis showed that lycopene supplementation mildly reduced the gene expression of androgen metabolizing enzymes [17β-hydroxysteroid-dehydrogenases (17β-HSD), 5α-reductase 2, and Cyp7B1] and androgen targets (prostatic steroid binding protein C1 and C3 chain, as well as cystatin-related protein 2 and seminal vesicle secretion protein IV). Moreover, local expression of IGF-I was reduced in the lateral (0.60-fold) and dorsal (0.43-fold) lobes. Lycopene also led to a down-regulation of inflammatory cytokines in the lateral lobe. Overall, this study highlighted the effectiveness of lycopene supplementation in reducing local prostatic androgen signaling, IGF-I expression, and basal inflammatory signals in normal prostate tissue.

Meanwhile, a short term supplementation trial has been conducted to evaluate the interrelations between lycopene, tomato powder or phytofluene (a carotenoid precursor of lycopene) consumption with the androgen status using the eight weeks old F344 rats (Campbell et al., 2006). All rats were castrated (or sham-operated), and provided with a daily oral supplementation of phytofluene or lycopene (0.7 mg/d) or fed with a 10% tomato powder supplemented diet (AIN 93G) for four days. Rats were killed by carbon dioxide asphyxiation, and the testes and prostate-seminal complex were collected and dissected to measure the accumulation of carotenoid. Serum testosterone and dihydrotestosterone (DHT) were quantified using radioimmunoassay kits. Total RNA was extracted, reversed-transcribed, and the mRNA expression was measured using the RT-PCR method with SYBR green fluorescence dye. Castrated rats with a short-term intake of lycopene or tomato powder exhibited a significantly ($p < 0.05$) greater prostatic 17β-HSD 4 mRNA expression than the sham-operated, control-fed rats, potentially indicating the reduction of local prostatic androgen signaling. Multiple linear regression analyses showed that lycopene or tomato powder fed rats demonstrated a greater PPARγ or fatty acid binding protein 3 (FABP3) mRNA expression, respectively, in the prostate than the castrated control-fed rats ($p < 0.05$). Overall, the findings revealed the short term supplementation of tomato carotenoids had significantly altered the androgen status and reduced the PCa risk.

Another trial evaluating the efficacy of lycopene and β-carotene against androgen-independent prostate tumor cell growth has been advocated (Yang, Yen, Huang, & Hu, 2011). PC-3 cells were injected into the athymic nude mice, and the mice were orally administered with a low or a high dose of lycopene (1 and 16 mg/kg) or β-carotene (16 mg/kg) for seven weeks. Results showed that the tumor volumes of mice treated with high dose of lycopene and β-carotene were reduced by 67% ($p < 0.001$) and 62% ($p < 0.001$), respectively, as compared to the control group. A decrease of 65% ($p < 0.001$) and 71% ($p < 0.001$) in tumor weight was detected among the mice treated with high dose lycopene and β-carotene. High dosage supplementation of lycopene significantly reduced the vascular endothelial growth factor (VEGF) level in plasma. While, high dosage supplementation of lycopene and β-carotene significantly decreased the expression of proliferating cell nuclear antigen in tumor tissues and increased the levels of IGFBP-3 in plasma. The study demonstrated that the anticancer activities of lycopene and β-carotene were likely related to the reduction of proliferation, and interfered with the IGF-1 signaling in PC-3 bearing nude mice.

3.2. Human study

A randomized, single-blinded crossover study was conducted to examine the differential effects of tomato matrix on 45 targeted genes expression of cancer prostatic cells (Talvas et al., 2010). Thirty healthy, non-smoking men (aged 50–70 y old) were randomly assigned into either one week yellow tomato paste (YT) (200 g/d, 0 mg lycopene) or red tomato paste (RT) (200 g/d, 16 mg lycopene) diet after a two week washout period. In a parallel design, the YT group received purified lycopene supplementation (16 mg/d), while the RT group received a placebo supplementation for one week. The serum was obtained at the baseline and after the interventions for incubation with LNCaP to measure the expression of 45 target genes. Consumption of RT ($p < 0.05$) and LYC ($p < 0.05$) resulted in a significant increase in the serum lycopene concentration as compared to the YT and placebo groups. After the first washout period, the LNCaP cells incubated with sera from the RT group showed a significant up-regulation of IGFBP-3 (growth factor) and Bax/Bcl-2 (apoptosis) ratio, and down-regulation of cyclin-D1 (cell cycle), p53 (oncogene), and NF-E2-related factor (Nrf-2) (oxidative stress). Meanwhile, cell incubated with sera from the purified lycopene group led to a significant up-regulation of IGFBP-3, c-fos (oncogene), and uPAR (metastasis) as compared with the placebo group.

Chan et al. (2011) have undertaken a three-month randomized, double-blind, placebo-controlled clinical trial (The Molecular Effects of Nutritional Supplements Trial) to establish the prostate tissue gene expression profiles among men with low burden PCa. In the study, active surveillance (AS) was chosen for the disease management, receiving either 30 mg/d lycopene, 3 g/d fish oil (containing 1098 eicosapentaenoic acid and 549 mg docosahexaenoic acid) or placebo capsules. An ultrasound-guided four-core research biopsy has been performed at baseline and after the intervention to procure fresh tissue for RNA analysis, followed by microarray analyses. Changes in the IGF-1 and COX-2 gene expressions were assessed using the quantitative RT-PCR. Results revealed no significant difference from baseline to the three-months in term of IGF-1 or IGF-1R expression level between the placebo and lycopene supplement arms ($p = 0.93$), nor in COX-2 expression between the fish oil and placebo arms ($p = 0.99$). This finding revealed that short term intervention with lycopene or fish oil supplement did not show significant effect on normal prostate expression of IGF-1 and COX-2 among men on AS for low-burden PCa. The application of genome-wide microarray technique should be implemented to examine the global gene expression profiles and bioactivity of these nutrients for the progression of PCa.

Following the null findings as reported by Chan et al. (2011), Magbanua et al. (2011) have further evaluated the effects of lycopene (30 mg/d) and fish oil (3 g/d) supplementation on global prostate gene expression and pathway analyses of morphologically normal prostate tissue before and after the three-month intervention, and between the diet groups (high *versus* low for tomato or fish consumption) at the baseline. Biopsy processing, RNA amplification, cDNA microarray analysis, differential expression and canonical pathway analysis were performed. Global gene expression analysis showed no significant individual genes were associated with the high dietary intake of tomato or fish consumption at baseline or after three-month of supplementation with lycopene or fish oil. Among men who routinely consumed more tomato ($p = 0.008$) and fish ($p = 0.029$), the exploratory pathway analyses of rank-ordered genes revealed the modulation of androgen and estrogen metabolism as compared to men who consumed less. Additionally, canonical pathway analysis demonstrated the modulation of Nrf2-mediated oxidative response to both lycopene ($p = 0.001$) and fish oil ($p = 0.012$) supplemented arms as compared to the placebo group, where fish oil arm showed a better modulation of arachidonic acid metabolism ($p = 0.014$), as compared to placebo group. The findings provided a platform to reveal the potential pathway that may be modulated by tomato and fish consumption, or lycopene and fish oil supplementation. Discovery of the molecular mechanisms in affecting the gene expression would contribute to the preventive treatment of PCa.

4. Putative mechanisms for the action of lycopene in PCa

The molecular mechanisms on how phytochemicals affect gene expression provide an insight into the development, prevention and treatment of PCa. Both *in vivo* and human intervention studies have been undertaken to determine the biological pathway connecting tomato and lycopene, and their impacts on the gene expression regulation of PCa. Numerous efforts have been made to establish a causal

relation between tomato and lycopene consumption with PCa, and a few putative mechanisms have been suggested to explain the pathway on how lycopene could affect the physiological state and the prostate tumorigenesis.

4.1. Lycopene consumption, steroid androgen signaling and PCa

Herzog et al. (2005) have proposed the role of lycopene treatment for the reduction of steroid androgen signaling in the dorsolateral prostate (Fig. 1). During the mechanism, 5α-reductase 2 and 17β-HSD are responsible for the conversion of dehydroepiandrosterone (DHEA), androstenedione and testosterone into the most potent natural androgen, 5α-dihydrotestosterone (5α-DHT). Lycopene plays a major role in regulating 17β-HSD, 5α-reductase 2, and Cyp7B1 expressions in the prostate tissues. It up-regulates 17β-HSD type IV to convert androst-5-ene-3β,17β-diol to DHEA, and reduces the direct conversion of androst-5-ene-3β,17β-diol to testosterone. Generally, DHEA is a ligand of estrogen receptor β (ERβ). It decreases the proliferation of prostate epithelium cells (Weihua, Lathe, Warner, & Gustafsson, 2002), and used as the PCa preventive agent (Kim et al., 2002). Cyp7B1 is another enzyme that typically inactivates 3β-Adiol (Gemzik, Green, & Parkinson, 1992), a DHT metabolite that functions as the major endogenous estrogen in the prostate (Weihua et al., 2001). Lycopene administration was shown capable to reduce the activation of testosterone, with a decrease of 5α-reductase 2 expressions in the dorsal and lateral lobes of prostate. Moreover, the reduction of Cyp7B1 expression after the lycopene supplementation suggests a diminished inactivation and excretion of 5α-androstane-3β,17β-diol (3β-Adiol). This increase of DHEA and 3β-Adiol levels might contribute to the beneficial effect of lycopene consumption on PCa.

4.2. Antiproliferative activity of lycopene through PPARγ-LXR-ABCA1 pathway on androgen-dependent PCa cells

The potential of lycopene in activating PPARγ–LXRα–ABCA1 pathway by promotion of the antiproliferative effect on human prostate tumor cell has been proposed by Yang, Lu, Chen, & Hu (2012b) (Fig. 2). Generally, PPARγ and LXRα are the members of NRs, which bind with a ligand in the cytoplasm, and translocated to the nucleus (Whiteside & Goodbourn, 1993). Both of them exist as heterodimers with retinoid X receptor (RXR) partners, and LXRα is normally presented as a direct target gene (Chawla et al., 2001; Olefsky, 2001). Previous report has shown that LXRs could regulate intracellular cholesterol levels by mediating the expression of ABCA1, and modulate cholesterol efflux and reverse cholesterol transport (RCT) from the peripheral tissues (Zhang et al., 2008). High-density lipoprotein (HDL) is important to mediate the activity of RCT and regulate the net movement of cholesterol from peripheral tissues back to the liver (Miller, La Ville, & Crook, 1985). ApoA1, the major

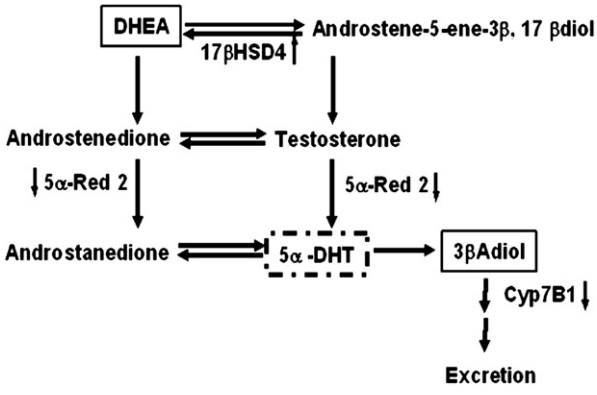

Fig. 1. Lycopene effect on steroid metabolism and signaling (Bélanger et al., 2003).

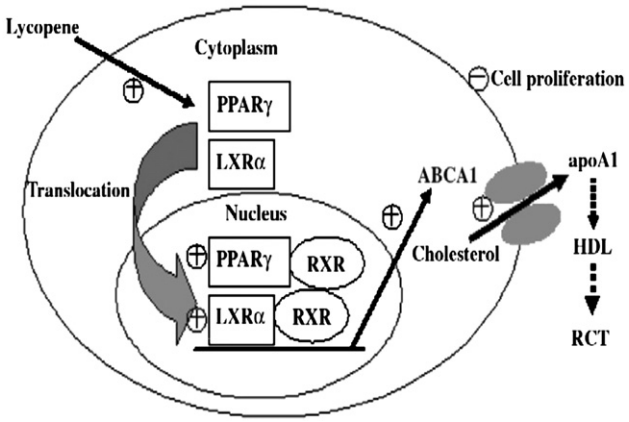

Fig. 2. Proposed mechanisms by which lycopene inhibit the proliferation of PCa cells (Yang et al., 2012b).

apolipoprotein of HDL, is important in mediating cholesterol efflux (Tsubakio-Yamamoto et al., 2008). Lycopene reacts as an exogenous agonist to trigger the biological reaction of PPARγ and LXRα. The antiproliferative effect of lycopene on androgen dependent PCa cells is associated with an up-regulation of the PPARγ–LXRα–ABCA1 pathway. It may increase the ABCA1 and apoA1 expressions and decrease the total intracellular cholesterol levels in the LNCaP cells. Therefore, it is suggested that lycopene may involve in the PPARγ–LXR–ABCA1 pathway by mediating the cholesterol efflux in the PCa cells.

5. Conclusion

The prescription of artificial drugs is the usual practice for the protective control and treatment of PCa. Long term drug prescription is accompanied by a variety of unavoidable side-effects. A growing exploitation has been devoted towards the beneficial use of agricultural-based phytochemical, lycopene. Lycopene has emerged to be a unique candidate in the regulation of DNA repair system, testosterone signaling, inflammatory cytokine secretion, intercellular gap junction interaction, PPARγ–LXRα–ABCA1 pathway, cell cycle and integrin, IGF-IR, activate endogenous antioxidant enzyme, and the migration and invasion of tumor signaling for PCa. The understanding of these individual genomic differences would facilitate a more precise PCa clinical trial delineating the diet × gene interactions. In future, it is plausible that major investigations will be made to utilize the discovery of nutritional intervention on gene regulation and expression into the health preventive strategies.

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