Dietary Lycopene Modulates Prostate Cancer Biomarker Genes in Androgen-Independent Human Prostate Cancer (PC-3) Cell Line

by

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ABSTRACT OF THESIS

Dietary Lycopene Modulates Prostate Cancer Biomarker Genes in Androgen-Independent Human Prostate Cancer (PC-3) Cell Line

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In the United States prostate cancer is the most commonly diagnosed cancer and is the second leading cause of death from malignancies in men. Dietary components have been recently targeted in the prevention and treatment of cancer, in combination with existing medical treatments for prostate cancer. Lycopene is a fat soluble red-orange carotenoid primarily consumed in foods containing tomatoes and tomato-derived products including tomato sauce, tomato paste and ketchup, with relatively smaller amounts in dried apricots, watermelon and pink grapefruit. Our objective is to determine whether lycopene treatment modulates prostate cancer biomarker genes in hormone-refractory human prostate cancer (PC-3) cell lines using Oligo GEArray® DNA Microarray which contains 263 genes involved in the prognosis and diagnosis of prostate cancer. Cell viability experiments determined that 25μM lycopene was the highest non-toxic treatment dose and therefore was selected for further experiments. Microarray image analysis demonstrated a decrease in the expression of transforming growth factor beta-2 (TGFβ-2), cyclin dependent kinase–9 (CDK-9), epidermal growth factor receptor (EGFR), B-cell lymphoma–2 (BCL-2), B-cell lymphoma–2 like 1 (BCL2L1), insulin-like growth factor 1 receptor (IGF1R), cyclin dependent kinase–7 (CDK-7), and breast cancer 1 (BRCA1) genes after the treatment of PC-3 cells with 25μM lycopene. Percent down-regulation calculated for TGFβ-2, CDK-9, EGFR, BCL-2, BCL2L1, IGF1R, CDK-7, and

BRCA1 genes was 79%, 68%, 59%, 54%, 52%, 48%, 43%, and 38%, respectively, in lycopene-treated versus untreated samples. The modulated expressions of EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, and BCL-2 genes were validated using Real-Time Polymerase Chain Reaction (Real-Time PCR) and the results demonstarted a down-regulation of 72%, 61%, 56%, 56%, 44%, 34%, and 30%, respectively. Among all modulated prostate cancer biomarker genes, EGFR demonstrated the most consistent down-regulation in expression in our microarray and Real-Time PCR analyses. Protein expression analysis demonstrated that lycopene treatment also decreased EGFR protein expression in lycopene-treated PC-3 cells by 36%. These results indicate that the treatment of PC-3 cells with lycopene consistently modulates several prostate cancer biomarker genes. The present study clearly indicates that EGFR is down-regulated at the mRNA and protein levels after treatment with 25μM lycopene. Therefore, the results suggest that lycopene may be beneficial in delaying or preventing the progression of prostate disease.

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Chapter I INTRODUCTION

According to statistics from the National Cancer Institute, in 2007 an estimated 218,000 new diagnoses and approximately 27,000 deaths will occur due to prostate cancer in the United States, making prostate cancer the most commonly diagnosed cancer and the second leading cause of death from malignancies in men. The risk of prostate cancer is often cateogorized by age, family history and race. Individuals at highest risk include men over the age of 65 years and those with a family history of prostate cancer. Also, men of African-American decent are 61% more likely to develop prostate cancer and are 2.5 times more likely to die compared to Caucasian men (Clinton & Giovannucci 1998, National Cancer Institute).

The prostate serves as an accessory gland of the male reproductive tract, is located at the base of the bladder and produces secretions that aid in sperm function (Clinton & Giovannucci 1998). Screening for prostate disease often involves a prostate-specific antigen (PSA) blood test and digital rectal exam. If screening indicates a high risk for cancer, treatment for prostate cancer follows the course of localized treatment like surgery often combined with androgen ablation while monitoring tumor growth, spread and prostate specific antigen (PSA) level. However, after these therapies have been completed without termination of the cancer, prostate cancer becomes more advanced and is referred to as hormone-refractory prostate cancer involving androgen-independent cancer cells (Garnick and Fair 1998, American Cancer Society).

In addition to the existing clinical treatments for prostate cancer, dietary components have been recently targeted in the prevention and treatment of cancer. Several food components including resveratrol, capsaicin, and quercetin, have decreased

the cell proliferation rate and increased cell death in various *in-vitro* studies with prostate cancer cell lines (Benitez et al. 2006, Mori et al. 2006, Nair et al. 2004).

Several studies report a promising anti-cancer effect of lycopene, a carotenoid found in tomato and tomato products. Lycopene is a fat soluble red-orange carotenoid not synthesized by the human body and primarily consumed in foods containing tomatoes and tomato-derived products including tomato sauce, tomato paste and ketchup, with relatively smaller amounts in dried apricots, watermelon and pink grapefruit (Krinsky & Johnson 2005, Rao & Rao 2007, Rafi et al. 2007). Lycopene consists of a 40 carbon and 13 double bond structure and is one of the most abundant carotenoids found in several human tissues. In the prostate, the concentration of lycopene is also the highest among all dietary carotenoids (Khachik et al. 2002). Studies have shown that mechanical and thermal processing increases the bioavailability of lycopene (Dewanto et al. 2002, Van het Hof et al. 2000). Therefore, lycopene found in tomato paste is more bioavailable compared to fresh tomatoes (Gartner et al. 1997). It is naturally present in raw tomatoes in the *trans* configuration. However, more than 50% of lycopene in human tissues and blood can be found in the *cis* configuration, suggesting c*is*-lycopene isomers are more bioavailable than *all-trans* isomers (Boileau et al. 2002, Bhuvaneswari & Nagini 2005).

Recently much attention has focused on the role of lycopene in the prevention and progression of prostate cancer. *In-vitro* and animal studies showed that lycopene more potently inhibited the growth of an androgen-independent cell line than an androgen-dependent cell line and decreased tumor growth in nude mice (Tang et al. 2005). Epidemiological and clinical studies suggest that consumption of fruits and vegetables rich in lycopene may serve as a protective agent against prostate cancer. Clinical studies

show that the consumption of over-the-counter lycopene supplements decreased PSA level and increased serum lycopene levels in men with high-grade prostate intraepithelial neoplasia (Mohanty et al. 2005) and that lycopene consumed in meals increased serum and prostate lycopene concentrations while also decreasing serum PSA levels in patients with prostate cancer (Chen et al. 2001). Lycopene intake was also associated with a reduced risk of prostate cancer and the intake of tomato sauce with even greater reduction in prostate cancer risk (Giovannucci et al. 2002).

Our objective is to determine whether lycopene treatment modulates prostate cancer biomarker genes in hormone-refractory human prostate cancer (PC-3) cell lines using Oligo GEArray® DNA Microarray which contains 263 genes involved in the prognosis and diagnosis of prostate cancer. Our microarray results indicate that treatment of PC-3 cells with lycopene significantly down-regulated transforming growth factor beta-2 (TGFβ-2), cyclin dependent kinase–9 (CDK-9), epidermal growth factor receptor (EGFR), B-cell lymphoma–2 (BCL-2), B-cell lymphoma–2 like 1 (BCL2L1), insulin-like growth factor 1 receptor (IGF1R), cyclin dependent kinase–7 (CDK-7), and breast cancer 1 (BRCA1) genes which are genes involved in human prostate cancer cell proliferation. Real-Time PCR also validated the down-regulation of EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, and BCL-2 genes. Our results clearly show that EGFR is down-regulated at the mRNA and protein levels after treatment with 25μM lycopene. Protein expression analysis by Western blot further demonstrates that lycopene treatment also decreases Epidermal Growth Factore Receptor (EGFR) protein expression in lycopene-treated PC-3 cells. These results suggest that lycopene may be beneficial in preventing or delaying the progression of prostate disease by modulating prostate biomarker genes,

especially the epidermal growth factor receptor (EGFR) gene, which is associated with the development of prostate cancer, as well as the progression to androgen-independent or hormone-refractory prostate cancer with EGFR expression (Hernes et al. 2004, Shah et al. 2006).

Chapter II LITERATURE REVIEW

II.A. Cancer in the United States

In the United States, cancer is the most common cause of death only to be preceded by heart disease. In 2007 alone, the American Cancer Society states that approximately 1,444,920 new cancer cases are expected to be diagnosed as well as 559,650 anticipated cancer-related deaths. The survival rate of cancer has risen to 66% during 1996 to 2002 compared to 51% in 1975 to 1977 due in part to screening, early detection and improved therapies. However, among cancers diagnosed in men and women, prostate, breast, lung/bronchus, colon/rectum, and urinary bladder cancers continue to lead the list of new cancer cases. Lung/bronchus, prostate, and colon/rectum cancers top the list in cancer-related deaths in men, while lung/bronchus, breast and colon/rectum cancers top the list for women (American Cancer Society).

The causes of cancer have been associated with external factors like tobacco, chemicals and radiation, as well as internal factors including inherited mutations, hormones, immune conditions and mutations in metabolism. While 5% of cancers are hereditary, most are a consequence of damage or mutation to genes that are responsible for cell growth and cell division. About one-third of the cancer deaths this year will be related to being overweight, obese, lack of physical activity and poor nutrition but with preventative screening, early detection and a change of lifestyle nearly half of all new cancer cases may be avoided (American Cancer Society).

II.B. Cell Cycle

The cells in a normal human healthy body regulate homeostasis between cell proliferation and apoptosis in order to maintain the size of each tissue organ and meet the needs of our body. In other words, the normal cells of the body work together to control and keep the human body system healthy and functioning well (Weinberg 1996). However, cancer cells do not maintain this state of homeostasis. They also possess the ability to travel away from their original site and invade other tissues in the body. Therefore, cancer cells are characterized by their uncontrolled growth and spread of abnormal cells (Weinberg 1996, American Cancer Society).

II.C. Benign and Malignant Tumors

The accumulation of abnormal cells will give rise to tumors. Benign tumors are less life-threatening compared to malignant tumors since the cells of a benign tumor do not migrate to other areas of the body. This type of tumor is localized and may be surgically removed if necessary. On the other hand, a more serious condition is the formation of a malignant tumor. The cells of this type of tumor are not localized and are the cause of many cancers. Malignant tumors are invasive, spread to other surrounding tissues and become more aggressive with time, ultimately disrupting the tissues and organs needed for survival. Their ability to travel away from their original site and cause secondary growth is known as metastasis (Darnell et al. 1990, Weinberg 1996).

The cells of a benign tumor function similar to normal cells and originate from one of three embryonic cell layers: endoderm, ectoderm or mesoderm, while the cells of malignant tumors characteristically appear less differentiated, have little structure, may

lack necessary enzymes to function, and consist of an abnormal and unstable number of chromosomes (Darnell et al. 1990). Malignant tumors that arise from the endoderm or ectoderm are known as carcinomas and those from the mesoderm are known as sarcomas which also include leukemia (Darnell et al. 1990).

II.D. Prostate Cancer

Overall statistics show that lung cancer is the leading cause of death among all cancer types in men and women and estimates remain consistent in 2007 followed by cancer deaths due to female breast, prostate and colon/rectal cancers. However, leading the list for new cases in 2007 is prostate cancer for men and breast cancer for women followed by lung/bronchus and colon/rectum cancers in both sexes (American Cancer Society).

The risk of prostate cancer is often cateogorized by age, family history and race. Individuals at highest risk include men over the age of 65 years and those with a family history of prostate cancer. Also, men of African-American decent are 61% more likely to develop prostate cancer and are 2.5 times more likely to die compared to Caucasian men (Clinton & Giovannucci 1998, National Cancer Institute).

The prostate serves as an accessory gland of the male reproductive tract. It is located at the base of the bladder and produces secretions that aid in sperm function (Clinton & Giovannucci 1998). Although symptoms are rare early in the disease, at a more advanced stage men may experience weak urine flow, the inability to urinate, difficulty stopping the urine flow, frequent urination, the presence of blood in the urine or burning with urination (American Cancer Society). However, these symptoms are not

restricted to advanced disease but may also be common in benign conditions (America Cancer Society).

II.E. Prostate Cancer Screening

Preliminary screening for prostate cancer involves the prostate-specific antigen (PSA) blood test and digital rectal exam starting at the age of 50 but at the age of 45 for populations at high risk (America Cancer Society). The PSA blood test measures the level of prostate-specific antigen a protein released by the prostate cells. A PSA level at or below 4ng/ml is considered normal but a rise above normal indicates the risk of cancer being present even when the size of the tumor is too small to detect (Garnick and Fair 1998). A PSA blood screening can help detect an early risk for prostate cancer, while a digital rectal exam can only identify tumors that are already larger in size (Garnick and Fair 1998, America Cancer Society).

PSA screening has long been controversial due its non-specificity. As many as 25% of men with cancer will test normal for PSA level, while more than half of cancer-free men may test above normal (Garnick and Fair 1998). However, since prostate cancer progresses at a slower rate than most other cancers, it is known as a disease of older men. Therefore, early PSA screening may be successful in detecting cancer at an early stage when treatment may receive a better response. Observing the future rise or fall in the prostate cancer death rate will truly be the only way to confirm the efficacy of early detection and treatment (Hanks and Scardino 1996).

II.F. Prostate Cancer Treatment

Early treatment of prostate cancer often follows the course of localized treatment including surgery, removal of the affected area on the prostate, external beam radiation, or radioactive seed implants (Garnick and Fair 1998, America Cancer Society). Metastatic disease is often treated with hormonal therapy, chemotherapy, radiation or a combination of the three (American Cancer Society). Local radiation aims to destroy the tumor and may be combined with hormonal therapy also known as androgen ablation (Garnick and Fair 1998, American Cancer Society). During androgen ablation the production of testosterone and related male hormones which are thought to support the growth of prostate cancer are blocked, aiming to stop the growth and/or inhibit the action of androgens (Garnick and Fair 1998). This combination of therapy may control the prostate cancer for some time, shrinking the tumor and alleviating symptoms of prostate cancer (American Cancer Society). However, after these therapies have been completed without complete termination of the cancer, prostate cancer becomes more advanced and is referred to as hormone-refractory prostate cancer involving androgen-independent cancer cells.

II.G. Nutrition and Prostate Cancer

In addition to the existing treatments for prostate cancer, dietary components have been recently targeted in the prevention and treatment of cancer. Due to a prolonged pre-clinical phase or latency period of prosate cancer, researchers are conducting *in-vitro* studies investigating the possible health benefits of various food components with prostate cancer as the target. Several food components including resveratrol, a

polyphenol found in grapes and red wine, capsaicin, a major pungent ingredient in red peppers and quercetin, a flavanoid found in fruits and vegetables have decreased the cell proliferation rate and increased cell death in various prostate cancer cell lines (Benitez et al. 2006, Mori et al. 2006, Nair et al. 2004). Another animal study involving the oral administration of pomegranate fruit extract to athymic nude mice implanted with a prostate cancer cell line resulted in a decreased tumor growth and serum PSA level (Malik & Mukhtar 2006). Other *in-vitro* studies involving theaflavins found in black tea and epigallocathechin-3-gallate (EGCG) found in green tea have been noted for inducing cell cycle arrest and apoptosis in androgen-dependent and androgen-independent cell lines (Kalra et al. 2007, Gupta et al. 2003, Adhami et al. 2003).

II.H. Carotenoids

Carotenoids are a family of compounds consisting of over 600 fat-soluble plant pigments (Krinsky & Johnson 2005, Rao and Rao 2007). There are approximately 40 carotenoids present in the human diet and 20 carotenoids identified in blood and tissues (Rao and Rao 2007). Among the carotenoids found in the human plasma abundant sources are primarily noted in fruits and vegetables. Ninety-percent of the dietary carotenoids present in the human plasma include β–carotene present in apricot, carrots, and spinach, α–carotene found in cooked carrots, β–cryptoxanthin existing in tangerine and papaya, Lutein & Zeaxanthin most commonly found in spinach, kale and broccoli and Lycopene consumed from tomato and tomato products (Krinsky & Johnson 2005).

Several studies have researched the action of carotenoids and their ability to prevent diseases such as cancer, HIV, cataracts, cardiovascular disease, and age-related

macular degeneration. Carotenoids have been noted for their effects on the immune response as well as their ability to improve pro-vitamin A activity, gap junction communication, antioxidant function, and xenobiotic and drug metabolism (Rao and Rao 2007).

II.I. Lycopene Food Sources and Bioavailability

Lycopene is a fat soluble red-orange carotenoid not synthesized by the human body primarily consumed in fruits and vegetables namely tomatoes and tomato-derived products including tomato sauce, tomato paste and ketchup (Krinsky & Johnson 2005, Rao & Rao 2007). Relatively smaller amounts are found in watermelon, papaya, guava and pink grapefruit (Krinsky & Johnson 2005, Rao & Rao 2007).

Food	Lycopene content (mg/100g wet wt)
Tomatoes, fresh	0.88-4.20
Tomatoes, cooked	3.70
Tomato sauce	6.20
Tomato paste	5.40-150.00
Tomato soup – condensed	7.99
Tomato juice	5.00-11.60
Tomato ketchup	12.71
Watermelon, fresh	2.30-7.20
Papaya, fresh	2.00-5.30
Pink guava	5.40
Pink grapefruit	3.36

Table 1. Lycopene content of various food items**.** (Bhuvaneswari & Nagini 2005)

II.J. Lycopene Structure

Lycopene consists of a 40 carbon and 13 double bond structure and is one of the most abundant carotenoids found in several human tissues (Bhuvaneswari & Nagini 2005). It is naturally present in raw tomatoes in the *trans* configuration. However, more

than 50% of lycopene in human tissues and blood can be found in the *cis* configuration, suggesting c*is*-lycopene isomers are more bioavailable than *all-trans* isomers (Boileau et al. 2002, Bhuvaneswari & Nagini 2005). Studies further support this by demonstrating that mechanical and thermal processing increases the bioavailability of lycopene (Dewanto et al. 2002, van het Hof et al. 2000). Therefore, lycopene found in tomato paste is more bioavailable compared to fresh tomatoes (Gartner et al. 1997). Unlike its raw counterparts such as fresh tomatoes, tomato juice and fresh fruits, cooked tomato products including tomato sauce, paste and ketchup contain more lycopene (Bhuvaneswari & Nagini 2005).

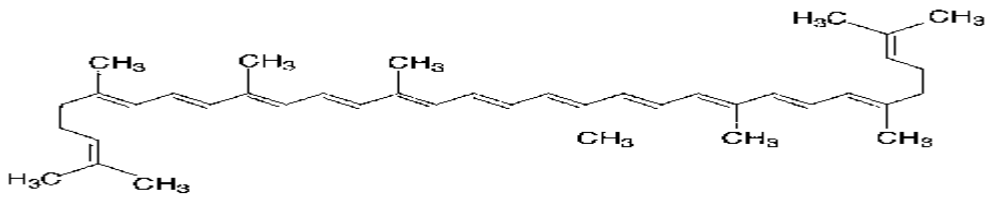

Figure 1. Structure of Lycopene

II.K. Lycopene Absorption, Distribution, & Metabolism

Due to the inability of the body to produce its own stores of lycopene, the human body must rely on the consumption of dietary lycopene. The body allows effective absorption of lycopene and distribution to various tissues of the body (Bhuvaneswari & Nagini 2005, Parker 1989). Once lycopene is released from the food matrix, it is incorporated into lipid micelles. Passive diffusion then allows lycopene to be absorbed into the intestinal mucosa, followed by uptake by the liver. Finally, circulation to various tissues occurs via lipoproteins LDL, HDL, and VLDL (Bhuvaneswari & Nagini 2005, Parker 1989).

A study conducted by Khachik et al. (2002) indicates that among all carotenoids and their metabolites, lycopene is one of the most abundant carotenoids found in human tissues and is the most abundant carotenoid found in the prostate.

It has been proposed that the metabolism of lycopene begins with the oxidation at the 1,2 and 5,6 positions to form lycopene 1,2-epoxide and lycopene 5,6-epoxide. The instability of 5,6-epoxide allows it to undergo cyclization and produce two forms of 2,6 cyclolycopene-1,5-epoxide, while 1,2-epoxide remains stable in its own form. In addition, their two corresponding cyclic diols, 2,6 cyclolycopene-1,5-diols have been detected in human serum and consist of a novel five-membered ring end-group with three asymmetric centers at C-2, C-5 and C-6. The origin of lycopene's metabolites in human serum is still remains uncertain because the concentrations of the 2,6 cyclolycopene-1,5-diols and their epoxide precursors are extremely low in tomatoes and tomato products (Linshield et al. 2007, Khachik et al. 2002).

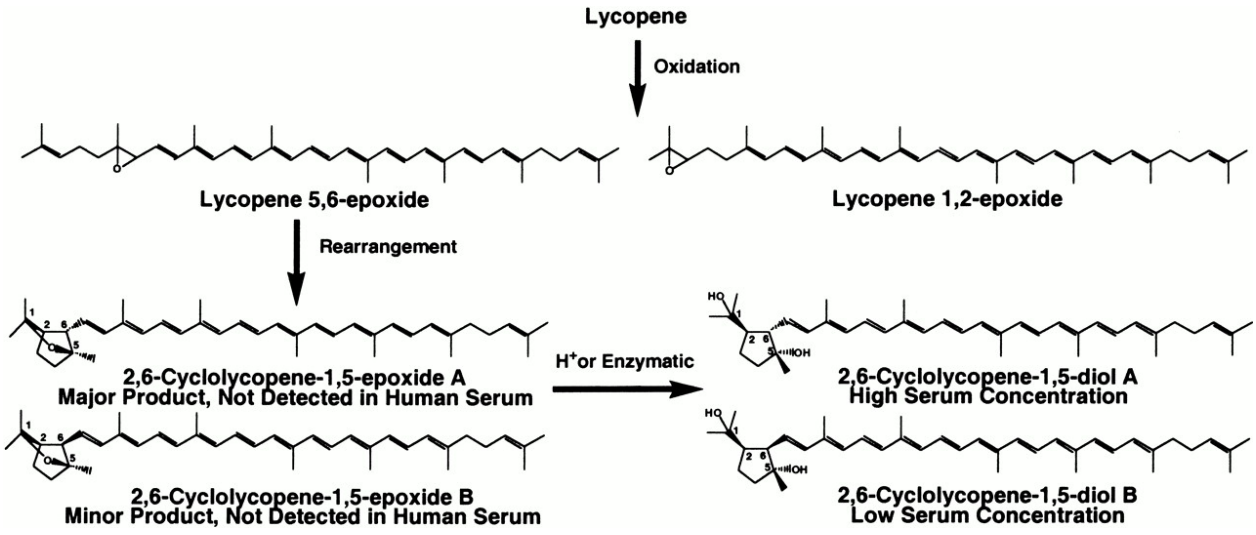

Figure 2. Proposed metabolic pathway of lycopene in humans. (Khachik et al. 2002)

II.L. Recommended Intake

Currently there is no dietary reference intake (DRI) for lycopene (nutriton.gov) but studies show that typical dietary intake of lycopene in U.S. is about 2-5mg/day (Krinsky & Johnson 2005) and excessive intake can result in lycopenaemia, a coloration of skin due to prolonged intake (La Placa et al. 2000). Typical over-the-counter supplements such as Centrum® Multivitamin contain approximately 300µg of lycopene.

II.M. Health Benefits of Lycopene

As a rich source of lycopene, tomatoes have been studied for their various health benefits. Some studies suggest its therapeutic potential in relation to coronary heart disease by improving lipid profile levels (Agarwal and Rao 1998, Rao 2002, Blum et al. 2006). Moreover, lycopene demonstrates antioxidant function by improving levels of serum enzymes and the lipid peroxidation rate involved in antioxidant activities after 60 days of lycopene supplementation (Bose and Agarwal 2007). An association between carotenoid consumption and lung cancer risk was also studied. While following the incidence of lung cancer and collecting food item dietary questionnaires, the risk for lung cancer in male smokers was decreased in response to the consumption of fruits and vegetables rich in lycopene, followed by lutein/zeaxanthin and β-cryptoxanthin (Holick et al., 2002). As a significant part of the Mediterranean diet, which is high in olive oil and fruits and vegetables like tomatoes, eggplant and peppers, as well as low in saturated fat, tomato consumption suggests a decreased relative risk for oral, pharyngeal, esophageal and colorectal cancers in Italian cancer subjects. This further implies the anti-carcinogenic and antioxidant effects of lycopene (La Vecchia 2002).

II.N. Lycopene and Prostate Cancer

Recently much attention has focused on the role of lycopene in the prevention and progression of prostate cancer. Several studies report a promising anti-cancer effect of lycopene, a carotenoid found in tomato and tomato products. *In-vitro* and animal studies showed that lycopene more potently inhibited the growth of an androgen-independent cell line than an androgen-dependent cell line and decreased tumor growth in mice (Tang et al. 2005).

Epidemiological and clinical studies suggest that consumption of fruits and vegetables rich in lycopene may serve as a protective agent against prostate cancer. Clinical studies show that oral lycopene supplementation (8 mg/day) decreased PSA level and increased serum lycopene levels in men with high-grade prostate intraepithelial neoplasia (Mohanty et al. 2005) and that consuming 30 mg/day of lycopene from meals increased serum and prostate lycopene concentrations while also decreasing serum PSA levels in patients with prostate cancer (Chen et al. 2001). A collection of food frequency questionnaires associated lycopene intake with reduced risk of prostate cancer and the intake of tomato sauce with even greater reduction in prostate cancer risk (Giovannucci et al. 2002).

II.O. Prostate Cancer Biomarker Genes

Among the numerous genes of the human genome, proto-oncogenes are responsible for promoting growth as opposed to tumor suppressor genes whose function is to prevent or inhibit growth (Weinberg 1996). Mutations to these types of genes affect the activity of cells consequently causing disruption to the well balanced system in the

human body. For example, proto-oncogenes contribute to cancer during excessive multiplication and over-activation, while tumor-suppressor genes become carcinogenic when their ability to suppress growth is lost and inappropriate growth continues (Weinberg 1996).

While measuring PSA levels and digital rectal exams are the most routine practices for diagnosis of prostate cancer, results are not always accurate and cannot predict the stage of a tumor (Charkrabarti et al. 2002). The development and progression of cancer is due to various environmental and genetic factors that include chromosomal rearrangements and gene mutations. These alterations affect cell proliferation, inhibition of apoptosis, invasion, angiogenesis and metastasis, further affecting hormone signaling pathways, oncogenes, tumor suppressor genes, growth factor genes and angiogenic regulators (Calvo et al. 2005, Weinberg 1996). Therefore, research today has initiated the identification of a number of genes to be used as diagnostic and prognostic markers for prostate cancer. In order to analyze hundreds or thousands of genes in diseased and normal tissues as well as cell lines, microarray analysis is often utilized in biomarker discovery (Bull et al. 2001). By combining analyses from microarrays with existing screening methods, histological studies and proteomics, results will provide a more comprehensive report about the initiation of prostate cancer and its progression.

II.O.1. TGFβ-2 Gene

Transforming growth factor beta-2 (TGFβ-2) acts as a tumor promotor especially in androgen-independent prostate cancer. It may contribute to angiogenesis,

augmentation of cell mobility and the inhibition of the immune system, thereby producing an ideal environment for cancer development (Blanchere et al. 2002).

II.O.2. CDK-7 and CDK-9 Genes

Cyclin-dependent kinases (cdks) play an important role in regulating the cell cycle and also RNA transcription. In malignant cells, the over-expression of CDKs or the loss of expression of CDK inhibitors may cause excessive growth. However, cyclin dependent kinases – 7 and 9 play a role in RNA transcription rather than the cell cycle. Few studies have examined the inhibition of Cdk-7 and Cdk-9 but have suggested that inhibition may affect mRNAs including those encoding anti-apoptotic proteins, cell cycle regulation and the p53 and nuclear factor-kappa B pathway (Shapiro 2006).

II.O.3. EGFR Gene

Epidermal Growth Factor Receptor (EGFR) has been significantly associated with prostate cancer progression. For example, in one study in patients with prostate cancer who had undergone surgical therapy and who were classified as EGFR-positive, eventually relapsed compared to EGFR-negative patients (DiLorenzo et al. 2002). Several other studies also attribute the development of prostate cancer, as well as the progression to androgen-independent or hormone-refractory prostate cancer with EGFR expression (Hernes et al. 2004, Shah et al. 2006).

II.O.4. BCL-2 and BCL2L1 Genes

B-cell lymphoma-2 (BCL-2) is well-known for its anti-apoptotic effect. However, its over-expression enables prostate cancer cells to survive in a hormone-refractory environment. Therefore, its over-expression is much more prevalent in androgen-independent prostate cancer compared to localized prostate cancer (Yoshino et al. 2006). BCL2L1 gene holds similar anti-apoptotic function to the BCL-2 gene. Its over-expression is associated with resistance to androgen ablation, chemotherapies, and radiotherapy (Yamanaka et al. 2005).

II.O.5. IGF1R Gene

Similar to EGFR, over-expression of Insulin-like Growth Factor-I Receptor (IGF1R) induces growth, neoplastic transformation, and tumorigenesis contributing to the progression of prostate cancer and possibly metastasis (Hellawell et al. 2002, Loughran et al. 2005).

II.O.6. BRCA1 Gene

The Breast Cancer 1 gene (BRCA1) is most commonly linked to hereditary breast cancer. However, this tumor suppressor gene, accompanied by an elevated PSA level, is now also linked to an increased risk for prostate cancer in males younger than 65 years old. Linkage to chromosome 17 has been detected in hereditary breast cancers and some hereditary prostate cancers, resulting in BRCA1 mutations in men with a familial history of breast and/or ovarian cancer (Dong 2006, Horsburgh et al. 2005, Thompson et al. 2002).

Chapter III HYPOTHESIS AND OBJECTIVES

III.A. Hypothesis

If lycopene modulates the expression of prostate cancer biomarker genes, then lycopene may decrease the risk, delay, or prevent the progression of prostate cancer

III.B. Objectives

III.B.1. To investigate the effects of lycopene on the expression of 263 prostate cancer biomarker genes in hormone-refractory human prostate cancer (PC-3) cell line using Oligo GEArray® DNA Microarray technology

III.B.2. To validate the expression of the modulated genes at the mRNA level using Real-Time PCR.

III.B.3. To investigate the expression of Epidermal Growth Factor Receptor (EGFR) at the protein level by investigating protein expression using Western blot.

Chapter IV MATERIALS AND METHODS

IV.A. Reagents and Cell Culture

A human prostate cancer cell line (PC-3) was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and grown in RPMI Medium 1640 supplemented with 10% fetal bovine serum (FBS), penicillin (100U/ml), and streptomycin (100µg/ml). Lycopene, RPMI and Tri-reagent were purchased from Sigma (St. Louis, MO). The Real-Time PCR kit and EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, BCL-2 and GAPDH primers and probes were obtained from Applied Biosystems (Foster City, CA). Human anti-EGFR was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

IV.B. Cell Viability (MTT)

MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) is a pale yellow substrate that is reduced by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not reduce significant amounts of MTT. Human prostate cancer cell line (PC-3) was cultured in a 96-well flat bottom plate at a concentration of 10,000 cells per well. After 48 hours of preconditioning, cells were treated with various concentrations of lycopene, 3.125µM to 200µM, for 18 hours. Thereafter, the culture medium was aspirated and 200µl of MTT dye (1mg/ml in phosphate-buffered saline [PBS]) was added to the cultures and further incubated for 3 hours at 37ºC. The formazan crystals made due to dye reduction by viable cells were dissolved using acidified isopropanol (0.1 N HCl). An index of cell viablity was calcuated by measuring the optical density of color produced by MTT dye

reduction at 570nm (BioRad Model 680 Microplate Reader, BioRad Laboratories, California).

IV.C. RNA isolation

Based on results from the MTT assay for cell viability, human prostate cancer cell line (PC-3) was treated with 25μM lycopene for an 18 hour period at 37°C. Subsequently, RNA was isolated from control (untreated) and 25μM lycopene-treated PC-3 cells based on protocol established in our laboratory. Lycopene-treated and untreated cells were washed twice with sterile phosphate buffer saline (PBS). The cells suspended in PBS were then centrifuged at 4°C at 5000rpm for 5 minutes. The supernatant liquid was discarded and the cell pellet was treated with tri-reagent and chloroform to lyse the cells and separate the homogenate into aqueous and organic phases. Isopropanol was added to precipitate RNA from the aqueous phase followed by washing with ethanol and nuclease free water. RNA quality was determined by gel electrophoresis and RNA quantity was obtained by measuring RNA absorption at 260nm. The UV spectrophotometer also calculated the RNA/Protein ratio.

IV.D. Protein Isolation

PC-3 Cells were cultured in RPMI media either alone or with 25μM lycopene. After 18 hours the cells were washed twice with sterile phosphate buffer saline (PBS) and solubilized in cold lysis buffer. After incubation on ice for 30 minutes lysates were centrifuged at 12,500rpm for 30 minutes at 4°C. Supernatant was collected for each sample and protein concentration was estimated.

IV.E. GEArray® Focused DNA Microarray

RNA isolated from untreated and lycopene-treated PC-3 cells were reverse-transcribed to cDNA, transcribed to cRNA using the SuperArray TrueLabeling-AMP^{TM} 2.0 kit (Superarray Bioscience Corp., Frederick, MD) and finally cRNA was also labeled with Biotin (Roche, Indianapolis, IN) according to manufacturer's instruction. Each biotin-labeled cRNA was then allowed to hybridize with Oligo GEArray® DNA Microarrays containing 263 genes involved in the prognosis and diagnosis of prostate cancer for 24 hours (Superarray Bioscience Corp., Frederick, MD) followed by gene detection using the Chemiluminescent Detection Kit (Superarray Biosciences Corp., Frederick, MD). Arrays were then exposed to x-ray film for image development and images were uploaded to GEArray® Expression Analysis Suite 2.0 computer software. The software reported gene expression normalized to the GAPDH housekeeping gene. Microarray experiments were conducted in triplicates and an average percent change in gene expression between untreated and lycopene-treated PC-3 cells was calculated.

IV.F. Real-Time PCR

Quantitative gene expressions of EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, and BCL-2genes were performed on the iCycler MYIQ Real-Time PCR detection system (Bio-Rad, Hercules, CA) using one step real-time PCR (Applied Biosystems, Foster City, CA). Total RNA was isolated using Tri-reagent (Sigma-Aldrich, St. Louis, MO). Primers and probes (Taqman™) for EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, and BCL-2 genes were purchased from Applied Biosystems (Foster City, CA). TaqMan reaction master mix was prepared using 2 µl RNA (200ng/µl), 19.25 µl PCR-grade RNase-DNase free water, 25 µl TaqMan® One-step RT-PCR Master Mix

(Applied Biosystems, Foster City, CA), 2.5 µl TaqMan® primers and probes, and 1.25 µl TaqMan® enzyme with a total volume of 50 µl. Amplification was carried out with the following parameters: One cycle at 55°C for 15 minutes, one cycle at 95°C for 3 minutes, and forty cycles at 60°C for 30 seconds each. The expressions of EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, and BCL-2 genes after treatment with 25µM lycopene compared to control (untreated) were estimated using the comparative C_T method ($\Delta\Delta C_T$). The threshold cycles (C_T) for EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, BCL-2, and GAPDH were determined for lycopene (25µM) and control samples. In this experiment, a comparison was made between the expression levels of BRCA, EGFR, IGF1R, BCL-2, CDK-7, CDK-9, and TGFβ-2 and GAPDH in the 25µM lycopene-treated and control samples. The mean C_T value of each sample was calculated and standard deviations were determined for each mean C_T value. The ΔC_T values were calculated by subtracting the average C_T value of GAPDH from the average C_T value of EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, and BCL-2 for each sample. The $\Delta\Delta C_T$ value is then calculated by subtracting the ΔC_T of lycopene (25µM) from the ΔC_T value of control using manufacturer's instructions (Applied Biosystems, Foster City, CA).

The relative expressions of the target genes in the 25µM lycopene-treated and untreated samples were calculated using $2^{-\Delta\Delta CT}$. The values of treated samples were expressed as *n*-fold difference relative to the expression of control samples (Applied Biosystems, Foster City, CA). Real-Time PCR experiments were conducted in triplicates and an average percent modulation in gene expression between untreated and lycopene-treated PC-3 cells was also calculated.

IV.G. Western blot

The human prostate cancer cells (PC-3) were cultured with 25μM lycopene for 18 hours. At the end of incubation, cells were rapidly washed with ice-cold PBS and solubilized in cold lysis buffer containing 10 mM Tris-base, 5 mM EDTA, 50 mM NaCl, 1% Triton X-100, 5 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM sodium orthovanadate, 10 μg/ml leupeptin, 25 μg/ml aprotinin, 1 mM sodium pyrophosphate and 20% glycerol. After incubation for 30 minutes on ice, lysates were centrifuged at12, 500 rpm for 15 minutes at 4°C and supernatants were collected and protein concentration in samples was estimated using spectrophotometer compared to known standard protein concentrations.

Equal amount of protein (100μg) from each sample was loaded on SDS-polyacrylamide electrophoresis gel (8% separating gels) and resolved for 5 hours at 120 V in buffer containing 95 mM Tris-HCl, 960 mM glycine, and 0.5% SDS. After electrophoresis the proteins were transferred to Hybond enhanced chemiluminescence nitrocellulose membrane (Amersham-Pharmacia Biotech, NJ, USA) at 200 mA for 3 hours in a buffer containing 25 mM Tris-base, 192 mM glycine, and 20% methanol. After transfer, the membrane was blocked in PBST (20 mM Sodium Phosphate buffer, pH 7.6, 150 mM NaCl, 0.1% Tween 20) containing 5% nonfat dry milk for 1 hour at room temperature and incubated with primary antibody in the blocking solution at 4°C overnight. Thereafter, the membrane was washed four times with PBST, incubated with secondary antibody in the blocking solution for 1 hour at room temperature and washed four times with PBST for 5 minutes every time. Specific bands were detected by enhanced chemiluminescence's detection system (Amersham-Pharmacia Biotech, NJ,

USA), the membrane was exposed to X-ray film and band intensity was measured using BioRad Quantity One 1D Analysis Software.

Chapter V RESULTS

V.A. MTT Assay for Cell Viability

The effect of lycopene treatment on the cell viability of PC-3 human androgen-independent prostate cancer cells

In order to determine the highest concentration of lycopene that is non-toxic to the human androgen-independent prostate cancer cell line, PC-3 cells were treated with various concentrations of lycopene (3.125 - 200µM). After 18 hours, a cell viability assay was performed using MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) dye. Optical density was measured to calculate an index of cell viability. Figure 3 below indicates that treatment with 25µM lycopene was the highest non-toxic dose to the PC-3 cells, closely followed by 12.5µM. Therefore, 25µM lycopene treatment was used for further experiments. Cell death was evident at lycopene concentrations of 200µM, 100µM and 50µM, while an unexpected increase in cell growth was observed at lowest lycopene concentrations, 6.25µM and 3.125µM.

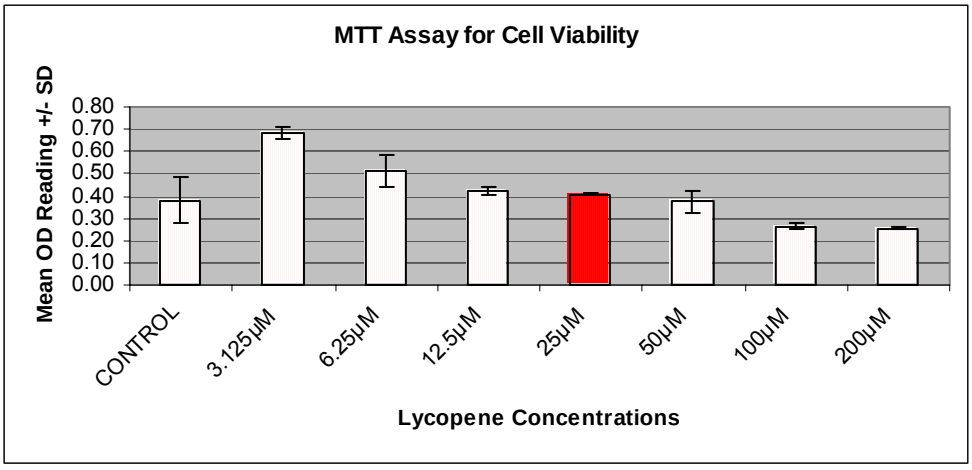

Figure 3. MTT assay for cell viability indicates lycopene treatments of 200µM, 100µM and 50µM result in a decrease in cell viability. The highest non-toxic dose was determined to be 25µM and therefore was used for further experiments.

V.B. Isolation of RNA

RNA was isolated from untreated and 25µM lycopene-treated PC-3 cells based on protocol established in our laboratory. RNA quality was determined by 1% agarose gel electrophoresis and RNA quantity was obtained by measuring RNA absorption at 260nm. Good quality RNA is indicated by an RNA/protein ratio between 1.6 and 2.0 measured by spectrophotometer at a wavelength of 260nm. RNA quality was indicated by the visible separation of two distinct bands of RNA, 28S RNA and 18S RNA**,** respectively.

RNA to Protein ratio determined using spectrophotometer

RNA Sample	WL 260nm	WL 280nm	RNA/Protein Ratio	Concentration
Control	**0.291**	**0.157**	**1.847**	**2.910µg/µl**
Lycopene 25µM	**0.380**	**0.219**	**1.739**	**3.8µg/µl**

Table 2. The calculated RNA to protein ratio of control and 25µM lycopene RNA samples each scored within the ideal range of 1.6 to 2.0 indicating good quantity RNA.

RNA quality determined by 1% agarose gel electrophoresis

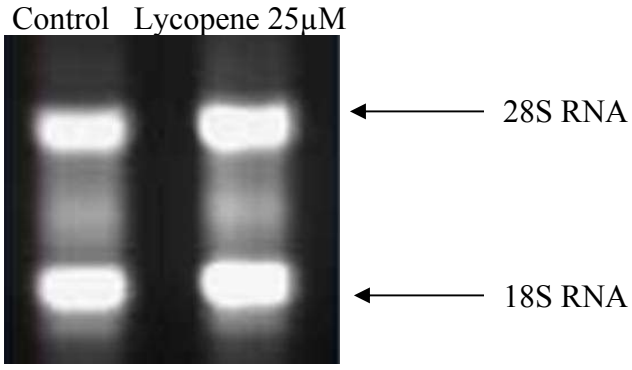

Figure 4. RNA isolated from control (untreated) and 25µM lycopene-treated PC-3 cells. Gel electrophoresis indicates the ideal separation of 28S and 18S bands.

V.C. GEArray® Focused DNA Microarray

Lycopene Modulates Prostate Cancer Biomarker Genes

Gene	Average Percent Down-Regulation
TGFβ-2	79%
CDK-9	68%
EGFR	59%
BCL-2	54%
BCL2L1	52%
IGF1R	48%
CDK-7	43%
BRCA1	38%

Table 3. Average percent down-regulation of prostate cancer biomarker genes after 25μM lycopene treatment.

Microarray experiments were conducted in triplicates and an average percent change in gene expression between untreated and lycopene-treated PC-3 cells was calculated using the following equation:

$$\text{Average Percent Down-Regulation} = \frac{\text{Average Gene Expression (Control)} - \text{Average Gene Expression (25μM lycopene)}}{\text{Average Gene Expression (Control)}} \times 100$$

RPS27A 1	RPS27A 2	AGR2 3	AGTR2 4	AIG1 5	AKAP1 6	AKT1 7	APC 8	APOC1 9	GAPDH 10	GAPDH 11	GAPDH 12
RPS27A 13	AR 14	BAK1 15	BAX 16	BCL2 17	BCL2L1 18	BMP6 19	BRCA1 20	CANT1 21	CASP1 22	CASP3 23	CASP7 24
CAV1 25	CCND1 26	CD164 27	CD44 28	CDH1 29	CDH10 30	CDH12 31	CDH13 32	CDH18 33	CDH19 34	CDH20 35	CDH7 36
CDH8 37	CDH9 38	CDK2 39	CDK3 40	CDK4 41	CDK5 42	CDK6 43	CDK7 44	CDK8 45	CDK9 46	CDKN1A 47	CDKN1B 48
CDKN1C 49	CDKN2A 50	CDKN2B 51	CDKN2C 52	CDKN3 53	CHGA 54	CHGB 55	CLDN3 56	CLN3 57	CLU 58	COL1A1 59	COL6A1 60
CYB5 61	CYC1 62	DAB2IP 63	DAPK1 64	DES 65	DNCL1 66	E2F1 67	EGF 68	EGFR 69	EGR3 70	ELAC2 71	ELL 72
ENO1 73	ENO2 74	ENO3 75	ERBB2 76	ERK8 77	ESR1 78	ESR2 79	EZH1 80	EZH2 81	FASN 82	FGF1 83	FGF10 84
FGF11 85	FGF12 86	FGF13 87	FGF14 88	FGF16 89	FGF17 90	FGF18 91	FGF19 92	FGF2 93	FGF20 94	FGF21 95	FGF22 96
FGF23 97	FGF3 98	FGF4 99	FGF5 100	FGF6 101	FGF7 102	FGF8 103	FGF9 104	FHIT 105	FLJ12584 106	FLJ25530 107	FOLH1 108
GAGEB1 109	GAGEC1 110	GGT1 111	GNRH1 112	GRP 113	GSTP1 114	HIF1A 115	HIP1 116	HK2 117	HK3 118	HRAS 119	HUMCYT2A 120
IGF1 121	IGF1R 122	IGF2 123	IGFBP3 124	IGFBP6 125	IL12A 126	IL1A 127	IL1B 128	IL2 129	IL24 130	IL29 131	ILK 132
INHA 133	INSL3 134	INSL4 135	ITGA1 136	JUN 137	K6HF 138	KAI1 139	KLK1 140	KLK10 141	KLK11 142	KLK12 143	KLK13 144
KLK14 145	KLK15 146	KLK2 147	KLK3 148	KLK4 149	KLK5 150	KLK6 151	KLK7 152	KLK8 153	KLK9 154	KRT1 155	KRT2A 156
MAP2K4 157	MAP3K1 158	MAPK1 159	MAPK10 160	MAPK11 161	MAPK12 162	MAPK13 163	MAPK14 164	MAPK3 165	MAPK4 166	MAPK6 167	MAPK7 168
MAPK8 169	MAPK9 170	MIB1 171	MMP2 172	MMP9 173	MSMB 174	MTSS1 175	MYC 176	NCOA4 177	NFKB1 178	NFKBIA 179	NKX3-1 180
NOX5 181	NR0B1 182	NR0B2 183	NR1D1 184	NR1D2 185	NR1H2 186	NR1H3 187	NR1H4 188	NR1I2 189	NR1I3 190	NR2C1 191	NR2C2 192
NR2E1 193	NR2E3 194	NR2F1 195	NR2F2 196	NR2F6 197	NR3C1 198	NR3C2 199	NR4A1 200	NR4A2 201	NR4A3 202	NR5A1 203	NR5A2 204
NR6A1 205	NTN4 206	ODZ1 207	PALM2-AKAP2 208	PAP 209	PART1 210	PATE 211	PAWR 212	PCA3 213	PCNA 214	PGR 215	PIAS1 216
PIAS2 217	PIK3CG 218	PLAU 219	PLG 220	PPID 221	TMEM37 222	PRKCA 223	PRKCB1 224	PRKCD 225	PRKCE 226	PRKCG 227	PRKCH 228
PRKCI 229	PRKD3 230	PRKCQ 231	PRKCZ 232	PRKD1 233	PRKD2 234	PRL 235	PSAP 236	PSCA 237	PTEN 238	RARB 239	RASSF1 240
RB1 241	RNASEL 242	RNF14 243	ROBO2 244	SERPINA3 245	SHBG 246	SLC2A2 247	SLC33A1 248	SLC43A1 249	SOX2 250	SRC 251	SRD5A2 252
HSPCB 253	STEAP 254	STEAP2 255	TGFA 256	TGFB1 257	TGFB1I1 258	TGFB2 259	TGFB3 260	TIMP3 261	TNF 262	Pol1 263	PUC18 264
B2M 265	Blank 266	Blank 267	TNFSF10 268	TP53 269	TPM1 270	TPM2 271	18SrRNA 272	AS1R3 273	AS1R2 274	AS1R1 275	AS1 276
B2M 277	B2M 278	ACTB 279	TRPC6 280	TRPS1 281	TYK2 282	VEGF 283	BAS2C 284	BAS2C 285	BAS2C 286	BAS2C 287	BAS2C 288

Figure 5. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Layout

The Oligo® GEArray Human Prostate Cancer Biomarkers Microarray purchased from SuperArray Bioscience Corporation (Frederick, MD) allows for the investigation of 263 genes used in the prognosis and diagnosis of prostate cancer. The genes are placed on the array in groups according to biological function and allows for simple comparison.

V.C.1 Lycopene Down-regulates the Expression of the TGFβ-2 gene

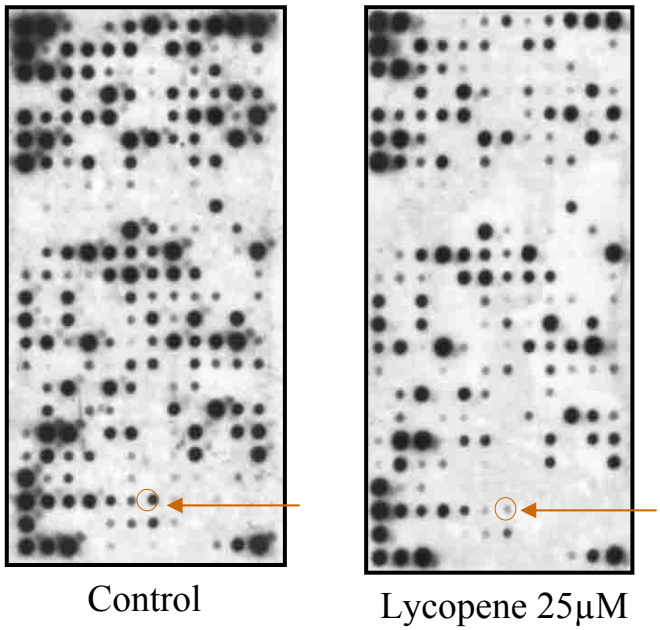

Figure 6. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in TGFβ-2 gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The TGFβ-2 gene, located at position 259 on the Human Prostate Cancer Biomarkers Microarray, is a tumor promoter especially in androgen-independent prostate cancer (Blanchere et al., 2002). The expression of this gene was down-regulated by an average of 79% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

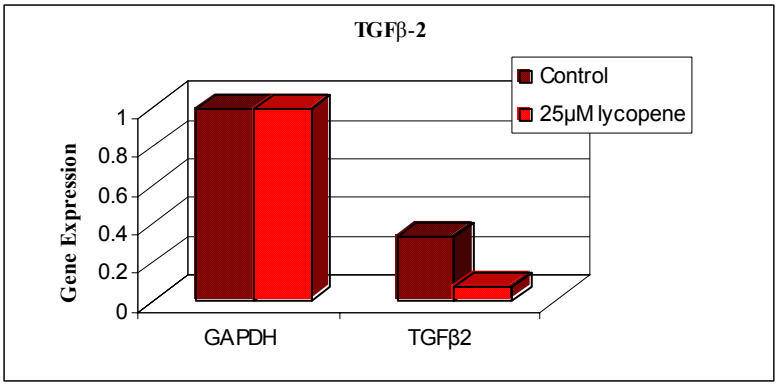

Figure 6a. Lycopene Down-regulates the Expression of the TGFβ-2 gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the TGFβ-2 gene decreases by 79%, when comparing control to 25µM lycopene treatment.

V.C.2. Lycopene Down-regulates the Expression of the CDK-9 gene

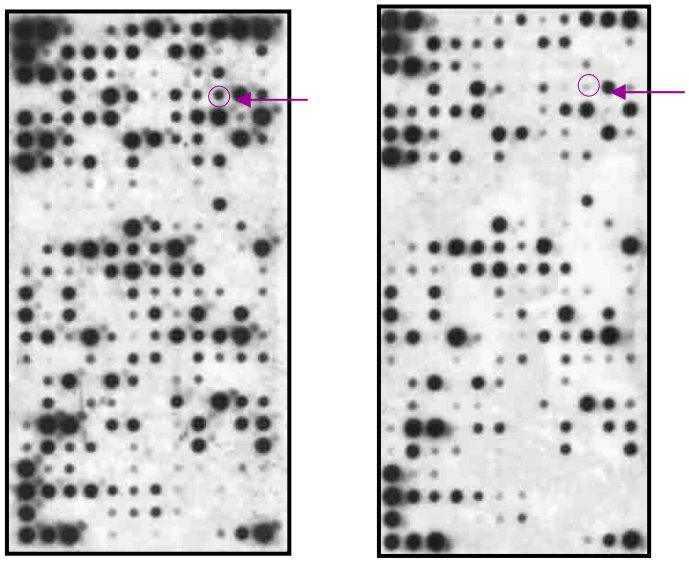

Figure 7. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in CDK-9 gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The CDK-9 gene, located at position 46 on the Human Prostate Cancer Biomarkers Microarray, is similar to CDK-7 which plays a role in cell cycle regulation and RNA transcription (Shapiro 2006). The expression of this gene was down-regulated by an average of 68% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

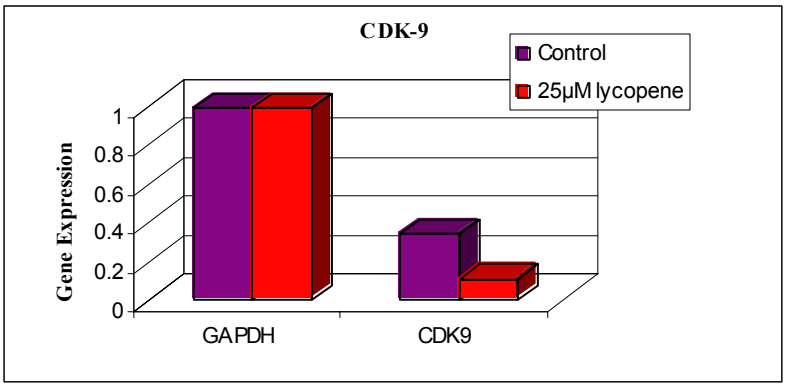

Figure 7a. Lycopene Down-regulates the Expression of the CDK-9 gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the CDK-9 gene decreases by 68%, when comparing control to 25µM lycopene treatment.

V.C.3. Lycopene Down-regulates the Expression of the EGFR gene

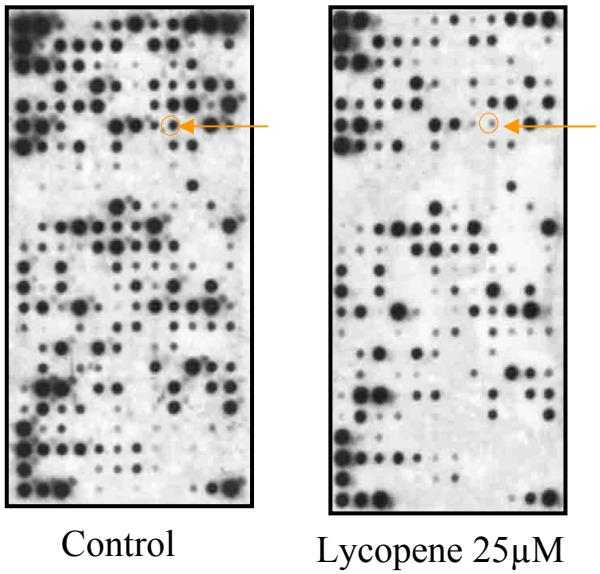

Figure 8. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in EGFR gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The EGFR gene, located at position 69 on the Human Prostate Cancer Biomarkers Microarray, is associated with the development of prostate cancer and expressed in androgen-independent or hormone-refractory prostate cancer (DiLorenzo et al. 2002, Hernes et al. 2004, Shah et al. 2006). The expression of this gene was down-regulated by an average of 59% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

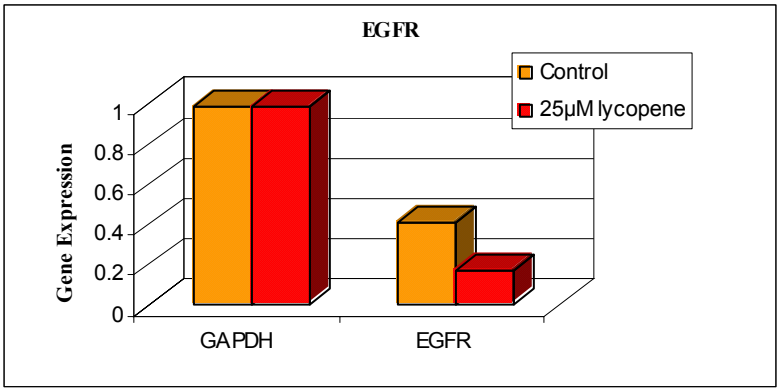

Figure 8a. Lycopene Down-regulates the Expression of the EGFR gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the EGFR gene decreases by 59%, when comparing control to 25µM lycopene treatment.

V.C.4. Lycopene Down-regulates the Expression of the BCL-2 gene

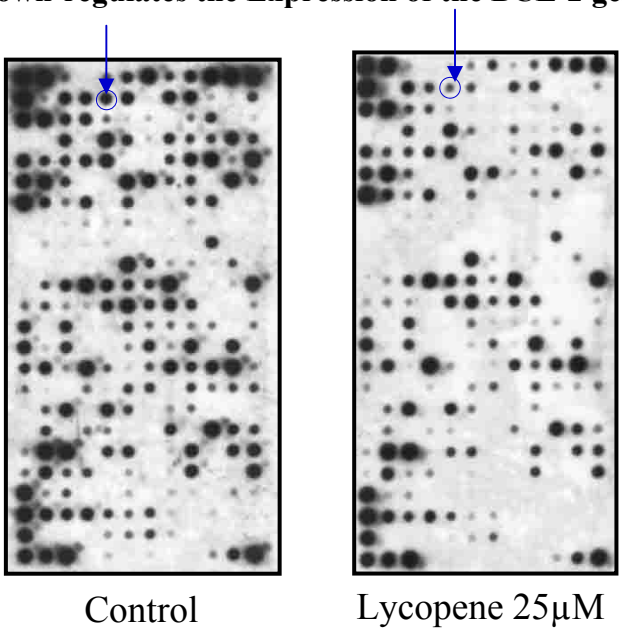

Figure 9. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in BCL-2 gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The BCL-2 gene, located at position 17 on the Human Prostate Cancer Biomarkers Microarray, inhibits apoptosis and its over-expression enables prostate cancer cells to survive in hormone-refractory prostate cancer (Yoshino et al. 2006). The expression of this gene was down-regulated by an average of 54% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

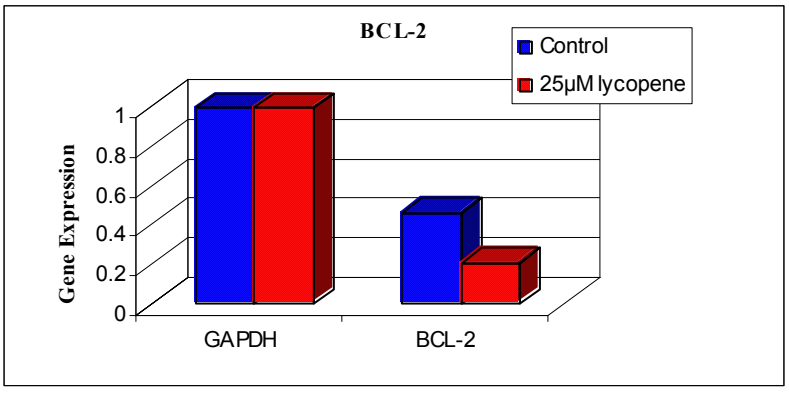

Figure 9a. Lycopene Down-regulates the Expression of the BCL-2 gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the BCL-2 gene decreases by 54%, when comparing control to 25µM lycopene treatment.

V.C.5. Lycopene Down-regulates the Expression of the BCL2L1 gene

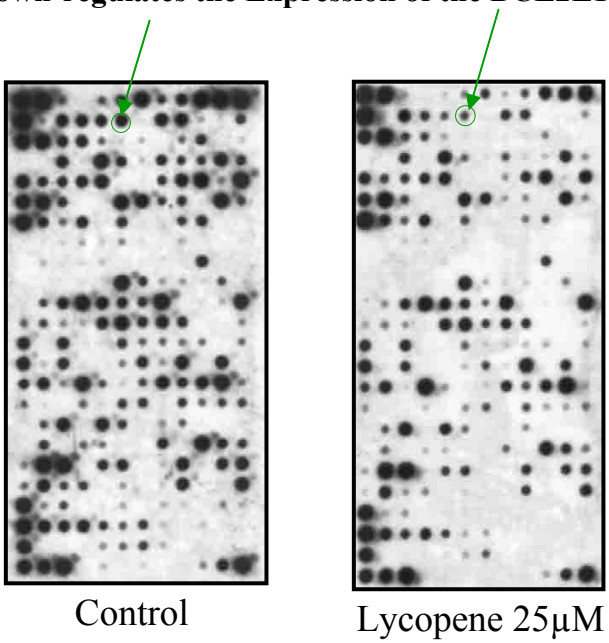

Figure 10. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in BCL2L1 gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The BCL2L1 gene, located at position 18 on the Human Prostate Cancer Biomarkers Microarray, has similar anti-apoptotic function to the BCL-2 gene (Yamanaka et al. 2005). The expression of this gene was down-regulated by an average of 52% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

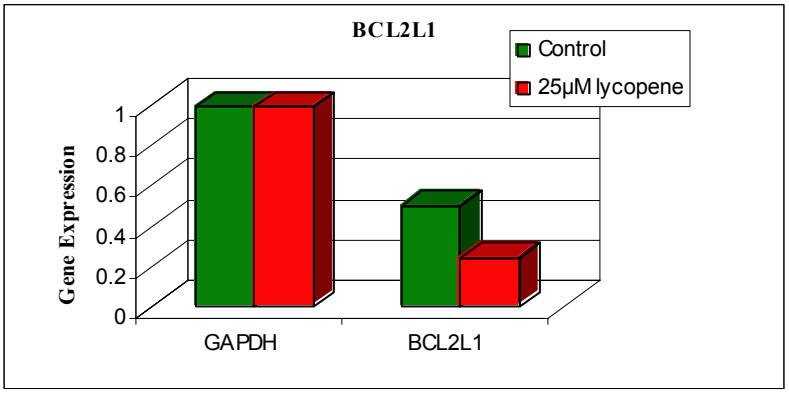

Figure 10a. Lycopene Down-regulates the Expression of the BCL2L1 gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the BCL2L1 gene decreases by 52%, when comparing control to 25µM lycopene treatment.

V.C.6. Lycopene Down-regulates the Expression of the IGF1R gene

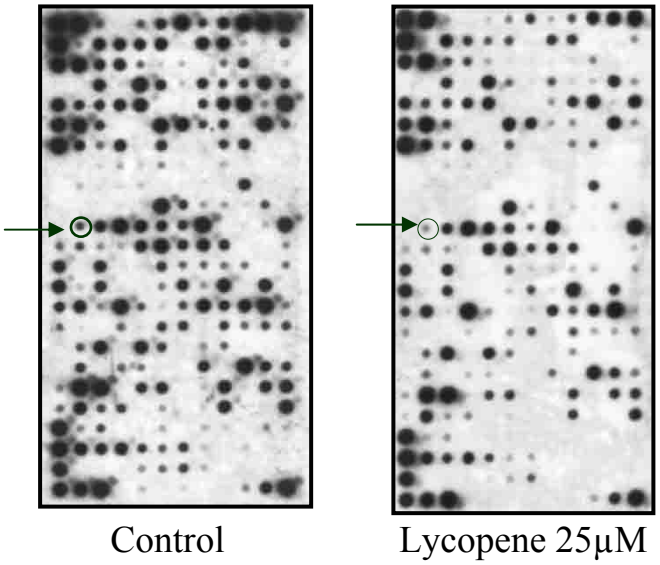

Figure 11. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in IGF1R gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The IGF1R gene, located at position 122 on the Human Prostate Cancer Biomarkers Microarray, contributes to the progression of prostate cancer and possibly metastasis (Hellawell et al. 2002 & Loughran et al. 2005). The expression of this gene was down-regulated by an average of 48% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

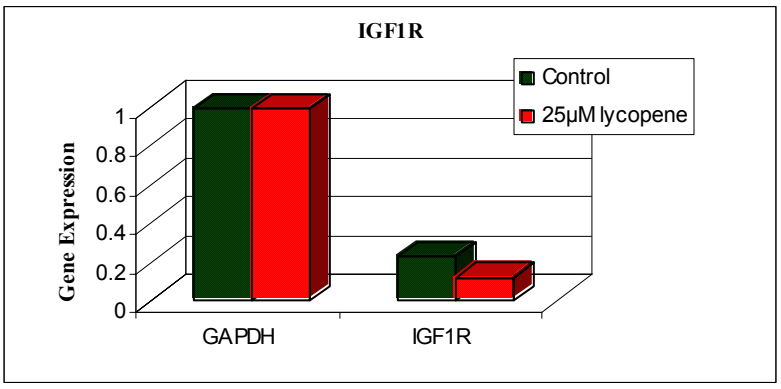

Figure 11a. Lycopene Down-regulates the Expression of the IGF1R gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the IGF1R gene decreases by 48%, when comparing control to 25µM lycopene treatment.

V.C.7. Lycopene Down-regulates the Expression of the CDK-7 gene

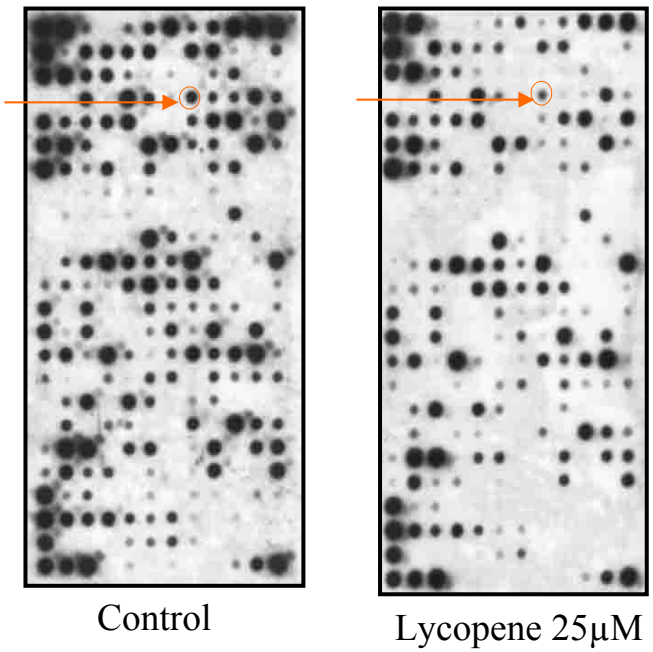

Figure 12. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in CDK-7 gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The CDK-7 gene, located at position 44 on the Human Prostate Cancer Biomarkers Microarray, plays a role in cell cycle regulation and RNA transcription (Shapiro 2006). The expression of this gene was down-regulated by an average of 43% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

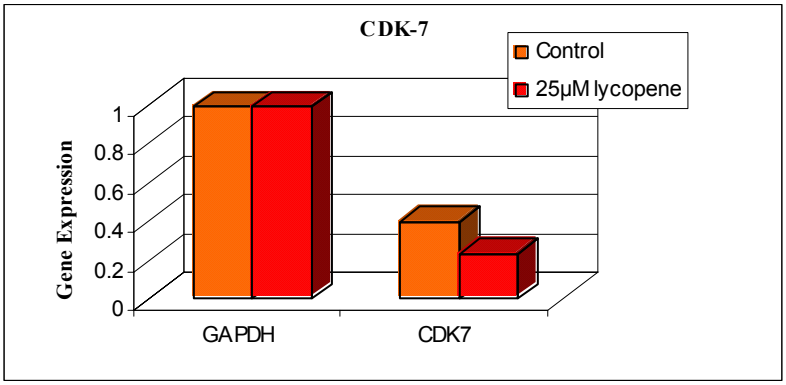

Figure 12a. Lycopene Down-regulates the Expression of the CDK-7 gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the CDK-7 gene decreases by 43%, when comparing control to 25µM lycopene treatment.

V.C.8. Lycopene Down-regulates the Expression of the BRCA1 gene

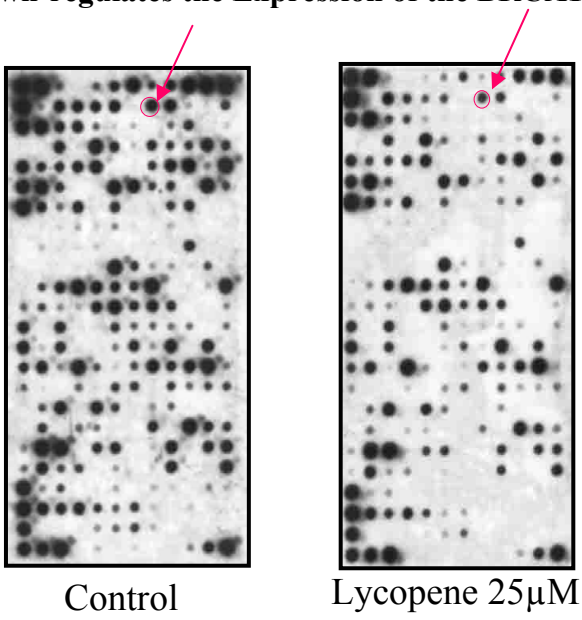

Figure 13. Oligo GEArray® Human Prostate Cancer Biomarkers Microarray. A down-regulation in BRCA1 gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

The BRCA1 gene, located at position 20 on the Human Prostate Cancer Biomarkers Microarray, is linked to hereditary prostate cancer in males younger than 65 years old (Dong 2006, Horsburgh et al. 2005, Thompson et al. 2002). The expression of this gene was down-regulated by an average of 38% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

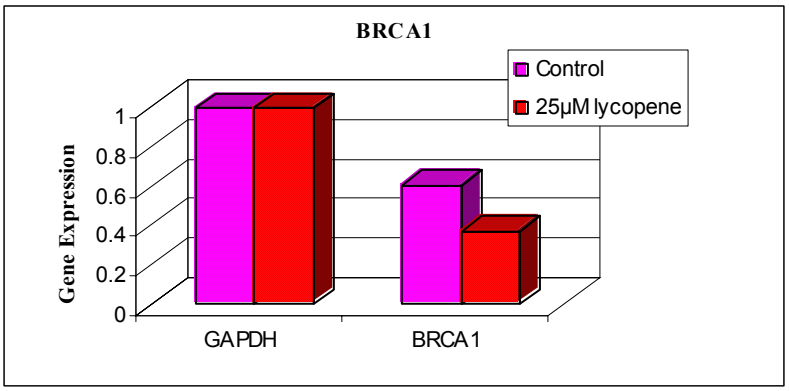

Figure 13a. Lycopene Down-regulates the Expression of the BRCA1 gene. The expression of the GAPDH housekeeping gene remains unchanged, while the expression of the BRCA1 gene decreases by 38%, when comparing control to 25µM lycopene treatment.

V.D. Real-Time PCR

Lycopene Modulates Prostate Cancer Biomarker Genes Using Real-Time PCR

Gene	Average Percent Down-Regulation
EGFR	72%
IGF1R	61%
BRCA1	56%
CDK-9	56%
TGFβ-2	44%
CDK-7	34%
BCL-2	30%

Table 4. Average percent down-regulation of prostate cancer biomarker genes after 25μM lycopene treatment.

In order to validate and obtain more sensitive modulation of gene expression, Real-Time PCR experiments were conducted in triplicates and an average percent modulation in gene expression between untreated and lycopene-treated PC-3 cells was also calculated using the following equation:

$$\text{Average Percent Down-Regulation} = \frac{\text{Average Gene Expression (Control)} - \text{Average Gene Expression (25μM lycopene)}}{\text{Average Gene Expression (Control)}} \times 100$$

Real-Time PCR quantifies differences in the expression level of a specific gene in control and 25μM lycopene samples. Unlike traditional PCR methods, the amount of product generated is detected as the reactions occur, not at completion. The results are highly sensitive and precise and are expressed as fold difference in expression levels.

V.D.1. Lycopene Down-regulates the Expression of the EGFR Gene using Real-Time PCR

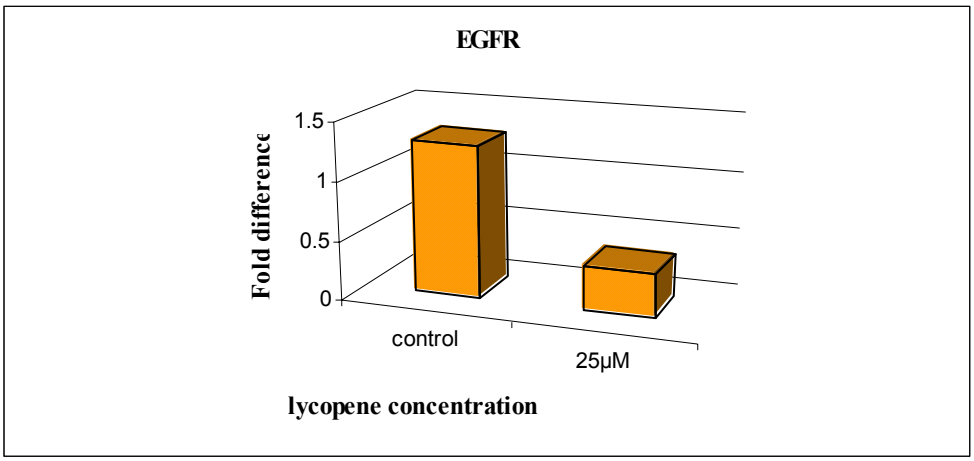

Figure 14. Fold difference in EGFR gene expression was measured when comparing control (untreated) versus 25μM lycopene treatment.

Real-Time PCR demonstrated an average 0.365 fold difference in EGFR gene expression when comparing the RNA of control (untreated) versus 25μM lycopene-treated. The expression of this gene was down-regulated by an average of 72% when comparing control (untreated) to lycopene 25μM-treated PC-3 cells. Among the 7 genes validated by Real-Time PCR, the highest down-regulation was demonstrated by EGFR.

V.D.2. Lycopene Down-regulates the Expression of the IGF1R Gene using Real-Time PCR

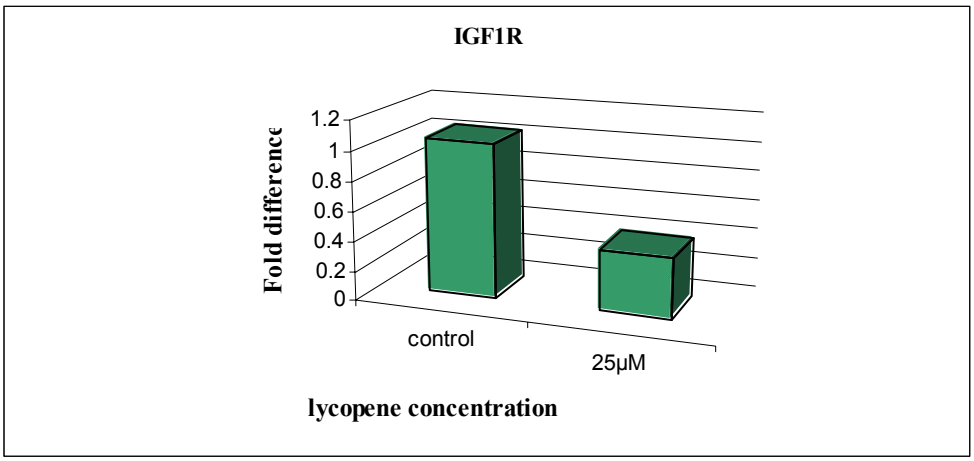

Figure 15. Fold difference in IGF1R gene expression was measured when comparing control (untreated) versus 25μM lycopene treatment.

Real-Time PCR demonstrated an average 0.402 fold difference in IGF1R gene expression when comparing the RNA of control (untreated) versus 25μM lycopene-treated. The expression of this gene was down-regulated by an average of 61% when comparing control (untreated) to lycopene 25μM-treated PC-3 cells.

V.D.3. Lycopene Down-regulates the Expression of the BRCA1 Gene using Real-Time PCR

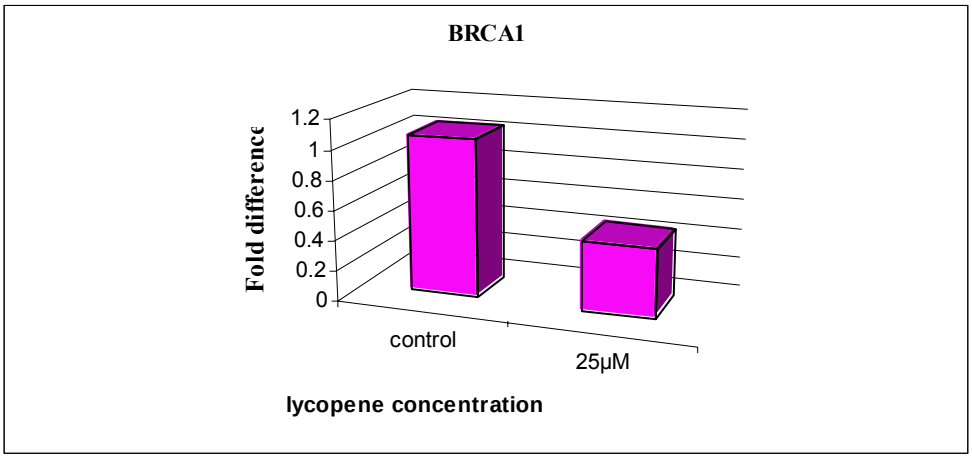

Figure 16. Fold difference in BRCA1 gene expression was measured when comparing control (untreated) versus 25µM lycopene treatment.

Real-Time PCR demonstrated an average 0.462 fold difference in BRCA1 gene expression when comparing the RNA of control (untreated) versus 25µM lycopene-treated. The expression of this gene was down-regulated by an average of 56% when comparing control (untreated) to lycopene 25µM-treated PC-3 cells.

V.D.4. Lycopene Down-regulates the Expression of the CDK-9 Gene using Real-Time PCR

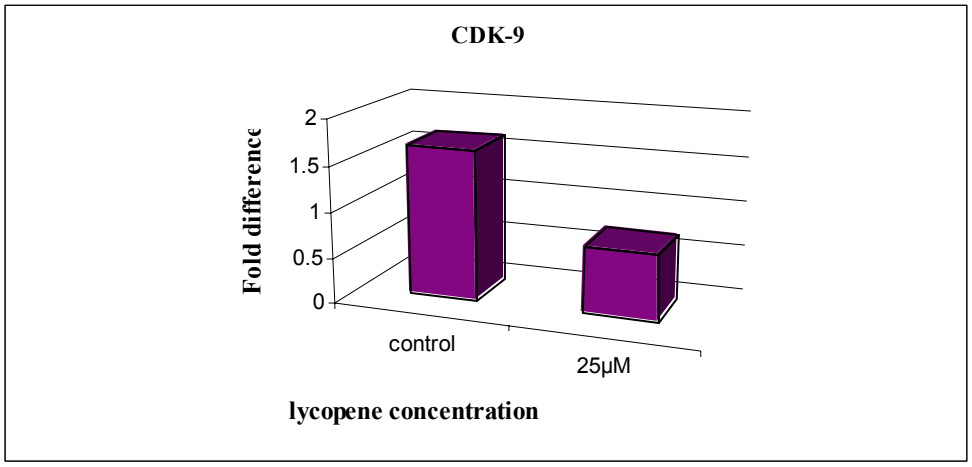

Figure 17. Fold difference in CDK-9 gene expression was measured when comparing control (untreated) versus 25μM lycopene treatment.

Real-Time PCR demonstrated an average 0.725 fold difference in CDK-9 gene expression when comparing the RNA of control (untreated) versus 25μM lycopene-treated. The expression of this gene was down-regulated by an average of 56% when comparing control (untreated) to lycopene 25μM-treated PC-3 cells.

V.D.5. Lycopene Down-regulates the Expression of the TGFβ-2 Gene using Real-Time PCR

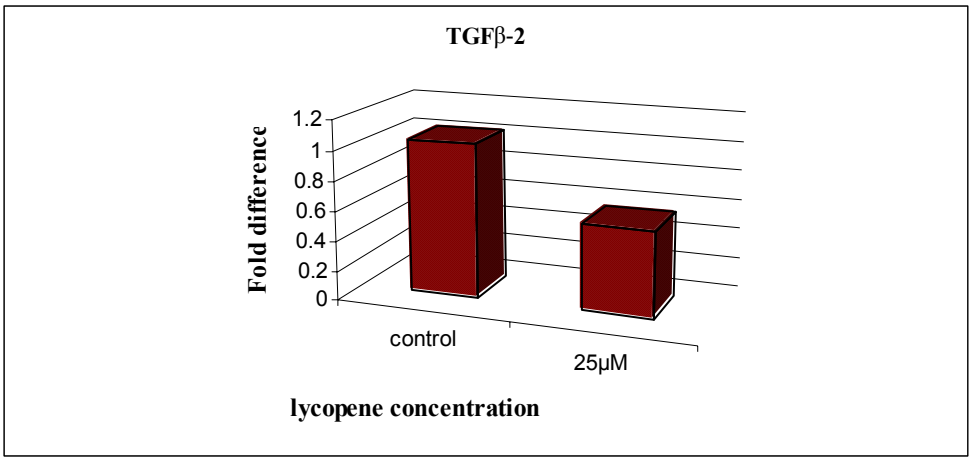

Figure 18. Fold difference in TGFβ-2 gene expression was measured when comparing control (untreated) versus 25μM lycopene treatment.

Real-Time PCR demonstrated an average 0.575 fold difference in TGFβ-2 gene expression when comparing the RNA of control (untreated) versus 25μM lycopene-treated. The expression of this gene was down-regulated by an average of 44% when comparing control (untreated) to lycopene 25μM-treated PC-3 cells.

V.D.6. Lycopene Down-regulates the Expression of the CDK-7 Gene using Real-Time PCR

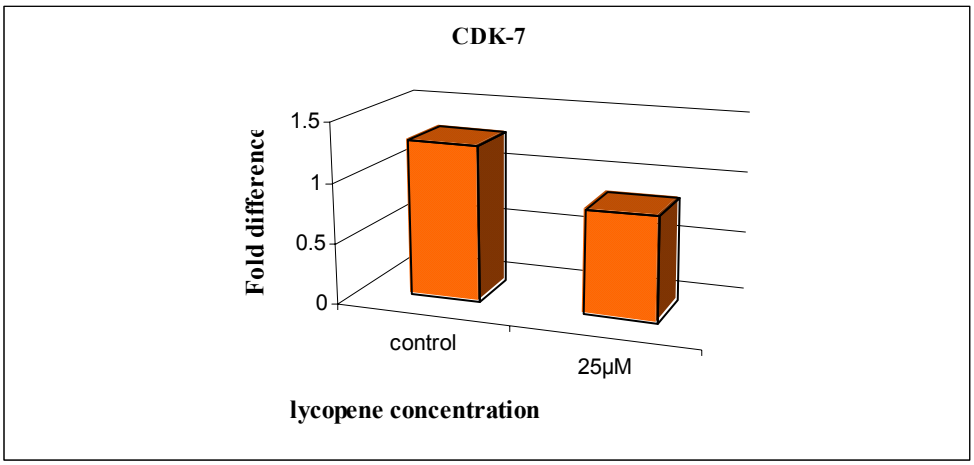

Figure 19. Fold difference in CDK-7 gene expression was measured when comparing control (untreated) versus 25μM lycopene treatment.

Real-Time PCR demonstrated an average 0.855 fold difference in CDK-7 gene expression when comparing the RNA of control (untreated) versus 25μM lycopene-treated. The expression of this gene was down-regulated by an average of 34% when comparing control (untreated) to lycopene 25μM-treated PC-3 cells.

V.D.7. Lycopene Down-regulates the Expression of the BCL-2 Gene using Real-Time PCR

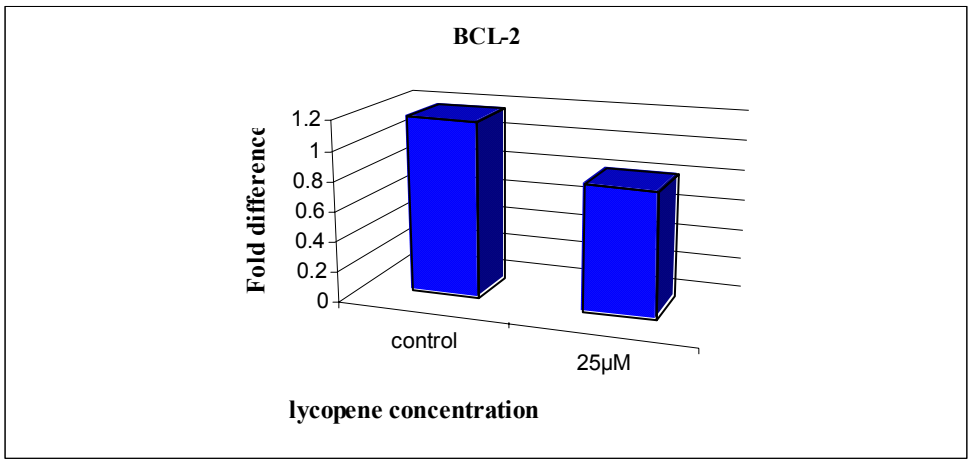

Figure 20. Fold difference in BCL-2 gene expression was measured when comparing control (untreated) versus 25μM lycopene treatment.

Real-Time PCR demonstrated an average 0.83 fold difference in BCL-2 gene expression when comparing the RNA of control (untreated) versus 25μM lycopene-treated. The expression of this gene was down-regulated by an average of 30% when comparing control (untreated) to lycopene 25μM-treated PC-3 cells.

V.E Western blot

Lycopene Decreases the Expression of EGFR Protein Expression using Western blot

Due to a valid decrease in gene expression from both microarray and Real-Time PCR experiments, in order to further investigate the decreased expression of EGFR at the protein level, the effect of Lycopene (25μM) on EGFR protein expression was studied by Immunoblot. The intensity of proteins were analyzed using BioRad Quantity One 1D Analysis software. Lycopene (25μM) decreased the protein expression of EGFR. The protein expression of housekeeping β-actin showed little or no change, while the protein expression of EGFR decreased by 36% when comparing control (untreated) to 25 μM lycopene treatment.

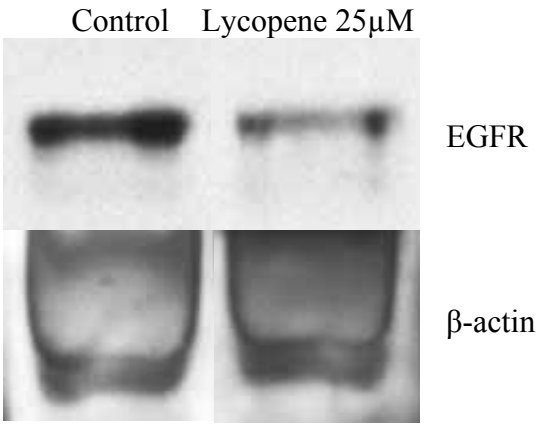

Figure 21. A down-regulation in EGFR protein expression was measured when comparing control (untreated) versus 25μM lycopene treatment, the expression of β-actin housekeeping remains virtually unchanged.

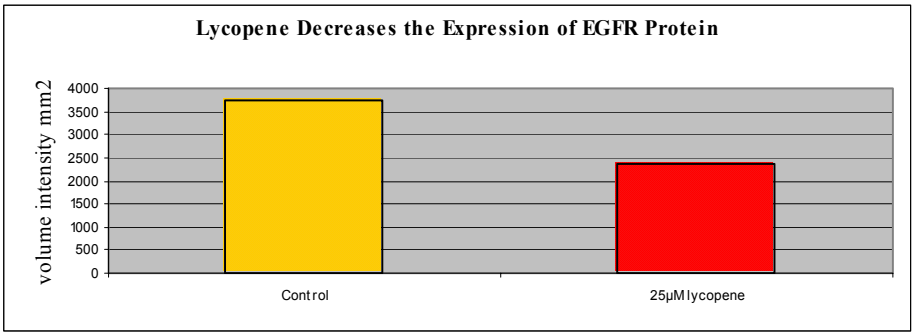

Figure 21a. Lycopene Down-regulates the Protein Expression of EGFR by 36% when comparing control to 25μM lycopene treatment.

CHAPTER VI DISCUSSION

Prostate cancer is often referred to as the disease of older men due to its increased incidence at ages greater than 65 years. In 2007 alone, prostate cancer will be responsible for approximately 218,000 new diagnoses and approximately 27,000 deaths (American Cancer Society). Its repuation as the most commonly diagnosed cancer and the second leading cause of death from malignancies in men, combined with its long latency period, raises questions of concern regarding the uncertainty and the lack of successful therapies.

The first line of defense after early detection of prostate cancer is most often by radiation and/or surgical removal of the tumor, if medically beneficial for the patient. However, during a more advanced stage in disease, a combination of chemotherapy and hormonal therapy is not uncommon (Garnick and Fair 1998, American Cancer Society). As with other cancer treatments, success rates are uncertain. The combination of therapy may control prostate cancer for some time by shrinking the tumor and alleviating symptoms of prostate cancer (American Cancer Society). Once these therapies have been completed, relapses may occur, leading to a more advanced stage of prostate cancer referred to as hormone-refractory prostate cancer involving androgen-independent cancer cells.

In an effort to delay or prevent the progression of prostate cancer, researchers now study the possible health benefits of various food components on prostate cancer either prior to or in addition to current clinical therapies. Several studies involving tea have demonstrated that compounds like theaflavins found in black tea and epigallocathechin-3-

gallate (EGCG) found in green tea are promising chemopreventive agents. These studies have been successful in inducing cell cycle arrest and apoptosis in androgen-dependent and androgen-independent cell lines (Kalra et al. 2007, Gupta et al. 2003, Adhami et al. 2003). *In-vitro* studies using PC-3 cells with soy and radiation inhibits tumor cell growth and potentiates radiation-induced cell dealth (Raffoul et al. 2007). An *in-vivo* study using mice with prostate tumors were pre-treated with soy isoflavones combined with radiation treatments resulted in the inhibition of prostate cancer tumor cell growth and increased tumor cell death (Raffoul et al. 2007). Several food components including resveratrol, a polyphenol found in grapes and red wine, capsaicin, a major pungent ingredient in red peppers and quercetin, a flavanoid found in fruits and vegetables have decreased the cell proliferation rate and increased cell death in various prostate cancer cell lines (Benitez et al. 2006, Mori et al. 2006, Nair et al. 2004).

The objective of this thesis is to determine whether the carotenoid lycopene, commonly found in tomato and tomato products, modulates prostate cancer biomarker genes associated with cell proliferation, differentiation, angiogenesis and apoptosis in hormone-refractory human prostate cancer (PC-3) cell line. If lycopene is successful in modulating the expression of prostate cancer biomarker genes, then lycopene may decrease the risk, delay, or prevent the progression of prostate cancer.

Previous research studies demonstrated that lycopene more potently inhibits the growth of an androgen-independent cell line than an androgen-dependent cell line and decreased tumor growth in mice (Tang et al. 2005). Several clinical studies have also demonstrated that oral lycopene supplementation (Kukuk et al. 2001, Mohanty et al. 2005), consumption of tomato based entrees (Bowen et al. 2002, Chen et al. 2001), and

increased lycopene intake reported in dietary questionnaires (Lu et al. 2001, Giovannucci et al. 2002) showed an inverse association between lycopene and prostate cancer by either increasing serum lycopene and prostate concentrations and/or decreasing serum PSA levels.

In order to begin investigating the chemopreventive properties of lycopene, a cytotoxicity (MTT) experiment was conducted to obtain the highest non-toxic dose for use in further experiments with the androgen-independent (PC-3) cell line. The MTT assay for cell viability studied the effect of lycopene concentrations, 3.125μM to 200μM, and indicated that 25μM lycopene was the highest non-toxic dose. Cells were then cultured and treated with 25μM lycopene for future RNA and protein isolations.

After isolating ideal quality RNA from control (untreated) and lycopene treated PC-3 cells, microarray experiments were conducted for gene expression studies. A microarray containing 263 genes associated with human prostate cancer cell proliferation, differentiation, angiogenesis, apoptosis and involved in the prognosis and diagnosis of prostate cancer was obtained from Superarray Bioscience Corporation. Results from the Oligo GEArray® Human Prostate Cancer Biomarkers Microarray indicated the consistent down-regulation of TGFβ-2, CDK-9, EGFR, BCL-2, BCL2L1, IGF1R, CDK-7, and BRCA1 genes after triplicate microarray experiments.

Transforming growth factor beta-2 (TGFβ-2) acts as a tumor promoter especially in androgen-independent prostate cancer (Blanchere et al., 2002) and our results demonstrated an average 79% down-regulation after lycopene treatment. Studies have suggested that CDK-7 and CDK-9 inhibition may affect mRNAs including those encoding anti-apoptotic proteins, cell cycle regulation and the p53 and nuclear factor-

kappa B pathway (Shapiro 2006) and our results indicated a down-regulation of CDK-7 and CDK-9 genes by 68% and 43%, respectively. EGFR expression has been attributed to the progression of prostate cancer to hormone-refractory prostate cancer and our findings show an average of 59% decrease in gene expression after lycopene treatment (Hernes et al. 2004, Shah et al. 2006). BCL-2 and BCL2L1 are known for their similar anti-apoptotic effect (Yoshino et al. 2006, Yamanaka et al. 2005). After treatment with lycopene both genes exhibited a down-regulation in gene expression by 54% and 52%, respectively. IGF1R expression is also associated with the progression of prostate cancer and metastasis (Hellawell et al. 2002, Loughran et al. 2005). This gene displayed a 48% down-regulation in gene expression after treatment with lycopene. BRCA1 gene holds a link to hereditary breast and prostate cancer in individuals younger than 65 years old (Dong 2006, Horsburgh et al. 2005, Thompson et al. 2002). Our studies indicated the consistent down-regulation of the BRCA1 gene by 38% after lycopene treatment.

Microarray results served as preliminary data by screening the effect of lycopene on hundreds of biomarker genes associated with prostate cancer. Microarray results indicated a consistent decrease in the gene expression of 8 prostate cancer-associated genes. However, Real-Time PCR experiments provided more sensitive and reliable results in gene expression and validated the modulation of EGFR, IGF1R, BRCA1, CDK-9, TGFβ-2, CDK-7, and BCL-2 genes at the mRNA level. Real-Time PCR experiments further validated the down-regulation of the 7 genes. However, among the 7 down-regulated prostate cancer biomarker genes validated by Real-Time PCR, Epidermal Growth Factor Receptor (EGFR) was the most down-regulated gene exhibiting a 72%

decrease in EGFR expression. The remaining genes reported a down-regulation of 61%, 56%, 56%, 44%, 34%, and 30%, respectively.

With the consistent down-regulation of EGFR observed through microarray studies and further validation using Real-Time PCR, our final objective was to investigate the modulation of EGFR expression at the protein level. The effect of lycopene (25μM) on EGFR protein expression was studied by Immunoblot. Immunoblot studies also indicated a 36 % decrease in EGFR protein expression after treatment with lycopene. Therefore, results from microarray, Real-Time PCR and Immunoblot experiments indicate the potential chemopreventive effect of lycopene (25μM) on the EGFR gene which is often involved in the progression of prostate cancer to a more advanced disease.

The activation of the EGFR signaling pathway is ultimately responsible for the growth, proliferation, metastasis and inhibition of apoptosis in tumors (Rocha-Lima et al. 2007, Venook 2005) and due to varying degrees of expression detected in several types of tumors, EGFR is a realistic target in treatment. The expression of EGFR has been observed most in head and neck tumors, followed by renal, lung, breast, colon, ovarian, prostate, glioma, pancreas and bladder tumors (Rocha-Lima et al. 2007, Herbst and Shin 2002). Research conducted on the phyllodes tumor of the prostate has also demonstrated EGFR gene amplification, EGFR overexpression and in some tumor cases even protein overexpression (Wang et al. 2007). Additional studies have also observed that the expression levels of EGFR serve as a good prediction of clinical outcome especially in head and neck, ovarian, cervical, bladder and esophageal cancers. However, in other cancers including breast, colorectal and non-small cell lung cancer, EGFR expression

appears to only have moderate or low prognostic value but research is on-going (Rocha-Lima et al. 2007).

In addition to hormonal or androgen-ablation therapies, docetaxel chemotherapy is considered a primary defense for androgen-independent prostate cancer used occasionally with paclitaxel chemotherapy (Gross et al. 2007, Busby et al. 2006). However, in most recent studies researchers investigate the effects of these standard clinical interventions in combination with monoclonal antibodies but more often with tyrosine kinase inhibitors (Gross et al. 2007, Busby et al. 2006, Rocha-Lima et al. 2007).

Many studies have targeted EGFR in prostate cancer attributed to the structure of EGFR which consists of an extracellular ligand-binding domain, a hydrophobic membrane-spanning region and an intracellular tyrosine kinase domain. Therefore, clinically, EGFR inhibition methods involve monoclonal antibody-mediated blockade of the extracellular-binding domain and small-molecule inhibition of the intracellular kinase domain (Rocha-Lima et al. 2007, Venook 2005).

Cetuximab (Erbitux) and Panitumumab (ABX-EGF) are the two most studied anti-EGFR monoclonal antibodies. Cetuximab, approved for use in other countries for colorectal and squamous cell carcinoma of the head and neck, is known to bind to EGFR, block ligand binding and induce receptor internalization and degradation, resulting in a decrease in EGFR expression by blocking the cell cycle, inducing cell death and inhibiting angiogenesis. Panitumumab, like Cetuximab, inhibits EGFR ligand binding, induces receptor internalization, and prevents tyrosine kinase phosphorylation but does not induce degradation (Rocha-Lima et al. 2007). However, while these antibodies seem

to be a reasonable agent for treatment, both are still under various phases in research (Rocha-Lima et al. 2007).

Erlotinib (Tarveca) and gefitnib (Iressa) are EGFR tyrosine kinase inhibitors which inhibit phosphorylation of the EGFR tyrosine kinase, inhibiting proliferation, tumor angiogenesis and inducing apoptosis (Rocha-Lima et al. 2007). A clinical study administering docetaxel and erlotinib was safe and well-tolerated in elderly patients with androgen-independent prostate cancer. However, survival was comparable to doxetaxel alone and more thorough study was required (Gross et al. 2007). Other combinations like paclitaxel and tyrosine kinase inhibitor AEE788 showed inhibition of EGFR phosphorylation, decreasing tumor incidence and tumor weight, while also preventing lymph node metastasis in multridrug-resistant prostate cancer nude mice (Busby et al. 2006). An *in-vitro* study targeting EGFR in various prostate cancer cell lines indicated the combined use of docetaxel, tyrosine kinase inhibitor gefitinib, as well as hedgehog signaling pathway inhibitor cyclopamine increased apoptosis in prostate cancer cells compared to the use of only one therapeutic agent (Mimeault et al. 2007). However, when used alone, derivatives of gefitinib were less potent EGFR inhibitors (Telliez et al. 2007).

Substantial attempts have been made in developing novel therapeutic agents that target growth factor pathways in cancer, especially in prostate cancer. However, uncertainty regarding the lack of successful interventions raises questions of concern for a disease with such slow progression but powerful impact. It is evident that standard clinical therapies, targeted agents and natural food components like lycopene may possibly enhance overall treatment by either preventing or delaying prostate disease.

From this research thesis it can be concluded that lycopene has potential chemopreventive properties and the results from the Oligo GEArray® Human Prostate Cancer Biomarkers Microarray and Real-Time PCR suggest that several prostate cancer biomarker genes are involved in the development and progression of hormone-refractory or androgen-independent prostate cancer. The effect of lycopene on EGFR protein expression only further supports the potential use of lycopene as an effective agent targeting the EGFR growth factor pathway in cancer. As of today, no significant research has been conducted on lycopene's effect on EGFR expression. Only significant findings of an inverse relationship between lycopene and prostate cancer in general have been studied. Therefore, the down-regulation of EGFR at the mRNA and protein levels in lycopene-treated (25μM) androgen-independent (PC-3) cells suggests the potential of lycopene as an additional therapeutic agent either used alone or in combination with current standard therapies. Future studies focusing on the combined effects of lycopene, chemotherapy and EGFR inhibitors on prostate cancer are warranted.

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CHAPTER VII APPENDICES

APPENDIX 1:
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 1

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
RPS27A	1.01	0.96	CDK5	0.82	0.43
RPS27A	1.01	1	CDK6	0.15	0.12
AGR2	0.74	0.47	CDK7	0.79	0.46
AGTR2	0.18	0.12	CDK8	0.62	0.15
AIG1	0.51	0.17	CDK9	0.67	0.17
AKAP1	0.81	0.31	CDKN1A	0.96	0.84
AKT1	1	0.59	CDKN1B	0.77	0.28
APC	0.7	0.19	CDKN1C	0.89	0.75
APOC1	0.89	0.45	CDKN2A	0.72	0.58
GAPDH	1.04	0.85	CDKN2B	0.67	0.57
GAPDH	1.03	0.95	CDKN2C	0.82	0.75
GAPDH	0.99	1	CDKN3	0.94	0.86
RPS27A	1.01	0.98	CHGA	0.09	0.13
AR	0.64	0.56	CHGB	0.09	0.1
BAK1	0.85	0.9	CLDN3	0.64	0.26
BAX	0.87	0.62	CLN3	0.96	0.67
BCL2	0.85	0.44	CLU	1.03	0.92
BCL2L1	0.91	0.51	COL1A1	0.46	0.18
BMP6	0.14	0.1	COL6A1	0.97	0.9
BRCA1	0.92	0.62	CYB5A	0.93	0.83
CANT1	0.97	0.64	CYC1	0.97	0.98
CASP1	0.28	0.04	DAB2IP	0.75	0.6
CASP3	0.21	0.07	DAPK1	0.08	0.12
CASP7	0.72	0.28	DES	0.12	0.11
CAV1	0.97	0.87	DYNLL1	1	0.93
CCND1	0.98	1.01	E2F1	0.99	0.81
CD164	0.82	0.66	EGF	0.62	0.26
CD44	0.82	0.51	EGFR	0.71	0.27
CDH1	0.36	0.24	EGR3	0.15	0.1
CDH10	0.17	0.13	ELAC2	0.99	0.95
CDH12	0.16	0.11	ELL	0.66	0.31
CDH13	0.06	0.09	ENO1	0.98	0.97
CDH18	0.39	0.12	ENO2	0.85	0.89
CDH19	0.79	0.31	ENO3	0.42	0.45
CDH20	0.14	0.11	ERBB2	0.83	0.82
CDH7	0.16	0.07	MAPK15	0.12	0.13
CDH8	0.14	0.09	ESR1	0.75	0.52
CDH9	0.12	0.13	ESR2	0.13	0.11
CDK2	0.82	0.72	EZH1	0.16	0.12
CDK3	0.15	0.12	EZH2	0.66	0.34
CDK4	0.97	0.92	FASN	0.83	0.42

APPENDIX 1 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression – Set 1

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
FGF1	0.1	0.06	IL12A	0.84	0.72
FGF10	0.1	0.08	IL1A	0.74	0.33
FGF11	0.13	0.08	IL1B	1	0.94
FGF12	0.11	0.13	IL2	0.13	0.08
FGF13	0.14	0.16	IL24	0.11	0.06
FGF14	0.22	0.17	IL29	0.23	0.09
FGF16	0.15	0.11	ILK	0.95	1
FGF17	0.28	0.14	INHA	0.38	0.26
FGF18	0.11	0.06	INSL3	0.49	0.34
FGF19	0.09	0.12	INSL4	0.3	0.25
FGF2	0.23	0.12	ITGA1	0.28	0.12
FGF20	0.11	0.07	JUN	0.87	0.83
FGF21	0.06	0.04	K6HF	1	0.92
FGF22	0.11	0.08	CD82	0.79	0.52
FGF23	0.07	0.08	KLK1	0.93	0.72
FGF3	0.17	0.13	KLK10	0.78	0.63
FGF4	0.13	0.17	KLK11	0.07	0.03
FGF5	0.13	0.11	KLK12	0.06	0.04
FGF6	0.16	0.14	KLK13	0.32	0.19
FGF7	0.09	0.04	KLK14	0.8	0.73
FGF8	0.08	0.04	KLK15	0.12	0.1
FGF9	0.12	0.09	KLK2	0.83	0.7
FHIT	0.12	0.11	KLK3	0.12	0.1
ARMC9	0.85	0.63	KLK4	0.15	
FLJ25530	0.13	0.05	KLK5	0.7	0.42
FOLH1	0.13	0.07	KLK6	0.4	0.18
PAGE1	0.1	0.11	KLK7	0.38	0.19
PAGE4	0.08	0.15	KLK8	0.37	0.14
GGT1	0.1	0.15	KLK9	0.34	0.18
GNRH1	0.12	0.13	KRT1	0.07	0.05
GRP	0.17	0.07	KRT2A	0.36	0.29
GSTP1	0.97	0.9	MAP2K4	0.86	0.87
HIF1A	0.7	0.17	MAP3K1	0.19	0.15
HIP1	0.26	0.14	MAPK1	0.76	0.63
HK2	0.26	0.14	MAPK10	0.14	0.09
HK3	0.14	0.05	MAPK11	0.1	0.05
HRAS	0.52	0.32	MAPK12	0.32	0.15
KRT2B	0.2	0.11	MAPK13	0.82	0.48
IGF1	0.15	0.15	MAPK14	0.31	0.1
IGF1R	0.48	0.3	MAPK3	0.96	0.94
IGF2	0.84	0.71	MAPK4	0.14	0.07
IGFBP3	1	0.97	MAPK6	0.92	0.76
IGFBP6	0.94	0.88	MAPK7	0.12	0.18

APPENDIX 1 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 1

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
MAPK8	0.69	0.62	PATE	0.06	0.12
MAPK9	0.84	0.82	PAWR	0.84	0.38
MIB1	0.36	0.16	PCA3	0.18	0.09
MMP2	0.98	1	PCNA	1	0.96
MMP9	0.52	0.33	PGR	0.79	0.75
MSMB	0.16	0.07	PIAS1	0.63	0.38
MTSS1	0.52	0.17	PIAS2	0.37	0.24
MYC	0.97	0.72	PIK3CG	1	0.97
NCOA4	0.81	0.48	PLAU	1	1.01
NFKB1	0.96	0.86	PLG	0.19	0.31
NFKBIA	0.99	1.03	PPID	0.75	0.54
NKX3-1	0.43	0.38	TMEM37	0.83	0.63
NOX5	0.45	0.31	PRKCA	0.08	0.12
NR0B1	0.12	0.12	PRKCB1	0.22	0.08
NR0B2	0.07	0.09	PRKCD	0.95	0.65
NR1D1	0.44	0.15	PRKCE	0.18	0.13
NR1D2	0.15	0.07	PRKCG	0.85	0.69
NR1H2	0.65	0.25	PRKCH	0.89	0.73
NR1H3	0.69	0.46	PRKCI	0.36	0.15
NR1H4	0.13	0.1	PRKD3	0.89	0.8
NR1I2	0.61	0.44	PRKCQ	0.54	0.3
NR1I3	0.39	0.19	PRKCZ	0.63	0.3
NR2C1	0.55	0.22	PRKD1	0.07	0.11
NR2C2	0.59	0.43	PRKD2	0.23	0.15
NR2E1	0.14	0.14	PRL	0.08	0.12
NR2E3	0.47	0.42	PSAP	0.18	0.11
NR2F1	0.95	0.9	PSCA	0.91	0.62
NR2F2	0.18	0.14	PTEN	0.13	0.08
NR2F6	0.94	0.78	RARB	0.17	0.04
NR3C1	0.64	0.35	RASSF1	0.92	0.84
NR3C2	0.12	0.1	RB1	0.98	0.92
NR4A1	0.33	0.13	RNASEL	0.37	0.33
NR4A2	0.39	0.24	RNF14	0.49	0.26
NR4A3	0.06	0.06	ROBO2	0.04	0.09
NR5A1	0.06	0.05	SERPINA3	0.08	0.13
NR5A2	0.14	0.11	SHBG	0.19	0.14
NR6A1	0.14	0.13	SLC2A2	0.06	0.12
NTN4	0.65	0.4	SLC33A1	0.2	0.13
ODZ1	0.14	0.1	SLC43A1	0.16	0.12
PALM2-AKAP2	0.46	0.16	SOX2	0.09	0.08
REG3A	0.35	0.17	SRC	0.32	0.13
PART1	0.11	0.09	SRD5A2	0.27	0.15

APPENDIX 1 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 1

GENES	CONTROL	LYCOPENE 25μM
HSPCB	1	0.91
STEAP1	0.93	0.83
STEAP2	0.81	0.6
TGFA	0.87	0.74
TGFB1	0.6	0.49
TGFB1I1	0.41	0.2
TGFB2	0.66	0.2
TGFB3	0.19	0.09
TIMP3	0.09	0.08
TNF	0.16	0.11
Pol1	0.12	0.12
PUC18	0.11	0.13
B2M	0.94	0.84
Blank	0.16	0.17
Blank	0.08	0.11
TNFSF10	0.08	0.12
TP53	0.26	0.1
TPM1	0.37	0.21
TPM2	0.6	0.42
18SrRNA	0.19	0.07
AS1R3	0.05	0.11
AS1R2	0.12	0.11
AS1R1	0.1	0.08
AS1	0.11	0.09
B2M	0.95	0.85
B2M	0.96	0.89
ACTB	0.98	0.99
TRPC6	0.14	0.29
TRPS1	0.17	0.15
TYK2	0.09	0.12
VEGF	0.12	0.12
BAS2C	0.05	0.12
BAS2C	0.11	0.13
BAS2C	0.31	0.24
BAS2C	0.86	0.79
BAS2C	0.95	1

APPENDIX 2:
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 2

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
RPS27A	1	1	CDK6	0.06	0.04
RPS27A	0.99	1.03	CDK7	0.13	0.07
AGR2	0.11	0.06	CDK8	0.09	0.04
AGTR2	0.03	0.08	CDK9	0.16	0.07
AIG1	0.09	0.03	CDKN1A	0.62	0.37
AKAP1	0.27	0.09	CDKN1B	0.17	0.03
AKT1	0.64	0.2	CDKN1C	0.55	0.28
APC	0.12	0.11	CDKN2A	0.2	0.09
APOC1	0.32	0.18	CDKN2B	0.2	0.09
GAPDH	0.98	0.68	CDKN2C	0.3	0.14
GAPDH	0.99	0.73	CDKN3	0.75	0.49
GAPDH	0.95	0.76	CHGA	0.07	0.12
RPS27A	1	1	CHGB	0.05	0.08
AR	0.21	0.12	CLDN3	0.19	0.09
BAK1	0.46	0.19	CLN3	0.68	0.28
BAX	0.2	0.1	CLU	0.9	0.75
BCL2	0.23	0.07	COL1A1	0.07	0.08
BCL2L1	0.34	0.1	COL6A1	0.92	0.58
BMP6	0.03	0.04	CYB5A	0.81	0.46
BRCA1	0.5	0.25	CYC1	0.97	0.71
CANT1	0.72	0.26	DAB2IP	0.31	0.12
CASP1	0.05	0.05	DAPK1	0.04	0.09
CASP3	0.04	0.03	DES	0.03	0.11
CASP7	0.08	0.04	DYNLL1	0.94	0.76
CAV1	0.9	0.67	E2F1	0.58	0.21
CCND1	0.98	0.95	EGF	0.09	0.03
CD164	0.52	0.09	EGFR	0.18	0.09
CD44	0.48	0.09	EGR3	0.05	0.04
CDH1	0.12	0.04	ELAC2	0.9	0.66
CDH10	0.04	0.06	ELL	0.07	0.04
CDH12	0.07	0.04	ENO1	1	0.97
CDH13	0.02	0.04	ENO2	0.46	0.18
CDH18	0.09	0.07	ENO3	0.12	0.07
CDH19	0.21	0.06	ERBB2	0.64	0.41
CDH20	0.01	0.04	MAPK15	0.05	0.1
CDH7	0.01	0.02	ESR1	0.31	0.15
CDH8	0.02	0.03	ESR2	0.05	0.07
CDH9	0.02	0.03	EZH1	0.07	0.06
CDK2	0.75	0.26	EZH2	0.08	0.07
CDK3	0.03	0.05	FASN	0.29	0.06
CDK4	0.88	0.78	FGF1	0.01	0.05
CDK5	0.38	0.07	FGF10	0.01	0.02

APPENDIX 2 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 2

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
FGF11	0.09	0.04	IL12A	0.29	0.14
FGF12	0.02	0.03	IL1A	0.13	0.09
FGF13	0.01	0.05	IL1B	0.9	0.72
FGF14	0.07	0.07	IL2	0.05	0.11
FGF16	0.08	0.12	IL24	0.03	0.1
FGF17	0.1	0.15	IL29	0.03	0.08
FGF18	0.02	0.07	ILK	0.86	0.61
FGF19	0.05	0.09	INHA	0.06	0.04
FGF2	0.07	0.07	INSL3	0.14	0.05
FGF20	0.07	0.07	INSL4	0.02	0.03
FGF21	0.02	0.04	ITGA1	0.07	0.06
FGF22	0.01	0.01	JUN	0.47	0.45
FGF23	0.06	0.03	K6HF	0.57	0.21
FGF3	0.03	0.04	CD82	0.21	0.16
FGF4	0.02	0.03	KLK1	0.61	0.29
FGF5	0.06	0.04	KLK10	0.25	0.09
FGF6	0.08	0.06	KLK11	0.02	0.11
FGF7	0.09	0.06	KLK12	0.03	0.09
FGF8	0.04	0.04	KLK13	0.04	0.04
FGF9	0.05	0.12	KLK14	0.45	0.19
FHIT	0.06	0.07	KLK15	0.01	0.03
ARMC9	0.26	0.12	KLK2	0.48	0.28
FLJ25530	0.03	0.05	KLK3	0.03	0.08
FOLH1	0.01	0.01	KLK4	0.05	0.11
PAGE1	0.06	0.06	KLK5	0.18	0.18
PAGE4	0.01	0.06	KLK6	0.04	0.07
GGT1	0.02	0.11	KLK7	0.1	0.1
GNRH1	0.08	0.12	KLK8	0.13	0.07
GRP	0.07	0.1	KLK9	0.09	0.09
GSTP1	0.88	0.4	KRT1	0.02	0.03
HIF1A	0.27	0.11	KRT2A	0.05	0.04
HIP1	0.03	0.1	MAP2K4	0.62	0.39
HK2	0.01	0.05	MAP3K1	0.02	0.05
HK3	0.05	0.05	MAPK1	0.31	0.15
HRAS	0.07	0.02	MAPK10	0.04	0.07
KRT2B	0.02	0.02	MAPK11	0.02	0.1
IGF1	0.04	0.04	MAPK12	0.07	0.13
IGF1R	0.13	0.04	MAPK13	0.29	0.09
IGF2	0.49	0.17	MAPK14	0.07	0.06
IGFBP3	1	0.85	MAPK3	0.72	0.49
IGFBP6	0.86	0.45	MAPK4	0.02	0.07

APPENDIX 2 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 2

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
MAPK6	0.37	0.08	REG3A	0.1	0.09
MAPK7	0.02	0.01	PART1	0.05	0.08
MAPK8	0.33	0.11	PATE	0.06	0.06
MAPK9	0.46	0.3	PAWR	0.25	0.09
MIB1	0.07	0.05	PCA3	0.06	0.07
MMP2	0.92	0.82	PCNA	0.87	0.53
MMP9	0.1	0.06	PGR	0.28	0.1
MSMB	0.05	0.04	PIAS1	0.12	0.04
MTSS1	0.06	0.05	PIAS2	0.06	0.06
MYC	0.64	0.16	PIK3CG	0.98	0.92
NCOA4	0.21	0.11	PLAU	1	1.03
NFKB1	0.48	0.16	PLG	0.17	0.1
NFKBIA	0.82	0.49	PPID	0.18	0.13
NKX3-1	0.05	0.04	TMEM37	0.28	0.15
NOX5	0.02	0.03	PRKCA	0.05	0.07
NR0B1	0.01	0.03	PRKCB1	0.04	0.06
NR0B2	0.05	0.04	PRKCD	0.52	0.21
NR1D1	0.09	0.06	PRKCE	0.06	0.06
NR1D2	0.08	0.1	PRKCG	0.37	0.15
NR1H2	0.16	0.07	PRKCH	0.31	0.1
NR1H3	0.27	0.11	PRKCI	0.05	0.02
NR1H4	0.06	0.09	PRKD3	0.3	0.3
NR1I2	0.12	0.1	PRKCQ	0.1	0.05
NR1I3	0.09	0.1	PRKCZ	0.11	0.04
NR2C1	0.11	0.07	PRKD1	0.05	0.03
NR2C2	0.06	0.08	PRKD2	0.09	0.04
NR2E1	0.02	0.03	PRL	0.02	0.05
NR2E3	0.07	0.07	PSAP	0.04	0.07
NR2F1	0.52	0.48	PSCA	0.51	0.26
NR2F2	0.02	0.06	PTEN	0.04	0.09
NR2F6	0.61	0.33	RARB	0.04	0.1
NR3C1	0.15	0.13	RASSF1	0.67	0.42
NR3C2	0.06	0.06	RB1	0.95	0.95
NR4A1	0.05	0.06	RNASEL	0.07	0.03
NR4A2	0.08	0.06	RNF14	0.04	0.02
NR4A3	0.06	0.08	ROBO2	0.04	0.06
NR5A1	0.02	0.03	SERPINA3	0.04	0.14
NR5A2	0.02	0.03	SHBG	0.03	0.12
NR6A1	0.05	0.02	SLC2A2	0.03	0.05
NTN4	0.12	0.06	SLC33A1	0.04	0.04
ODZ1	0.03	0.04	SLC43A1	0.04	0.08
PALM2-AKAP2	0.08	0.05	SOX2	0.05	0.07

APPENDIX 2 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 2

GENES	CONTROL	LYCOPENE 25μM
SRC	0.05	0.06
SRD5A2	0.02	0.06
HSPCB	0.99	0.95
STEAP1	0.7	0.43
STEAP2	0.3	0.24
TGFA	0.56	0.3
TGFB1	0.1	0.08
TGFB1I1	0.08	0.06
TGFB2	0.16	0.07
TGFB3	0.04	0.04
TIMP3	0.05	0.06
TNF	0.02	0.05
Pol1	0.02	0.04
PUC18	0.01	0.03
B2M	0.91	0.87
Blank	0.05	0.03
Blank	0.04	0.05
TNFSF10	0.04	0.04
TP53	0.04	0.04
TPM1	0.12	0.03
TPM2	0.13	0.05
18SrRNA	0.2	0.04
AS1R3	0.06	0.03
AS1R2	0.06	0.04
AS1R1	0.02	0.03
AS1	0.04	0.05
B2M	0.89	0.87
B2M	0.94	0.85
ACTB	1.01	1.03
TRPC6	0.33	0.06
TRPS1	0.09	0.05
TYK2	0.07	0.03
VEGFA	0.1	0.03
BAS2C	0.09	0.05
BAS2C	0.07	0.05
BAS2C	0.28	0.16
BAS2C	0.89	0.75
BAS2C	0.99	1

APPENDIX 3:
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 3

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
RPS27A	0.96	1.01	CDK6	0.1	0.13
RPS27A	0.94	0.97	CDK7	0.28	0.17
AGR2	0.12	0.18	CDK8	0.15	0.1
AGTR2	0.13	0.11	CDK9	0.19	0.08
AIG1	0.08	0.15	CDKN1A	0.55	0.41
AKAP1	0.35	0.2	CDKN1B	0.13	0.03
AKT1	0.63	0.38	CDKN1C	0.32	0.18
APC	0.14	0.18	CDKN2A	0.16	0.09
APOC1	0.19	0.21	CDKN2B	0.24	0.08
GAPDH	0.83	0.84	CDKN2C	0.34	0.25
GAPDH	0.84	0.81	CDKN3	0.76	0.57
GAPDH	0.91	0.8	CHGA	0.09	0.08
RPS27A	1	0.98	CHGB	0.08	0.15
AR	0.06	0.16	CLDN3	0.21	0.14
BAK1	0.48	0.36	CLN3	0.6	0.46
BAX	0.29	0.08	CLU	0.83	0.66
BCL2	0.31	0.13	COL1A1	0.1	0.05
BCL2L1	0.26	0.1	COL6A1	0.79	0.52
BMP6	0.07	0.04	CYB5A	0.78	0.39
BRCA1	0.38	0.24	CYC1	0.91	0.72
CANT1	0.58	0.32	DAB2IP	0.17	0.06
CASP1	0.02	0.11	DAPK1	0.09	0.14
CASP3	0.07	0.07	DES	0.09	0.15
CASP7	0.21	0.04	DYNLL1	0.97	0.8
CAV1	0.59	0.33	E2F1	0.67	0.43
CCND1	0.71	0.77	EGF	0.21	0.17
CD164	0.42	0.09	EGFR	0.34	0.14
CD44	0.43	0.09	EGR3	0.11	0.15
CDH1	0.12	0.13	ELAC2	0.77	0.67
CDH10	0.07	0.11	ELL	0.04	0.03
CDH12	0.07	0.12	ENO1	1	1
CDH13	0.02	0.11	ENO2	0.42	0.23
CDH18	0.02	0.06	ENO3	0.16	0.08
CDH19	0.27	0.16	ERBB2	0.61	0.48
CDH20	0.14	0.09	MAPK15	0.09	0.1
CDH7	0.01	0.02	ESR1	0.38	0.2
CDH8	0.03	0.02	ESR2	0.12	0.12
CDH9	0.02	0.02	EZH1	0.13	0.11
CDK2	0.4	0.22	EZH2	0.23	0.17
CDK3	0.12	0.03	FASN	0.37	0.19
CDK4	0.84	0.82	FGF1	0.01	0.13
CDK5	0.27	0.2	FGF10	0.01	0.05

APPENDIX 3 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 3

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
FGF11	0.06	0.03	IL12A	0.59	0.35
FGF12	0.01	0.01	IL1A	0.22	0.18
FGF13	0.02	0.05	IL1B	0.89	0.79
FGF14	0.08	0.12	IL2	0.09	0.09
FGF16	0.09	0.08	IL24	0.1	0.13
FGF17	0.17	0.1	IL29	0.11	0.1
FGF18	0.1	0.11	ILK	0.84	0.73
FGF19	0.16	0.13	INHA	0.03	0.01
FGF2	0.11	0.1	INSL3	0.06	0.03
FGF20	0.11	0.09	INSL4	0.11	0.02
FGF21	0.01	0.12	ITGA1	0.14	0.03
FGF22	0.01	0.08	JUN	0.52	0.32
FGF23	0.01	0.01	K6HF	0.45	0.46
FGF3	0.02	0.02	CD82	0.28	0.15
FGF4	0.03	0.04	KLK1	0.5	0.29
FGF5	0.07	0.14	KLK10	0.24	0.14
FGF6	0.11	0.15	KLK11	0.08	0.06
FGF7	0.14	0.14	KLK12	0.02	0.12
FGF8	0.1	0.14	KLK13	0.05	0.06
FGF9	0.14	0.15	KLK14	0.2	0.09
FHIT	0.12	0.09	KLK15	0.02	0.01
ARMC9	0.49	0.19	KLK2	0.48	0.18
FLJ25530	0.01	0.08	KLK3	0.11	0.07
FOLH1	0.1	0.05	KLK4	0.09	0.12
PAGE1	0.01	0.01	KLK5	0.26	0.18
PAGE4	0.01	0.01	KLK6	0.11	0.15
GGT1	0.1	0.02	KLK7	0.11	0.08
GNRH1	0.07	0.1	KLK8	0.12	0.14
GRP	0.1	0.14	KLK9	0.06	0.11
GSTP1	0.87	0.68	KRT1	0.02	0.11
HIF1A	0.32	0.15	KRT2A	0.04	0.07
HIP1	0.17	0.16	MAP2K4	0.68	0.49
HK2	0.14	0.17	MAP3K1	0.03	0.02
HK3	0.08	0.15	MAPK1	0.24	0.09
HRAS	0.12	0.17	MAPK10	0.11	0.11
KRT2B	0.08	0.05	MAPK11	0.13	0.12
IGF1	0.01	0.01	MAPK12	0.14	0.13
IGF1R	0.07	0.03	MAPK13	0.33	0.21
IGF2	0.32	0.12	MAPK14	0.18	0.07
IGFBP3	0.98	0.9	MAPK3	0.67	0.39
IGFBP6	0.81	0.69	MAPK4	0.04	0.13

APPENDIX 3 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 3

GENES	CONTROL	LYCOPENE 25μM	GENES	CONTROL	LYCOPENE 25μM
MAPK6	0.46	0.22	REG3A	0.15	0.11
MAPK7	0.01	0.08	PART1	0.13	0.1
MAPK8	0.4	0.08	PATE	0.1	0.12
MAPK9	0.52	0.29	PAWR	0.33	0.13
MIB1	0.17	0.05	PCA3	0.09	0.1
MMP2	0.9	0.75	PCNA	0.78	0.52
MMP9	0.17	0.15	PGR	0.31	0.2
MSMB	0.16	0.15	PIAS1	0.08	0.1
MTSS1	0.17	0.17	PIAS2	0.08	0.03
MYC	0.65	0.36	PIK3CG	0.9	0.8
NCOA4	0.29	0.12	PLAU	1.05	1.04
NFKB1	0.57	0.25	PLG	0.13	0.13
NFKBIA	0.72	0.59	PPID	0.37	0.16
NKX3-1	0.04	0.03	TMEM37	0.33	0.18
NOX5	0.02	0.02	PRKCA	0.1	0.12
NR0B1	0.01	0.14	PRKCB1	0.11	0.12
NR0B2	0.13	0.16	PRKCD	0.47	0.22
NR1D1	0.15	0.11	PRKCE	0.08	0.13
NR1D2	0.13	0.12	PRKCG	0.22	0.18
NR1H2	0.26	0.19	PRKCH	0.36	0.14
NR1H3	0.3	0.25	PRKCI	0.03	0.01
NR1H4	0.14	0.15	PRKD3	0.32	0.22
NR1I2	0.18	0.15	PRKCQ	0.09	0.11
NR1I3	0.11	0.15	PRKCZ	0.18	0.19
NR2C1	0.1	0.1	PRKD1	0.04	0.17
NR2C2	0.08	0.15	PRKD2	0.13	0.13
NR2E1	0.02	0.03	PRL	0.11	0.13
NR2E3	0.08	0.14	PSAP	0.12	0.11
NR2F1	0.76	0.77	PSCA	0.41	0.25
NR2F2	0.15	0.11	PTEN	0.1	0.16
NR2F6	0.63	0.31	RARB	0.01	0.13
NR3C1	0.29	0.16	RASSF1	0.57	0.48
NR3C2	0.04	0.16	RB1	0.9	0.73
NR4A1	0.14	0.13	RNASEL	0.02	0.07
NR4A2	0.17	0.17	RNF14	0.14	0.03
NR4A3	0.09	0.15	ROBO2	0.02	0.03
NR5A1	0.04	0.12	SERPINA3	0.14	0.14
NR5A2	0.01	0.01	SHBG	0.13	0.13
NR6A1	0.02	0.01	SLC2A2	0.12	0.14
NTN4	0.21	0.16	SLC33A1	0.14	0.09
ODZ1	0.08	0.13	SLC43A1	0.12	0.09
PALM2-AKAP2	0.14	0.14	SOX2	0.1	0.11

APPENDIX 3 (Continued):
Oligo GEArray® Human Prostate Cancer Biomarkers Microarray Gene Expression - Set 3

GENES	CONTROL	LYCOPENE 25μM
SRC	0.07	0.1
SRD5A2	0.01	0.07
HSPCB	1.01	0.94
STEAP1	0.51	0.31
STEAP2	0.41	0.14
TGFA	0.56	0.32
TGFB1	0.26	0.12
TGFB1I1	0.13	0.11
TGFB2	0.18	0.13
TGFB3	0.12	0.14
TIMP3	0.08	0.13
TNF	0.09	0.1
Pol1	0.05	0.05
PUC18	0	0.1
B2M	0.89	0.68
Blank	0.03	0.04
Blank	0.02	0.01
TNFSF10	0.01	0.01
TP53	0.06	0.08
TPM1	0.12	0.08
TPM2	0.12	0.11
18SrRNA	0.09	0.09
AS1R3	0.09	0.12
AS1R2	0.05	0.06
AS1R1	0.05	0.06
AS1	0.01	0.06
B2M	0.87	0.63
B2M	0.85	0.7
ACTB	1	1
TRPC6	0.09	0.13
TRPS1	0.09	0.07
TYK2	0.09	0.1
VEGFA	0.13	0.11
BAS2C	0.13	0.08
BAS2C	0.11	0.07
BAS2C	0.19	0.21
BAS2C	0.55	0.68
BAS2C	0.86	0.99