WHAT IS THE RELATIONSHIP BETWEEN A SOY-RICH DIET AND THE INCIDENCE OF PROSTATE CANCER: A SYSTEMATIC EVIDENCE-BASED LITERATURE REVIEW?

A Research Project by

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We hereby recommend that the research project prepared under our supervision by Joshua W. Burrow entitled What is the Relationship Between a Soy-Rich Diet and the Incidence of Prostate Cancer: A Systematic Evidence-Based Literature Review be accepted as partial fulfillment for the degree of Master of Physician Assistant.

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ABSTRACT

Introduction Prostate cancer (PC) is the most common non-cutaneous cancer in the United States male population, and the second most common cause of cancer mortality. It has been proposed that dietary differences in Asian and Western men may be partially responsible for the lower incidence of PC among Asian men. The assumption that the Asian diet may be prostate-healthy is based on the fact that it contains many soy products. The purpose of this study was to determine whether there is a relationship between the consumption of a soy-rich diet and PC incidence.

Methodology A systematic review of evidence-based literature was conducted by examining peer-reviewed articles from the following databases: MEDLINE FirstSearch, MEDLINE PubMed, and Cochrane Library. MeSH terms utilized included prostate cancer / carcinoma, soy, soy-rich diet, prostate cancer incidence, genistein, daidzein, and equol.

Results The findings suggest there are benefits related to the consumption of a soy-rich diet, but none were shown to be conclusively preventive.

Conclusion More *in vivo* research must be completed before a statistically significant relationship between the consumption of a soy-rich diet and the incidence of PC can be substantiated.

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CHAPTER I

INTRODUCTION

The incidence of prostate cancer in the US is a matter of concern for clinicians on a daily basis. According to recent statistics, prostate cancer is now the most common non-cutaneous cancer in the United States male population, and the second most common cause of death of cancer overall, following cancer of the lung.[1] These statistics become even more significant as research suggests this problem will only worsen as the projected life expectancy increases in the next millennium.[1] It is estimated that approximately 218,890 new cases will be diagnosed in 2007 and result in the eventual death of approximately 27,050 men.[2] Also according to the American Cancer Society, for the general population, a man in his lifetime, has a 16.6% chance of being diagnosed with prostate cancer and a 3% chance of dying from the disease.[2]

The etiology of PC is quite complicated due to the fact that few risk factors have been positively associated with the disease. Those risk factors which may be relevant are: age, diet, family history, geographic location, race, and testosterone deficiency.[1] Prostate cancer is typically found upon either Digital Rectal Examination (DRE) of a nodular prostate or by elevated serum Prostate Specific Antigen (PSA). PSA is a glycoprotein produced in the cytoplasm of benign and malignant prostatic cells; its level correlates to the amount of prostate tissue, benign or malignant.[1] When PSA levels are increased, it is indicative of inflammation or PC development. It is thought that there are other markers that may indicate the development of PC that have yet to be determined.

With statistics such as these it is clear that effective treatment of PC is not enough, it is imperative that a tested and proven cancer prevention method be determined. It is for this reason that scientists, nutritionists, clinicians, and researchers strive to identify preventative agents for PC.

Scientists and researchers believe that diet may be the best way to prevent PC. Since it has been determined that there is a decreased incidence of PC among Asian men compared to Western men, their diet has been of utmost concern. The greatest differences between Asian and Western diets are an increased consumption of soy-rich foods, decreased consumption of red meats and dairy products, and a decreased consumption of a diet high in fat. These dietary differences have led scientists and researchers to ask the following questions: 1) Which soy components are responsible for reducing the incidence of PC and 2) what effects do these components have on PC? Phytochemicals in soy include isoflavones which are naturally occurring compounds found in plants that have strong biological activity in the body. They are relatively safe and exert multiple effects in the body including estrogen receptor activation, antiestrogenic actions, inhibition of growth factor signaling via tyrosine kinases, induction of apoptosis, induction of cell differentiation, inhibition of angiogenesis, and induction of genetic damage.[3]

CHAPTER II

PURPOSE OF STUDY

The purpose of this study is to determine whether the scientific literature substantiates a relationship between the consumption of a diet rich in soy and the incidence of PC.

CHAPTER III

METHODOLOGY

This research study was completed by performing a systematic review of evidence-based literature. The comprehensive literature search was carried out using the following databases: MEDLINE FirstSearch, MEDLINE PubMed, and Cochrane Library from 1980 - 2006. MeSH (Medical Subject Heading) terms included prostate cancer / carcinoma, soy, soy-rich diet, prostate cancer incidence, genistein, daidzein, and equol. Articles chosen for review were based on their level of evidence, publication in peer-reviewed journals, relevance of the data, and publication in the English language. The inclusion criteria used to select articles for this review were: 1) Men between the ages of 19 – 89 years old that had not been diagnosed with any other type of cancer besides PC at the onset of the trials and 2) the evidence level of the article had to be either a Level 1 or Level 2. A total of three Level 1 articles and twenty-one Level 2 articles met the inclusion criteria. Exclusion criteria utilized were articles that were low in evidence and African American men (due to their higher rates of PC).

There were three levels of evidence used to classify each study. Level 1 evidence included double-blinded randomized control trials and large meta-analysis studies. Level 2 studies were non-blinded randomized control trials, case-control studies, cross-sectional studies, prospective studies, cohort studies, and randomized crossover intervention studies. Level 3 included literature reviews and meta-analysis.

CHAPTER IV

LITERATURE REVIEW

The distinctive epidemiologic contrast between Asian and American prostate cancer incidence and mortality has resulted in a body of research focused on identifying specific factors which account for the much lower incidence and mortality of prostate cancer among the Asian population. The fact that the rate of advanced prostate cancer in the United States outpaces the Asian rate by a factor of 15, coupled with reports documenting an increased incidence of prostate cancer as Asian men immigrate to the United States and assume a Western diet have allowed researchers to isolate diet as a key factor in the prevention and management of prostate cancer.[4-6]

One distinct difference between the diet of American and Asian men is the consumption of soy-based products.[5] The traditional Asian diet provides for a much higher concentration of isoflavones which is a primary component of soy. In fact, the quantity of isoflavones in soy is much greater than what is found in other plant-based foods.[7] Asian men consume a variety of soy-rich foods in the form of tofu, natto (fermented soybeans), soy milk and bean sprouts among others.[8] Isoflavone concentration in blood or urine is the manner in which soy intake is measured. In particular, the level of genistein in the blood or urine is associated with soy intake as genistein is the more predominant isoflavone found in soy.[8] Several researchers confirm the point that Asian men tend to have a higher soy isoflavone concentration. For example, Sonoda reports a mean concentration of isoflavones in Japanese men, is 492.7 nmol/liter compared to a value of 33.2 nmol/liter in men in the UK.[8] As well, Lamartiniere et al report higher blood and urine genistein levels in Asian men as compared to American men.[5] In this regard, considerable focus has been placed on soy as an important preventative and management agent for prostate cancer.

Researchers associate the isoflavones contained in soy, such as genistein, daidzein, and glycitein, with the proposed chemo-preventative nature of soy.[9] Isoflavones are phytoestrogens which are plant-derived, non-steroidal compounds.[9] These compounds demonstrate estrogen-like biological activity. Kucuk asserts that these potential chemopreventive agents, known as soy isoflavones, function to reduce the proliferation of prostate cancer cells through varied mechanisms such as "antioxidant, anti-inflammatory, anti-hormonal and anti-angiogenic effects."[10]

Level 1 Evidence

Few Level 1 studies have been employed to investigate the relationship between soy isoflavones and their potential role in preventing or arresting the development of prostate cancer. Although such studies are few in number, their contributions serve as a foundation for continued research and scientific inquiry.

Adams et al built upon a pre-existing randomized, controlled, double blinded parent study which was conducted over a 12-month period.[11] This parent study, titled the Soy Isoflavone Prevention Trial (SIP), enrolled 128 participants of whom 85% where male. The participants were randomly divided into two groups. Each group was asked to consume a daily soy protein drink throughout the 12-month project. One group's protein drink packet contained 83mg/day isoflavones, similar to the soy intake associated with a conventional Asian diet. The other group's daily protein packet lacked soy isoflavones yielding a non-soy product. In four month intervals through the 12-month time frame, fasting labs were obtained and frozen. Overall, 112 participants completed the SIP parent study.[11]

Adams et al utilized the collections from the SIP trial to explore the PSA and genistein concentration levels of participants. By contacting the original 112 participants, Adams et al were able to acquire consent to further investigate the previously collected blood sera. Of the 112 original study participants, 81 men ranging in age from 50-80 years agreed to the further examination of their blood collections. Adams et al evaluated the baseline and 12-month blood sera collections for the rate of PSA elevation and genistein concentration; PSA is an intermediate marker for prostate cancer while genistein concentration is a marker for isoflavone or soy intake.[11]

The results of this continued study demonstrated no connection between PSA and genistein concentrations. Instead, the data revealed an equal increase in PSA levels among participants from both groups over the 12-month study period. The researchers of this study recognize that PSA is an intermediate, not early, marker for prostate cancer and therefore may not have effectively correlated the impact of a soy-rich diet on earlier stages of prostate cancer. Furthermore, the authors of the study acknowledge that while 83mg/day of isoflavones did not prevent elevation of PSA values, it may affect tumor cell proliferation in a manner that is not detectable by PSA testing.[11]

In contrast to Adams et al, Schroder reports a 2.6 fold increase in PSA doubling time through the use of a self-designed dietary supplement containing soy isoflavones, lycopene, selenium, and antioxidants.[12] This randomized, double-blind placebo controlled crossover study consisted of 49 male enrollees who had been diagnosed and curatively treated for prostate cancer by way of radical prostatectomy or radiotherapy. The trial occurred over two 10-week periods separated by a 4-week washout session. The participants were divided into two groups, Group I and Group II respectively. During the first 10-week period, Group I ingested two placebo pills

twice daily. Following the same regime, Group II consumed the soy-rich dietary supplement. During the second 10-week phase, the groups alternated roles. Throughout the course of the trial, PSA blood sampling occurred in two week intervals.[12]

Analysis of the participants' PSA levels revealed a significant lengthening of the PSA doubling time. During the supplementation phases of the study, PSA doubling time extended to 1150 days compared to the 445 day doubling time recorded during the placebo period.[12]

While Schroder does not discuss a specific mechanism by which the soy-rich dietary supplement hindered the elevation of PSA concentrations, he does indicate that the supplement was intended to modify the manner in which prostate cancer progresses into an advanced disease stage.[12] Schroeder asserts that the reported increase in PSA doubling time by a factor of 2.6 could be clinically significant if the prolonged PSA elevation time could be directly correlated with a comparable decrease in tumor progression.[12] It is this correlation that requires further study.

Meta-analysis studies are also of the utmost importance when examining the impact of a soy-rich diet on the incidence and progression of prostate cancer. Yan and Spitznagel conducted a quantitative evaluation of epidemiologic studies which evaluated soy consumption and prostate cancer risk. A total of eight studies met the review criteria set by Yan et al; the review included cohort and case controlled studies originating in North America and Asian countries.[7]

The meta-analysis conducted by Yan et al examined the association of soy consumption and prostate etiology. The collective studies utilized soy isoflavone concentrations as a measure of soy intake. The soy isoflavone of interest among the studies was genistein because it is the predominant isoflavone found in soy. Overall, Yan et al conclude that a soy-rich diet and prostate cancer etiology are inversely related stating that soy yields a 30% reduction in prostate

cancer risk.[7] The authors further claim that soy provides 4-times more protection against prostate cancer than any other dietary factors.[7]

While the results are promising in terms of the influence soy has on prostate health, the meta-analysis does not point to the mechanism by which soy prevents prostate cancer cells from developing or progressing. Yan et al indicate that several of the reviewed studies evaluated the PSA of either pre-surgical, post-primary treatment, or healthy trial participants, however, a decisive correlation between PSA reduction and soy could not identified. Although Yan et al referred to a study which showed a link between soy intake and a prolonged PSA doubling time.[7] This is similar to the assertion Schroder makes based on the results of his randomized, double-blind placebo controlled crossover study as discussed previously.[12]

Level 2 Evidence

Cohort Studies

Of the identified articles, only one was a cohort study. This study conducted by Hedlund et al served to investigate the connection between habitual diet, isoflavone metabolism and the impact these factors may have as preventative agents for prostate cancer.[13] Daidzein is one of the common isoflavones found in soy, along with genistein. The authors of this cohort study report that the metabolism of daidzein, within the intestinal flora, results in specific compounds or metabolites such as equol. Past research suggests that equol possesses a higher bioactivity than other soy metabolites leading many to further evaluate its role in prostate health. Hedlund et al support this assertion as they report that equol is 10-fold more effective in reducing the growth of both normal and malignant prostate epithelial cells *in vitro*.[13]

Interestingly, not all individuals produce equol. The production of this metabolite is highly regular among Asians who consume a traditional high-soy diet yet only 1 in 3 Caucasians actually produce equol.[13] This disparity is one of the focuses of the study as Hedlund et al strived to demonstrate an association between a long-term, high-soy diet and an increased production of equol. The secondary aims of the study involve the determination of whether soy isoflavonoids are better reflected in blood plasma or prostatic fluid and whether other dietary influences stimulate the production of equol.[13]

Hedlund et al recruited healthy men from 8 Colorado based Seventh Day Adventist churches. This specific population is known to typically consume a high soy diet. The age of the participants ranged between 19 and 65 years. Additional subjects were recruited from the University of Colorado Health Sciences Center. Each participant completed a dietary survey which assessed their monthly dietary habits including references to 34 different soy-based foods. The survey also collected the past medical history of participants. Exclusionary factors consisted of the following: history of prostate disease; surgical resection of intestines or stomach; and intake of antibiotics within 2 months leading up to the study. Based on the results of the survey, 45 men aligned with the pre-determined inclusion criteria. The participants were divided into two categories titled long-term, high-soy consumers and long-term, low-soy consumers. Twenty-five high-soy consumers were defined, all of which were Seventh Day Adventists. These participants had a two year history of consuming 30 mg or more of soy isoflavones per day. Twenty low-soy consumers were identified. Half of the members of this group were Seventh Day Adventists. These individuals had an intake of less than 5 mg of soy isoflavones per day over a two year period.[13]

The week long study began with the collection of blood samples to establish baseline levels of isoflavonoids and PSA. Next, the volunteers consumed a 330 mL soy beverage once daily for the duration of one week. After the 1 week interval, participants returned for post-soy blood sampling and prostate fluid collection. Comparisons were then made between the baseline and post-soy collections of both groups.[13]

Findings indicate that concentrations of soy isoflavonoids, except for genistein, were higher in the prostatic fluid than in the blood plasma.[13] Because elements of the prostatic fluid pass through the basal and epithelial cells of the prostate which are the cells that are linked to malignant development, Hedlund et al suggest that the isoflavonoids may impact prostate health on two levels. First, the isoflavonoids may directly restrain prostatic cell growth.[13] The other suggested mechanism by which isoflavonoids may affect cellular proliferation is through an antioxidant response in which toxins are prevented from building up as readily in the prostate.[13] While this study provided researchers with information regarding the manner in which isoflavonoids may work in the prostate to reduce cellular proliferation, the results did not support the assumption that a long-term, high-soy diet provides for more isoflavones and their metabolites such as equol. In fact, there was minimal difference in concentrations of daidzein and equol among the high-soy and low-soy groups.[13] As well, findings indicate that the baseline and post-soy diet PSA values of both study groups were similar.[16] Despite research that asserts that soy may reduce PSA or minimize the speed with which it increases in males with a history of prostate cancer, the results of Hedlund et al suggest that soy isoflavones do not influence the PSA concentrations of healthy men. One final unexpected result noted by Hedlund et al is the possible association between the production of equol and the consumption of meat. The authors

of this study suggest further research regarding this finding as it seems to conflict with previous research that implies an inverse relationship between equol production and fat intake.[13]

Case Control Studies

Undoubtedly, the most common type of study used to investigate the association between soy and prostate cancer is a case-control study; ten articles were selected. The first case study is a Japanese study in which Sonoda et al attempted to demonstrate the positive impact of a traditional Japanese diet on the incidence of prostate cancer. In this study, 140 cases from four geographical regions of Japan were obtained. The cases were of men ranging in age between 59 and 73 who had a definite diagnosis of adenocarcinoma of the prostate between the dates of January 1996 and September 2002.[8] A control group was selected from the same geographic locations and consisted of individuals diagnosed with a variety of conditions such as oral diseases, cataracts, and benign kidney disorders.[8] Exclusionary criteria for the control group included the following: history of prostate disease or cancer; dietary restriction; and PSA level greater than 5.0ng/ml.[8]

Through a dietary interview process, the average daily consumption, 5 years prior to diagnosis, of specific food and beverages was measured. Soy-based foods such as "tofu (bean curd), natto (fermented soybeans), miso soup (soybean paste soup), aburaage (fried bean curd), kinako (soy flour), yuba (dried bean curd), tonyu (soybean milk), soy sauce, green soybeans and bean sprouts were of particular interest."[8] Green tea, coffee, black tea and alcohol were also analyzed; however, Sonoda et al concluded that minimal associations between these beverages and a decrease or increase in prostate cancer risk existed.[8]

The fundamental outcome of this study is that the data supported the hypothesis that a soy-rich diet as found in Japan correlates with decreased prostate cancer risk. Sonoda et al refer to other research that points to the isoflavones from the soy products as the mechanisms of cancer protection. Such defense is achieved through the inhibition of cellular proliferation and via stimulation of malignant cell apoptosis.[8]

A case control study conducted in 12 cities throughout China by Lee et al presents a similar outcome to Sodona et al, suggesting that soy isoflavones decrease the risk of prostate cancer. In the study of Lee et al, a food dietary questionnaire was utilized to assess the soy consumption of 133 case patients with newly diagnosed prostate cancer and 256 control subjects between 1989 and 1992.[14]

A case-control study conducted in Taipei, Taiwan between August 1996 - July 1998 by Chen et al primarily investigated the impact of a common folk vegetarian diet, called Zhai or Sue, had on prostate cancer incidence. The study compared survey data related to food consumption, socio-economic factors, occupational exposures, medical history, tobacco and alcohol usage, and level of physical activity. All participants were patients of a Veteran Administration Hospital in Taiwan. The case group consisted of 237 men, 50 years or older, who had a confirmed case of adenocarcinoma of the prostate. The control group contained 481 males, ages 50 years or greater, who had no history of malignancy, benign hyperplasia, cardiovascular disease, hormone dysfunction, inguinal hernia, or kidney stones.[4]

Through analysis of the food frequency questionnaire, which came from a previous study conducted by Sung et al, the researchers determined that the low-fat Zhai vegetarian diet common to Taiwan consisting of soybean products, plain rice, wheat, beans, nuts, and pickles provides protection against prostate cancer. The study reports that the impact of the vegetarian

diet is more pronounced in men with a BMI of $\leq$ 25kg m^{-2}.[4] In fact, Chen et al associate the diet with a 50% decrease in prostate cancer risk in thin males. In addition to the influence of the Zhai diet, the study correlated an increased risk of prostate cancer with men who have a higher income status, are married, physically active, and have a lower BMI.[4]

The third case-control study was published in April 2006 from Hedlund, Bokhoven, Johannes, Nordeen and Ogden titled *Long-Term Dietary Habits Affect Soy Isoflavone Metabolism and Accumulation in Prostatic Fluid in Caucasian Men 1, 2* and is similar to the cohort study conducted by Hedlund et al in June 2005. This earlier study was charged with evaluating the link between habitual diet and the role isoflavone metabolites play in preventing prostate cancer. While the 2005 study assessed both plasma and prostatic fluid isoflavone concentrations and determined that the isoflavonoids may impact prostate health through a reduction of cellular proliferation, the study really only focused on one specific soy metabolite called equol.[13] More recently, new soy metabolites such as dihydrodaidzein (DHD), O-desmethylangolensin (ODMA), have been identified thus leading Hedlund et al (2006) to investigate the influence these newer metabolites have on prostate health.

The objective of the 2006 study was to examine the influence that isoflavones and their metabolites have on cellular proliferation of benign epithelial cells of the prostate and the actual concentrations of genistein, daidzein, dihydrodaidzein (DHD), O-desmethylangolensin (ODMA), and equol in actual study participants. As well, the study examined the impact that these same isoflavonoids had on cancerous prostate cells. Cellular growth or proliferation was measured by way of DNA content comparisons throughout the duration of the study. [15]

Hedlund et al utilized the study data that was collected in the 2005 Seventh Day Adventist cohort study in which participants were placed in either a high-soy or low-soy consumption group, instructed to ingest a soy supplement for a period of one week, and after the established 7-day time frame, blood and prostatic fluid samples were obtained from each participant.[13] Next, Hedlund et al acquired both benign and malignant prostate cells. Through a defined method, the cells were exposed to 5 isoflavonoids (genistein, equol, ODMA, daidzein, and DHD) which mirrored the concentrations of the 2005 participants.[15]

The final outcomes of the 2006 case-control study offer several important points. First, genistein seems to be the most effective isoflavone in terms of decreasing cellular growth of benign prostate cells.[15] Hedlund et al suggest that this antiproliferation effect of benign cells is connected to the induction of apoptosis or programmed cell death and obstruction of cell cycle progression. Furthermore, the data demonstrates that although not all men consuming a soy-rich diet produce equol during digestion, those that do manufacture this soy metabolite present with extremely high prostatic fluid concentrations which is inversely related to prostate disease. This is similar to the findings regarding daidzein which presented with low plasma concentrations but adequate prostatic fluid concentrations leading researchers to associate daidzein with a chemo-protective affect. ODMA is one of the newer daidzein metabolites, it has not been included in past research; however, the results of Hedlund et al suggest that ODMA may serve to prevent growth of benign prostate cells via an alternative mechanism or perhaps through a synergistic effect with other isoflavonoids. Lastly, another new metabolite of daidzein called DHD showed little influence on cellular proliferation of benign prostate cells.[15] Remarkably, several of the isoflavenoids yielded very different responses in connection with malignant prostate cells.

While DHD showed little influence on the growth rate of benign cells of the prostate it proved to be the most substantial deterrent to the proliferation of prostate cancer cells.[15]

The case-control study conducted by Hedlund et al offers valuable insight regarding the impact soy isoflavonoids may have on the proliferation of benign and malignant prostate cells, particularly the newly identified metabolites. Perhaps most importantly, this study demonstrated that the concentration levels of such isoflavonoids needed to impact cellular growth are attainable through the consumption of a high-soy diet.[15]

While many studies focus on the chemoprevention aspect of soy isoflavones, Wang et al opted to examine how soy isoflavones, such as genistein, serve to inhibit the growth of prostate cancer cells. Specifically, Wang et al investigated the combination of external beam radiation therapy and genistein both *in vitro* and *in vivo*.

The *in vitro* studies utilized human prostate cancer cells that were androgen-dependent and androgen-independent. The *in vivo* studies employed nude mice which were implanted with human prostate cancer cells. By exposing both the *in vitro* and *in vivo* malignant prostate cells to genistein and then irradiating them, Wang et al discovered that when genistein was applied 24 hours prior to radiation exposure and routinely thereafter, the combination hindered the synthesis of cellular DNA.[16] When DNA synthesis is inhibited, the prostate cancer cells lose their ability to proliferate which means the cancer is unable to progress or grow.[16]

The authors of this study postulate that the inhibitory effect seen with the combination of genistein and radiation may be the result of several factors including the blockage of the cell cycle and stimulation of apoptosis. Regardless of the mechanism, both the *in vitro* and *in vivo* studies demonstrated the inhibition of cellular division when the cells were exposed to genistein and radiation.[16] Such results were not found when genistein was applied independently. In fact,

when genistein was used alone researchers report that it reduced the growth rate of the primary tumor, but appeared to induce metastatic spread of the disease to regional lymph nodes. This conclusion of Wang et al comes from evaluating the size of the para-aortic lymph nodes of the nude mice. The regional nodes were 40% larger when genistein was used alone as compared to the control group or the group exposed to a combination of genistein and radiation.[16] Based on their data, Wang et al assert that genistein in combination with external beam radiation therapy is the more effective treatment approach for prostate cancer patients than utilizing genistein alone.

The mechanism by which soy isoflavones are thought to affect the development and progression of prostate cancer is not singular. The influence soy isoflavones have over cellular proliferation is complex and not entirely understood. In 2005, X. Wang et al published a study which examined the impact of genistein on non-tumorigenic epithelial cells of the prostate. This study focused on genistein's influence over the signaling of estrogen receptors as previous studies have shown that the signaling of estrogen receptors, specifically estrogen receptor beta, may provide protection to the prostate.[17]

According to X. Wang et al, estrogen receptor signaling is accomplished either through the transformation of estrogen receptor genes or by stimulating signaling pathways.[17] The extracellular signal-regulated kinase cascade (ERK1/2) is of particular importance as it functions in cellular growth and maturation.[17] To explore genistein's ability to signal estrogen receptors and thus minimize prostate cell proliferation, X. Wang et al exposed human prostate epithelial cells to a range of genistein concentrations. The range of 0-100 μmol/L allowed for the analysis of genistein concentrations found in the blood plasma of soy-consuming individuals (1-2.4 μmol/L) as well as supraphysiologic concentrations of soy (>25 μmol/L).[17]

The results of the study show that high levels of genistein, greater than 25 μmol/L are required to reduce the signaling of estrogen receptors for the purpose of growth inhibition. It is suggested that high levels of genistein can modulate the signaling of these receptors and reduce cellular growth by obstructing receptor tyrosine kinase action.[17] The inhibition of the receptor tyrosine kinases can lead to programmed cellular death also known as apoptosis.

In contrast to the supraphysiologic level results, the quantity of genistein associated with relevant concentrations of individuals who subsist on soy seems to stimulate the proliferation of cells and extracellular signal-regulated kinase cascade (ERK1/2) activity.[17] This study offers important insight into method by which genistein impacts cellular growth, but this study is relevant to non-malignant cells of the prostate and its relevance to carcinogenesis are still being evaluated.[17]

In a 2005 published report, Huang et al analyzed the role of genistein as an inhibitor in adhesion formation and cellular invasion or metastatic spread. Matrix metalloproteinases (MMP) are known to regulate cellular invasion.[18] Previous studies have shown that one specific MMP called MMP-2 appears to become up-regulated as prostate cancer is in a progression stage.[18] Huang et al illustrate that genistein serves to inhibit MMP-2 in a variety of cell lines including cells of human prostate. Furthermore, the study demonstrated that genistein inhibits both p38 mitogen-activated protein kinase (MAPK) and transforming growth factor β (TGF-β) activity. The p38 MAPK is needed to stimulate TGF- β which then activates MMP-2. As discussed previously, MMP-2 is shown to increase during the advancement of prostate cancer. Most importantly, the impact of genistein on p38 MAPK, TGF- β and thus MMP-2 is seen at concentrations relevant to daily genistein levels of soy-consuming populations.[18]

Prospective Studies

The most recent results regarding the relationship between soy and prostate cancer are derived from a study conducted by Kurahshi et al. The data from this population-based study, which evaluated soy-based food consumption of 43,509 Japanese male participants ranging in age from 45-74 years, was published in March 2007.[19] Between 1995 and 2004, 307 of the 43,509 participants were diagnosed with prostate cancer, of these diagnoses, 220 were categorized as localized disease, 74 were advanced cases and 13 had an unclassified stage.[19] The results of this study show that genistein and diadzein offer a minimal reduction in prostate cancer risk overall. More significantly, the research demonstrates a dose dependent relationship between soy consumption and localized stages of prostate cancer, thus upholding the assertions of many other researchers who claim that soy functions in a chemoprotective capacity.[19] However, this effect did not carry over to advanced stages of the disease. In fact, the data suggests that one particular soy-based food, miso soup, was associated with increase risk of advanced adenocarcinoma of the prostate.[19] In the end, Kurahashi et al acknowledge the conflict between soy's impact on localized versus advanced stages of prostate cancer and suggest that the isoflavones may delay the progression of disease rather than prevent advancement from occurring.[19] The authors support further study of isoflavones, their mechanism of influence and the timing of their influence on both localized and advanced prostate cancer.

A prospective study published in 2002 employed two models which centered on evaluating the impact lifelong soy consumption has on the incidence of prostate cancer and disease grade. The soy phytoestrogen evaluated was genistein as it is the principal isoflavone of soy.[5] The first model involved exposing Lobund-Wistar rats whose cancer was chemically induced. The specimens received a diet containing either 0 mg genistein/kg, 25 mg genistein/kg,

or 250 mg genistein/kg.[5] Lamartiniere et al concluded that life long exposure to dietary levels of genistein offers a chemo-preventative effect based on the fact that rats exposed to 25 and 50 mg genistein/kg developed prostate cancer at a rate of 77.8% and 63% respectively compared to the 86.4% incidence rate of the control group.[5] Perhaps an even more substantial outcome of life long genistein exposure is the dose dependent reduction of invasive prostate cancer. Rats exposed to 0 mg genistein/kg demonstrated an incidence rate of 77.3% for invasive disease while rats that ingested 25 mg genistein/kg exhibited a rate of 61.1% and those that received 250 mg genistein/kg displayed a 44.4% incidence value.[5]

The second model compared genistein exposure and its impact on the progression of existing prostate disease. The study used mice that developed spontaneous prostate cancer. Fifty percent of the mice developed well-differentiated adenocarcinoma of the prostate while the remaining mice suffered from moderately and poorly differentiated disease grades.[5] The mice consumed either 0, 100, 250 or 500 mg genistein/kg from weeks 5-6 to weeks 28-30.[5] The end result of the study showed that genistein has a dose dependent influence on the development of advanced prostate cancer.[5]

In addition to the two models, Lamartiniere et al analyzed the prostates of transgenic (TRAMP) and non-transgenic mice and found that the signaling pathways for growth hormone and sex steroid is higher in the transgenic population. However, when the transgenic mice received dietary levels of genistein in their food, the activation of these signaling pathways decreased. From this, the authors hypothesize that genistein serves to suppress or slow these processes which play a role in cellular proliferation and thus the development of adenocarcinoma of the prostate.[5]

A cancer research prospective study conducted by Mentor-Marcel served to further investigate the role of soy isoflavones in chemoprevention. In this study, TRAMP mice, which spontaneously develop prostate cancer, were fed a soy-free diet and received phytoestrogen supplementation with genistein at levels of 0, 100, 250 and 500 mg per kg.[6] The mice received this diet between 5-30 weeks of age. At autopsy, the organs of the mice including the prostate and lymph nodes were weighed. Associations were made between the weights of the prostate and the degree of disease progression.[6] The data collected from this prospective study indicate that genistein does inhibit the initiation of advanced stage prostate cancer.[6] The intent of the study was not to determine the mechanism by which the genistein influence occurs but the authors offered several possible mode of influence such as the suppression of tyrosine kinases or transduction signals.[6]

Another prospective study conducted my Mentor-Marcel et al acknowledges the preventative effect of genistein on prostate cancer incidence. The authors delve deeper into this topic by examining the mechanism by which soy isoflavones offer protection against prostate cancer. The study strives to prove the hypothesis that genistein may protect against malignancy progression through the modulation of a specific protein called osteopontin.[20] Osteopontin is derived from macrophages which permeate from prostate cancer cells, specifically in more aggressive tumors which present with an elevated Gleason score.[20] Mentor-Marcel et al refer to previous studies that suggest an association between Osteopontin and disease progression and metastatic spread.[20]

Through the evaluation and comparison of data from various groups of TRAMP mice which were fed specific concentrations of genistein, Mentor-Marcel et al suggest that genistein does have a dose dependent inhibitory influence on Osteopontin in poorly differentiated prostate

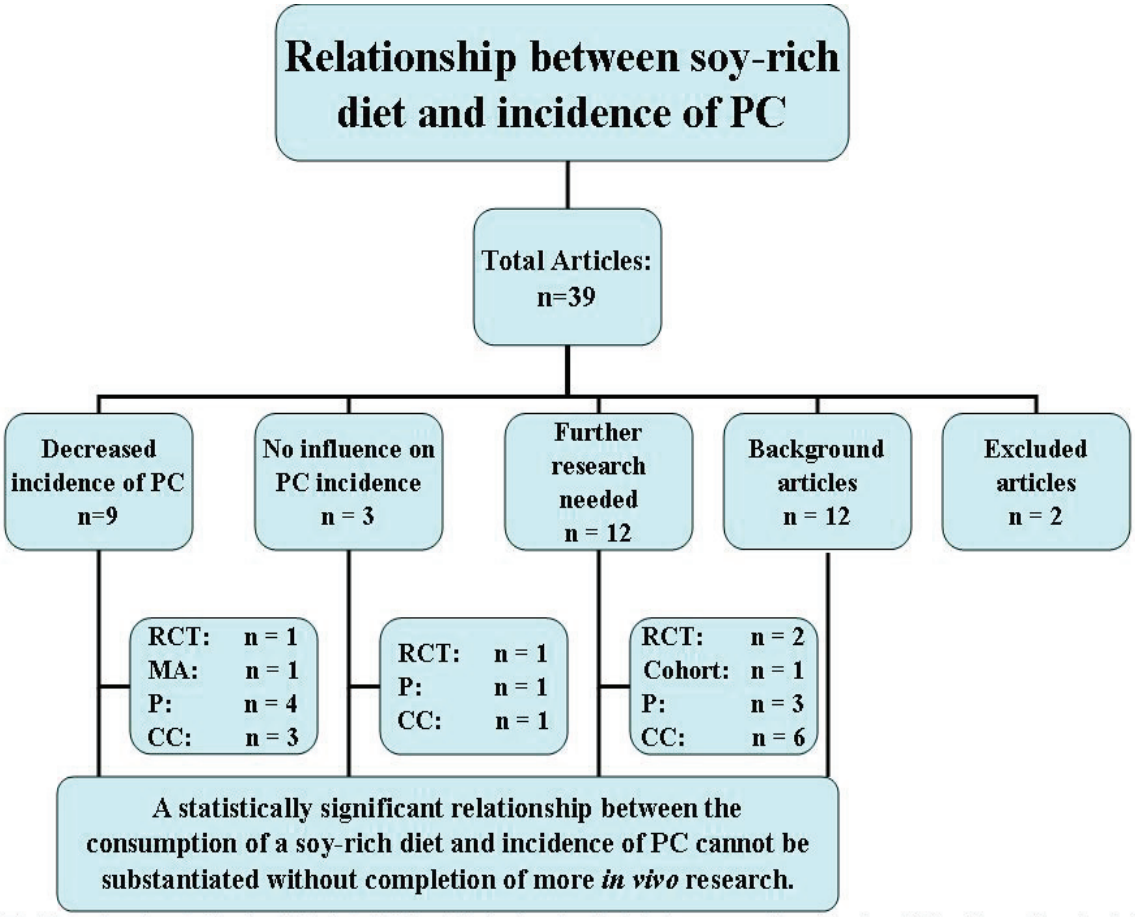
cancers categorized with a Gleason score of 6. As well, by comparing the weight of lymph nodes, the researchers documented a decreased rate of metastatic spread in advanced disease and postulate that genistein is associated with the result.[20] However, the effect on Osteopontin expression and metastasis was not identified in less advanced or aggressive cancers having a Gleason score of less than 6.[20]

Because prostate cancer development and progression is closely linked to androgenic hormones, Yu et al conducted a study which functioned to evaluate the affect of soy isoflavones on the expression of prostate androgens. Specifically, the study focused on prostate androgen-regulated transcript 1 (PART-1). This transcript is known to be elevated in malignant tissue as opposed to benign tissue. Its ability to serve as a prostate cancer biomarker due to its sensitive and early response is of significant value as it could be used as a tool in future chemoprevention studies. As well, it could have the potential to assist in the diagnosis of androgen-dependent prostate cancer, something that the current diagnostic screening method of PSA testing is not always consistent in doing.[21] The results of this study support the theory of Yu et al in regard to soy having an inhibitory influence on PART-1 in association with androgen-dependent prostate cancer.[21] Another isoflavone known as daidzein failed to demonstrate a down-regulating effect as significant of as that of genistein.[21]

CHAPTER V

RESULTS

The results of this evidence based literature review revealed the following: nine Level 1 and 2 studies showed a decreased incidence of PC, while three studies concluded that soy demonstrated no chemo-preventative influence. The majority of studies (12) implied further research was necessary to establish a conclusive association of soy consumption and a reduced incidence of prostate cancer as shown in Figure 1.



RCT - Randomized Control Trial; MA - Meta Analysis; P - Prospective Study; CC - Case Control Study

Figure 1. Systematic Evidence-Based Literature Review Results

CHAPTER VI

DISCUSSION

Evidence in the Literature

Based on the review of literature it is clear that soy isoflavones such as genistein and daidzein possess some degree of chemoprotection against the initiation and advancement of the more aggressive forms of prostate cancer. Most of the studies agree that this effect can be induced using dietary levels of soy similar to the concentrations found in individuals consuming a traditional soy-based Asian diet whose daily intake ranges between 39-47 mg isoflavones.[3] This level is compared to the much lower concentrations of Americans; less than 5mg isoflavones per day.[3]

Weaknesses in the Literature

The current research of soy's role in the reduction of prostate cancer has contributed significant findings, however, there is still more investigation required, particularly in the area of soy's method of influence. Many of the studies were conducted *in vitro* which leads one to question if such results are possible in human specimens. Examining the impact of soy on advanced prostate disease is difficult to accomplish *in vivo* as spontaneous cases of prostate cancer are not common among animals.[6] Even in the cases of prostate cancer in animals, the form of cancer does not fully resemble that of humans.[6] This does present a challenge for researchers as they continue to investigate the role of a soy-based diet on the incidence of advanced adenocarcinoma of the prostate.

Gaps in the Literature

The research data is less clear regarding the mechanism(s) by which the protective effect of soy occurs. The studies provide many hypotheses related to the derivative of soy's influence. The explanations include soy's ability to inhibit the signaling of tyrosine kinases, Osteopontin, matrix metalloproteinases, prostate androgen-regulated transcript (PART-1) and estrogen receptors such as ERK1/2.[5, 6, 17, 18, 21] Others believe that soy isoflavones contribute a protective influence via the induction of apoptosis and obstruction of cell cycle.[15]

The importance of continued research is apparent. Prostate cancer is the most commonly diagnosed cancer in men.[6] In the United States, this disease tends to be more advanced, leading to higher incidences of metastatic disease and mortality. Additional therapies aside from surgery, radiation therapy, and hormone replacement are needed to, at the very least, aid in reducing the progression of prostate cancer. A dietary modification of soy could serve as an effective alternative concomitant therapy for prostate disease.[22] Yet the concentration of isoflavones needed to initiate the protective effect of soy is still in question. Some researchers believe the effect can be achieved with dietary levels while others report that supra-levels of soy are required to gain the desired chemoprotective effect. While the current body of research is very promising, continued study is needed and such study should also include the investigation of possible negative effects of soy consumption such as genetic damage and other possible toxicities.[3]

CHAPTER VII

CONCLUSION

There was confounding amongst the Level 1 studies in that Adams et al indicated no association between soy and prostate health while Schroeder et al demonstrated, through the implementation of a soy supplement, an extension of the PSA doubling time to 1150 days compared to the 445 day doubling time associated with a low-soy diet.[11, 12] Such results offer promise for the treatment and prevention of PC. In considering the twenty-one Level 2 studies, seven showed a decreased incidence of PC while two did not associate soy consumption with a decrease in PC incidence.

The Adventist Health Study 2 is an ongoing health study designed to answer whether the consumption of soy products really helps prevent PC. The results of this study will not be available until 2011, at which time we will have a clearer understanding of the effects of soy products on prostate cancer. It is safe to say that more *in vivo* research must be completed before a statistically significant relationship between the consumption of a soy-rich diet and the incidence of PC can be substantiated.

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Appendix A: Included Articles

Author	Study Year	Title of Article	Research Addresses	Demographics	Type of Study	Findings / Results	Level of Evidence
Adams et al	2004	Soy Isoflavones Do Not Modulate Prostate-Specific Antigen Concentrations in Older Men in a Randomized Controlled Trial.	Whether a 12-month soy isoflavone supplementation would alter serum PSA concentrations in healthy older men.	128 participants enrolled. 112 participants completed the study. Only 81 men consented to allow their sera to be analyzed for PSA.	Randomized, double blinded, parallel-arm, control study.	No evidence that a 12 month, 83 mg/day isoflavone treatment alters serum PSA concentration or velocity in seeming healthy men ages 50 - 80 years. On the other hand, it is possible that isoflavones affect earlier stages in the cancer process or have other effects on tumor growth not reflected in PSA levels. This study suggested that an isoflavone intervention does not slow tumor growth or other prostate conditions that affect circulating PSA concentration.	Level 1
Schroder et al	2005	Randomized, Double-Blind, Placebo-Controlled Crossover Study in Men with Prostate Cancer and Rising PSA: Effectiveness of a Dietary Supplement.	The effect of a dietary supplement in comparison with placebo on the rate of increase of prostate-specific antigen (PSA).	49 pts w/ hx of prostate cancer and rising PSA levels after radical prostatectomy (n=34) or radiotherapy (n=15) were treated for 10 weeks with 4 week washout periods. The treatment consisted of soy, isoflavones, lycopene, silymarin and antioxidants as main ingredients. Changes in the rate of incr of PSA was the primary parameter of efficacy.	Randomized, double blinded control study.	The soy-based dietary supplement utilized in this study was shown to delay PSA progression after potentially curative treatment in a significant fashion. The data suggest that there was an increase in the doubling time of the PSA levels with the study participants consuming the supplement; therefore, hopefully prolonging the progression (delay) of tumor growth.	Level 1
Yan et al	2005	Meta-analysis of soy food and risk of prostate cancer in men	A systematic review evaluating the epidemiologic studies available to date that related soy consumption to the risk of prostate cancer in men. Secondly, the purpose was to provide a quantitative evaluation in a standardized form permitting a numerical analysis across the studies.	Thorough Medline search for English-language publications, supplemented with hand-searching of articles' bibliographies and non indexed medical and professional journals, on epidemiologic studies of soy and prostate cancer. 2 cohorts & 6 case-controls met the inclusion / exclusion criteria.	Meta-Analysis (Review Article)	Consumption of soy food showed a lower risk of prostate cancer in men.	Level 1

Allen et al	2001	Soy Milk Intake in Relation to Serum Sex Hormone Levels in British Men	The aim of this study was to assess the relationship between dietary soy intake and sex hormone levels. Speculation was that the isoflavones found in soy milk may affect the progression / development of prostate cancer.	696 British Caucasian men with a wide range of soy intakes. Subjects were divided into 3 categories of soy milk intake (none, <= 0.25 pint/day, >=0.50 pint/day). The men were meat eaters, lacto-ovo-vegetarians, and vegans. Meat eaters ate atleast 3 meat servings / week. Vegetarians did not eat meat or fish, but did consume dairy products and / or eggs. Vegans did not eat any animal products. Subjects were excluded if they had a self reported history of cancer or were taking meds that influenced hormone levels. Soy milk intake was measured using a validated semi-quantitated food frequency questionnaire, and serum hormone concentrations were measured by immunoassay.	Case control study. Cross-sectional Analysis	Soy milk intake was not associated with serum sex hormone concentrations (such as testosterone, free testosterone, androstanediol glucuronide, sex hormone-binding globulin, or luteinizing hormone) among free-living Western men.	Level 2

Bektic et al	2003	Androgen Receptor Regulation by Physiological Concentrations of the Isoflavonoid Genistein in Androgen-Dependent LNCaP Cells is Mediated by Estrogen Receptor Beta.	The potential of genistein to modulate androgen receptor (AR) expression and transcriptional activity in the human androgen-sensitive prostate cancer cell line LNCaP.	LNCaP, PC-3, & DU-145 culture cells were obtained from the American Type Culture Collection. Androgen receptor (AR) expression at mRNA and protein level was analyzed by real-time RT-PCR and immunoblot, respectively. In conditioned media, PSA was measured by a microparticle enzyme immunoassay (MEIA). Binding of genistein to the AR was tested in a radioligand-binding assay and reporter gene co-transfection assay was employed to investigate AR activity.	Prospective Study	Using concentrations of genistein that have been detected in sera of Asian men on regular soy-diet, they found down-regulation of androgen receptor at both mRNA and protein level. The relative binding affinity to the androgen receptor (AR) was below 4% when compared to methyltrienologe (R1881), and there was no modulation of AR transcriptional activity by genistein concentrations up to 1 microM. Inhibition of PSA secretion after genistein treatment was demonstrated. It was postulated that the genistein action on AR is mediated through Estrogen Receptor - Beta (ER-Beta). Using physiological concentrations of genistein, they showed down-regulation by genistein in prostate cancer cells occurring via ER-Beta. They believe that this results in a modified response to hormonal stimuli and may help to explain the low incidence of prostate cancer in the Asian population.	Level 2
Dillingham et al	2005	Soy Protein Isolates of Varying Isoflavone Content Exert Minor Effects on Serum Reproductive Hormones in Healthy Young Men 1, 2	The purpose of this study was to investigate the effects of soy protein of varying isoflavone content on a wide profile of serum reproductive hormones in a sample of healthy young men between 20 - 40 years old. The study included a focus on the isoflavone component of soy by investigating soy protein isolates high and low in isoflavone content in relation to a milk protein isolate.	35 men, ages 20 - 40 years old, with a BMI of 19 - 29 kg/m2 consumed milk protein isolate (MPI), low-isoflavone protein isolate (SPI) (low-iso SPI; 1.64 +/- 0.19 mg isoflavone/day), and high-iso SPI (61.7 +/- 7.35 mg isoflavones/day) for 57 days each. There were 28 day washout periods between each 57 day study. 24 hour urine samples were drawn and serum was collected on days 1, 29, and 57 of each treatment.	Randomized crossover intervention study. (Non-blinded study).	Soy protein, regardless of isoflavone content, decreased dihydrotestosterone (DHT) and DHT/testosterone with minor effects on other hormones, providing evidence of some effects for soy protein on hormones.	Level 2

Fang et al	2005	Reversal of Hypermethylation and Reactivation of *p16INK4a, RARBeta,*and *MGMT* Genes by Genistein and Other Isoflavones from Soy	This article addresses whether genistein reverses the DNA hypermethylation and reactivation of genes responsible for the development of prostate and many other cancers.	"For the time-course study, cells were treated with 5 micromol/L of genistein in fresh culture medium on days 0, 2, 4, and 5. Prostate cancer cell lines LNCaP and PC3 were obtained from the American Type Culture Collection and were grown in RPMI 1640 containing 10% fetal bovine serum. KYSE 150, LNCaP, and PC3 cells were treated with 10 or 20 micromol/L of genistein for 6 days as described above."	Case-control study	"Genistein (2-20 micromol/L) reversed DNA hypermethylation and reactivated *RARBeta, p16INK4a, and MGMT* KYSE 510 cells. Genistein also inhibited cell growth at these concentrations. Reversal of DNA hypermethylation and reactivation of RARBeta by genistein were observed in KYSE 150 cells and prostate cancer LNCaP and PC3 cells. Genistein (20 - 50 micromol/L) dose-dependently inhibited DNA methyltransferase activity, showing substrate- and methyl donor-dependent inhibition. Biochanin A and daidzein were less effective in inhibiting DNA methyltransferase activity, and reactivating RARBeta, and in inhibiting cancer cell growth. In combination with trichostatin, sulforaphane, or 5-aza-d-Cyd, genistein enhanced reactivation of these genes and inhibition of cell growth." "The above results indicate that genistein and related soy isoflavones reactivate methylation-silenced genes, partially through a direct inhibition of DNA methyltransfersase, which may contribute to the chemopreventative activity of dietary isoflavones."	Level 2
Handayani et al	2006	Soy Isoflavones Alter Expression of Genes Associated with Cancer Progression, Including Interleukin-8, in Androgen-Independent PC-3 Human Prostate Cancer Cells 1	This study investigated the effects of soy isoflavone concentrate (ISF) on growth and gene expression profiles of PC-3 human prostate cancer cells.	In vitro study done using the PC-3 cell line. NovaSoy was dissolved in dimethyl sulfoxide (DMSO) and PC-3 cells were treated w/ varying ISF concentrations for 48 hrs. Control cultures were treated with DMSO vehicle alone with a final concentration of 0.1%.	Case control study.	ISF decreased viability and caused a dose-dependent inhibition of DNA synthesis in PC-3 cells. DNA synthesis was inhibited by 50% at 52mg/L. "The data shows that ISF inhibits the growth of PC-3 cells through modulation of the cell cycle progression and expression of genes involved in cell cycle regulation, metastasis, and angiogenesis".	Level 2

Hedlund et al	2005	Long-Term Dietary Habits Affect Soy Isoflavone Metabolism and Accumulation in Prostatic Fluid in Caucasian Men1,2	The purpose of this study as to investigate the influence of long-term dietary habits on daidzein metabolism in healthy Caucasian men (19 - 65 years old), and secondly to compare plasma and prostatic fluid concentrations of 5 isoflavonoids: genistein, daidzein, equol, dihydrodaidzein, and O-desmethylangloensin.	25 men b/w ages 19 - 65 y/o who had consumed >=30 mg of soy isoflavones/day for at least 2 years (All were Seventh Day Adventist(SDA)). 20 men between ages 19 - 65 years old who had consumed long-term low amounts of soy (<=5mg soy isoflavones/day for at least 2 years (10 were SDA and 10 non-SDA)).	Cohort Study	Long-term dietary habits can significantly affect the intestinal metabolism of daidzein (specifically the conversion to equol). Also, it is likely that the high concentrations of isoflavonoids in prostatic fluid are related to the ability of soy to reduce prostate cancer risk.	Level 2
Huang et al	2005	Genistein Inhibits p38 Map Kinase Activation, Matrix Metalloproteinase Type 2, and Cell Invasion in Human Prostate Epithelial Cells	This study shows that p38 mitogen-activated protein kinase (MAPK) is necessary for transforming growth factor B (TGF-B)-mediated increases in matrix metalloproteinase type 2 (MMP-2) activity and cell invasion. MMP's degrade extracellular matrix proteins & mediate cell invasion, & metastatic behavior in a variety of cell types. MMP-2 has been shown to be upregulated during prostate cancer cell progression, in the human prostate. Also in this study, it was shown that genistein inhibits activation of p38 MAPK, MMP-2, and cell invasion.	PC-3, PC-3M, DU-145 cell lines, HPV, transformed normal and cancerous cell lines were utilized and maintained at 37 degrees C. in a humidified atmosphere of 5% CO2, w/ weekly media changes. All cells were monitored for *Mycoplasma* Cell viability was determined by counting the number of tryptan blue-excluding cells under an inverted microscope, using a hemocytometer. Several experiments were completed utilizing transinfection models, zymography, cell invasion assays, cell lysis and Western blot analysis.	Case-control study	This study showed that p38 MAPK is necessary for TGF-B-mediated induction of MMP-2 and cell invasion in prostate cancer. It was also shown that genistein blocks activation of p38 MAPK, thereby inhibiting processes closely linked to metastasis and does so at concentrations associated w/ dietary soy consumption. Lastly, it is believed that if genistein is exerting this activity in humans, then it would support a causal relationship to epidemiologic findings. Further investigation is needed to support this belief.	Level 2

Kurahashi et al	2007	Soy Product and Isoflavone Consumption in Relation to Prostate Cancer in Japanese Men.	This article attempts to determine if there is any preventative effect against prostate cancer through a population-based prospective study.	A validated questionaire was completed by 43,509 Japanese men ages 45 - 74 years and generally have high intake of isoflavones and low incidence of prostate cancer. During follow-up, 307 men were newly diagnosed w/ PC, of which 74 cases were advanced, 220 cases were organ localized, and 13 cases were of undetermined stage.	Prospective Study	"Intakes of genistein, daidzein, miso soup, and soy food were not associated with total PC; however, these four items decreased the risk of localized PC. Whereas, positive associations were seen b/w isoflavones and advanced PC. Dose-dependent decreases in risk of localized cancer were seen in men > 60 years. Overall, isoflavone intake was associated with a decreased risk of localized PC."	Level 2
Lamartiniere et al	2002	Genistein Chemoprevention: Timing and Mechanisms of Action in Murine Mammary and Prostate	Genistein in dietary physiological amounts could regulate biochemical reactions of the prostate; therefore, inhibiting prostate cancer development. This was completed in two different rat models.	Lobund-Wistar rats were exposed to 0, 25, 250 mg genistein /kg AIN-76A diet at different stages & intervals. 14 TRAMP mice fed 250 mg genistein/kg diet. 14 TRAMP and nontransgenic mice were fed AIN-76A diet only.	Prospective Study	Physiological amounts of genistein can protect against chemically induced and spontaneously developing prostate cancers. Dietary genistein regulates, with specificity, sex steroid receptor and growth factor ligand and receptor mRNA expression. They speculate that these gene products contribute to the chemoprevention of prostate cancer by genistein - leading to their belief that postpubertal genistein exposure protects against prostate cancer development & can regulate sex steroid and growth factor signaling in animal models & possibly in men.	Level 2
Lee et al	2003	Soy and Isoflavone Consumption in Relation to Prostate Cancer Risk in China	The effect of soy food consumption and isoflavones (genistein & daidzein) on the risk of prostate cancer in China.	133 cases and 265 age and residential community matched controls between ages 50 - 89 (interviewed in person).	Case-control study	A reduced risk of prostate cancer associated with the consumption of soy foods and isoflavones.	Level 2
Mentor-Marcel et al	2005	Dietary Genistein Improves Survival and Reduces Expression of Osteopontin in the Prostate of Transgenic Mice with Prostatic Adenocarcinoma (TRAMP)1	Osteopontin (OPN) may have a role in the transition from clinically insignificant tumors to metastatic prostate cancer (PC). This study was done to test whether Genistein will exert its preventative effect by inhibiting OPN expression.	80, 68, and 30 TRAMP mice were fed AIN-76A diet containing 0, 250, & 500 mg genistein /kg body weight. Organ weights were obtained.	Prospective Study	Genistein had a dose-dependent, significant inhibitory effect on OPN transcript levels in prostates displaying advanced prostate cancer (PD; score = 6; $P = 0.05$). This study was consistent with the idea that dietary genistein may delay the progression from benign to malignant tumors by inhibiting the OPN expression.	Level 2

Mentor-Marcel et al	2001	Genistein in the Diet Reduces the Incidence of Poorly Differentiated Prostatic Adenocarcinoma in Transgenic Mice (TRAMP)1.	The hypothesis that dietary genistein might prevent prostate cancer progression in a transgenic mouse model (TRAMP).	Starting at 5-6 weeks of age: transgenic male mice were fed a phytoestrogen-free diet (AIN-76A) containing 0, 100, 250, or 500 mg of genistein per kg AIN-76A (25, 10, 17, and 7 mice/group respectively). The mice remained on this diet until 28 - 30 weeks of age. Mice were weighed weekly, and at necropsy, selected organs were weighed and prepared for histopathological evaluation. Serum levels of genistein were obtained and compared with those found in Asian men on a regular soy diet.	Prospective Study	The percentage of transgenic male mice that developed poorly differentiated prostatic adenocarcinoma was reduced in a dose-dependent manner by dietary genistein.	Level 2
Nagata et al	2001	Effect of Soymilk Consumption on Serum Estrogen and Androgen Concentrations in Japanese Men	The effects of soy consumption on serum levels of steroid hormones in men.	35 teachers and students in nurse school were randomly assigned to either a control group or experimental group. The control group was required to consume 400 mL of soymilk daily for 8 weeks, while the control group maintained their usual diet. Both groups were asked to maintain their usual lifestyle. Serum samples were drawn every 2 weeks from nonfasting subjects and analyzed. Subject's weights were also recorded.	Randomized control study. (Non-blinded study).	The results showed that there was a significant difference between the two groups in terms of changes in the serum estrone concentrations which tended to decrease in soy-supplemented group and increase in the control group over time. It is therefore believed that soymilk consumption may modify circulating estrone concentrations in men. It is not entirely clear whether decreased estrone concentrations lead to a reduction in the risk of prostate cancer. It's only been suggested that estrogens may be involved in the development of prostate cancer.	Level 2

Sonoda et al.	2004	A case-control study of diet and prostate cancer in Japan: possible protective effect of traditional Japanese diet.	Whether the intake of a traditional Japanese diet, consisting of soy products and other foods, is related to the risk of prostate cancer.	84 cases were obtained from the Department of Urology of Tsukuba University Hospital in Ibaraki and 56 cases form the Department of Urology of Sapporo Medical University Hospital in Hokkaido. All cases has confirmed histological diagnosis of adenocarcinoma of the prostate. Controls were selected from the Department of Oral Surgery, Ophthalmology, or Dermatology of the same hospital as cases and matched to each case by age (+/- 5 yrs).	Case-control study	Natto (fermented soybeans) is especially rich in phytoestrogens and they have a significant protective effect against prostate cancer. Also, energy intake increased the risk of prostate cancer to some extent. There was insufficient evidence to draw a conclusion regarding protective effects of vegetables against prostate cancer. Green tea showed no significant reduction in risk for prostate cancer. The traditional Japanese diet, consisting of soybean products and fish, may be protective against prostate cancer	Level 2
Spentzos et al	2003	Minimal Effect of a Low-Fat/High Soy Diet for Asymptomatic, Hormonally Naïve Prostate Cancer Patients	To investigate the effects of a low-fat diet or a low-fat diet with the addition of soy in asymptomatic, hormonally naïve prostate cancer patients with rising PSA levels.	18 invaluable patients who had undergone primary therapy for biopsy-proven prostatic adenocarcinoma. Patients had to exhibit rising PSA values above the postradiation / postprostatectomy nadir value with the second higher than the first. They had to have a PSA >= 0.5ng/ml if treated with prostatectomy & 1.0 ng/ml if treated by radiation therapy.	Prospective Study	A low-fat diet supplemented w/ soy did not result in a significant decline in PSA levels. The addition of soy protein had a medial effect on the time to progression (TTP). Also, a possibly undesirable effect associated with the administration of soy was an increase in IGF-I serum levels.	Level 2

Wang et al	2006	Genistein modulates prostate epithelial cell proliferation via estrogen - and extracellular signal-regulated kinase-dependent pathways.	This study examines the molecular mechanisms by which genistein modulates proliferation of the nontumorigenic prostate epithelial cell line RWPE-1. RWPE-1 cells are nontumorigenic human prostate epithelial cells.	Human prostate epithelial cell line (RWPE-1) was maintained in a keratinocyte serum-free medium and supplemented with bovine pituitary extract and epidermal growth factor in a humidified incubator. Cells were plated and treated with genistein. Incubated for another 24 hours. After incubation, cells proliferation was determined. Additional proliferation tests were completed. Additional components to this experiment were done as follows: immunoblotting, annexin/propidium iodide staining, morphology, and statistical analysis.	Case-control study	Low concentrations of genistein (0 - 12.5 micromol/L) significantly increased cell proliferation and extracellular signal-regulated kinase (ERK 1/2) activity (P< .01) in nontumorigenic human prostate epithelial cell line (RWPE-1) cells, while higher concentrations (50 and 100 micromol/L) of genistein significantly inhibited cell proliferation and ERK1/2 activity (P<.001). A similar biphasic effect of genistein on MEK1 activity, and ERK1/2, was also observed. It is thought that genistein modulates RWPE-1 cell proliferation and signal transduction via an estrogen-dependent pathway involving ERK1/2 activation.	Level 2
Wang et al	2006	Prostate Cancer Treatment is Enhanced by Genistein *In vitro* and *In Vivo* in a Syngeneic Orthotopic Tumor Model.	This article addresses the combination of genistein and irradiation to treat RM-9 orthotopic prostate cancer cells placed in syngeneic C57BL/6 mice with a fully functional immune system (*in vitro study)*.	4 groups of 6 or 7 mice; group 1 = control group, group 2 = genistein treatment only, group 3 = radiation treatment only, and group 4 = genistein and radiation treatment combined.	Case-control study	"It was shown that genistein combined with prostate tumor irradiation caused a greater inhibition of tumor growth and increased control of spontaneous metastasis to para-aortic lymph nodes, increasing the survival of the mice. On the other hand, treatment w/ genistein alone increased metastasis to lymph nodes. The study was repeated in orthotopic RM-9 prostate tumors in syngeneic C57BL/6 mice and the same results were observed." Article is excluded since it deals with the combo of genistein and irradiation in the treatment of prostate cancer.	Level 2

Yu et al	2003	Genistein and daidzein downregulation prostate androgen - regulated transcript-1 (PART-1) gene expression induced by dihydrotestosterone in human prostate LNCaP cancer cells	The effect of soy isoflavones on the expression of prostate androgen regulated transcript 1 (PART-1) - which is a newly discovered androgen-induced gene that may represent a novel androgen -dependent prostate cancer tumor marker, in human prostate cancer cells in vitro.	Genistein, daidzein, and glycitein were used to determine whether there was any inhibition of the androgen-induced expression of the PART-1 gene. Each, at different concentrations, were added to either LNCaP, DU 145, and PC-3 human prostate cancer cell lines, and incubated for 24 hours.	Prospective Study completed *in vitro.*	"Genistein at 50 micro-mol/L completely inhibited PART-1 gene expression induced by 5 alpha-dihydrotestosterone (DHT) at 0.1 and 1 nmol/L. Daidzein also dose-dependently inhibited the expression of the PART-1 transcript induced by DHT, but its effect was less dramatic than that of genistein. Glycitein did not inhibit DHT-induced expression of the PART-1 transcript". "The findings of this study suggest that PART-1 may serve as a candidate biomarker for evaluating the efficacy of soy products on androgen-dependent prostate cancer prevention."	Level 2
Chen et al	2005	Diet, vegetarian food, and prostate carcinoma among men in Taiwan	Associations between consumption of low-fat folk style vegetarian food, BMI, income, being married, coffee consumption, and physical activity levels with prostate cancer in a case-controlled Taiwan study population.	237 histology-confirmed prostate carcinoma patients at the Division of Urology, Department of Surgery at a veterans medical centre in Taipei, Taiwan between Aug. 1996 - July 1998, with 481 controls that were frequency matched by age, for their consumption of vegetarian food, namely soybean products, rice, wheat protein and other vegetables. All subjects were >= 50 years of age.	Case-control study	The study showed that prostate carcinoma cases were more likely to occur in educated individuals who engaged in more physical activities and had a lower BMI. The seemingly protective effect related to the increased intake of folk vegetarian food with very low fat content is particularly significant for thin Asian men (in this study population).	Level 2

Hedlund et al	2005	Prostatic Fluid Concentrations of Isoflavonoids in Soy Consumers Are Sufficient to Inhibit Growth of Benign and Malignant Prostatic Epithelial Cells In Vitro.	"There were 4 goals of this study: (1) to compare the dose-dependent effects of 5 isoflavonoids on the growth of benign prostatic epithelial cells (PrEC) to the in vivo concentrations present in plasma and prostatic fluid (PF) of caucasian men consuming soy; (2) to assess the relative potencies of several additional isoflavonoids on PrEC and LNCaP cell growth (including glycitein, 6H-ODMA, 3HD, 6HD, 8HD, and C4HE); (3) to determine if these compounds act by blocking cell cycle progression or by inducing apoptotic cell death; (4) to determine if these compounds are likely to act in an additive or synergistic manner with each other when used at concentrations present in actual PF samples."	25 men between ages 19 - 65 years old who had consumed >=30 mg / day of soy isoflavones for at least 2 years (All were Seventh Day Adventist (SDA)). 20 men between ages 19 - 65 years old who had consumed long-term low amounts of soy (<=5mg soy isoflavones/day for atleast 2 years (10 were SDA and 10 non-SDA)). All men consumed a soymilk-based beverage daily for 7 days containing 42-60 mg isoflavones. Approx. 5-6 hours after consumption of the last soy drink, blood was drawn and prostatic fluid (PF) collected by prostatic massage. See article for demographics regarding isoflavonoids, cell cultures, growth assays, flow cytometric analysis of apoptosis via active caspase 3, & flow cytometric analysis of cell cycle.	Case-control study	"PF concentrations of genistein, equol, and daidzein (but not ODMA or DHD) were often within the ranges that reduce PrEC growth in vitro. Profound differences in sensitivities were observed with LNCaP cells. The hydroxydaidzeins, C4HE, and 6H-ODMA had significant inhibitory effects at 0.00010 on PrEC growth (but not LNCaP). Glycitein had significant effects on both. Reductions in cell growth were typically associated with both changes in cell cycle distribution and Caspase 3 activation. When 5 isoflavonoids were used in combination at concentrations present in PF samples, synergistic effects were observed. The profound differences in sensitivities of PrEC to these compounds along with their synergistic effects suggest that multiple metabolites in vivo may be optimal for preventing prostate cancer."	Level 2
Jacobsen et al	1998	Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States).	Whether there is a relationship between soy milk, a beverage containing isoflavones, and prostate cancer incidence.	225 incident cases of prostate cancer in 12,395 California Seventh Day Adventist.	Prospective Study	Frequent consumption of soy milk was associated with a 70% reduction of the risk of prostate cancer (p=0.03).	Level 2

Bektic et al	2005	Molecular Effects of the Isoflavonoid Genistein in Prostate Cancer	This article reviews the current literature on the molecular mechanisms of genistein in relation to its effects on prostate cancer cells.	N/A	Literature Review	The authors of this article believe that genistein may be an anticarcinogenic along with sharing many other positive effects. It is also thought that a definitive statement that genistein is a chemopreventive and/or therapeutic agent cannot be made at this time; although, there is sufficient evidence for protective effects to warrant further investigation and clinical trials.	Level 3 - Background Article
Branca et al	2005	Health Effects of Phytoestrogens	This is a literature review of phytoestrogens and their effects on the health of humans. The article provides some detail as to how the phytoestrogens are broken down by the gut and how they may work in humans.	N/A	Literature Review	There is a lot of speculation/belief, and little scientifically proven evidence, as to the complete understanding of how phytoestrogens work in the human body. Accurate selection of compounds and their doses has not been established.	Level 3 - Background Article
Chan et al	2005	Role of Diet in Prostate Cancer Development and Progression	This article is a review, summarizing data for some of the most consistently observed dietary associations for prostate cancer. It also briefly considers the possible post-diagnostic effects of nutrition on prostate cancer progression/survival. And, lastly the article comments on current areas of controversy for future research focus.	N/A	Review Article of many studies	"Soy is a rich resource for isoflavones, specifically genistein and daidzein, and equol, which can interrupt cell growth pathways and angiogenesis, and therefore might slow the development and progression of cancer. Isoflavones have also been shown to influence the production, metabolism, and excretion of testosterone and estrogens, hormones that can play important roles in the development and spread of prostate cancer. Unfortunately, there is little soy consumption in the Western populations, where many of the large epidemiologic studies with the largest follow-up, most cases, and detailed dietary histories have been conducted. In the few epidemiologic studies, in which soy or its active phytochemicals have been examined, results have often suggested and inverse association, though not always statistically significant".	Level 3 - Background Article
Ganry et al	2004	Phytoestrogens and prostate cancer risk	This review article evaluates the effects of phytoestrogens on prostate cancer risk by reviewing analytical epidemiologic data.	N/A	Literature Review	"Few studies showed any protective effect between phytoestrogen intake and prostate cancer risk.	Level 3 - Background Article

Holzbeierlein et al	2005	The role of soy phytoestrogens in prostate cancer	This review addresses the effects of soy at the molecular level as well as to review the in-vivo effects.	N/A	Literature Review	Isoflavones, genistein in particular, appear to have an inhibitory effect on prostate cancer cell proliferation, although the mechanisms for this effect are unclear. More studies in humans needs to be performed.	Level 3 - Background Article
Klein et al	2004	Update on chemoprevention of prostate cancer	This article reviews the results of the Prostate Cancer Prevention Trial, the design of other large scale trials, and advances in understanding of the molecular mechanisms underlying the effect of other promising agents.	N/A	Literature Review	This article does not discuss the effects of soy on the incidence of prostate cancer. This article suggest that "several studies have shown that the major components of soy, including genistein, daidzein, and their metabolites inhibit benign and malignant prostatic epithelial cell growth, downregulate androgen -regulated genes, and reduce tumor growth in some animal models. They also state that recent work suggest that the previous mentioned effects are mediated in part by inhibition of insulin-like growth factor I, reducing in cell-cycle arrest and induction of apoptosis, and by proteasome inhibition." They also mention that one study has shown that "supplemental dietary soy or isoflavones do not appear to have any effect on serum PSA levels in men with hypercholesterolemia, despite a beneficial effect LDL levels."	Level 3 - Background Article
Kucuk et al	2002	Chemoprevention of prostate cancer	Chemoprevention of prostate cancer by administering natural chemicals; such as, soy isoflavones - Genistein	N/A	Backgorund Article	In other studies and Phase I of this authors study, *in vitro* effects of genistein on PSA synthesis and secretion of prostate cancer cells were demonstrated. When LNCaP cells were treated *in vitro* with genistein, there was a significant decrease in the amount of PSA secretion. Considering that the effects of soy isoflavone supplements on serum PSA were not known in the clinical setting, a Phase II clinical trial was conducted to define the effects of soy isoflavone supplementation on prostate cancer and serum PSA. Significant activity was found of soy isoflavones in patients with advanced prostate cancer.	Level 3 - Background Article

McCarty et al	2006	Isoflavones made simple - Genistein's agonist activity for the beta-type estrogen receptor mediates their health benefits.	This article is a literature review showing how genistein is a relatively potent agonist for estrogen receptor beta (ERbeta).	N/A	Literature Review	High intakes of soy protein can actually boost serum IGF-I--> now known to have important cancer promotional activity. "ERbeta expression tends to be lost as prostate cancer progresses - may mean that as a therapy for pre-existing prostate cancer, genistein will have at best transient efficacy; thus its greater potential may be for chemoprevention."	Level 3 - Background Article
Miltyk et al	2003	Lack of significant genotoxicity of purified soy isoflavones (genistein, daidzein, and glycitein) in 20 patients with prostate cancer	The assessment of the potential genotoxicity of a purified soy unconjugated isoflavone mixture in men with prostate cancer.	20 male volunteers > 40 years old with stages B, C, or D prostate neoplasia recruited from the Research Triangle area of North Carolina.. Subjects were required to be >= 3 weeks post-surgery (major) and 4 weeks post-radiation or fully recovered. Other exclusion criteria was required. Each subject was given a purified isoflavone mixture (70% unconjugated isoflavones containing genistein, daidzein, & glycitein). Initial dose was 300 mg genistein for 28 d, and then 600 mg for 56 days.	Prospective Study completed *in vitro* and *in vivo.*	It was observed that isoflavones (unconjugated) were capable of inducing genetic damage *in vitro*. Although, genetic damage was not observed in subjects treated with a purified soy unconjugated isoflavone mixture.	Level 3 - Background Article
Santillo et al	2006	Role of Vitamins, Minerals and Supplements in the Prevention and Management of Prostate Cancer	This article is a review of the current literature on the use of vitamin E, vitamin A, selenium, zinc, soy, lycopene, pomegranate juice, green tea, and omega-3 fatty acids in the complimentary / alternative treatment of prostate cancer patients and those at risk for the disease.	N/A	Literature Review	The authors of this article believe that there is no definitive proof that any of the nutritional supplements discussed can impact the course of prostate cancer or its development. They also believe that by simply taking a standard daily multivitamin a patient should receive sufficient amounts of vitamins and minerals with out risking the over utilization of vitamins, minerals, and supplements which can lead to numerous side effects."	Level 3 - Background Article

Usui et al	2006	Pharmaceutical Prospects of Phytoestrogens	This review article focuses on the molecular properties and pharmaceutical potential of phytoestrogens. Very little is mentioned in regards to prostate cancer.	N/A	Literature Review	"The classical phytoestrogens, such as genistein and daidzein have a higher affinity to ER beta than alpha, and ER beta is strongly expressed in the prostate. Phytoestrogens might act as antioxidants and/or inhibit blood vessel growth, which is essential for tumor expansion." "The ability to metabolize phytoestrogens to more potent compounds affects the estrogenicity of soy-related products."	Level 3 - Background Article
Whittemore et al	1995	Prostate Cancer in Relation to Diet, Physical Activity, and Body Size in Blacks, Whites, and Asians in the United States and Canada.	This study attempted to evaluate the roles of diet, physical activity patterns, body size, and migration characteristics on risk in blacks, whites, and Asian-Americans in LA, San Francisco, Hawaii, Vancouver, and Toronto.	A population-based case control of PC among blacks, whites, and Asian-Americans in LA, San Francisco, Hawaii, Vancouver, and Toronto was completed using a questionaire and personal interview w/ 1655 black, white, Chinese-American, and Japanese-American case pts diagnosed during 1987 - 1991 w/ PC and 1645 population-based control subjects matched by age, ethnicity, and region of residence. Sera samples were taken from 1127 control subjects and analyzed for PSA levels to permit comparison with case patients for serological evidence of PC.	Population-based case control study	"A positive statistically significant association of PC risk and total fat intake was found for all ethnic groups combined. The data derived from this study support a causal role in PC for saturated fat intake, but suggest that other factors are largely responsible for interethnic differences in risk."	Level 3 - Background Article

Wietrzyk et al	2005	Phytoestrogens in Cancer Prevention and Therapy - Mechanisms of their Biological Activity.	This article provides a brief overview of the effects (preventative, antitumor as well as carcinogenic and tumor-stimulating) of phytoestrogens on various tumor types.	N/A	Literature Review	This article discusses various outcomes of soy research. Outcomes such as: "a low-fat diet with subsequent addition of soy supplement did not result in a significant decline in PSA levels. The addition of soy protein had a modest effect on time-to-progression (TTP). A potentionally undesirable effect associated with the administration of soy was an increase in IGF-I serum levels." "Also the molecular mechanism(s) by which genistein elicits its effects on prostate cancer cells has not been fully elucidated." "It was found that genistein regulated the expression of genes that are critically involved in the regulation of cell growth, cell cycle, apoptosis, cell signaling transduction, angiogenesis, tumor cell invasion, and metastasis." "The combo of soy phytochemical concentrate (SPC) and black tea synergistically inhibited tumorigenicity, final tumor weight and metastases to lymph nodes *in vivo.* The combo of SPC and green tea synergistically inhibited the final tumor weight and metastasis and significantly reduced serum concentrations of both testosterone and DHT *in vivo;* leading one to believe that such a combo could be an effective nutritional regimen in prostate cancer prevention."	Level 3 - Background Article
<u>Level Of Evidence</u>							
Level 1	=	RCT's					
Level 2	=	Cohorts					
Level 3	=	Background					

Appendix B: Excluded Articles

Author	Study Year	Title of Article	Research Addresses	Demographics	Type of Study	Findings / Results	Level of Evidence
Denmark-Wahnefried et al	1999	Prostate cancer and diet	This article is a review of an article written by Fair et al, entitled "Cancer of the prostate: a nutritional disease."	N/A	Literature Review	This review article implies that the Fair et al should be more careful when using the term "linoleic acid" when implying omega-3 fatty acids. Also, there was an implication made that the most abundant food source of linoleic acid in the American diet was fish oil, when indeed it is vegetable oils. This article was excluded due to it having nothing to do with Soy and its effect on the incidence of prostate cancer.	EXCLUDED ARTICLE
Singh et al	2006	Prostate Cancer Chemoprevention by Silibinin: Bench to Bedside	This article addresses chemoprevention of prostate cancer by the use of Silibinin, a polyphenolic flavonoid isolated from the seeds of milk thistle *(Silybum marianum)*.	N/A	Literature Review	This review article was excluded due to it having nothing to do with Soy and its effect on the incidence of prostate cancer. Silibinin does appear to have some promising effects on the chemoprevention of prostate cancer.	EXCLUDED ARTICLE

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