Evaluation of Soy and Black Raspberry Constituents for Prostate Cancer Prevention and Therapy

BY

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THESIS

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To my parents, David and Betty Eskra, for their love, support and lifelong sacrifices that made completion of this dissertation possible.

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LIST OF ABBREVIATIONS

ABC	ATP-binding cassette transporter
ADT	Androgen deprivation therapy
AR	Androgen receptor
BAX	Bcl-2 associated protein X
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra large
BCRP	Breast cancer resistance protein
BPH	Benign prostatic hyperplasia
BRB	Black raspberry
BSA	Bovine serum albumin
CBZ	Cabazitaxel
CI	Combination index
CIS	Carcinoma *in situ*
CRPC	Castration resistant prostate cancer
Cy-3-Rut	Cyanidin-3-rutinoside
CYP	Cytochrome P450 enzyme
Daid	Daidzein
DAPI	4',6-diamidino-2-phenylindole
DBOMF	Dibenzoxymethylfluorescein
DHT	Dihydrotestosterone
DMSO	Dimethyl sulfoxide
DX	Docetaxel
EA	Ellagic acid
EDC	N-ethyl-N-(dimethyl-aminopropyl)-carbodiimide
EDTA	Ethylenediaminetetraacetic acid

LIST OF ABBREVIATIONS (continued)

EdU	5-ethynyl-2'-deoxyuridine
EQ	Equol
ER	Estrogen receptor
EtOH	Ethanol
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDP	Guanosine diphosphate
Gen	Genistein
GI	Gastrointestinal
GTP	Guanosine triphosphate
HPLC	High performance liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
IP	Intraperitoneal
IU	International units
IV	Intravenous
LDS	Lithium dodecyl sulfate
LHRH	Luteinizing hormone releasing hormone
LPH	Lactase phlorizin hydrolase
MAP	Microtubule associated protein
Mcl-1	Myeloid Cell Leukemia 1
MDR	Multidrug resistance
MNU	Methylnitrosourea
MNU + T	Methylnitrosourea plus testosterone
MRP1	Multi-drug resistance related protein 1
MS	Mass spectrometry
ND	Not determined

LIST OF ABBREVIATIONS (continued)

NBL	Noble rats
ND	Not determined
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	N-hydroxysuccinimide
PARP	Poly ADP ribose polymerase
PBS	Phosphate-buffered saline
PCA	Protocatechuic acid
PCNA	Proliferating cell nuclear antigen
P-gp	P-glycoprotein
PIN	Prostatic intraepithelial neoplasia
PSA	Prostate specific antigen
PSMA	Prostate specific membrane antigen
PTX	Paclitaxel
PVDF	Polyvinyl difluoride
RU	Response units
SCID	Severe combined immunodeficiency
SD	Standard deviation
SPR	Surface plasmon resonance
SRB	Sulforhodamine B
T + E2	Testosterone plus 17β-estradiol
TBST	Tris-buffered saline tween
TCA	Trichloroacetic acid
TGD	Tumor growth delay
UroA	Urolithin A
WT	Wild type
WU	Wistar-Unilever rats

CONTRIBUTION OF AUTHORS

This dissertation represents unpublished work conceptualized, designed, and performed by the author, Jillian N. Eskra, in collaboration with a number of individuals whose contributions are described below.

CHAPTER I. INTRODUCTION

- Figures 1, 2, 3, 5, 8, 9, 11 and 14 are used with permission from applicable sources. License agreements for each figure can be found in Appendix D.

CHAPTER III. EFFECTS OF BLACK RASPBERRIES AND THEIR CONSTITUENTS ON RAT PROSTATE CARCINOGENESIS AND HUMAN PROSTATE CANCER CELL GROWTH

- Michael Schlicht – Animal studies, necropsies, and tissue collection
- Zhongfa Liu – Measurement of protocatechuic acid in rat prostate tissue
- Maarten Bosland – Animal studies, necropsies, and prostate pathology

CHAPTER IV. IN VITRO EFFECTS OF BLACK RASPBERRIES AND THEIR CONSTITUENTS ON EFFICACY OF TAXANE CHEMOTHERAPY

- Alaina Dodge – Assistance with cell proliferation assays
- Monday Akpanabiatu – Preparation of black raspberry extract

CHAPTER V. MICROTUBULE POLYMERIZING EFFECTS OF ELLAGIC ACID AND IMPACT ON THE EFFECTIVENESS OF TAXANE CHEMOTHERAPY

- Alaina Dodge – Assistance with polymerization assays and western blots
- Michael Schlicht – Animal study, necropsies, and tissue collection
- Maarten Bosland – Animal study, necropsies, and tissue collection

CHAPTER VI. COMBINATION EFFECTS OF SOY ISOFLAVONES AND TAXANE CHEMOTHERAPY

- Michael Schlicht – Animal study, necropsies, and tissue collection
- Maarten Bosland – Animal study, necropsies, and tissue collection

SUMMARY

The preventive and therapeutic effects of soy and black raspberries constituents on prostate cancer were assessed in this dissertation using *in vitro* and *in vivo* experimental approaches.

Black raspberries (BRBs) are an abundant source of bioactive compounds and have especially high levels of ellagic acid and anthocyanins, polyphenolic compounds with anti-cancer activity. Numerous experimental and clinical reports indicate that BRBs can inhibit tumor initiation and progression of several cancer types, including oral, esophageal, colon, mammary and skin cancers (1). However, no publications have reported effects of BRB as an anti-cancer agent for prostate cancer. Based on the described mechanisms of BRBs and their bioactive constituents (2), we hypothesized that BRBs could prevent the development of prostate cancer and would enhance efficacy of chemotherapeutic drugs used to treat late stage prostate cancer.

In Chapter III, we evaluate the chemopreventive capacity of BRBs and their constituents against prostate cancer using *in vitro* and *in vivo* methods. Following dietary administration of BRBs to Noble rats, we were able to detect a BRB metabolite (protocatechuic acid) in rat prostate tissue, demonstrating bioavailable levels of BRB compounds. In spite of this, dietary BRBs did not inhibit prostate carcinogenesis in two different rat models. Ellagic acid inhibited prostate cell growth *in vitro*, but only at high concentrations. No substantial *in vitro* effects on prostate cancer cell proliferation, colony formation, or migration were observed for BRB extract, cyanidin-3-rutinoside or protocatechuic acid. We found that anthocyanin uptake by prostate cancer cells is low, which may account for the absence of inhibitory effects *in vitro.*

We next conducted the experiments described in Chapters IV and V, in order to investigate the combination effects of BRBs constituents and taxane chemotherapy for CRPC. We did not observe any enhancement or inhibition of taxane cytotoxicity by BRB extract or protocatechuic acid *in vitro*. We demonstrate for the first time that ellagic acid can interfere with the dynamics of

microtubules, the target of taxane chemotherapeutic drugs, and may reduce taxane efficacy. However, dietary administration of ellagic acid did not reduce effectiveness of docetaxel in a xenograft mouse model. Considering the collective results from Chapters III, IV and V, it appears that BRB constituents are not effective for preventing to treating prostate cancer, which is likely due to low bioavailability of active compounds to prostate tissue.

In Chapter VI we investigated the combination effects of taxane chemotherapy with soy isoflavones genistein, daidzein and the metabolite equol. Isoflavones have pleiotropic anti-cancer activity and several studies have shown that genistein can increase sensitivity of cancers to radiation and chemotherapy (3). Therefore, we hypothesized that soy isoflavones would synergistically enhance the efficacy of taxanes for castration-resistant prostate cancer. Genistein, daidzein and equol did not have any effect on tubulin polymerizing activity or growth inhibitory effects of taxanes on prostate cancer cell lines. However, genistein inhibited CYP3A4 and drug efflux transporter activity, suggesting that it may reduce metabolism of taxanes and inhibit drug efflux by tumors *in vivo* to increase cytotoxicity of taxanes. We explored this possibility using a xenograft mouse model with 22Rv1 human prostate cancer cells, but we did not find any evidence of enhancement of inhibition of taxane efficacy or toxicity with dietary administration of genistein.

The goal of this dissertation was to investigate the overall hypothesis that bioactive dietary polyphenols can prevent the development and progression of prostate cancer, and may be used as adjuvants to enhance conventional treatment strategies. Disappointingly, we found no evidence to support this and our results suggest that low bioavailability of dietary compounds is a highly restrictive factor for their use in prostate cancer.

I. INTRODUCTION

A. Prostate Cancer

i. Epidemiology of prostate cancer

Prostate cancer is one of the most frequently diagnosed cancers and a major cause of cancer-related death among men worldwide, with the United States ranking high in rates of both incidence and mortality (Figure 1) (4-6). In 2016, over 180,000 new cases of prostate cancer are anticipated to occur in the U.S., accounting for 21% of new cancers in males, making prostate cancer the most common non-cutaneous malignancy in American men (7). Prostate cancer incidence is higher in black men (208.7 per 100,000) compared to other races (white: 123.0 per 100,000; Asian: 67.8 per 100,000; Hispanic: 112.1 per 100,000). Mortality rates are also significantly higher for black men (47.2 per 100,000) compared to others (white: 19.9 per 100,000; Asian: 9.4 per 100,000; Hispanic: 17.8 per 100,000) (7).

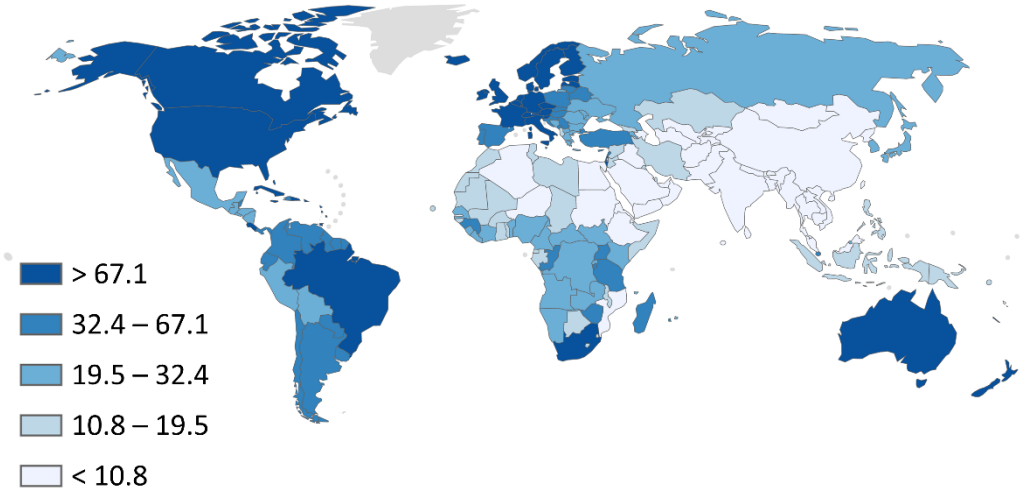

Figure 1. Worldwide prostate cancer incidence. Estimated age-standardized prostate cancer incidence rates per 100,000 men. Source: GLOBOCAN 2012. Reproduced with permission from Ferlay *et al* (5).

Although the causes of prostate cancer are not well understood, several risk factors have been associated with this malignancy. Risk of prostate cancer is most strongly related to age. Based on evidence from autopsy studies, the incidence of prostate cancer in men ages 30-40 is estimated to be less than 10%, which increases substantially to approximately 60% in men ages 70-80 (8). Geographical location and race/ethnicity also have a large impact on prostate cancer risk (9). Incidence is much lower in Asian populations, but increases in Asian immigrants to the U.S., possibly due to environmental or lifestyle changes (10). Dietary intake of red meat and high-fat dairy products has been linked to increased prostate cancer risk (11, 12), and a high body-mass index has been associated with risk of developing aggressive cancer (13, 14). However, the exact contributions of diet and obesity to prostate carcinogenesis are not clear. Additionally, exposure to sex hormones and inflammation of the prostate have been implicated as risk factors for prostate cancer (15, 16).

ii. Prostate pathophysiology

The prostate is a male reproductive gland situated around the urethra directly below the urinary bladder (Figure 2) that is responsible for production of seminal fluid containing prostate specific antigen (PSA), which can leak into the blood as a result of prostatic disease conditions. Circulating PSA expression levels are measured clinically in the detection of prostate cancer and to monitor treatment response and disease progression in prostate cancer patients (17). The prostate is divided into four zones (anterior, peripheral, central and transition zone) and consists of epithelial, basal, and neuroendocrine cell types surrounded by stromal myofibroblasts (18, 19). Growth and size of the prostate changes with age and are regulated by exposure to endogenous androgens and androgen receptor (AR) signaling (20). The prostate is vulnerable to disease, particularly prostatitis, benign prostatic hyperplasia (BPH), and cancer

(21). BPH is a non-neoplastic enlargement of the prostate, which usually develops from the transition zone (22). BPH is not considered a risk factor for prostate cancer or a precancerous lesion, and is frequently co-identified in men with prostate cancer (23), whereas prostatitis, an acute or chronic inflammation of the prostate gland, may be a predisposing factor in prostate carcinogenesis (24).

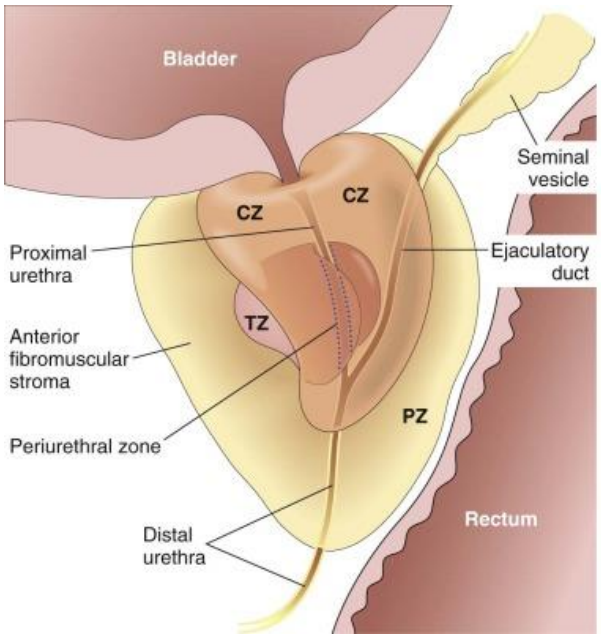

Figure 2. Anatomy of the adult prostate
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iii. <u>Pathogenesis and progression of prostate cancer</u>

Prostate cancer is a slowly developing malignancy that arises through successive genetic alterations. The majority of cancers identified in the prostate are adenocarcinomas that arise from epithelial cells (25). Progression to malignancy is generally considered to be a multistep process, whereby inflammation leads to development of prostatic intraepithelial neoplasia (PIN),

followed by adenocarcinoma (Figure 3) (26). PIN is a premalignant lesion which is typically found in the peripheral zone, preceding the development of cancer (27). The latency period between appearance of the first neoplastic lesions and the emergence of aggressive or metastatic tumors is often very long, possibly 10 years or more (28).

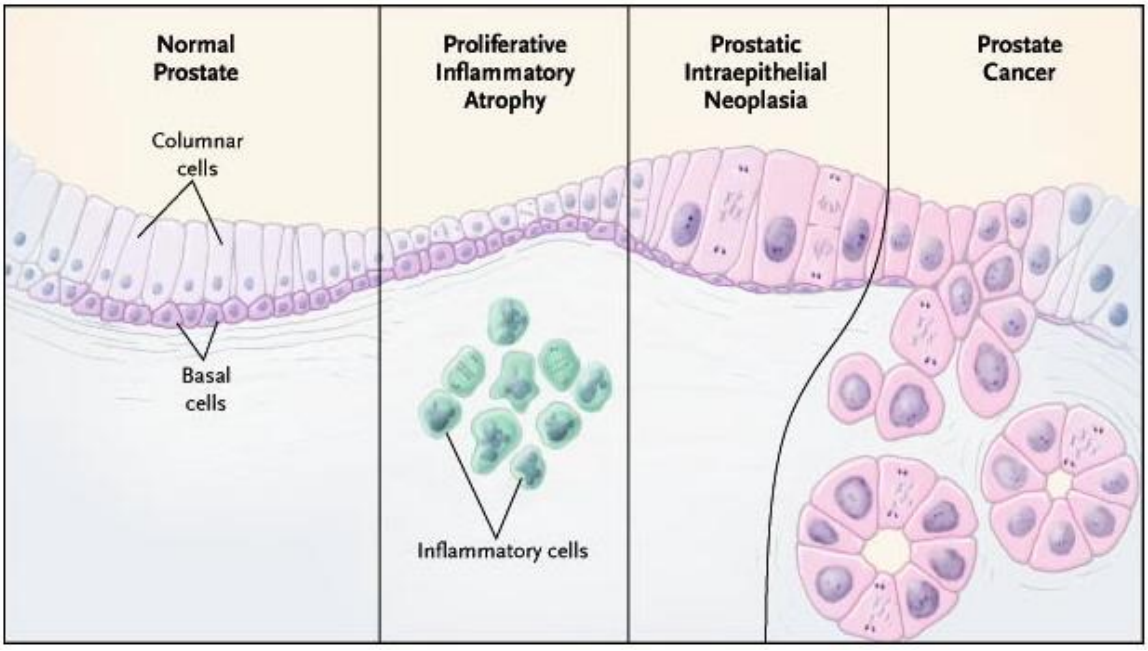


Figure 3. Multistep process of prostate carcinogenesis and cancer progression. Reproduced with permission from Nelson WG, De Marzo AM, and Isaacs WB. Prostate cancer. *N Engl J Med* 2003; 349: 366-81, Copyright Massachusetts Medical Society.

Once prostate cancer develops it continues to progress through multiple stages, gradually acquiring aggressive characteristics that warrant distinct treatment (Figure 4). Early, organ-confined prostate cancer is usually treated by radical prostatectomy and/or radiotherapy in the U.S., which is typically successful for 70-80% of patients (29, 30). However, a variable subset of patients will relapse within 5-10 years (31). Therapeutic agents after recurrence routinely

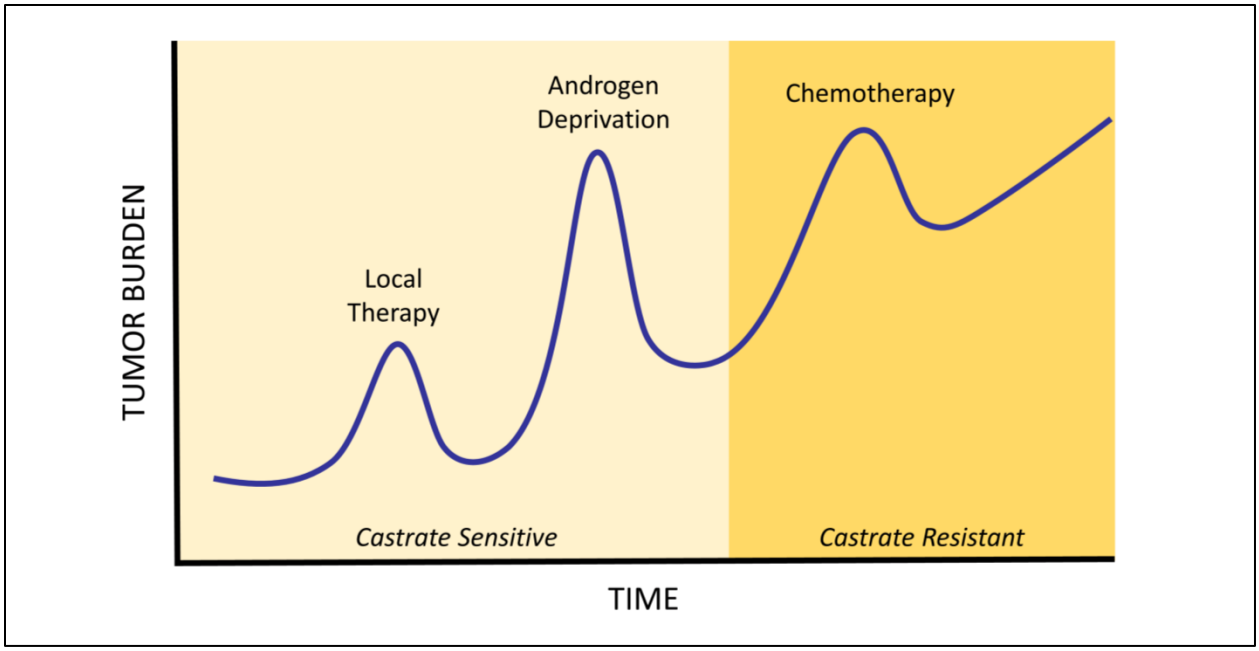


Figure 4. Treatment and progression timeline of prostate cancer

involve hormonal therapies referred to as androgen deprivation therapy (ADT), which includes treatment with anti-androgens or luteinizing hormone releasing hormone (LHRH) agonists and antagonists (32-34). Although initially effective, nearly all tumors will lose responsiveness to ADT and will gain the ability to proliferate in an androgen-depleted environment, becoming castration-resistant prostate cancer (CRPC)[1] (38, 39). Taxane-based chemotherapy is administered at this stage with docetaxel as the first-line treatment and cabazitaxel as a second-line option, but chemotherapy increases overall survival by only a few months (40-42). Recently

[1] The terms 'androgen-independent' and 'hormone-refractory' have been used interchangeably to describe CRPC, but based on the current understanding of androgen signaling, CRPC is the preferred term to describe 'prostate cancer that has progressed despite castrate levels of serum testosterone' (36, 37).

some subsequent treatments have become available that typically prolong life for another few months (see below).

iv. Androgen deprivation therapy

The concept of androgen deprivation therapy (ADT) was first introduced in the 1940s when it was demonstrated that advanced prostate cancer could be dramatically inhibited by eliminating androgens through castration (43). It is now commonly accepted that the initial stages of prostate cancer are driven by AR activity and that blocking AR signaling reduces local and metastatic tumor growth. Since the initial discovery, numerous hormonal therapeutic agents have emerged allowing ADT to remain a mainstay in the treatment of advanced prostate cancer. Clinically used hormonal therapies in the U.S. include LHRH agonist and antagonists, anti-androgens, and inhibitors of dihydrotestosterone (DHT) biosynthesis (Figure 5) (44). LHRH therapeutics act on the hypothalamus-pituitary axis to downregulate the expression of LHRH receptors in the pituitary gland, leading to suppression of testosterone production in the testes (45). A number of LHRH-targeting agents are available including leuprolide, goserelin, and triptorelin (46). Anti-androgens, including flutamide and bicalutimide, are AR antagonists that block actions of testosterone in the prostate by competitively binding to the AR, thereby preventing binding and activity of DHT (47). Androgen biosynthesis inhibitors act on enzymes involved in testosterone metabolism; agents included in this category include ketoconazole and abiraterone, a recently developed inhibitor of CYP17, and finasteride, an inhibitor of 5α-reductase, although this is used to treat BPH rather than prostate cancer. Androgen biosynthesis inhibitors, as well as second generation anti-androgens (enzalutamide), are usually administered after patients become resistant to initial hormonal therapies, but earlier treatment with these agents is being explored (48).

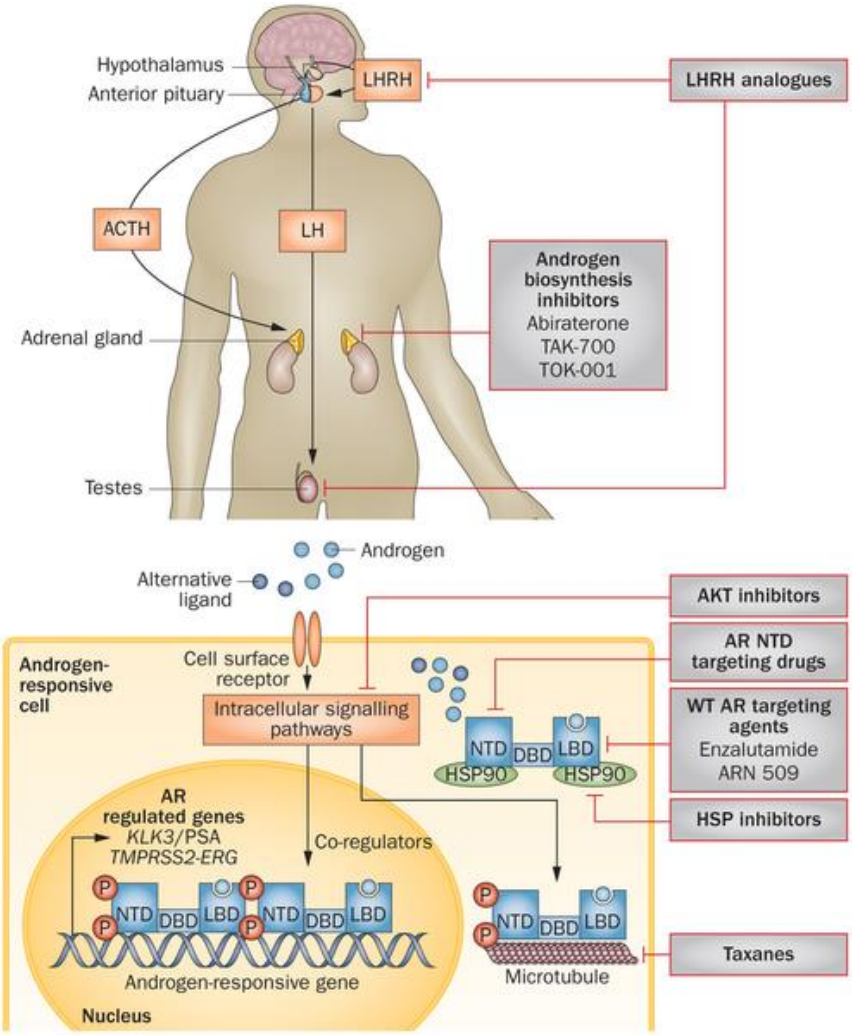


Figure 5. AR targeting treatment strategies in CRPC. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Clinical Oncology.* Wong Y, Ferraldeschi R, Attard G, and de Bono J. Evolution of androgen receptor targeted therapy for advanced prostate cancer. 11: 365-376, copyright 2014.

v. Treatment of castration-resistant prostate cancer

CRPC occurs following ADT and is indicated by rising PSA levels and/or symptoms of the development of metastatic disease, despite castrate levels of testosterone (less than 50 ng/mL) (49). The standard treatment for men with CRPC consists of taxane-based regimen of chemotherapy. Docetaxel is administered as first line therapy, with cabazitaxel as a second line option. Prior to the FDA approval of docetaxel for prostate cancer in 2004, there were no therapeutic options available for patients who failed ADT (50). The use of docetaxel has been successful in prolonging patient survival, however the survival benefit is on average only a few additional months (51). Drug failure may be attributable to rapid development of resistance to docetaxel, after which cabazitaxel can be administered (52). Cabazitaxel was shown to provide therapeutic benefit in CRPC patients who had been previously treated with docetaxel (53).

Since the approval of docetaxel, several novel agents have been approved for CRPC including the second generation taxane cabazitaxel, enzalutamide (second-generation anti-androgen), abiraterone (CYP17 inhibitor), radium-233 (alpha-emitting radiopharmaceutical), and Sipleucel-T (an immunotherapeutic approach). Despite the recent increase in treatment options, and potential combination or sequential regimens, CRPC patients ultimately succumb to the disease, with a median survival of 15 to 18 months post initial chemotherapy (54).

vi. Diet, supplements and prostate cancer

It is common for prostate cancer patients to make lifestyle changes and dietary modifications following their diagnosis. Studies have reported that nearly half of men with prostate cancer will make changes to their diets, typically increasing consumption of fruits and vegetables (55-58), in the hope to have a substantial impact on disease progression, recurrence and mortality.

Some studies have reported evidence that increasing dietary consumption of fruits and vegetables can provide therapeutic benefit for prostate cancer patients, but overall there is little solid evidence for this. In a small one-arm intervention trial of 14 participants with early-stage patients undergoing active surveillance, a plant-based diet plus stress reduction reduced serum PSA levels and rates of disease progression (59), but in a larger randomized study (93 participants) there was no effect on PSA levels or PSA velocity after 2 years of intervention (60). A nutritional intervention study in men with indolent prostate cancer demonstrated that dietary modifications induced changes in expression of genes related to carcinogenesis comparing pre- and post-intervention prostate biopsy specimens (61). Additionally, a diet high in fruits and vegetables was associated with reduced mortality in prostate cancer patients compared to patients on a “Western” style diet in the Physicians' Health Study (62).

B. Taxanes

i. Background

Taxanes belong to the broader class of microtubule-targeting drugs, which includes a number of chemically diverse compounds – many of which are derived from natural sources – that inhibit proliferation by disrupting microtubule dynamics (63). Currently, three taxanes are approved for clinical use: docetaxel (Taxotere, Sanofi-Aventis), paclitaxel (Taxol, Bristol-Myers Squibb), and cabazitaxel (Jevtana, Sanofi-Aventis). The first taxane to be identified was paclitaxel in 1971, as a result of a NCI-USDA program between 1960 and 1981 that collected and screened 115,000 plant extracts for anti-cancer activity (64). Crude bark extract of the Pacific yew tree (*Taxus brevifolia*) displayed cytotoxic effects, from which paclitaxel was isolated and identified as the active ingredient (65, 66). Paclitaxel entered clinical trials in 1984 and was approved in the following years for treatment of ovarian and breast cancer, and ultimately

became the most successful and profitable chemotherapeutic agent in history (64, 67). Due to the high demand, natural sources of paclitaxel were depleted and manufacturers began producing it semi-synthetically, which led to the eventual development of the other semi-synthetic taxanes docetaxel and cabazitaxel (68). All three of these drugs have a similar diterpenoid structure (Figure 6) and share a common mechanism of action, as described below (69).

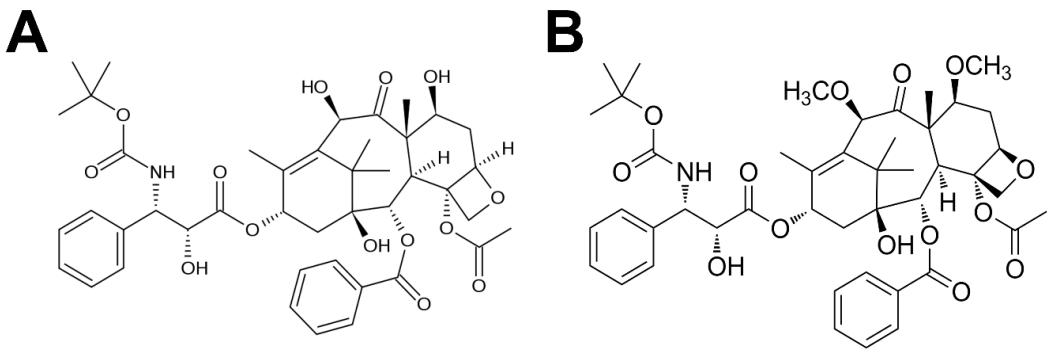


Figure 6. Chemical structures of docetaxel (A) and cabazitaxel (B).

ii. <u>Mechanisms of action</u>

Taxanes are microtubule stabilizing agents[2] that exert their antitumor activity by binding to β-tubulin. Microtubules are major components of the cytoskeleton that play a critical role in signaling, migration, intracellular trafficking, and most importantly they facilitate the separation of chromosomes during mitosis (70). Microtubules are composed of heterodimers of α- and β-tubulin subunits that polymerize to form a cellular network of hollow filaments (71). These

[2] Due to their mechanism of action, taxanes are also referred to as 'microtubule targeting agents', 'mitotic spindle poisons', 'microtubule inhibitors', and 'tubulin binding agents'.

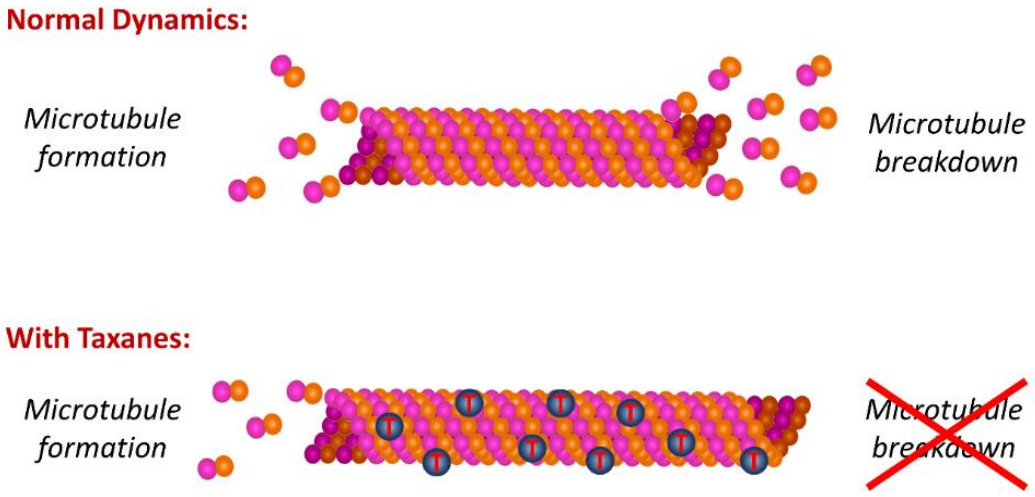


Figure 7. Taxanes inhibit normal microtubule dynamics. The binding of taxanes to microtubules, specifically to the β-tubulin component of the microtubules, prevents their ability to depolymerize and therefore stop cells from proceeding through the cell cycle and they arrest in G2/M phase (72).

filaments are highly dynamic and structures are continually undergoing assembly and disassembly – a process known as 'dynamic instability' (73). This process is essential for chromosomal alignment and segregation in metaphase and anaphase of mitosis. All members of the taxane family stabilize microtubules by binding to the taxoid-binding site of β-tubulin, which promotes polymerization and prevents disassembly of αβ-dimers (Figure 7) (74, 75). This suppression of microtubule dynamics impairs function of the mitotic spindle and cell cycle progression is blocked at the spindle-assembly checkpoint (Figure 8A) (76). The prevailing hypothesis has been that apoptosis is induced in mitotically arrested cells that cannot complete M phase (77, 78). But it has also been shown that cell death can occur following an abnormal exit from mitosis known as 'mitotic slippage' (79, 80). Treatment with low concentrations of

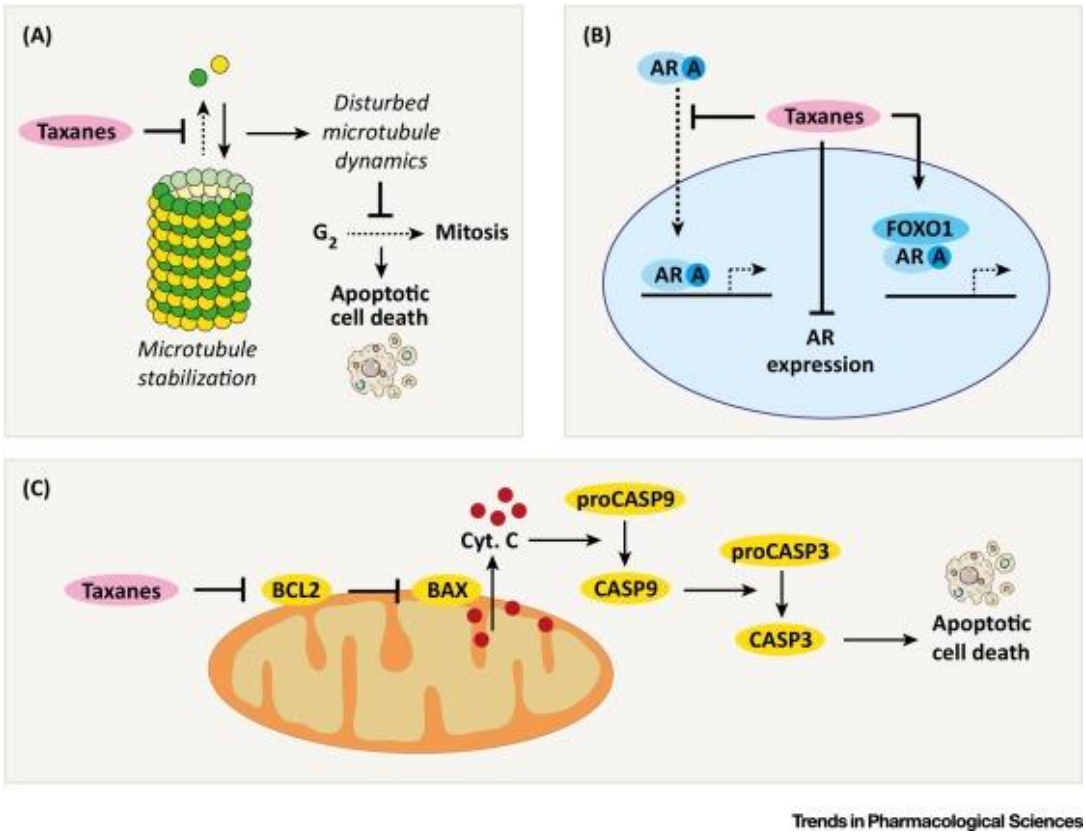


Figure 8. Mechanisms of action of the anti-tumor activity of taxanes. Taxanes have been described to exert their antitumor efficacy via distinct modes of action. (A) Taxanes bind to microtubules and thereby prevent their disassembly, resulting in G2/M cell cycle arrest and apoptosis (51, 81). (B) Alternatively, taxanes are able to inhibit androgen receptor (AR) transcriptional activity by constraining AR expression (82), blocking AR nuclear translocation (83), and facilitating FOXO1-mediated repression of AR transcriptional activity (84). (C) Finally, taxanes may inhibit the expression of anti-apoptotic Bcl-2, favoring apoptotic cell death through the relief of BAX mediated cytochrome C release (85). Figure and description reprinted from *Trends in Pharmacological Sciences*, Vol. 3, No. 6, Kroon J, Kooikman S, Cho NJ, Storm G, and van der Pluijm G, Improving Taxane-Based Chemotherapy in Castration-Resistant Prostate Cancer (2016), with permission from Elsevier.

taxanes induces the formation of multipolar spindles in mitotic cells, but cells proceed past the spindle-assembly checkpoint resulting in aneuploidy of daughter cells and subsequent cell death (Figure 9) (64, 86). The formation of multipolar spindles has been demonstrated mostly for docetaxel and paclitaxel (87-89), but a similar phenomenon has been observed by others in MCF-7 breast cancer cells (90) and by our group in 22Rv1 cells treated with 10 nM cabazitaxel (Figure 10).

More recently, an alternate mechanism of action on interphase microtubules by taxanes has been proposed to account for seemingly paradoxical effects of taxanes on non-dividing cells (91). Taxanes have toxic effects on neuronal cells with a low mitotic index and can cause severe neuropathy in treated patients, suggesting that cellular processes other than mitosis are affected by taxanes (92, 93). Indeed, studies have demonstrated that taxane-stabilized microtubules can induce apoptosis via alterations in normal cellular trafficking, signaling, and microtubule-mediated transport (94).

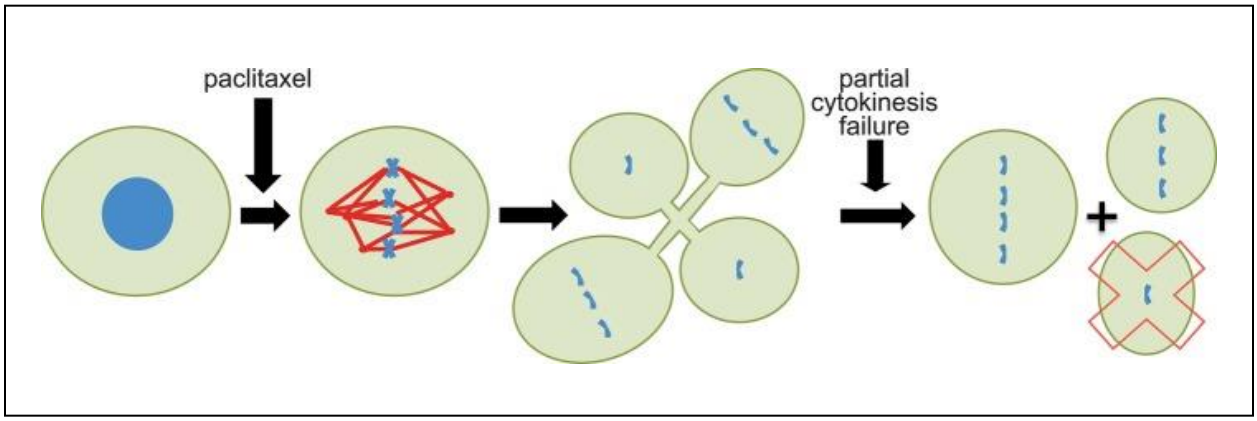


Figure 9. Paclitaxel kills tumor cells by inducing multipolar divisions. Cells entering mitosis in the presence of paclitaxel form abnormal spindles that contain additional spindle poles. Rather than mounting a long-term mitotic arrest, these cells enter anaphase and divide their chromosomes in multiple directions. However, a portion of the cytokinetic furrows often fail, and two or three daughter cells are usually produced. Chromosome segregation is randomized due to multipolar division followed by partial cytokinesis failure. The resultant daughter cells are aneuploid, and a portion of these die (red X), presumably due to loss of one or more essential chromosomes. Figure and description republished with permission of American Society for Cell Biology, from How Taxol/paclitaxel kills cancer cells, Beth A. Weaver, Vol. 25, 2014; permission conveyed through Copyright Clearance Center, Inc.

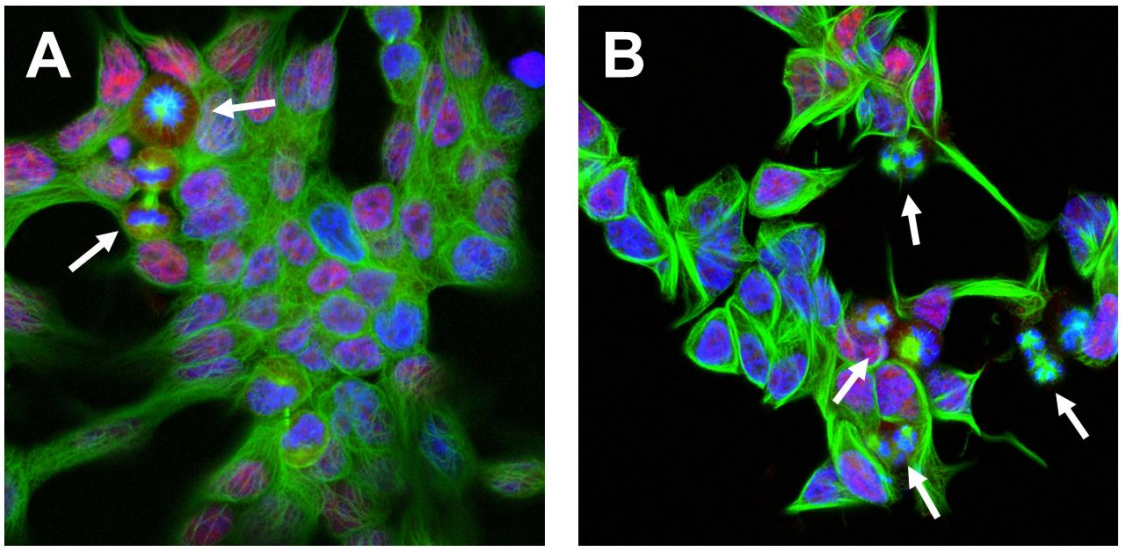


Figure 10. Cabazitaxel interferes with the cytoskeleton and mitotic spindle structure. 22Rv1 control (A) and 10 nM CBZ (B) treated cells. In control cells, microtubules appear as fine filaments throughout the cell. In mitotic control cells have well-formed bipolar spindles and duplicated chromosomes at opposite ends. Taxane treated induces formation of abnormal spindles; many cells will have multipolar or monopolar spindle. Microtubules, green; androgen receptor, red; nucleus, blue.

The inhibition of intracellular trafficking appears to be a particularly relevant function for inhibiting the growth of prostate cancer cells. Evidence from several recent studies suggests that the effectiveness of taxanes may be partially attributed to inhibitory effects on AR signaling pathways. It has been demonstrated that taxane-induced microtubule stabilization can interrupt nuclear translocation of the AR, induce FOXO1-mediated repression of AR transcriptional activity and downregulate AR expression (Figure 8B) (84, 95, 96); these effects prevent transcription of AR-regulated genes that drive prostate cancer growth and progression. Since CRPC tumors rely on androgen signaling, the inhibitory of effects of taxanes on the AR-axis may play a role in their efficacy against prostate cancer.

Finally, taxanes have been reported to alter signaling of the Bcl-2/Bax intrinsic apoptosis pathway (97). Members of the Bcl-2/Bax pathway include anti-apoptotic proteins Bcl-2, Bcl-xL, and Mcl-1, which interact with pro-apoptotic members such as Bax and Bak to regulate permeability of the outer mitochondrial membrane (98, 99). Taxanes can induce Bcl-2 phosphorylation, which blocks its inhibitory effect on Bax, thereby allowing Bax to initiate apoptosis through the release of cytochrome C from the mitochondria, triggering caspase cleavage (Figure 8C) (85).

iii. Mechanisms of taxane resistance

As with many chemotherapeutic agents, development of resistance is a major problem for CRPC patients receiving docetaxel or cabazitaxel treatment. Resistance to taxanes has been attributed to several factors (Figure 11) (100). Alterations in microtubules that reduce taxane binding affinity, including increased expression of the β-III-tubulin isoform and increased acetylation of tubulin, have been associated with reduced taxane efficacy and drug resistance (101-104). Increased expression and activity of ATP-binding cassette (ABC) drug transporters, such as P-glycoprotein (P-gp; also known as MDR-1 and ABCB1), multidrug resistance-related protein (MRP; also known as ABCC1), and breast cancer resistance protein (BCRP; also known as ABCG2) reduced intracellular accumulation of taxanes (105-107). Differential expression of apoptotic factors, for instance the upregulation of anti-apoptotic proteins Bcl-2 and Bcl-xL, allow cancer cells to circumvent apoptotic signals induced by taxanes (106, 108, 109). Finally, overexpression of AR splice variants that are constitutively active and capable of ligand-independent signaling may allow cells to overcome taxane-induced inhibition of AR activity (110-113).

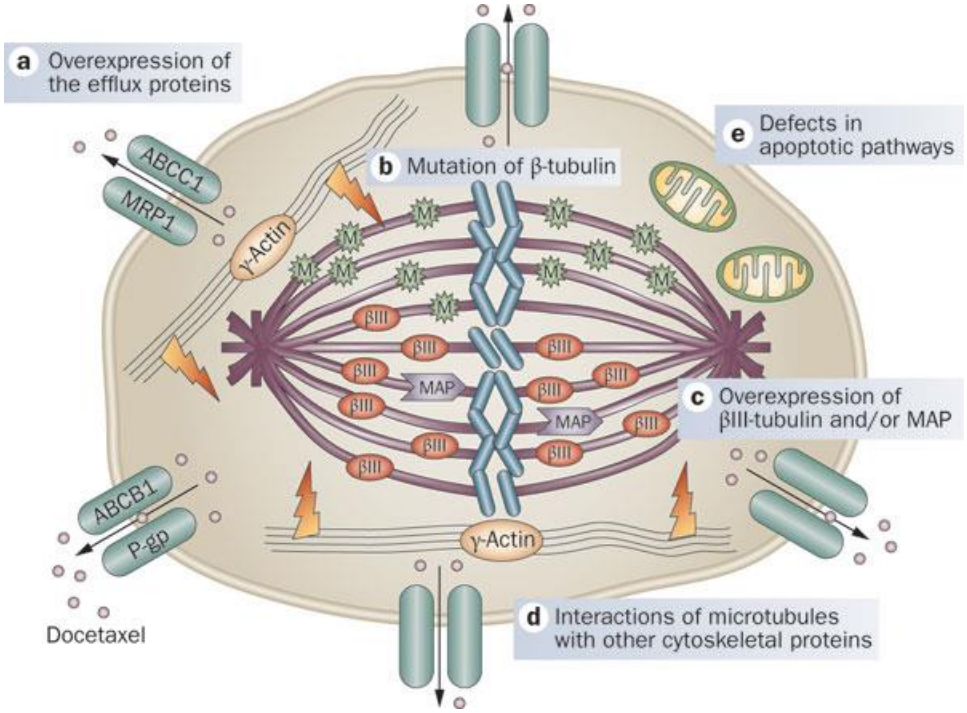


Figure 11. Cellular mechanisms of resistance to taxanes. Cellular mechanisms of resistance can be a result of decreased cellular drug accumulation due to a) overexpression of membrane-bound efflux proteins, such as ABC transporters including P-gp and MRP1 (107); b) a direct alteration of drug target by mutation (114); c) altered expression of tubulin isotypes (for example, overexpression of β-III-tubulin) or MAPs (115); d) changes to the microtubules induced by interactions with other cytoskeletal proteins (for example, γ-actin) (116); and/or e) defects in apoptotic pathways (108). Abbreviations; ABC, ATP-binding cassette; M, mutation; MAP, microtubule-associated protein; MRP1, multiple drug resistant protein 1; P-gp, P-glycoprotein. Reprinted with permission from Macmillan Publishers Ltd: *Nature Reviews Clinical Oncology*, Seruga B, Ocana A, and Tannock IF, Drug resistance in metastatic castration-resistant prostate cancer, copyright (2011).

C. Polyphenolic compounds

Polyphenols consist of a diverse group of plant compounds that may be beneficial in the prevention of diseases including cancer (Figure 12). Polyphenols have been widely studied for their antioxidant properties and ability to exert anti-cancer effects through several mechanisms including decreasing carcinogen-induced DNA damage, altering enzymatic activities, inhibiting cell cycle progression, and promoting apoptosis (117). Additionally polyphenolic compounds can reduce inflammation, angiogenesis, and multidrug resistance. Most polyphenols exist and are ingested in their glycosylated form (118). In the gastrointestinal tract, they are cleaved by β-glucosidase and lactase phlorizin hydrolase (LPH) enzymes to release their lipophilic aglycones (119, 120). It is generally believed that the aglycones are absorbed mainly by simple diffusion, whereas transport of glycosides by a sodium-dependent glucose transporter (SGLT-1) has been suggested (121-123).

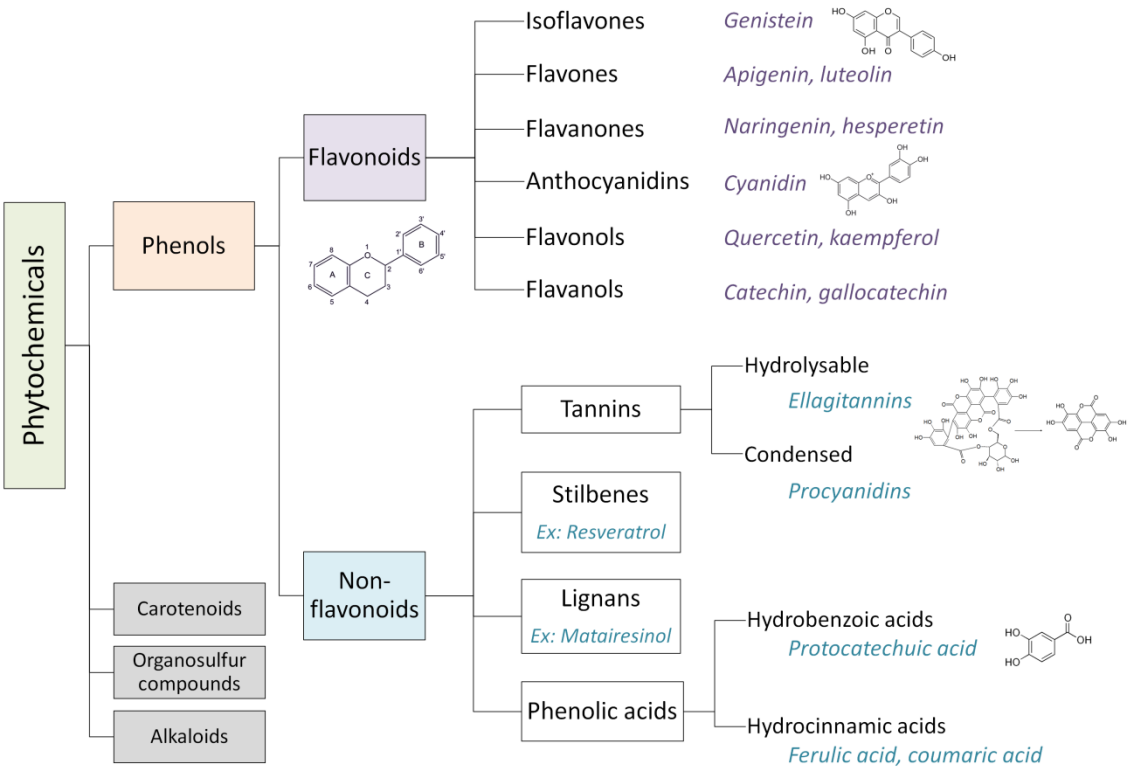


Figure 12. Types of polyphenolic compounds

D. Soy

i. Soy and prostate cancer

The incidence of prostate cancer in Asia is significantly lower than in Western countries (4, 124). Comparative geographic and molecular studies have demonstrated that these differences could be attributed to genetic and environmental factors or a combination of both (125-128). While there are many potential reasons for this disparity, several epidemiological studies have shown a strong association between the consumption of soy foods and a reduction in risk of prostate cancer (129, 130). Compared to the Western diet, which is characterized by a high content of animal fat and protein, many Asian diets contain much larger amounts of soy-derived foods that are abundant sources of dietary isoflavones such as genistein and daidzein. Traditional Asian foods made from soy include tofu, tempeh, and miso, which typically have a higher content of isoflavones than the soy products popular in western countries (ex: soy-based meat substitutes, soy milk, soy cheese) (131). A typical Japanese diet contains approximately 25–100 mg soy isoflavones per day, while a Western diet may contain only 2–3 mg (132), which is consistent with observations of circulating soy isoflavones levels in Asian men that are at least 10 times higher than those in American and European men (133-135). Furthermore, serum levels of genistein, daidzein and equol have been dose-dependently associated with reduced risk of prostate cancer in Japanese and Chinese men (136, 137).

Asian migrant studies have demonstrated that prostate cancer incidence rates for Asian immigrants are directly related to the length of time spent residing in the United States and that risk increases in subsequent generations of Asian-American men (138-140). Likewise, westernization of dietary habits in Asian countries correlates with increased incidence and mortality of prostate cancer (141), leading to the speculation that the protective effects of soy foods on prostate cancer risk may be lost with the adoption a westernized diet high in meat and

fat (140). These studies indicate that environmental or lifestyle factors, including diet, play a role in carcinogenesis, and therefore dietary interventions may provide a chemopreventive strategy for prostate cancer.

While numerous epidemiological studies support a role for soy in prostate cancer prevention, soy intervention trials have yielded mixed results. In men with prostate cancer, several studies demonstrated that soy consumption led to reduction in serum PSA levels (142-144), but such an effect was not found by others (145, 146). In a randomized, double-blind trial of 86 men with prostate cancer, daily intake of 80 mg soy isoflavones for up to six weeks prior to scheduled prostatectomy did not have a significant effect on serum levels of testosterone, estrogen, or PSA; however, changes in expression levels of genes involved in cell cycle and apoptosis were observed in prostate tissue of soy-treated men compared to control (147). In another randomized study, two years of supplementation with isoflavone-rich soy protein in 81 men at elevated risk of prostate cancer recurrence after a radical prostatectomy did not change the risk of recurrence compared to 78 casein-supplemented control subjects (148). A recent meta-analysis of eight randomized clinical trials on efficacy of soy isoflavones in men with prostate cancer reported that soy intake did not have significant effects on levels of PSA, testosterone, estradiol, or dihydrotestosterone (149). The contribution of soy in prostate cancer prevention remains unclear, as the epidemiology and laboratory studies (see below) suggest preventive benefit but randomized studies do not.

ii. Soy isoflavones

Isoflavones are naturally occurring polyphenolic compounds that are capable of exerting estrogen-like effects. The major isoflavones found in soy are genistein and daidzein, which are usually present in their glycoside form and converted to aglycones by intestinal bacteria during

digestion, but preparation of soy foods through processes like fermentation can also cleave the sugar moiety and release the aglycone (150-152). Daidzein can undergo further metabolic processing by gut bacteria to produce the metabolite equol (153, 154). Oxidative metabolism of isoflavones is catalyzed mainly by CYP1A2, with minor contributions by CYP2C8 and CYP3A4 (155).

The biological activity of isoflavones is attributed in part to their structural similarity to 17β-estradiol (Figure 13), which allows them to bind estrogen receptors (ER) and modulate ER signaling pathways (156). Genistein preferentially binds to ER-β over ER-α and has a higher affinity for ER than daidzein, likely due to the presence of an additional hydroxyl group on the A-ring of genistein (Figure 13) (157). However, metabolic transformation of daidzein to equol increases its affinity for estrogen receptor to levels similar to that of genistein (154).

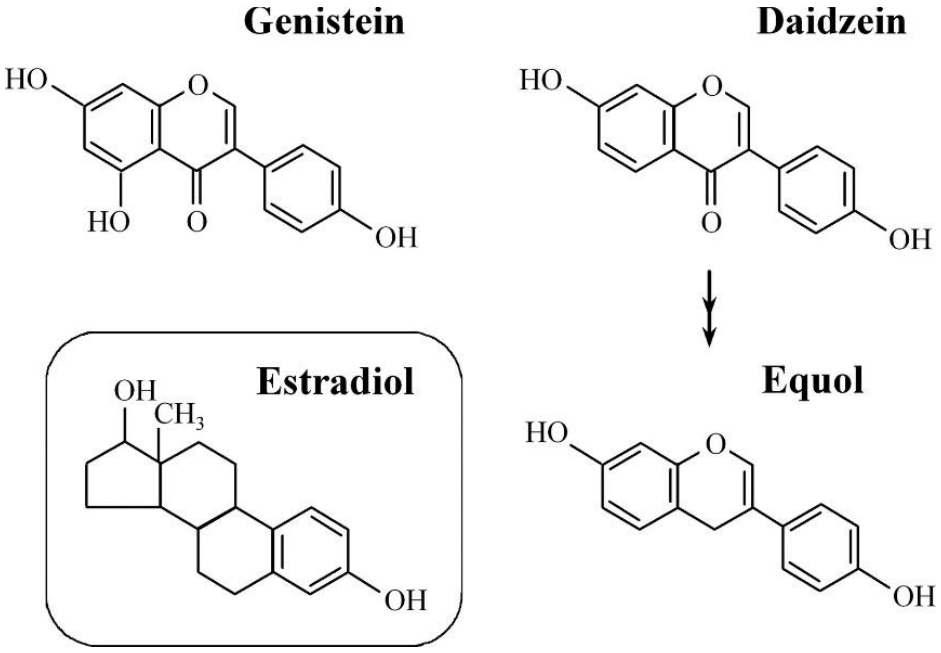


Figure 13. Chemical structures of soy isoflavones and estradiol

Genistein is the most abundant and biologically active isoflavone found in soy. Genistein was first identified as a potent inhibitor of protein tyrosine kinase activity (158), but numerous other mechanisms of action for genistein have been proposed (Figure 14) (159). Genistein has estrogenic effects, can modulate cell growth, alter signaling transduction, induce apoptosis and inhibit angiogenesis (160-164). It has antioxidant properties, can reduce cell invasion and prevent metastasis (165-171). Additionally, genistein can downregulate AR expression and signaling (172, 173).

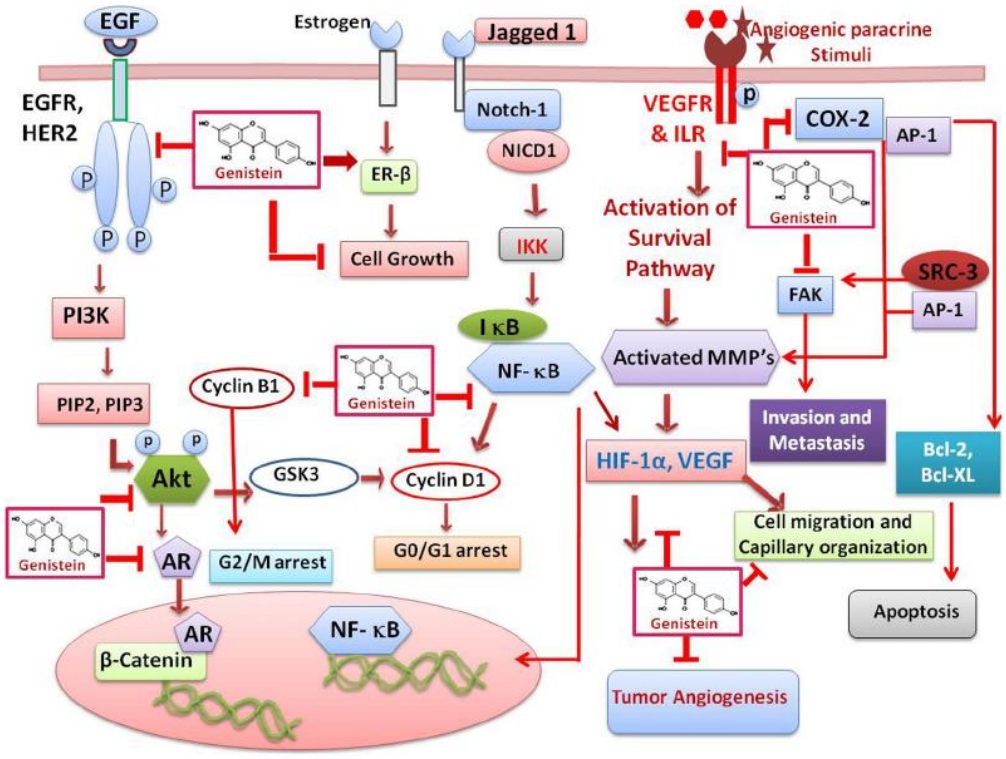


Figure 14. Pleiotropic effects of genistein. Reprinted from *Food Chemistry*, Russo *et al*, Understanding genistein in cancer: the "good" and "bad" effects: a review (2016), with permission from Elsevier.

Inhibitory effects of genistein on pathways associated with development and progression of prostate cancer have been demonstrated in numerous *in vitro* studies. Genistein inhibits growth of androgen-independent and -dependent prostate cancer cell lines (174-177) and induces apoptosis by blocking the nuclear translocation of NF-κB in LNCaP and PC-3 prostate cancer cells (178). Genistein has also been shown to promote apoptosis via activation of capsase-3 in DU145 and LNCaP prostate cancer cell lines (179). Genistein reduced incidence and development of prostate tumors in the TRAMP mouse model, and inhibited growth and angiogenesis of LNCaP xenografts in nude mice (180-182).

There is some evidence to suggest that genistein could be a potential adjuvant for taxane chemotherapy. Genistein enhanced the apoptotic effects of docetaxel (DX) on PC-3 cells *in vitro*, possibly mediated by inactivation of NF-κB (183, 184). Dietary genistein (at 0.1%) in an animal study enhanced the growth inhibitory effects of DX (I.V. injections of 5 mg/kg on three alternate days) on PC-3 cell xenografts in SCID mice (184). In a recently published paper, marked enhancement was reported by genistein (100 mg/kg, three times per week) on the growth inhibitory effect of cabazitaxel (CBZ; 5 mg/kg, I.P. once weekly) on xenografted PC-3 cells (185); this effect was also found *in vitro* on PC-3 cells and LNCaP-C4-2 cells well as on the apoptotic effects of CBZ on PC-3 cells. A fermented genistein product (genistein combined polysaccharide) also enhanced the *in vitro* apoptotic effect of DX on LNCaP cells and increased anti-proliferative activity of DX on 22Rv1 and PC-3 cells, but not in LNCaP cells (186). Additionally, there is some evidence to suggest that genistein interacts with tubulin and can alter microtubule dynamics (187, 188), although other studies do report this effect (189, 190) (current evidence is summarized in Table I). Since taxane cytotoxicity occurs as a consequence of their stabilizing effects on microtubules, microtubule targeting by genistein may either augment or disrupt this process. It is currently unclear what, if any, role soy isoflavones may play in microtubule stability and function.

Table I. Effects of genistein on microtubules

Reference	Agent	Model System	Effect on microtubules	Taxane combination effect?
Ahmed *et al,* 2011 (187)	ITB-301 (10 μM)	*In vitro* (cell-free) polymerization assay, SKOv3 & ES2 ovarian cancer cells	Induced microtubule depolymerization in cells and in cell-free assay	Not investigated
Liao *et al,* 2004 (189)	Genistein (100 μM)	MDA-MB-231 breast cancer cells	No effect on microtubule morphology	Reduced PTX-induced cytotoxicity and apoptosis. Inhibited PTX-induced G2/M accumulation.
Mukherjee *et al,* 2010 (188)	Genistein (25, 50, 75, 100, 150 μM)	*In vitro* (cell-free) polymerization assay, A549 non-small lung cancer cells	A549 cells: no effect of 25 μM Gen, dose dependent depolymerization at 50 and 100 μM visible; reduction in polymerized tubulin mass determined by western blotting. Inhibited polymerization *in vitro* IC_{50} = 87 μM. Genistein binds tubulin K_D = 15 μM.	Not investigated
Ponnathpur *et al*, 1995 (191)	Genistein (30 μM)	697/BCL-2 cells	Not investigated	Reduced PTX-induced cytotoxicity and apoptosis. Did not alter cell cycle effects of PTX,
Rusin *et al*, 2009 (190)	ITB-301 (5 & 25 μM) Genistein (100 μM)	DU145 prostate cancer cells	Gen had no effect on microtubules, ITB-301 induced microtubule depolymerization	Not investigated

ITB-301 (also known as G21) is a lipophilic glycoside derivative of genistein. PTX = paclitaxel

E. Black Raspberries

Berries are popularly consumed in cuisines worldwide and are an excellent source of vitamins, minerals, and many bioactive phytochemicals including flavonoids, tannins, carotenoids, anthocyanins, lignans and stilbenes – many of which possess antioxidant properties (192). Berry fruits such as blueberry, cranberry, black raspberry, and strawberry vary significantly in both their phytochemical content and composition. Several studies have demonstrated anti-cancer activity of berry extracts, but black raspberries (*Rubus occidentalis*, BRB) in particular have shown tremendous chemopreventive and therapeutic potential. Investigations with BRBs in cell culture, animal models, and a few human clinical trials have demonstrated that BRBs can influence cellular and molecular events associated with proliferation, apoptosis, inflammation, and angiogenesis (1). BRBs contain numerous polyphenolic compounds, but their anti-cancer effects have been attributed to the high concentration of ellagic acid and anthocyanins (193-196). BRBs are one of the highest dietary sources of anthocyanins (5 – 20 mg/g dry BRB weight) and ellagic acid (1.2 – 2 mg/g dry BRB weight) (197-199). In addition to being a rich source of anthocyanins, BRBs have a unique anthocyanin profile, predominately consisting of the cyanidin subtype: cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xylosylrutinoside, and cyanidin-3-sambubioside (Figure 15) (200). Collectively, the concentration and profile of these polyphenolic compounds suggest BRBs may exhibit superior anti-cancer properties compared to other berry varieties.

i. Black raspberries and cancer

Anti-cancer effects of BRBs have been demonstrated *in vitro*, *in vivo*, and in some clinical trials for several types of cancer. BRB preparations and extracts can inhibit cancer cell proliferation, induce apoptosis, and inhibit angiogenesis (1). BRBs inhibit proliferation of

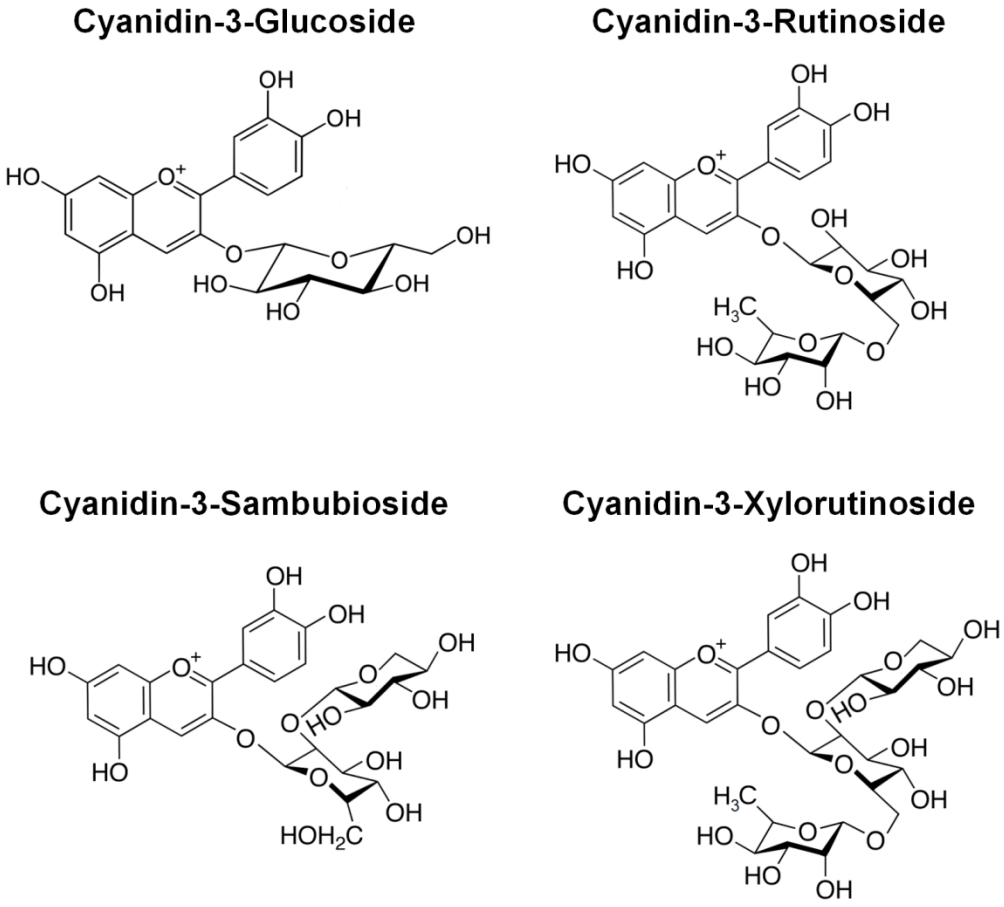


Figure 15. Chemical structures of black raspberry anthocyanins

esophageal (201), colon (200), endothelial (202), breast (200), lymphocytes (203), and cervical (204) cancer cells.

In vivo, BRBs are effective for preventing carcinogenesis induced by genotoxic agents and reducing oxidative stress. Dietary administration of BRBs inhibits tumor initiation as well as tumor promotion-progression in N-nitrosomethylbenzylamine (NMBA) induced esophageal cancer in rats (205-210). Likewise, BRBs inhibit tumor development in azoxymethane (AOM)-induced colon cancer in rats (211). Topical applications of a black raspberry extract reduced inflammation and carcinogenesis induced by UV-B radiation in SKH-1 hairless mice (212).

Additionally, oral administration of BRBs inhibited mammary tumor development in animal models (213-215). Dietary supplementation with 2.5% lyophilized BRBs or blueberries significantly reduced estrogen-induced mammary tumorigenesis in the ACI rats (214). However, blueberries were less effective than BRBs in reducing tumor volume and multiplicity, indicating that BRB-specific bioactive compounds may be potent inhibitors of carcinogenesis. Both BRBs and blueberries are rich in anthocyanins, but differ in their anthocyanin profiles; blueberries are rich in the delphinidin glycoside subtype (Figure 16), whereas BRBs contain mainly the cyanidin subtype (198). Inhibition of mammary tumorigenesis by BRBs notably demonstrates that biologically active berry constituents have the ability to reach a distant tumor site following oral administration, which justifies investigation of berries on other sites, including the prostate.

BRBs were well tolerated and had protective effects in several human clinical trials. Daily administration of rectal suppositories containing 720 mg BRB powder reduced the burden of rectal polyps in patients with familial adenomatous polyposis (216). In patients with colorectal cancer, BRB treatment decreased tissue expression of β-catenin, Ki67 and DNMT1 and increased levels of TUNEL and p16 (217). BRBs reduced a urinary marker of oxidative stress in patients with Barrett's esophagus, but did not reduce lesion size (218). The most promising effects of BRBs have been demonstrated in clinical trials examining the effects of topical BRB application in patients with oral cancers and precancerous lesions (219-221). A summary of clinical trials involving BRBs can be found in Table II. These clinical trials indicate that the bioactive constituents of BRBs may inhibit carcinogenesis in the GI tract, where they come into direct contact with the target tissues.

Table II. Summary of completed clinical trials with black raspberries

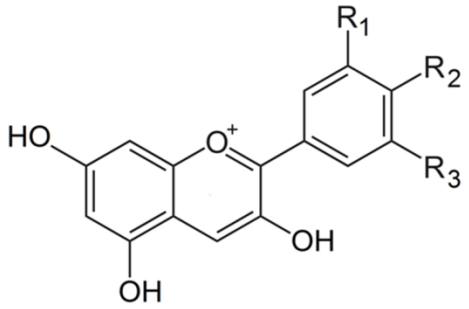
Lesion	Patients (n)	Route of Delivery	Treatment	Endpoints	Results	References
Oral intraepithelial neoplasia	20-40	Topical	10% w/w freeze-dried BRB bioadhesive gel applied 4x daily for 6-12 weeks	Histopathology, gene expression, micro vascular densities, loss of heterozygosity	↓ LOH prevalence Improvement in histologic grade for a subset of patients ↓ Vascular densities ↓ Epithelial COX-2 expression ↓ Expression of genes associated with RNA processing, growth factor recycling and inhibition of apoptosis	Mallery *et al,* 2008 (219); Shumway *et al*, 2008 (222); Mallery *et al*, 2007 (223); Mallery *et al,* 2014 (221)
Barrett's esophagus	20	Oral	32 or 45 g BRB powder in water, once daily for 26 weeks	Lipid peroxidation, DNA damage, tissue markers of proliferation, detoxification, and inflammation.	*Urinary markers:* ↓ 8-Iso-PGF2α No change in urinary 8-OHdG *Tissue markers:* ↑ GST-pi No change in Ki67 and NF-κB ↑ Patient body weight and BMI. No change in lesion size	Kresty *et al,* 2006 (218); Kresty *et al,* 2016 (218, 224)

Table II (continued) Summary of completed clinical trials with black raspberries

Lesion	Patients (n)	Route of Delivery	Treatment	Endpoints	Results	References
Colorectal cancer	20-28	Oral	20 g BRB powder 3x daily for 1 to 9 weeks	Genetic and epigenetic biomarkers, tissue markers of proliferation, apoptosis and angiogenesis, serum biomarkers	*Tissue expression*: ↓ DNMT1, Ki67 ↑ TUNEL *Plasma levels:* ↓ IL-8 ↑ GM-CSF Changes in DNA methylation observed in patients that were treated for at least 4 weeks	Pan *et al,* 2015 (225); Wang *et al*, 2011 (217); Mentor Marcel *et al*, 2012 (226)
Rectal polyps	14	Oral and rectal suppository	Oral placebo or 20 g BRB powder 3x per day, plus two BRB rectal suppositories (720 mg)	Polyp number and size	↓ Polyp burden No change in polyp number No added benefit of oral BRBs in addition to suppositories ↓ Cell proliferation, DNMT1 expression, *p16* promoter methylation in patients that responded to treatment.	Wang *et al,* 2014 (216)

ii. Anthocyanidins and anthocyanins

Anthocyanins are naturally occurring flavonoid compounds that provide blue, purple, and red coloring to a variety of fruits, vegetables and flowers (227). They constitute the largest group of water-soluble pigments and are commonly used as alternatives to synthetic dyes and as colorants in the food and beverage industry (228, 229).



Anthocyanidin	R_1	R_2	R_3
Pelagonidin	H	OH	H
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Peonidin	OMe	OH	H
Petunidin	OMe	OH	OH
Malvidin	OMe	OH	OMe

Figure 16. Anthocyanidin structure

When found as glycosides (bound to a sugar moiety) they are known as anthocyanins and their aglycones are called anthocyanidins. Anthocyanins belong to the larger polyphenolic group of flavonoids and share structural similarity as flavylium (2-phenylbenzopyrylium) salts (230). The most common anthocyanidins found in nature are cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin (Figure 16) (230), which provide the base structure for over 600

currently known types of naturally occurring plant anthocyanins (198). Members of this family differ in the number of hydroxyl groups, the degree of methylation, and the nature and number of the bonded sugars, as well as the position of attachment. Anthocyanidin aglycones are rarely found in nature due to their instability; they are sensitive to a number of factors including pH, temperature, light, oxygen, solvents, and the presence of other flavonoids, proteins or metal ions. Stability is slightly greater for anthocyanin glycosides, but glycosylation provides only modest protection, leaving anthocyanins susceptible to rapid degradation, especially under physiological conditions (231).

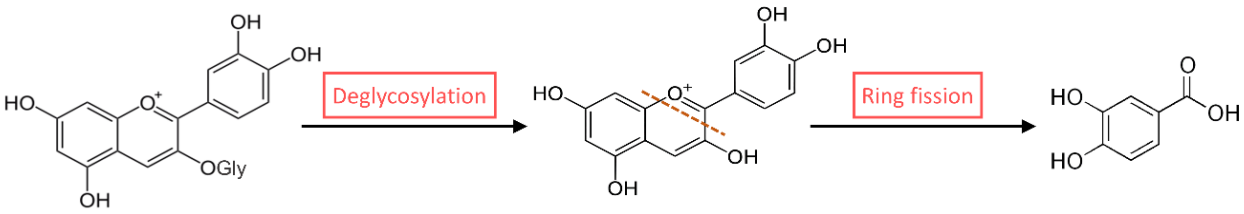


Figure 17. Degradation of anthocyanins

Anti-cancer activity of anthocyanins has been demonstrated in numerous *in vitro* and *in vivo* studies, along with reports of various potential mechanisms of action. Anthocyanin compounds have been reported to exert anti-mutagenic activity (232, 233), reduce oxidative DNA damage (234, 235), induce phase II detoxification enzymes (236-238), promote apoptosis (239-241), induce cell cycle arrest (242) and inhibit angiogenesis (243). The diversity of anthocyanin effects suggests they could be an attractive option for the prevention and treatment of cancer.

iii. *Ellagic acid*

Ellagitannins are naturally occurring compounds found in pomegranates, berries, walnuts and numerous other foods. Ellagic acid (EA) is released upon hydrolysis of ellagitannins. Ingested EA is further metabolized by colonic bacteria to yield dibenzopyran-6-one derivatives (urolithins), mainly urolithin A (UroA). Ellagitannins, ellagic acid, and urolithins exhibit potent antioxidant and anti-cancer activity in a variety of cell and animal models (195, 213, 244-248). EA has very low aqueous solubility (water solubility ≃ 9.7 µg/mL), which limits absorption and bioavailability (249). The solubility of urolithin metabolites is greater than that of EA due to the reduction in the number of hydroxyl groups, which improves absorption but diminishes their antioxidant capacity (246, 250, 251). Figure 18, demonstrates how antioxidant activity and absorption of EA and urolithins is correlated with their degree of hydroxylation.

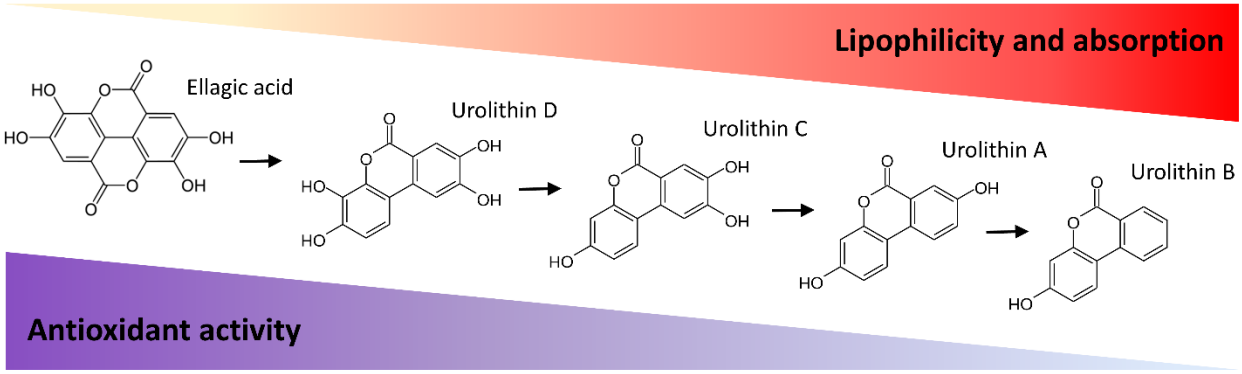


Figure 18. Ellagic acid and urolithin metabolites: relationship of structure to absorption and antioxidant activity.

II. MATERIALS AND METHODS

A. Materials

i. Reagents and chemicals

Cyanidin-3-rutinoside, cyanidin chloride, 3,3'-diethyloxacarbocyanin iodide, docetaxel, resazurin, rhodamine 123, rifampicin, sulforhodamine B, and vinblastine were purchased from Sigma-Aldrich (St. Louis, MO). Cabazitaxel (Sanofi, Bridgewater, NJ) was a generous gift from Dr. Robert Nagourny (Rational Therapeutics, Long Beach, CA). Ellagic acid was obtained from Indofine Chemical (Hillsborough, NJ). Daidzein, equol, genistein, ketoconazole, and protocatechuic acid were purchased from LKT Labs (St. Paul, MN). Urolithin A was obtained from Santa Cruz (Dallas, TX). Crystalline testosterone and 17β-estradiol were obtained from Steraloids (Newport, RI). Noble agar was purchased from Affymetrix (Cleveland, OH). Purified tubulin protein was purchased from Cytoskeleton (Denver, CO). Phenol-red free matrigel was obtained from Corning (Bedford, MA). DMSO, EtOH, formaldehyde, formic acid and trichloroacetic acid were obtained from Thermo Fisher Scientific (Waltham, MA).

ii. Antibodies

β-actin (catalog #3700), AR (#5153), BAX (#2772), Bcl-2 (#2870), Bcl-xL (#2764), Mcl-1 (#5453), caspase-3 (#9665), MDR1 (#13342), MRP1 (#14685), PARP (#9542), PCNA (#2586), PSMA (#12815), PSA (#2475), α-tubulin (#3873), β-tubulin (#2128), anti-mouse Alexa Fluor 488 (#4408), and anti-rabbit Alexa Fluor 555 (#4413) antibodies were obtained from Cell Signaling Technologies (Danvers, MA). CYP3A4 (#53850) and GAPDH (#365062) antibodies were

obtained from Santa Cruz Biotechnology (Dallas, TX). IRDye 800 anti-rabbit (#926-32211) and IRDye 680 anti-mouse (#926-68070) antibodies were obtained from Li-Cor Biotechnology (Lincoln, NE).

iii. Preparation of black raspberry extract

An ethanol/H_2O (80/20) soluble BRB extract was prepared from lyophilized black raspberry powder (Berri Products, Corbett, OR) which was stored at -20^{o}C. 200 g BRB powder was combined with 80% EtOH and stirred overnight at room temperature. The resulting mixture was vacuum-filtered. The solvents were removed to the extent possible by rotary evaporation at reduced pressure, yielding a syrup. The remaining solvent was removed under a nitrogen stream. Final weights of extracts were obtained and BRB extract were reconstituted in 100% EtOH and stored at -20^{o}C.

iv. Cell lines

22Rv1, LNCaP, VCaP, and PC-3 cells were obtained from American Type Culture Collection (Manassas, VA). C4-2 cells were a generous gift from Leland Chung (Cedars Sinai Medical Center). LAPC-4 cells were a generous gift from Dr. Karen Knudsen who had obtained them from Dr. Charles Sawyers (Memorial Sloan Kettering, New York, NY) and were provided within less than 20 passages of arrival from Dr. Sawyers. HepG2 and HepG2-CYP3A4 cells were a gift from Natalia Nieto (University of Illinois at Chicago).

B. *In vitro* methods

i. Cell culture

LAPC-4, LNCaP, C4-2, 22Rv1, and PC-3 cells were cultured in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FBS, 100 IU/mL penicillin, 100 µg/mL streptomycin. VCaP and HepG2 cells were cultured in DMEM (Life Technologies) supplemented with 10% FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin. Cells were maintained at 37^{o}C in a humidified 5% CO_2 incubator. Twenty four hours prior to experiments, media was replaced with phenol-red free RPMI or DMEM containing 10% dextran charcoal stripped FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin.

ii. Confocal microscopy

Cells were plated on Lab-Tek 4-well chamber slides (Nunc, Naperville, IL) and cultured to approximately 80% confluency. Cells were treated with compounds for 24 hours, fixed with 3.7% formaldehyde, washed with PBS, permeabilized for 10 minutes with 0.1% Triton-X 100, and blocked with 5% BSA for 30 minutes. Cells were then incubated overnight at 4^{o}C with primary antibodies, followed by incubation with fluorescent secondary antibodies for 2 hours at room temperature in a dark, humidified chamber. Cover slips were mounted on slides with ProLong Gold Antifade Mountant containing DAPI (Life Technologies). Fluorescent images were acquired using a Zeiss LSM 710 confocal laser scanning microscope using 40x or 63x objectives.

iii. DNA synthesis assay

Incorporation of EdU (5-ethynyl-2'-deoxyuridine) into DNA was measured to assess cell proliferation using the Click-iT EdU Microplate Assay (Invitrogen). EdU is an analog of thymidine and is incorporated into DNA during S-phase. Cells were labeled with EdU for 2 hours then fixed. Cells were treated with buffer containing $CuSO_4$ and Oregon Green 488 azide for 25 min, incubated with 5% BSA for 30 min, and then incubated with anti-Oregon Green antibody conjugated to horseradish peroxidase for 30 minutes, followed by incubation with Amplex Red for 15 minutes. Fluorescence intensity (excitation: 560, emission: 590) was measured with SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA).

iv. Sulforhodamine B assay

Cells were seeded on 96-well plates at a density of 2,000 cells per well 24 hours prior to addition of test compounds to culture media. Cells were incubated for 72 hours, then the sulforhodamine B (SRB) assay was performed to evaluate growth (252). Briefly, cells were fixed with 1% TCA and stained with 0.057% SRB following incubation with treatments. Wells were washed with 1% acetic acid and the remaining dye was solubilized in 10 mM Tris. Optical density at 510 nm was measured with the Synergy HTX plate reader (Biotek, Highland Park, VT). Results were expressed as percent proliferation relative to vehicle treated control cells. Each treatment condition was replicated 3 times in a single experiment, which was repeated at least 3 times.

v. Resazurin reduction assay

The resazurin reduction assay was used to as an alternative to the SRB assay to measure cell proliferation[3]. Reduction of non-fluorescent resazurin dye to fluorescent compound resorufin can be measured as an indication of cell viability. Cells were plated and treated under the same conditions as the SRB assay. Resazurin solution (10% w/v resazurin in PBS) was added to culture media to a final concentration of 1%, and cells were incubated for 24 hours at 37^{o}C in a humidified 5% CO_2 incubator. Resorufin fluorescence (excitation 530-560 nm, emission 580-600 nm) was measured with the SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA). Culture media containing resazurin without cells was used as a negative control, and 100% reduced resazurin served as a positive control.

vi. Western blot analysis

Protein expression levels were quantified by western blotting. Total protein was isolated from cells using RIPA buffer (Sigma, St. Louis, MO). Soluble proteins were separated from insoluble proteins by centrifugation, the pellet was collected and the insoluble fraction reconstituted in RIPA containing 3 M urea. Protein concentration was quantified using the bicinchoninic acid method with the Piece BCA Protein Assay Kit (ThermoFisher). Samples were mixed with LDS NuPAGE sample buffer and separated by electrophoresis through 4-12% NuPAGE Bis-Tris gels (Invitrogen, Thermo Fisher Scientific, Waltham, MA). Proteins were transferred to PVDF membranes. Membranes were incubated with blocking buffer (5% BSA or

[3] Cyanidin and Cy-3-Rut are brightly colored compounds, which alter the color of cells when taken up and subsequently interfere with optical density readings in the SRB assay. The resazurin reduction assay provides fluorescently based readings that are not affected by color of BRB compounds.

5% non-fat milk) for 30 minutes, and then probed with primary antibodies overnight at 4^{o}C. Membranes were incubated with fluorescent secondary antibodies and bands were detected using the Odyssey CLx Infrared Imaging System (Li-Cor).

vii. Anchorage independent growth

The effect of test compounds on anchorage independent growth of PC-3 cells was assessed by soft agar colony formation assay. The bottom of each well of 12-well culture dish was coated with 1 mL noble agar mixture (RPMI, 10% FBS, 0.6% agar). After the bottom layer solidified, 1 mL top agar medium mixture (RPMI, 10% FBS, 0.3% agar) containing 5 x 10^3 cells with varying concentrations of BRB extract, cyanidin-3-rutinoside, ellagic acid or protocatechuic acid was added and incubated at 37°C for 3 weeks. Culture media was replaced 3 times per week. At the end of the incubation period, colonies were stained with crystal violet and the number of colonies was counted using GelCount equipment and software (Oxford Optronix, Abingdon, UK).

viii. Wound healing assay

PC-3 cells were used to study cell migration due to their ability to form a monolayer when confluent. Cells were plated in 6 well plates and grown until confluent. A wound was created in the monolayer by scratching with a sterile 200 µL pipette tip. Cells were washed with PBS and fresh media containing treatments compounds was added to wells. Wound distance was measured after 0, 6, 12 and 24 hours. Distance was measured in the same field of view for each time point.

ix. Surface plasmon resonance

Binding affinity of compounds to tubulin was evaluated by SPR using Biacore T-2000 instrument (GE Life Sciences, Uppsala, Sweden). Purified tubulin protein (100 µg/mL in 10 mM sodium acetate pH 4.0) was immobilized on CM5 sensor chips (GE Life Sciences) by amine coupling using N-hydroxysuccinimide (NHS) and N-ethyl-N-(dimethyl-aminopropyl)-carbodiimide (EDC) using filtered (0.2 µm) PBS-P+ buffer (GE Life Sciences). A minimum of 9,000 response units (RU) were immobilized to the chip. For each experiment, 1 flow channel of the CM5 chip was prepared in the absence of tubulin for reference subtraction. The running buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.05% (vol/vol) Tween-20) was supplemented with 2% DMSO. Test compounds (serially diluted, 0-200 µM) were injected at a flow rate of 30 µL/min. SPR data is representative of duplicate injections acquired for three independent experiments. The equilibrium dissociation constants (K_D) were calculated from steady-state affinity after DMSO solvent correction.

x. Tubulin polymerization assay

Effects of compounds on tubulin polymerization were evaluated by monitoring incorporation of a fluorescence based reporter (Cytoskeleton, Denver, CO) into microtubules as polymerization occurs. Purified tubulin (2 mg/mL) was suspended in 80 mM PIPES pH 6.9, 2.0 mM $MgCl_2$, 0.5 nM EDTA, 1.0 GTP and 15% glycerol, and experiments compounds or vehicle controls were added. Fluorescence intensity (excitation: 360 nm, emission: 420 nm) was measured at 1 minute intervals for 60 minutes using the Synergy HTX plate reader (Biotek).

xi. Intracellular measurement of anthocyanins

Cells were plated and cultured to approximately 80% confluence, then treated for 4 hours with BRB extract, cyanidin, Cy-3-Rut, or vehicle control. Cells were harvested with trypsin, suspended in PBS and centrifuged. Cells were resuspended in 95% EtOH and 5% formic acid, lysed by 3 freeze/thaw cycles, and samples were stored at -80^{o}C. HPLC-MS/MS was carried out using an Agilent 6410 Triple Quad Mass Spectrometer (Agilent Technologies; Santa Clara, CA) coupled with an Agilent HPLC 1200 series chromatographic system.

xii. Efflux assay

Effects of BRB and soy compounds on P-glycoprotein activity was determined by measuring efflux of fluorescent substrates (calcein AM) of MDR1 and MRP1. Cells were plated and cultured to 80% confluence, then incubated at 37^{o}C with compounds in phenol-red free culture media. After 24 hours, cells were treated with cold media containing either calcein AM, and incubated for 1 hour at 4^{o}C to facilitate uptake of substrates. Cells were collected, washed with PBS, resuspended in phenol-red culture media and incubated at 37^{o}C. Cell culture media was collected at 0, 2 and 4 hours, and fluorescence intensity (excitation: 488 nm, emission 530 nm) of samples was measured using a Synergy HTX plate reader (Biotek).

xiii. CYP3A4 activity

Effects of BRB and soy compounds on CYP3A4 activity was evaluated using insect microsomes expressing human CYP3A4 enzyme and the Vivid CYP3A4 Green Screening Kit (Life Technologies). Microsomes were incubated with glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP+, and DBOMF (non-fluorescent CYP3A4 substrate, which

releases fluorescent compound when metabolized by CYP3A4) in presence test compounds or vehicle control. Fluorescence (excitation: 490 nm, emission: 520 nm) was measured using a Synergy HTX plate reader (Biotek).

C. *In vivo* methods

i. Institutional approval

Animal care and experiments were conducted in accordance with protocols approved by the Animal Care Committee (ACC) at the University of Illinois at Chicago. ACC approval notices for all animal studies can be found in Appendix A.

ii. Prostate cancer xenografts in nude mice

A subcutaneous xenograft model with 22Rv1 cells was used to study the effects of dietary consumption of genistein and ellagic acid on the efficacy of docetaxel (Figure 19). Six-week-old athymic nude mice (Hsd:Athymic Nude-*Foxn1*^{nu}) were obtained from Envigo (Indianapolis, IN). 22Rv1 cells were selected for this experiment because they are characteristic of CRPC tumors (see APPENDIX B)[4]. Cells were suspended in matrigel solution (4 mg/mL phenol-red free matrigel obtained from Corning in PBS). Cells were injected subcutaneously on both flanks, 1 x 10^6 cells in 200 µL per injection, while mice were anesthetized with ketamine (100 mg/kg) and

[4] VCaP cells were the preferred cell line for this experiment because they have the most features associated with CRPC tumors. However, in pilot studies assessing growth of VCaP and 22Rv1 xenografts in both nude and SCID mice, we encountered difficulty generating VCaP tumors.

xylazine (5 mg/kg). Mice were fed a standard chow diet (Harlan Teklad) until tumors reached approximately 500 mm^3, then were randomized to one of the following diets: AIN-93M diet (control groups), AIN-93M containing 250 mg/kg genistein (low Gen group), 500 mg/kg genistein (high Gen group), 2 g/kg ellagic acid (low EA group), or 4 g/kg ellagic acid (high EA group). The agents were added to a premix of sucrose that was subsequently mixed into a basal diet (containing less sucrose to accommodate the premix); the diets were stored at –20°C. One week after the start of modified diets, mice received docetaxel (15 mg/kg; 0.4 mL injection volume) or sham injections, administered at 3 day intervals. Docetaxel solution was prepared by diluting stock solution (22.5 mg/mL in 100% EtOH) in diluent (1 volume polysorbate and 18 volumes 5% glucose in sterile water) to a final concentration of 1.125 mg/mL. Sham injection solutions were prepared similarly, except DX was replaced with 100% EtOH. Tumor size was measured three times per week using calipers.

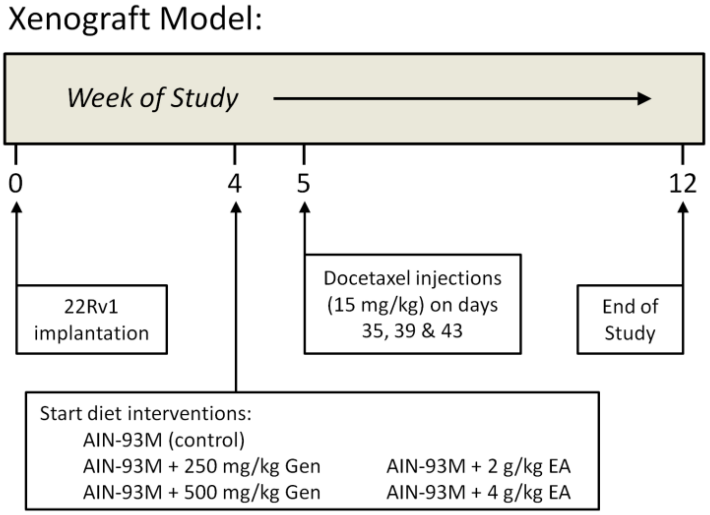


Figure 19. Human prostate cancer cell xenograft model

iii. Testosterone plus estradiol model of rat prostate carcinogenesis

12 week old male Noble (NBL) rats were obtained from an in-house breeding colony. Prostate cancer was induced as described by Özten *et al.* (253) (Figure 20). While under ketamine-xylazine anesthesia (100 mg/kg Ketamine [Henry Schein Animal Health, Dublin, OH] and 5 mg/kg Xylazine [Lloyd Laboratories, Shenandoah, IA]), each rat surgically received two Silastic tubing implants (Dow Corning, I.D. 0.078 inch; O.D. 0.125 inch) containing crystalline testosterone tightly packed over 2 cm length and one implant containing crystalline 17β-estradiol tightly packed over 1 cm length. Rats were randomly divided into three groups of 30 animals. One week after hormone implantation, rats were switched from a standard chow obtained from Harlan Teklad (currently Envigo, Madison, WI) to AIN-93M diet (control) or isoenergetic AIN-93M diets containing 5% or 10% lyophilized BRB powder at the expense of the starch component of the AIN-93M diet. The AIN-93M diet was also obtained from Harlan Teklad and was stored at 4^{o}C; the berry powder was mixed into the diet in-house using a Patterson-Kelly mixer and stored at -20^{o}C until fed freshly three times per week. Rats were euthanized when moribund or surviving for 48 weeks.

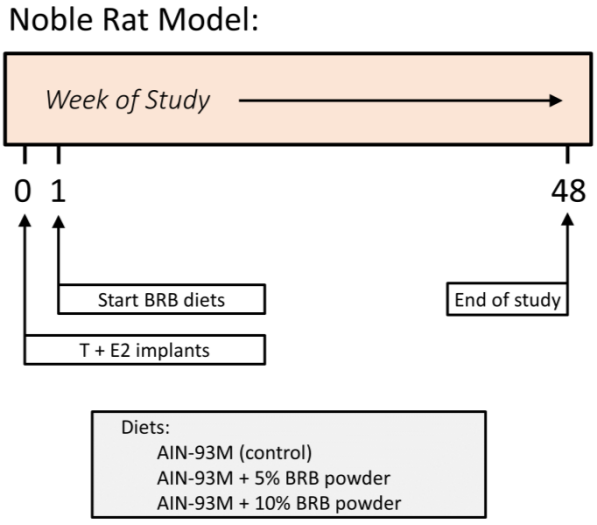


Figure 20. Testosterone plus estradiol model of rat prostate carcinogenesis

iv. MNU plus androgen model of rat prostate carcinogenesis

10-12 week old male Wistar-Unilever (WU) rats were obtained from Harlan Sprague-Dawley (Currently Envigo, Madison, WI). Rats were given a sequential treatment (Figure 21) of flutamide administered for 20 days, followed by a single subcutaneous injection with testosterone propionate suspended in corn oil on day 21 and a single intraperitoneal injection of N-methyl-N-nitrosourea (MNU; dissolved in a citrate-phosphate buffer pH 4.8 and diluted in saline to a dose of 30 mg/kg; NCI carcinogen repository, MRIGlobal, Kansas City, MO) 52-56 hours later. Two weeks later, each rat surgically received (ketamine-xylazine anesthesia) two subcutaneous Silastic tubing implants (ID 0.078”; OD 0.125”) containing crystalline testosterone tightly packed over 3 cm length. One week after MNU, rats were started on AIN-93M diet (control) or isoenergetic AIN-93M diets containing 5% or 10% lyophilized BRB powder (see above). Rats were euthanized when moribund or surviving for 56 weeks.

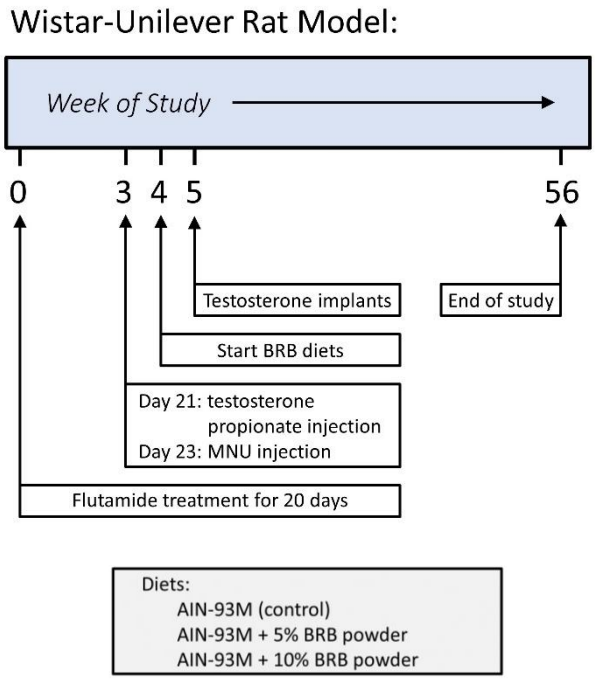


Figure 21. MNU plus androgen model of rat prostate carcinogenesis

v. Necropsy and histology

At necropsy, the accessory sex glands were excised with the urinary bladder from WU and NBL rats following euthanasia through exsanguination while under ketamine-xylazine anesthesia of by cervical dislocation after CO_2 inhalation and asphyxiation. Accessory sex glands, pituitaries, and all grossly observed lesions in other organs were fixed in 10% neutral buffered formalin. After fixation, the ventral prostate, dorsolateral prostate and anterior prostate plus seminal vesicles were dissected and processed to and embedded in paraffin wax. From the ventral prostate and grossly observed tumor masses one section was made and from all other accessory sex gland tissues step sections were prepared at 250 micrometer intervals, which were stained with hematoxylin and eosin as described by McCormick *et al*. (254). All prostate lobes and other accessory sex glands were evaluated histopathologically and the presence, type, and size of all lesions were scored, using previously published criteria (254-256).

The xenograft tumors from mice euthanized through exsanguination while under ketamine-xylazine anesthesia of by cervical dislocation after CO_2 inhalation and asphyxiation were also collected in formalin at necropsy for possible future histological examination.

vi. Prostatic levels of protocatechuic acid

Male NBL rats were randomized into three groups, each with 5 animals, and fed AIN-93M control diet, or isoenergetic diets containing 5% or 10% lyophilized BRB powder. Animals were euthanized after three weeks. Dorsolateral prostate tissue was removed immediately following euthanasia and flash frozen in liquid nitrogen and stored at -80^{o}C until analysis. Protocatechuic acid levels were measured using LC-MS/MS by Dr. Zhongfa Liu (Ohio State University, Columbus, OH) as previously described (257) following shipping on dry ice.

D. Data analysis

i. Statistical analysis of in vitro data

All experiments were performed at least 3 times. Results are expressed as mean ± standard deviation (SD), unless otherwise specified. Comparisons were performed using Student's t test or one way analysis of variance (ANOVA) followed by a post-hoc test when appropriate. Statistical analysis was performed with GraphPad. A p value <0.05 was considered significant.

ii. Combination index analysis

Combination treatment effects were quantified by combination index (CI) analysis based on the Chou-Talalay equation (258). Analysis was performed using CompuSyn software (ComboSyn, Paramus, NJ). CI values indicates an additive effect (CI = 1), synergism (CI < 1) or antagonism (CI > 1) in drug combinations.

iii. Analysis of animal experimental data

Differences in lesion incidence among the accessory sex glands were analyzed using Fisher's exact test (two group comparisons) and X^2 analysis (three group comparisons). Xenograft tumor volume was calculated using formula 2.1, and tumor growth delay (TGD) was calculated with formula 2.2 (259, 260). Outliers were identified using the extreme studentized deviate test (Grubbs' test) (261) and excluded from data analysis.

(2.1) Volume = length x (2 x width) x 0.523

(2.2) $$\text{TGD} = \left(\begin{array}{c}\text{time for treated group to reach} \\ \text{tumor vol. of 3,000 mm}^3\end{array}\right) - \left(\begin{array}{c}\text{time for control group to reach} \\ \text{tumor vol. of 3,000 mm}^3\end{array}\right)$$

III. EFFECTS OF BLACK RASPBERRIES AND THEIR CONSTITUENTS ON RAT PROSTATE CARCINOGENESIS AND HUMAN PROSTATE CANCER CELL GROWTH IN VITRO

A. Introduction

Despite advances in early detection and treatment modalities, prostate cancer remains one of the most frequently diagnosed cancer types and a leading cause of cancer related death among men in United States. In 2016, over 180,000 new cases of prostate cancer are anticipated to occur in the U.S., accounting for 21% of new cancers in American men (7). Considering the high prevalence of this disease and the lack of prevention options, strategies to reduce incidence and disease progression are urgently needed.

The use of dietary agents has long been considered an appealing approach to cancer prevention. Numerous laboratory studies have reported anti-cancer activity of various dietary constituents and phytochemicals, supporting the concept that increased consumption of bioactive compounds through dietary modification or supplementation could reduce cancer incidence. Additionally, the use of food products for cancer prevention is very practical because they are readily available, cost-efficient, and generally exhibit little to no toxicity (262).

There is an increasing amount of evidence establishing chemopreventive and chemotherapeutic potential of black raspberries (BRB; *Rubus occidentalis*). BRB preparations and extracts can inhibit cancer cell proliferation, induce apoptosis, and inhibit angiogenesis (1). The most compelling evidence supporting the use of BRBs as a chemopreventive agent has been demonstrated in studies of gastrointestinal (GI) cancers (2). Dietary administration of BRBs inhibited tumor initiation as well as tumor promotion-progression in the esophagus and colon in animal models of carcinogenesis (205, 208, 210, 211). Oral administration of BRBs also

inhibited mammary tumorigenesis in animal models (213-215), suggesting that biologically active berry constituents are systemically bioavailable following oral administration and have the ability to reach a distant tumor site. Additionally, BRBs were well tolerated and had protective effects in several human clinical trials.

BRBs contain numerous bioactive phytochemicals including anthocyanins, ferulic acid, ellagitannins, β-sitosterol, and quercetin, and several other nonnutritive compounds, as well as vitamins and minerals (196). The potent anticancer effects of BRBs have been attributed to their high concentration of ellagic acid and anthocyanins (193-195). BRBs contain approximately 5 – 20 mg of anthocyanins and approximately 1.5 – 2 mg of ellagic acid per gram dry weight (1, 197-199). Ellagic acid (EA) is a polyphenolic compound found in fruits and nuts such as pomegranates, berries and walnuts (Figure 37, Page 82). EA has potent antioxidant activity, inhibits cell migration, and reduces cell proliferation *in vivo* and *in vitro* (244, 246, 248). Anthocyanins are naturally occurring flavonoids that provide blue, purple, and red pigmentation to a variety of fruits and vegetables, including berries, grapes, apples, and purple cabbage (227, 263). They have growth inhibitory, anti-inflammatory, and antioxidant activity, which makes anthocyanin-rich foods, like BRBs, an attractive option for the prevention and treatment of cancer (240, 264, 265). In addition to being a rich source of anthocyanins, BRBs have a unique anthocyanin profile, predominately consisting of the cyanidin subtype (Figure 15, page 25) (198, 200).

Although BRBs and their constituents have been shown to prevent carcinogenesis at a number of organ sites, inhibition of prostate cancer by berries has not been evaluated (2). Based on the abundant literature demonstrating anti-cancer activity of BRBs and evidence that BRBs can inhibit tumor development systemically, we hypothesized that BRBs would prevent prostate carcinogenesis and inhibit proliferation of prostate cancer cells, and that these effects are predominately mediated by BRB anthocyanins. The overall goals of this study were to 1)

investigate the efficacy of dietary BRB consumption in prostate cancer chemoprevention, 2) evaluate growth inhibitory activity of BRB extract on prostate cancer cells, and 3) identify BRB constituent compounds responsible for the effects of BRBs on prostate cancer growth and development.

B. Results

i. Identification of protocatechuic acid in rat prostate following dietary administration of black raspberries

For dietary agents to be effective in preventing or treating cancer, they must first come into contact with the target tissue. Ingested food products are subject to many factors including digestion, absorption, metabolism, and excretion, which greatly impact tissue distribution of compounds. Therefore, it is critical to determine the bioavailability of dietary agents to target tissues before investigating efficacy *in vitro* or *in vivo*. Since tissue distribution of BRB metabolites has not been comprehensively evaluated, we set out to determine if the major anthocyanin metabolite, protocatechuic acid (PCA), could be identified in prostate tissue following dietary administration. To this end, we fed NBL rats diets containing 0, 5 or 10% lyophilized BRB powder for three weeks, then measured dorsolateral prostatic levels of PCA. We detected 3.64 ± 1.35 and 4.60 ± 1.92 ng/g PCA in rats fed 5% and 10% BRB diet, respectively, whereas PCA was virtually undetectable in rats fed a control diet (Table III). This observation demonstrates that at least one BRB metabolite is bioavailable to the rat prostate following oral exposure to BRBs, and therefore we proceeded to investigate effects of BRBs in animal and cell culture models.

Table III. Prostatic levels of protocatechuic acid

Treatment (% BRBs in diet)	0%	5%	10%
Number of rats	5	5	5
PCA level in dorsolateral prostate (ng/g wet tissue)[1]	0.5 ± 0.0 [2,3]	3.64 ± 1.35 [3]	4.60 ± 1.92 [3]

[1] Mean ± standard deviation. [2] PCA levels in the control group were below the limit of detection (1 ng/g); control values were arbitrarily set at 0.5 ng/g. [3] p = 0.012 (linear trend)

ii. Effects of black raspberries on the proliferation of prostate cancer cells

Numerous studies have demonstrated growth inhibitory properties of BRB extract and constituents in cell culture models, but only a few investigations involved prostate cancer cells lines. To study the effects of BRB extract, bioactive constituents, and metabolites on growth of prostate cancer cells, we used a panel of six prostate cancer cell lines to measure cell viability: C4-2, LAPC-4, LNCaP, 22Rv1, PC-3 and VCaP. These cell lines were selected to evaluate possible differential effects of BRB compounds on cells with varying growth rates and molecular characteristics (see Table VIII, APPENDIX B). Cells were treated with BRB extract (1 – 1,000 µg/mL), PCA (1 ng/mL – 10 µg/mL), Cy-3-Rut (1 – 100 µM), EA (0 – 30 µM) or appropriate vehicle controls for 72 hours and proliferation was measured by SRB assay. We chose to use Cy-3-Rut because it is the most abundant BRB anthocyanin. Results are expressed as percent relative to control. BRB extract (Figure 22A), Cy-3-Rut (Figure 22B) and PCA (Figure 22C) had no significant effect on proliferation of any of the prostate cancer cell lines. 10 µM EA inhibited proliferation of VCaP cells by 17.5 ± 5.7% and PC-3 cells by 44.2 ± 10.0%. 30 µM EA inhibited proliferation of LAPC-4 (21.7 ± 6.4%), VCaP (25.2 ± 7.4%), C4-2 (31.7 ± 7.0%), 22Rv1 (66.7 ± 11.1%), and PC-3 cells (82.7 ± 8.5%) (Figure 22D).

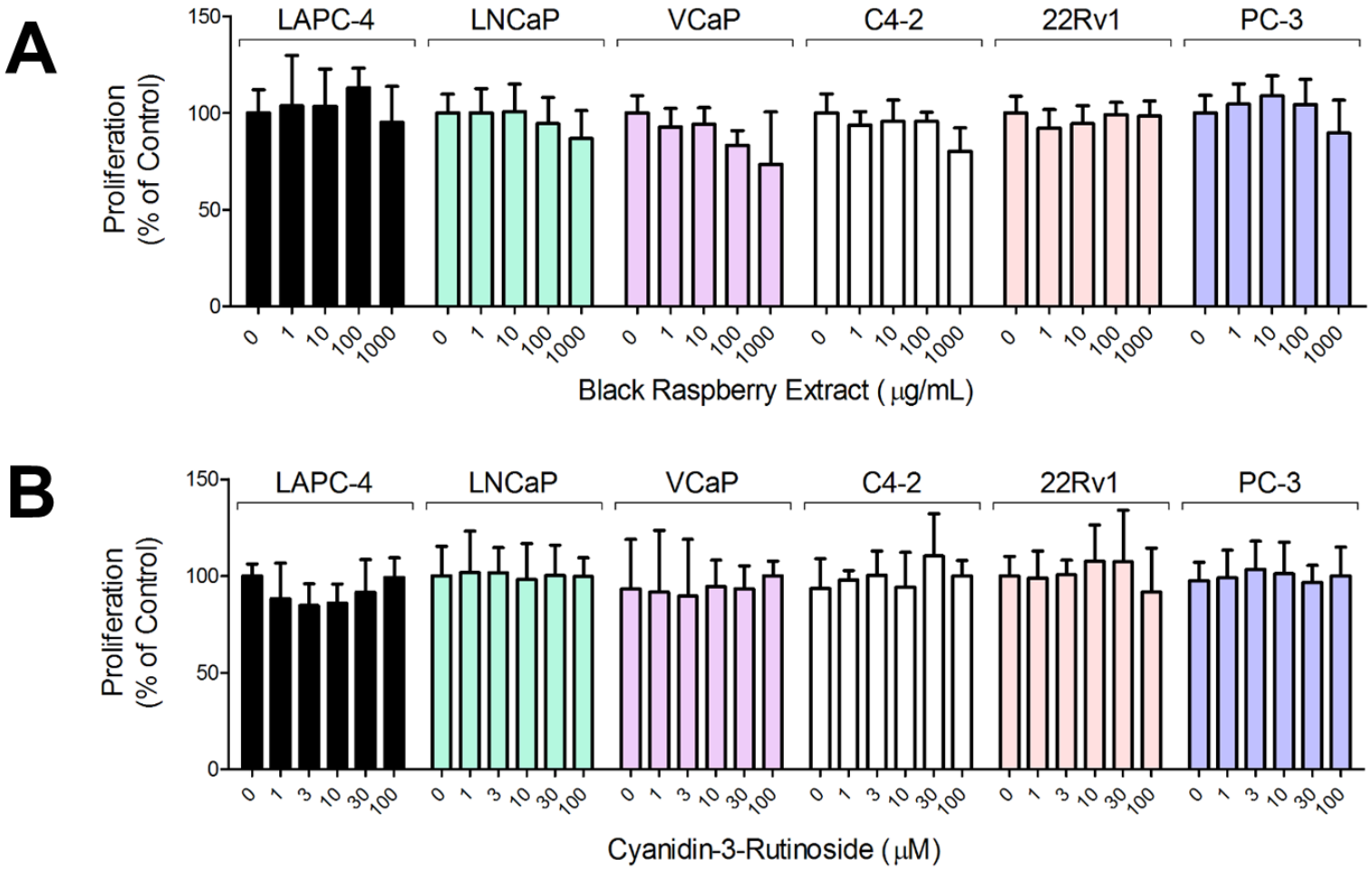


Figure 22. Effects of black raspberry extract and constituents on proliferation of prostate cancer cells.
Proliferation was measured in six prostate cancer cell lines using the SRB assay after 72 hours treatment with 1 – 1,000 mg/mL BRB extract (A), 1 – 100 μM cyanidin-3-rutinoside (B), 1 – 10,000 ng/mL protocatechuic acid (C), and 1 - 30 μM ellagic acid (D) and normalized to vehicle control treated cells. Data represent mean ± SD; * significantly different from vehicle control $p < 0.05$.

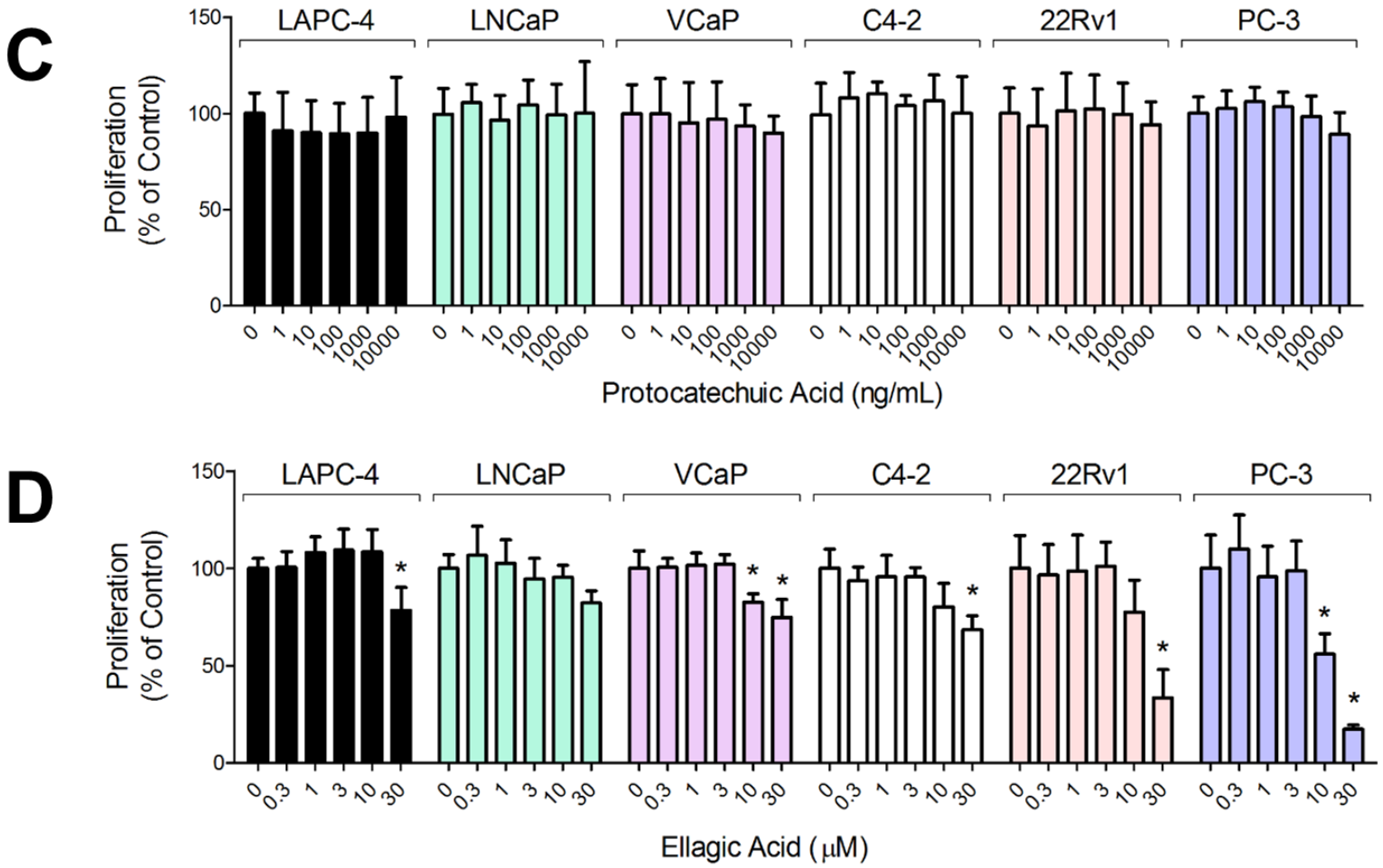


Figure 22 (continued). Effects of black raspberry extract and constituents on proliferation of prostate cancer cells. Proliferation was measured in six prostate cancer cell lines using the SRB assay after 72 hours treatment with 1 – 1,000 mg/mL BRB extract (A), 1 – 100 μM cyanidin-3-rutinoside (B), 1 – 10,000 ng/mL protocatechuic acid (C), and 1 - 30 μM ellagic acid (D) and normalized to vehicle control treated cells. Data represent mean ± SD; * significantly different from vehicle control $p < 0.05$.

iii. Black raspberry extract and constituents reduce anchorage-independent growth

Anchorage-independent growth is associated with progression and metastasis of solid tumors, and often involves the downregulation of E-cadherin (266, 267). Studies have shown that dietary BRBs can upregulate E-cadherin expression in colon of both humans and mice (217, 268). Therefore, we investigated the effects of BRBs on anchorage independent growth of prostate cancer cells using a soft-agar colony formation assay with PC-3 cells, which are the only cells in our panel that grew efficiently in soft-agar. Treatment with BRB extract significantly reduced the number of colonies compared to control (413.8 ± 31.2 colonies) at concentrations of 10 µg/mL (321.6 ± 26.6 colonies), 100 µg/mL (348.0 ± 32.3 colonies), and 1 mg/mL (209.3 ± 20.1 colonies), by 22.2%, 15.9%, and 49.4% respectively (Figure 23). EA inhibited colony formation by 21.2% and 39.8% at 1 µM (326.1 ±18.9 colonies) and 3 µM (249.3 ± 18.9 colonies). PCA inhibited colony formation by 32% at 10 µg/mL (281.3 ± 42.3 colonies). Cy-3-Rut did not inhibit colony formation at any concentration tested (1 – 30 µM).

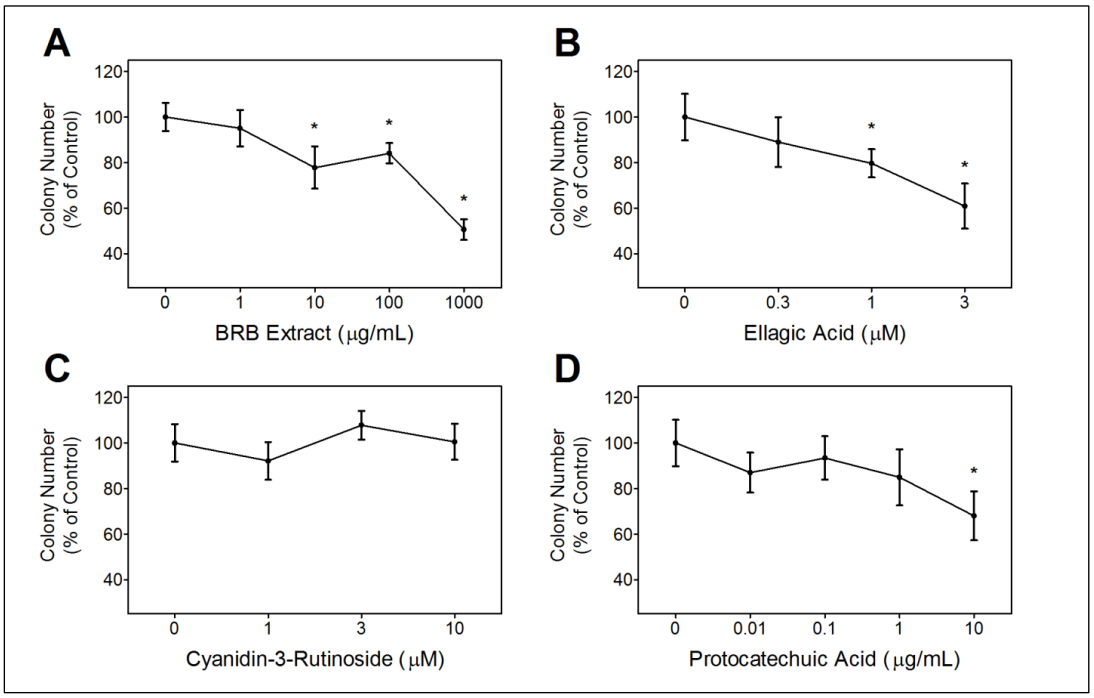


Figure 23. Effects of black raspberry extract and constituents on anchorage independent growth of PC-3 cells.

iv. BRB extract, EA and PCA do not inhibit migration of prostate cancer cells

Cell migration is a critical step in the metastatic process that enables cancer cells to invade surrounding tissue and disseminate from the primary tumor site (269, 270). *In vitro* studies have shown that BRBs extract and EA can inhibit cell migration of vascular endothelial cells (245, 271). Thus, we examined the effect of BRB extract, EA and PCA on migration of PC-3 cells using a wound healing assay (Figure 24). Confluent cells were treated with 100 µg/mL BRB extract, 10 µg/mL PCA or 3 µM EA for 24 hours prior to making a scratch in monolayer. As shown in Figure 24C no significant differences in rate of wound closure were observed for BRB extract, EA or PCA compared to vehicle control.

v. Anthocyanin uptake

Given the lack of growth inhibition by Cy-3-Rut (predominant BRB anthocyanin) on our prostate cancer cells, we speculated that anthocyanin uptake could be limited in these cells. Anthocyanins are large, bulky compounds, which require active transport to cross the cell membrane or metabolism to smaller anthocyanidin aglycones that can enter the cell by diffusion (119). To evaluate anthocyanin uptake in prostate cells, we treated 22Rv1 cells with Cy-3-Rut (30 µM), cyanidin (aglycone of Cy-3-Rut; 30 µM), BRB extract (1 mg/mL), or vehicle control, then measured intracellular levels of cyanidin and Cy-3-Rut by HPLC-MS/MS. Since we expected uptake of anthocyanins to be low based on literature reports, we treated cells with high concentrations to increase sensitivity of intracellular detection.

As expected, we observed high intracellular levels of cyanidin (44.82 ± 5.96 ng per 1 x 10^6 cells) in cells treated with cyanidin, compared with vehicle (0.43 ± 0.20 ng per 1 x 10^6 cells), Cy-3-Rut (0.43 ± 0.17 ng per 1 x 10^6 cells), and BRB treated cells (0.66 ± 0.32 ng per 1 x 10^6 cells)

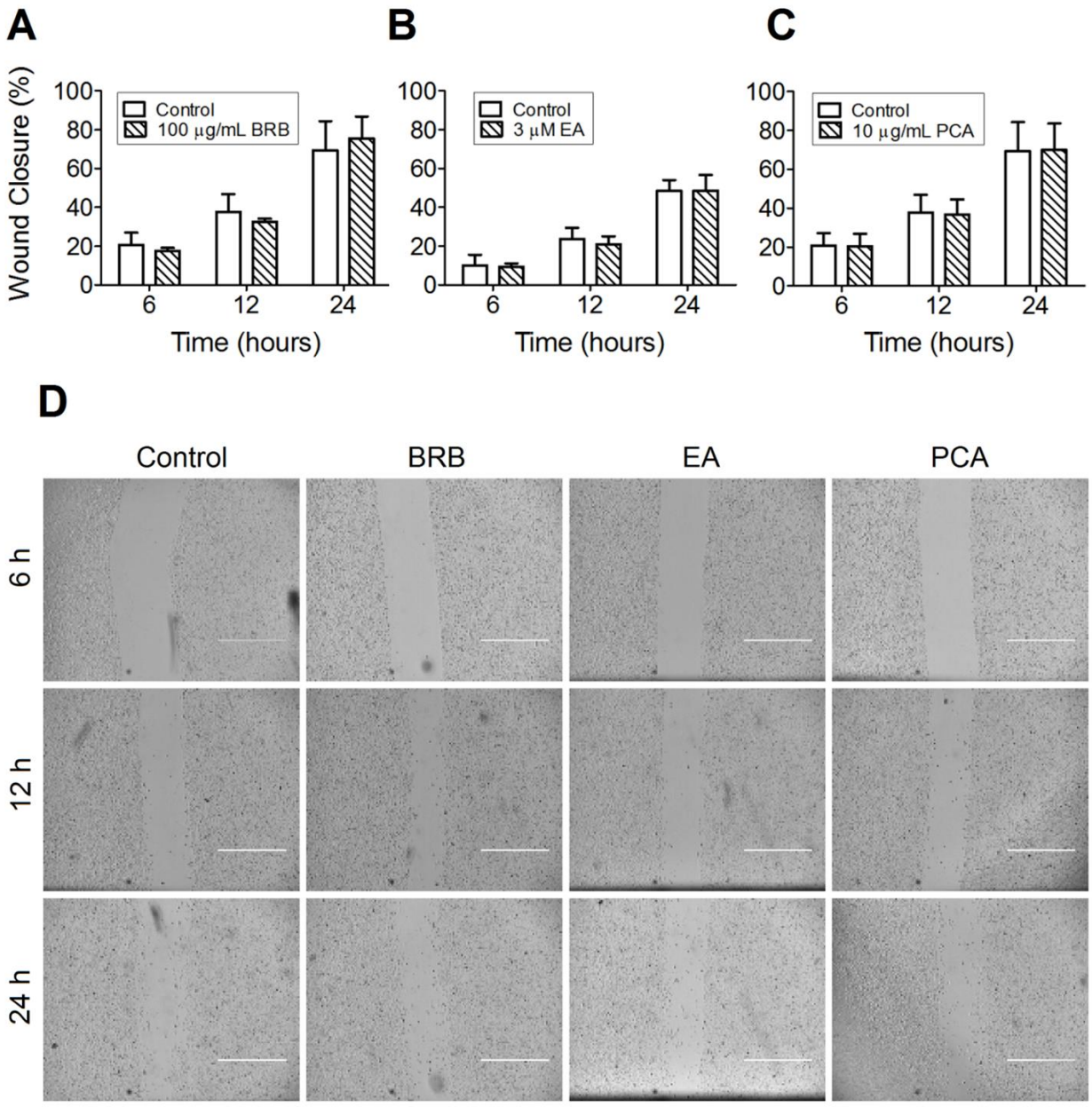


Figure 24. Black raspberry extract and constituents do not inhibit migration of prostate cancer cells. Migration of PC-3 cells was measured by wound healing assay following 24 hour incubation with 100 µg/mL BRB extract (A), 3 µM EA (B), and 10 µg/mL PCA (C) or vehicle control. Percent wound closure was calculated by normalizing to distance at 6, 12 and 24 hours to distance of wound opening at 0 hours. Data represent average of three experiments ± SD.

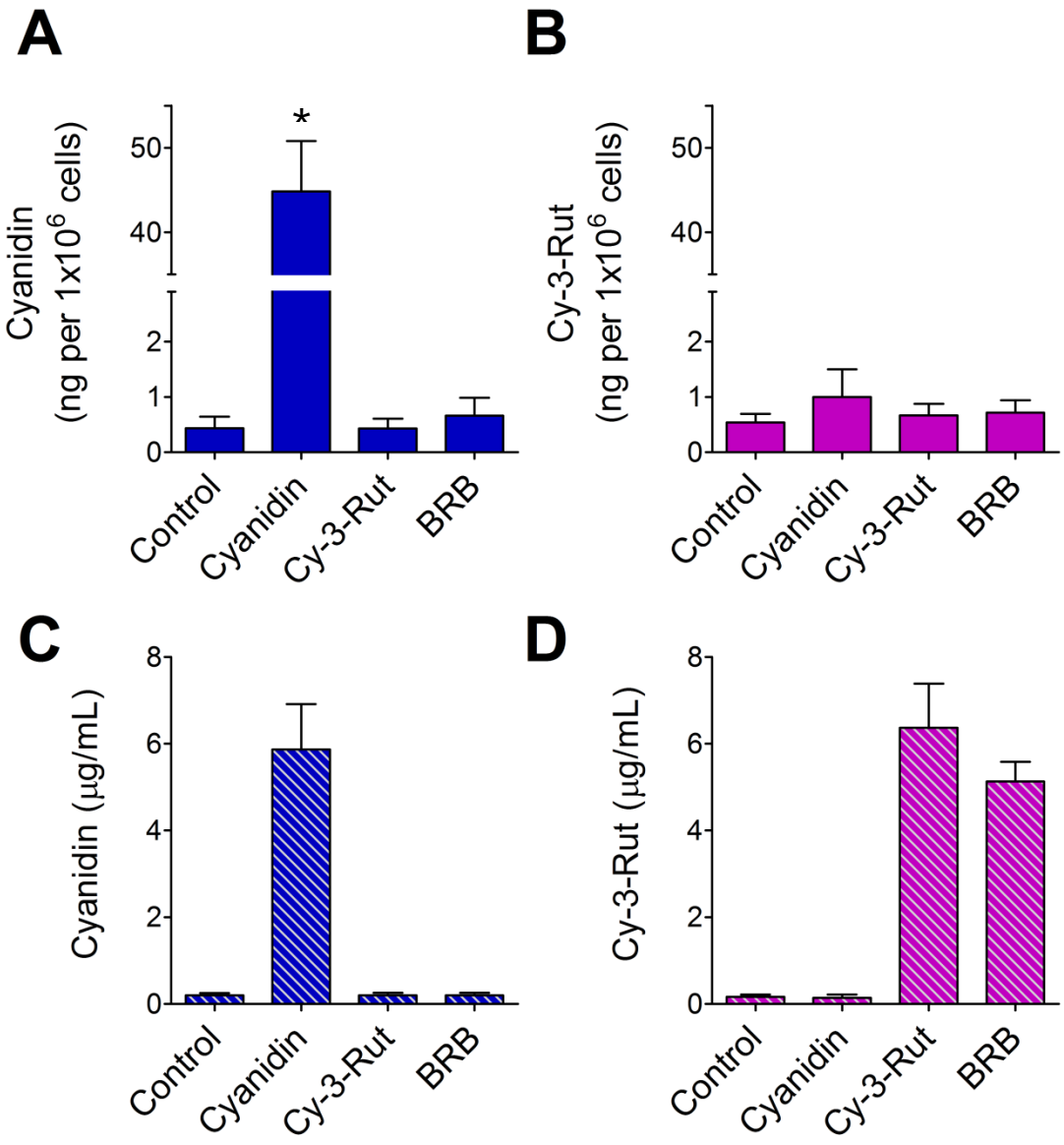


Figure 25. Differential uptake of cyanidin-3-rutinoside and its aglycone by prostate cancer cells. Intracellular levels of cyanidin (A) and cyanidin-3-rutinoside (B) were measured in 22Rv1 following incubation with vehicle control, cyanidin (30 µM), cy-3-rut (30 µM), or BRB extract (1 mg/mL). Measurement of cyanidin (C) and cyanidin-3-rutinoside (D) in cell culture media.

(Figure 25A), but significant intracellular levels of Cy-3-Rut were not detected in cells treated with Cy-3-Rut (0.67 ± 0.21 ng per 1 x 10^6 cells) or BRB extract (0.72 ± 0.23 ng per 1 x 10^6 cells), compared to control (Figure 25B). Levels of cyanidin and Cy-3-Rut in the cell culture media were quantified to verify that compounds were present in media. Cyanidin was detected in cyanidin-supplemented media (5.87 ± 1.05 μg/mL) and Cy-3-Rut was detected in media supplemented with both Cy-3-Rut (6.37 ± 1.02 μg/mL) and BRB extract (5.13 ± 0.45 μg/mL) (Figure 25 C & D); this confirms that cells were exposed to similar levels of cyanidin and Cy-3-Rut across treatment groups and differential intracellular levels are not attributable to differences in exposure levels.

vi. Prostate cancer cell growth is inhibited by cyanidin

Because we observed cellular uptake of cyanidin, but not Cy-3-Rut, we investigated effects of cyanidin on proliferation of our prostate cancer cells. Due to the intense color of cyanidin, we could not perform an SRB assay to assess proliferation as previously done with Cy-3-Rut. When present in cell culture media, uptake of cyanidin results in a blueish-purple “staining” of cells, which is visibly apparent with the naked eye and interferes with colorimetric readings in the SRB assay. Therefore, we performed a fluorescent-based resazurin reduction assay as an alternative method of measuring cell viability. 22Rv1, C4-2 and PC-3 cells were treated with Cy-3-Rut (1 – 100 μM) and cyanidin (1 – 100 μM). Similar to results of the SRB assay, Cy-3-Rut did not inhibit viability at any concentration in any of the cell lines (Figure 26A). In contrast, cyanidin significantly inhibited viability of all cell lines at 30 and 100 μM (Figure 26B). Additionally, we examined the effects of cyanidin and Cy-3-Rut on S-phase activity my measuring incorporation of a thymidine analog (EdU) into DNA of C4-2 cells. Consistent with results of the resazurin assay, we observed a reduction in EdU incorporation in cells treated with 30 and 100 μM

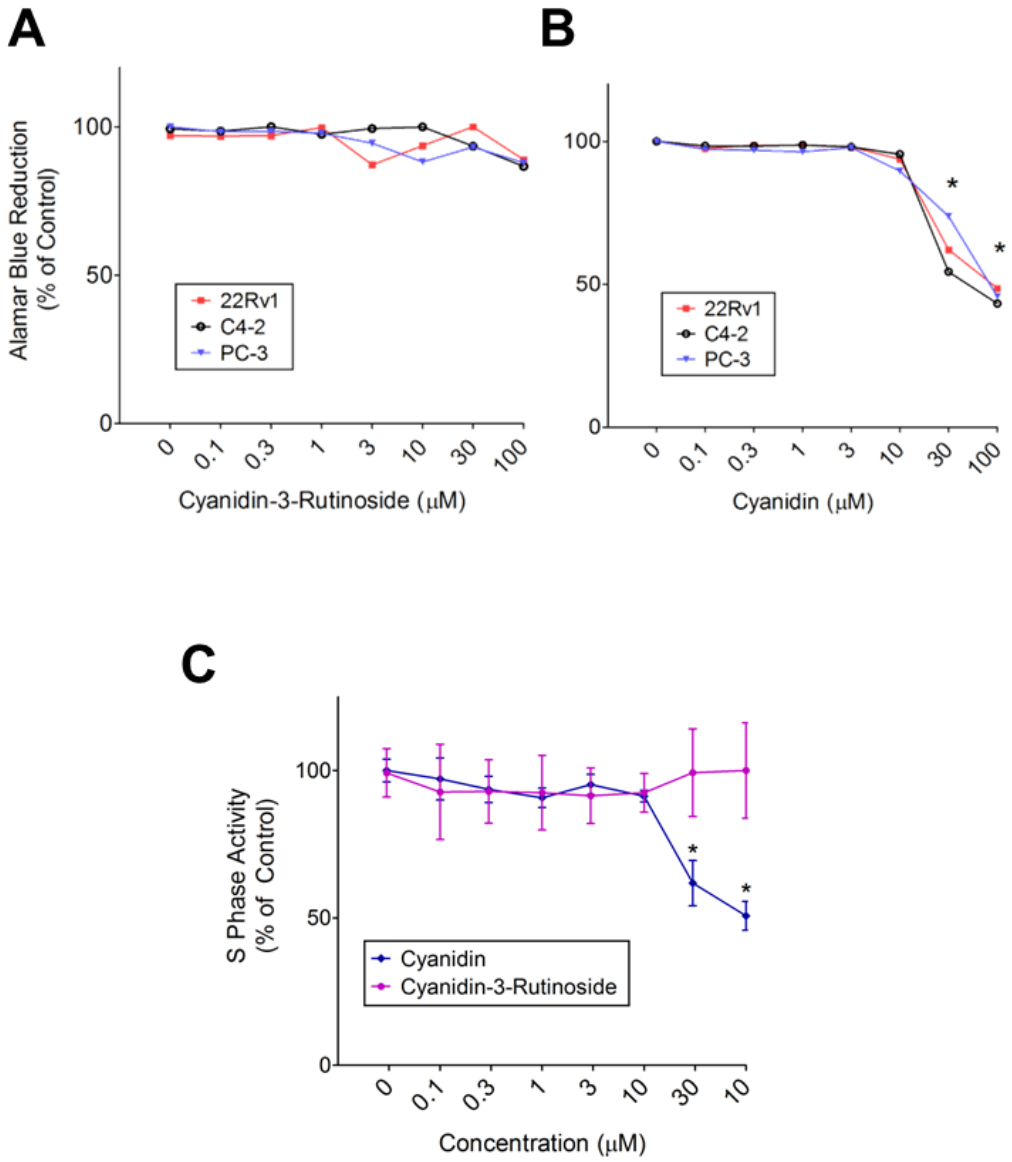


Figure 26. Cyanidin but not cyanidin-3-rutinoside inhibits growth of prostate cancer cells Viability of 22Rv1, C4-2 and PC-3 cells is inhibited by cyanidin (B) but not by Cy-3-Rut in a resazurin reduction assay (A). Likewise, cyanidin rather than Cy-3-Rut reduces S-phase activity in C4-2 cells (C).

cyanidin, indicating an inhibition of S-phase activity by cyanidin, whereas Cy-3-Rut had no effect at any concentration (Figure 26C).

vii. Dietary black raspberries are not protective against rat prostate carcinogenesis

Two rat models of prostate carcinogenesis were used to evaluate the chemopreventive efficacy of dietary BRBs as described in Chapter II, Section C.

Wistar-Unilever (WU) Rat Model: We studied the effect of feeding BRBs on prostate cancer induced by MNU and chronic testosterone (MNU + T) via subcutaneous Silastic implants in WU rats. Rats received either AIN-93M diet or AIN-93M supplemented with 5 or 10% lyophilized BRB powder. Overall incidence of grossly observed large prostate tumors, histologically confirmed as adenocarcinomas, was reduced from 52% (16 of 31) in the control group to 46% (14 of 30) and 25% (8 of 32) in the low and high dose BRB groups, respectively, which was significant ($p = 0.031$ X^2 test), as was the difference between the control and high dose groups (0.039, 2-sided Fisher test). However, detailed examination of step sections revealed no statistically significant difference in incidences of neoplastic lesions in any of the accessory sex glands, although there was an apparent, but non-significant, shift from large (>5 mm) to small (<5 mm) tumors ($p = 0.065$; Table IV).

Noble (NBL) Rat Model: We also studied the effect of feeding AIN-93M diet containing 5 or 10% lyophilized BRB powder on induction of prostate cancer in NBL rats by chronic testosterone plus 17β-estradiol (T + E2). As was the case in the MNU + T model, we did not observe any differences among the three groups in total cancer incidence in this model and prostate cancer multiplicity was also not affected by the BRB treatment (Table V).

Table IV. Results of prostate cancer induction study in WU rats fed BRBs

Group	1	2	3
Treatment (% BRB in diet)	0%	5%	10%
Effective number of animals	31	30	32
	Number (%) of rats:		
All Accessory Sex Glands Combined (dorsolateral and anterior prostate plus seminal vesicle):			
Grossly observed tumors at necropsy	16 (52) [a,b]	14 (47) [a]	8 (25) [a,b]
Adenocarcino(sarco)ma, All (with or without C.I.S.)	23 (74)	ND	23 (77)
Microscopic (< 5 mm)	6 (19)[c]	ND	12 (38) [c]
Macroscopic (> 5 mm)	17 (55) [c]	ND	11 (34) [c]
Adenocarcinoma + C.I.S.	26 (84)	ND	24 (75)
Carcinoma *in situ* (C.I.S.) only	3 (10)	ND	1 (3)
Dorsolateral Prostate Region (originating from dorsolateral or anterior prostate or seminal vesicle):			
Adenocarcino(sarco)ma, Macroscopic size	5 (16)	ND	6 (19)
Anterior Prostate/Seminal Vesicle Region (originating from anterior prostate or seminal vesicle):			
Adenocarcino(sarco)ma, All	6 (19)	ND	3 (9)
Microscopic (< 5 mm)	0	ND	1 (3)
Macroscopic (> 5 mm)	6 (19)	ND	2 (6)
Dorsolateral plus Anterior Prostate (clearly confined to these glands; w or w/o seminal vesicle lesions):			
Adenocarcinoma, Microscopic (with or without C.I.S.)	7 (23)	ND	8 (25)
Adenocarcinoma + C.I.S.	10 (32)	ND	11 (34)
Carcinoma *in situ* (C.I.S.) only	3 (10)	ND	3 (9)
Seminal Vesicle Only (clearly confined to this gland; w or w/o C.I.S. in dorsolateral/ anterior prostate):			
Adenocarcinoma, All (with or without C.I.S.)	4 (13)	ND	6 (19)
Microscopic (< 5 mm)	2 (6)	ND	5 (16)
Macroscopic (> 5 mm)	2 (6)	ND	1 (3)

[a] Trend: $p = 0.031$ (X^2 Test)

[b] Difference: $p = 0.039$ (2-sided Fisher Exact Test)

[c] Shift from large to small tumors: $p = 0.065$ (1-sided Fisher Test), $p = 1.30$ (2-sided Fisher Test)

Abbreviations: C.I.S. = Carcinoma *in situ*; ND = Not Determined

Table V. Results of prostate cancer induction study in NBL rats fed BRBs

Group	1	2	3
Treatment (% BRB in diet)	0%	5%	10%
Number of animals	30	30	30
When only one slide was examined:			
Number of rats with prostate carcinomas (%)	18 (60) [a,b]	24 (77)	26 (87) [a,b]
Mean number of carcinomas per cancer-bearing rat ± SD	1.72 ± 0.75	1.75 ± 0.85	1.88 ± 0.77
When six slides per prostate were examined:			
Number of rats with prostate carcinomas (%)	28 (93)	ND	30 (100)
Mean number of carcinomas per cancer-bearing rat ± SD	2.28 ± 1.01	ND	2.77 ± 1.17 [c]

[a] $p = 0.044$ (X^2 test); $p = 0.016$ for linear trend

[b] $p = 0.044$ for difference with the control group (2-sided Fisher exact test)

[c] $p = 0.10$ (2-sided t-test); $p = 0.099$ (2-sided t-test with Welch's correction for unequal SDs; $p = 0.08$ (2-sided Mann Whitney test) for difference with the control group

Abbreviations: SD = Standard Deviation; ND = Not Determined

C. Discussion

Numerous publications have demonstrated anti-cancer effects of black raspberries in GI cancers, including many examples of efficacy *in vitro* and in animal models, as well as several reports describing chemopreventive and therapeutic effects of BRBs observed in clinical trials. By contrast, there is relatively little known about the effects of BRBs on organs outside of the GI system. In the present study, we provide the first information of BRB effects on rat prostate carcinogenesis and several prostate cancer cell lines.

The effectiveness of BRBs is often attributed to their high anthocyanin content (193-195). We evaluated the effects of Cy-3-Rut, the most abundant BRB anthocyanin, on growth of prostate cancer cell lines using multiple proliferation assays. Contrary to our hypothesis, we found no effect on proliferation of prostate cancer cells by Cy-3-Rut (up to 100 μM). This result was observed consistently across cell lines and in three different proliferation assays, reducing the likelihood that these findings are an artifact of experimental design or is due to cell line- or assay-specific characteristics. Further investigation into the anti-cancer properties of Cy-3-Rut revealed no effect on anchorage-independent growth or *in vitro* migration of PC-3 cells. These results suggest that anthocyanins are not effective inhibitors of prostate cancer.

The lack of growth inhibitory effects of Cy-3-Rut prompted us to evaluate anthocyanin uptake by prostate cancer cells, with the new hypothesis that low Cy-3-Rut uptake by prostate cells was precluding inhibitory effects on cell growth. Our analysis of intracellular anthocyanin levels supported this theory by revealing that culturing 22Rv1 cells in media supplemented with BRB extract or Cy-3-Rut does not lead to intracellular accumulation of Cy-3-Rut or its aglycone (cyanidin). But when cells were treated with cyanidin directly, we observed robust intracellular levels of this compound, as well as reduced proliferation of 22Rv1, PC-3 and C4-2 cells at high concentrations. These findings suggest that prostate cancer cells are sensitive to effects of the

anthocyanidin aglycones, but insensitive to the parent anthocyanins (such as Cy-3-Rut) due to an inability to take up these compounds. Impaired uptake and activity of anthocyanins, relative to their aglycones, has been previously reported and is thought to be caused by hindrance due to the presence of bulky glycosidic moieties. In one study comparing the incorporation of several anthocyanins into endothelial cells, an inverse association between anthocyanin uptake and the number of glycoside groups was reported (272). Another study investigating protective effects of anthocyanins against DNA damage, cyanidin and delphinidin, but not their glycosides, were effective against lipid peroxidation, leading the authors to conclude that protective effects of anthocyanins are diminished by the presence of bulky sugar moieties (273).

In contrast to the ineffectiveness of Cy-3-Rut, we did observe growth inhibitory activity of EA on prostate cancer cells. EA significantly reduced viability in five of the six cell lines tested, having the greatest impact on the two most aggressive cell lines (22Rv1 and PC-3), suggesting that EA may preferentially target rapidly proliferating cells types. However, inhibitory concentrations of EA (>10 μM) in this study are unlikely achievable through dietary consumption of BRBs or other EA containing foods. On the other hand, in the colony formation assay EA inhibited anchorage-independent growth of PC-3 cells at concentrations as low as 1 μM. Inhibition of colony formation by the lower levels of EA used in this assay could be due to activity on mechanisms associated with metastatic and invasive growth. It has been indicated that EA can reverse epithelial-to-mesenchymal transition by modulating expression of factors involved in cell adhesion, metastasis, and chemotaxis (274-276). Alternatively, the effects at low EA doses may be a result of prolonged exposure to EA on proliferation (3 weeks in colony formation assay vs. 3 days in the SRB assay). Inhibitory effects on proliferation due to chronic exposure may also provide an explanation for reduction in colony number in BRB and PCA treated cells at concentrations that did not decrease proliferation after 72 hours.

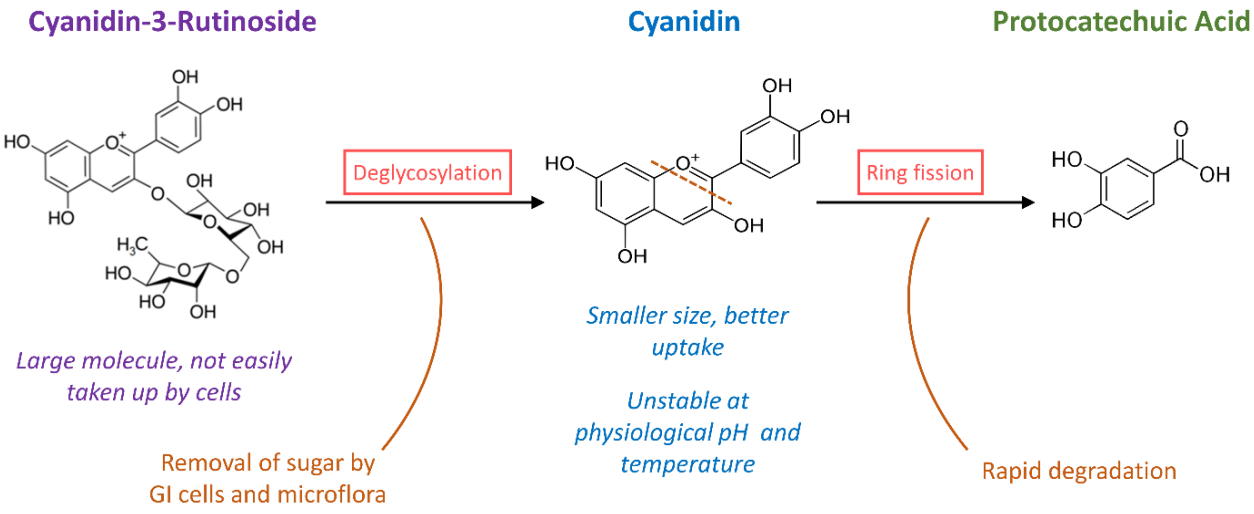


Figure 27. Deglycosylation and degradation of cyanidin-3-rutinoside

Considering the results of our anthocyanin uptake experiments, it is not surprising that we did not observe inhibitory effects *in vitro*, but this may not provide an explanation for absence of efficacy in our animal experiments. Based on the current understanding of anthocyanin metabolism, the inability of BRBs to prevent prostate cancer in our study is conceivably due to low tissue bioavailability. Both anthocyanins and their aglycones are very unstable under physiological conditions, rendering them subject to rapid metabolic modification and/or degradation (Figure 27) (230, 277). The instability of these compounds is immediately apparent when they are added to cell culture media by observing the characteristic loss in color associated with degradation (Figure 28). In the digestive tract, anthocyanins are deglycosylated either by gut microflora or by epithelial enzymes (such as β-glucosidase and LPH) or they can be taken up intact by glucose transporters and metabolized by intracellular β-glucosidases (119, 278-282). Following absorption, anthocyanins can undergo further hepatic modification to glucuronidated, methylated, or sulphated forms before entering circulation (283-285). Overall,

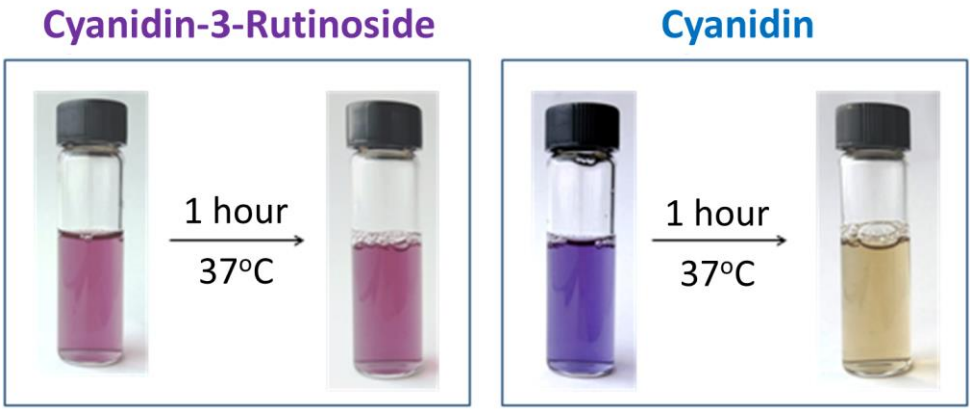


Figure 28. Anthocyanin degradation is associated with loss of color
Cyanidin (right) is less stable than Cy-3-Rut (left) in phenol red-free RPMI media at 37°C and is rapidly degraded, resulting in a color change. Although Cy-3-Rut is more stable than the aglycone under physiological conditions, it also subject to degradation within 24 hours.

the extensive metabolism and rapidly degradation of anthocyanins suggest poor systemic bioavailability (Figure 29). Concordantly, studies have demonstrated that anthocyanins are detectable at only very low plasma levels after ingestion, with phenolic degradation products appearing soon after ingestion (280, 282, 286). Intact anthocyanins (cyanidin-3-glucoside) have been identified in prostate tissue of animals fed an anthocyanin-enriched diet, but at only very low levels (1–3% of amount administered in the diet) that are likely insufficient to be biologically active (287, 288). Moreover, we observed very low intracellular levels of Cy-3-Rut and cyanidin in prostate cancer cells following exposure to Cy-3-Rut, suggesting that prostate cells may lack ability to transport or metabolize anthocyanins. Thus, even if anthocyanins can reach the prostate tissue through the circulation, they would likely lack chemopreventive efficacy.

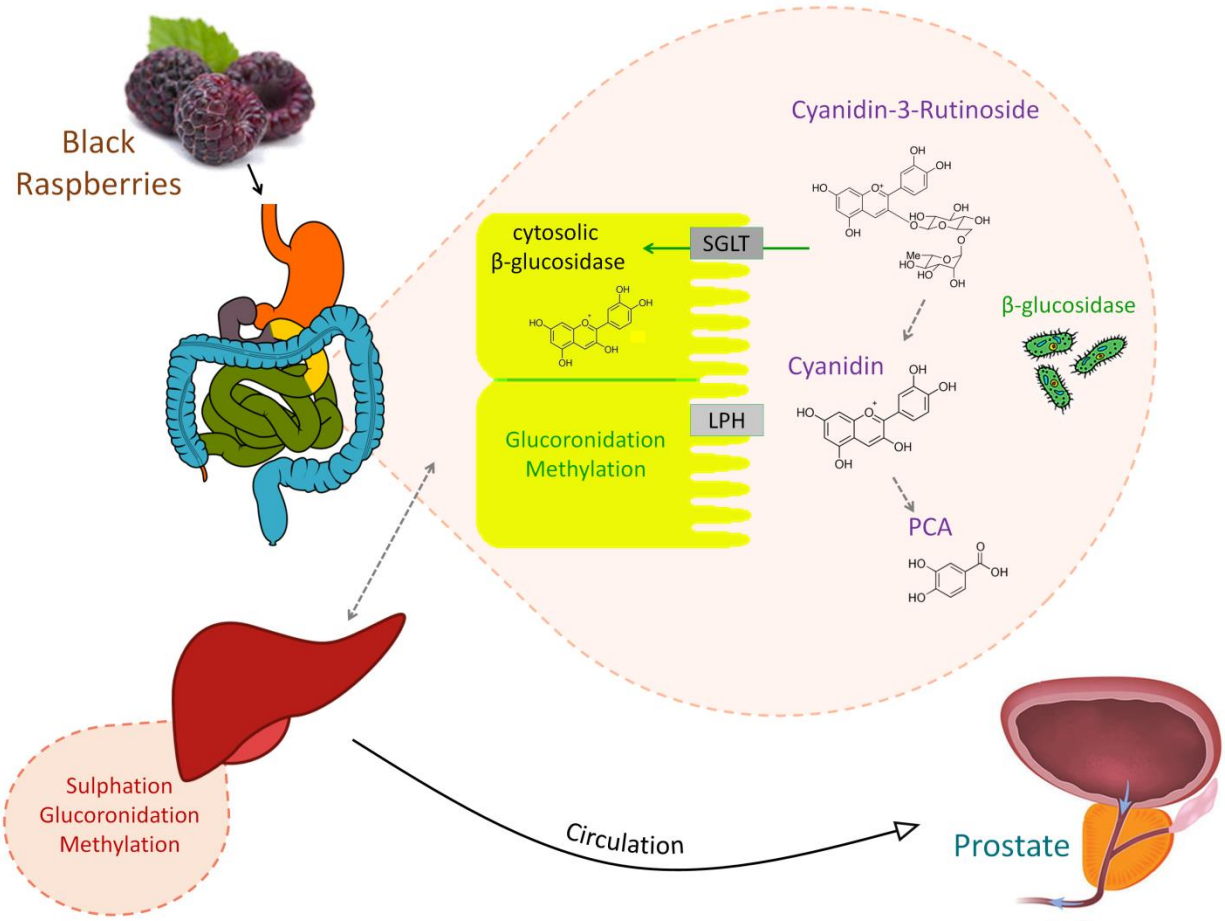


Figure 29. Digestion and metabolism of BRB anthocyanins

We were able to quantify PCA (up to 10 ng/g), the primary anthocyanin metabolite, in prostate tissue of rats following dietary administration, demonstrating that PCA is bioavailable to the prostate. However, levels of PCA obtained through dietary administration of BRBs were not sufficient to inhibit induction of prostate cancer in our study. Although it has been shown that PCA may play a role in cancer prevention via induction of apoptosis, inhibition of inflammation, and reduction of oxidative damage (289), concentrations of PCA necessary to elicit these

effects do not reflect levels that can be achieved through dietary consumption. In our *in vitro* studies, we tested a wide range of PCA concentrations and did not see effects on proliferation, migration or colony formation at concentrations comparable to those detected in prostates of NBL rats (1–10 ng/mL) or at higher concentrations (up to 10 μg/mL).

Bioavailability and metabolism of EA, although different from anthocyanins, may also contribute to the inability of BRBs to prevent prostate cancer. EA has greater stability compared to anthocyanins, but very low aqueous solubility (water solubility $\simeq$ 9.7 μg/mL), which limits absorption (249). Ingested EA is metabolized by colonic bacteria to dibenzopyran-6-one derivatives (urolithins), mainly urolithin A (UroA). The solubility of these metabolites is greater than that of EA due to the reduction in the number of hydroxyl groups, which improves absorption but diminishes their antioxidant capacity (246, 250, 251). Effects of UroA on prostate cancer cells were not evaluated in this part of the project, but were later investigated as a consequence of the outcome of this study and are reported in a Chapter V of this dissertation.

We were unable to determine prostatic levels of EA and UroA in this study due to limitations in quality and quantity of our rat prostate tissue available, however some information regarding prostate bioavailability of EA and its metabolites has been reported by others. Following oral administration of UroA (0.3 mg/mouse/dose), urolithin metabolites were detected in mouse prostate tissue, but neither EA nor urolithin metabolites were detected in mouse prostate tissue after feeding a diet supplemented with an ellagitannin-enriched pomegranate extract (0.8 mg/mouse/dose, containing approximately 0.03 mg EA) (290). In a clinical trial with 63 men consuming either walnuts (35 g/day, containing 210 mg EA) or pomegranate juice (200 mL/day, containing 279 mg EA) for three days, investigators were able to detect EA metabolites in the prostate tissue of only 8 of the 33 (24%) patients who consumed either walnuts or pomegranate juice and at very low levels (up to 2 ng/g UroA glucuronide conjugate) (291).

The absence of preventive effects in our animal experiments is in contrast to prior studies showing efficacy of dietary BRBs in prevention of estrogen-induced mammary carcinogenesis in ACI rats (213-215). Diets supplemented with 2.5% BRB powder resulted in a 69% reduction of tumor volume and a 37% reduction in tumor multiplicity, but did not affect tumor incidence (214). Increasing BRB dose to 5% did not further reduce tumor volume, but did significantly delay the appearance of tumors (215). Additionally, BRBs reduced expression of CYP1A1 and CYP1B1, enzymes which are involved in converting estradiol to DNA-damaging catechol metabolites, suggesting that the protective effects of BRBs in this model may be attributed to a reduction in genotoxic metabolites via altered estrogen metabolism (213). To date, all mammary tumor prevention studies with BRBs have been carried out in female ACI rats with hormone-induced mammary cancer. However, in a study investigating the effects of ellagic acid, the incidence of carcinogen-induced (7,12-dimethyl benzanthracene; DMBA) mammary tumors was not significantly reduced (292).

In our study, 5% and 10% dietary BRBs did not protect against either hormone-induced or carcinogen-induced rat prostate tumorigenesis. It is unclear why BRBs lacked efficacy in our study, but were protective against hormone-induced mammary cancer in rats. It is possible that the chemopreventive activity of BRBs or BRB constituents is mediated by effects on estrogen metabolism. It has been reported that anthocyanins, EA and urolithins have estrogen receptor modulating activity, and dietary BRBs were shown to modulate several enzymes involved in estrogen metabolism (213, 293, 294). Given that carcinogenesis in the ACI rat model is driven exclusively by chronic exposure to estradiol, the involvement of BRBs in estrogen metabolism provides a plausible explanation for their protective effect against mammary tumorigenesis (295). While it was been shown that estradiol exposure plays a role in prostate carcinogenesis in the NBL rat model, additional mechanisms, including inflammation and testosterone-induced proliferation, contribute to the development of prostate tumors in this model (255, 296). The

effect of BRBs on these factors remains unresolved. Another possible explanation for why BRBs did not inhibit prostate carcinogenesis in our study is that the carcinogenic stimulus of chronic exposure to high estradiol levels in NBL rats may have been substantial enough to obscure protective effects of BRBs. Previous studies have shown that the magnitude of BRBs effectiveness is dependent on the level of estradiol exposure; 2.5% BRBs reduced mammary tumor incidence induced by chronic low-dose estradiol implants (9 mg 17β-estradiol per implant), but did not reduce incidence when a higher dose of estradiol (27 mg 17β-estradiol per implant) was used to induce carcinogenesis (213, 214). BRB intervention reduced tumor volume and burden in both models, but effects were greater in animals exposed to low-dose estradiol, demonstrating that effects of a dietary BRB intervention were enhanced when estradiol doses were reduced. Also, our PCA results (see above) suggest that active BRB compounds may not have reached the prostate in concentrations that are sufficient for prevention of carcinogenesis.

In conclusion, BRBs did not inhibit the development of rat prostate carcinogenesis and did not inhibit prostate cancer growth at physiological concentrations *in vitro*. It seems likely that the chemopreventive effects of BRBs observed in studies of GI cancers are attributed to localized absorption and direct contact of BRB compounds with target tissue. BRB constituents that come in contact with these tissues can exert anti-cancer effects at the site of absorption. However, instability and low bioavailability of bioactive BRB compounds and their metabolites dramatically limits circulating levels, so that distant tissues, like the prostate, are not exposed to quantities sufficient for preventing cancer. While the potential of BRBs as preventive and therapeutic agents for GI cancers remains encouraging, our evidence indicates that BRBs are unlikely to be effective for preventing prostate cancer.

IV. IN VITRO EFFECTS OF BLACK RASPBERRIES AND THEIR CONSTITUENTS ON EFFICACY OF TAXANE CHEMOTHERAPY IN PROSTATE CANCER CELLS

A. Introduction

Despite the increasing number of therapeutic options available, managing prostate cancer continues to be challenge. Androgen deprivation therapy (ADT) has remained an effective mainstay for first-line therapy; however, although highly successful initially, nearly all tumors become resistant to ADT and progress to castrate-resistant prostate cancer (CRPC) (297, 298). The standard of care for CRPC includes a regimen of taxane-based chemotherapy, with docetaxel as first-line systemic treatment and cabazitaxel as potential second-line option (299-302). These secondary chemotherapeutic measures to control and cure advanced-stage prostate cancer lack substantial efficacy, increasing overall survival time by only a few months, and can severely impair the quality-of-life of the patient (41, 301, 303). As a consequence, many men with prostate cancer, particularly those with more advanced stage disease, chose to make dietary modifications or use some form of dietary supplements in addition to their standard of care therapy regimens (304-306).

The use of complementary and alternative medicine (CAM) is widespread among adults in the U.S. and is becoming increasingly common (307, 308). In particular, the use of dietary supplements as CAM is very prevalent in the general population and is especially high among cancer patients (309, 310). In a recent study, 61.2% of cancer patients reported using at least one dietary supplement (excluding multivitamins), while men with recurrent cancers were almost twice as likely to use supplements compared to other patients (311). This is comparable with another study in which 48% of men with prostate cancer reported use of at least one dietary supplement, which was about 10% higher than the prevalence in cancer-free men (312). The

use of dietary supplements often occurs concurrently with other medications. In a cohort of adults ages 62-85, 53.9% of participants reported the concomitant use of dietary supplements with prescription medication in 2005, which increased to 65.7% in 2011 (313). Despite the frequent use of supplements by cancer patients in combination with prescription medications, little information exists on potential beneficial or harmful interactions between most supplements and chemotherapy drugs.

The development of black raspberries (BRBs) as potential cancer prevention agents has gained much attention and BRB preparations are currently being investigated in several clinical trials (314). Anthocyanins, ellagic acid, ferulic acid and quercetin are some of the biologically active components of BRBs, and there is evidence that each alone exhibits anti-cancer and antioxidant properties (315-319). In addition to preventive and therapeutic effects, BRBs could potentially be used as adjuvants to chemotherapy. However, no studies have investigated the use of BRBs for this purpose. It remains unknown whether BRBs can be used to enhance the effectiveness of chemotherapy, but considering that many of the biological activities of BRBs target similar pathways as chemotherapeutic drugs, it is a plausible that BRB combination therapy could result in enhanced drug efficacy and reduced resistance.

In the present study, we investigated the ability of BRBs to modulate effects of taxane chemotherapeutic agents used in the treatment of CRPC. We hypothesized that treatment of prostate cancer cells with BRB extract would improve effectiveness of standard chemotherapy agents, docetaxel (DX) and cabazitaxel (CBZ), resulting in decreased proliferation and increased apoptosis, and increase sensitivity of prostate cells to chemotherapeutic agents by inhibiting mechanisms of taxane resistance. A secondary objective of this study was to rule out possible adverse effects, i.e., reduction of the anti-cancer activities of DX and CBZ by BRB compounds. Identification of any harmful effects or absence thereof is critically important from a clinical standpoint for patients who are consuming such supplements while on chemotherapy.

Thus, we evaluated co-treatment of BRB extract, protocatechuic acid (PCA), and ellagic acid (EA) with DX and CBZ in CRPC prostate cancer cells. The effects of BRB extract and PCA with taxanes are presented in this chapter, whereas results of EA in combination with taxanes are presented separately in Chapter V. Additionally, results from control experiments confirming the effectiveness of taxanes alone can be found in Appendix C.

B. Results

i. Black raspberry extract inhibits microtubule assembly in vitro

It is well established that microtubule polymerization by taxanes prevents disassembly of mitotic spindle structure, leading to G2/M arrest and apoptosis (64, 320). Effects of BRB constituents on microtubule dynamics have not been investigated, but it has been reported that an anthocyanin-containing bilberry extract can cause depolymerization of microtubules in MCF-7 breast cancer cells (321). We investigated the effects of DX, CBZ, BRB extract and PCA on tubulin polymerization using an *in vitro* (cell-free) assay. As expected, both DX and CBZ had a robust, dose-dependent effect on polymerization (Figure 60, Appendix C). Tubulin polymerization was prevented by BRB extract at 10, 100 and 1,000 μg/mL (Figure 30A). PCA (10 μg/mL) insignificantly increased the amount of polymerized tubulin from 25.6% (control) to 46.7% (Figure 30B). We next investigated the effects of BRBs and PCA in combination with CBZ. The combination of BRB extract (1 mg/mL) and CBZ (100nM) decreased polymerization by 24.1% compared to CBZ alone (Figure 31A). PCA (10 μg/mL) in combination CBZ resulted in a non-significantly increase in tubulin polymerization (Figure 31B).

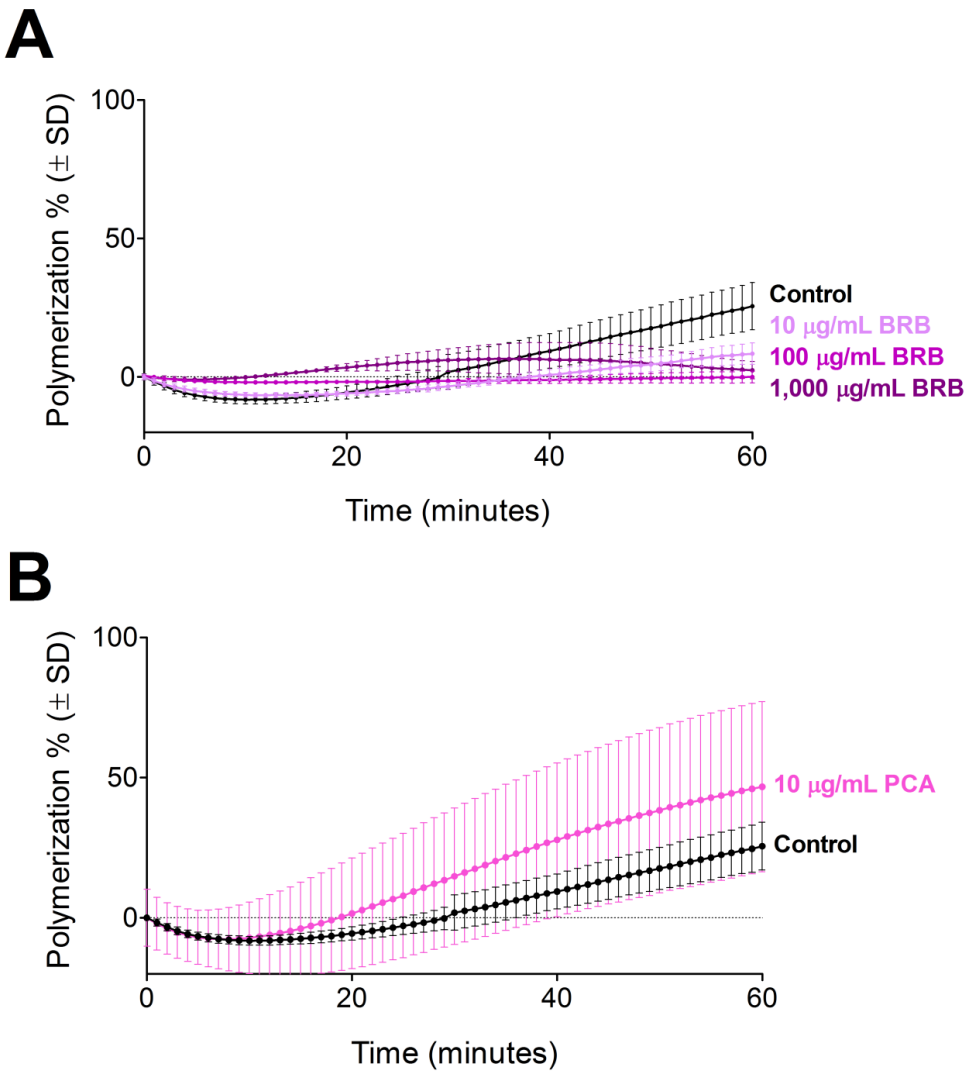


Figure 30. Effects of BRB extract (A) and PCA (B) on tubulin polymerization *in vitro*.

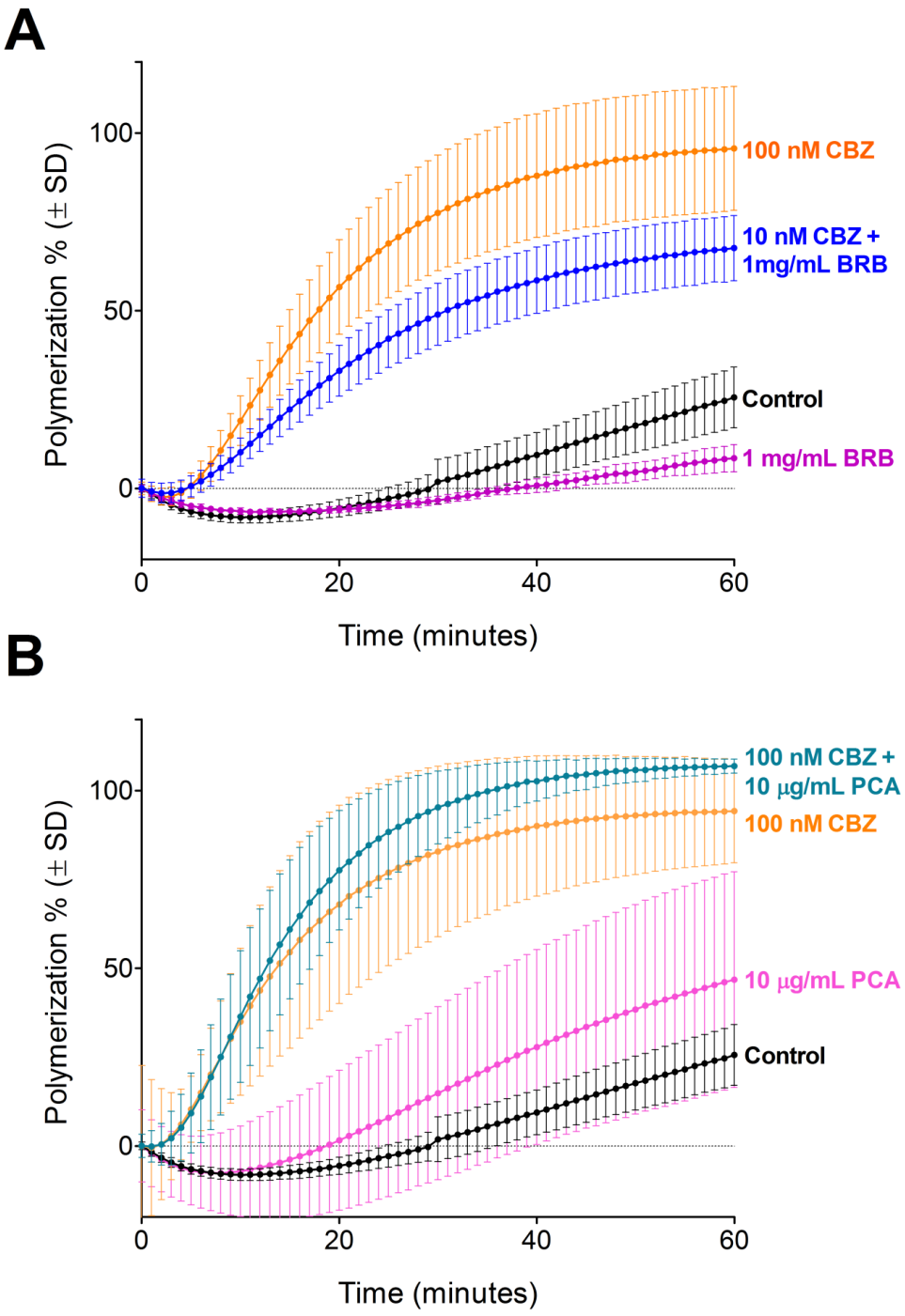


Figure 31. Combination effects of 100 nM CBZ with 1 mg/mL BRB extract (A) and 100 nM CBZ with 10 µg/mL PCA (B) on tubulin polymerization *in vitro*.

ii. Black raspberry extract does not alter microtubule assembly in 22Rv1 cells

Because BRB extract reduced efficacy of CBZ *in vitro*, we proceeded to study the effects of BRBs in combination with CBZ on microtubule assembly in 22Rv1 cells. First, the extent of microtubule polymerization was determined by the amount of soluble and polymerized tubulin via western blotting. 22Rv1 cells were treated with vehicle (EtOH), BRB (1 mg/mL), CBZ (10 nM) or BRB + CBZ for 24 hours, cells were lysed and the amount of tubulin present in supernatant (soluble) and pellets (polymerized) was analyzed. Unlike the results from the cell-free assay, BRB treatment did not reduce the amount of polymerized tubulin (Figure 32 A & B); polymerized tubulin in BRB treated cells was similar to control cells. Additionally, tubulin polymerization in cells that received BRB and CBZ combination treatment was not significantly different than that of cells treated with CBZ alone. We further evaluated effects of BRB and CBZ combination treatment on tubulin polymerization in 22Rv1 cells by confocal microscopy. Results were similar those of western blot analysis; there were no apparent differences in microtubule morphology were observed for control compared to BRB treated cells, or for CBZ treated compared to combination treated cells (Figure 32C).

iii. Neither black raspberry extract nor protocatechuic acid enhance cytotoxicity of taxanes

To determine if BRB extract and PCA alter cytotoxicity of taxanes, we measured cell proliferation in 22Rv1 and C4-2 cell lines, which are capable of androgen independent growth and resemble the aggressive clinical phenotypes of CRPC. In the SRB proliferation assay, BRB extract (1 mg/mL) or PCA (10 µg/mL) in combination with CBZ (0.01 nM – 1 µM) did not further reduce the growth of prostate cells compared to CBZ alone (Figure 33 A-D). Additionally, we investigated combination effects of BRBs and PCA with CBZ on DNA synthesis activity by measuring incorporation of a thymidine analog (EdU) into DNA of C4-2 cells. Cells were treated

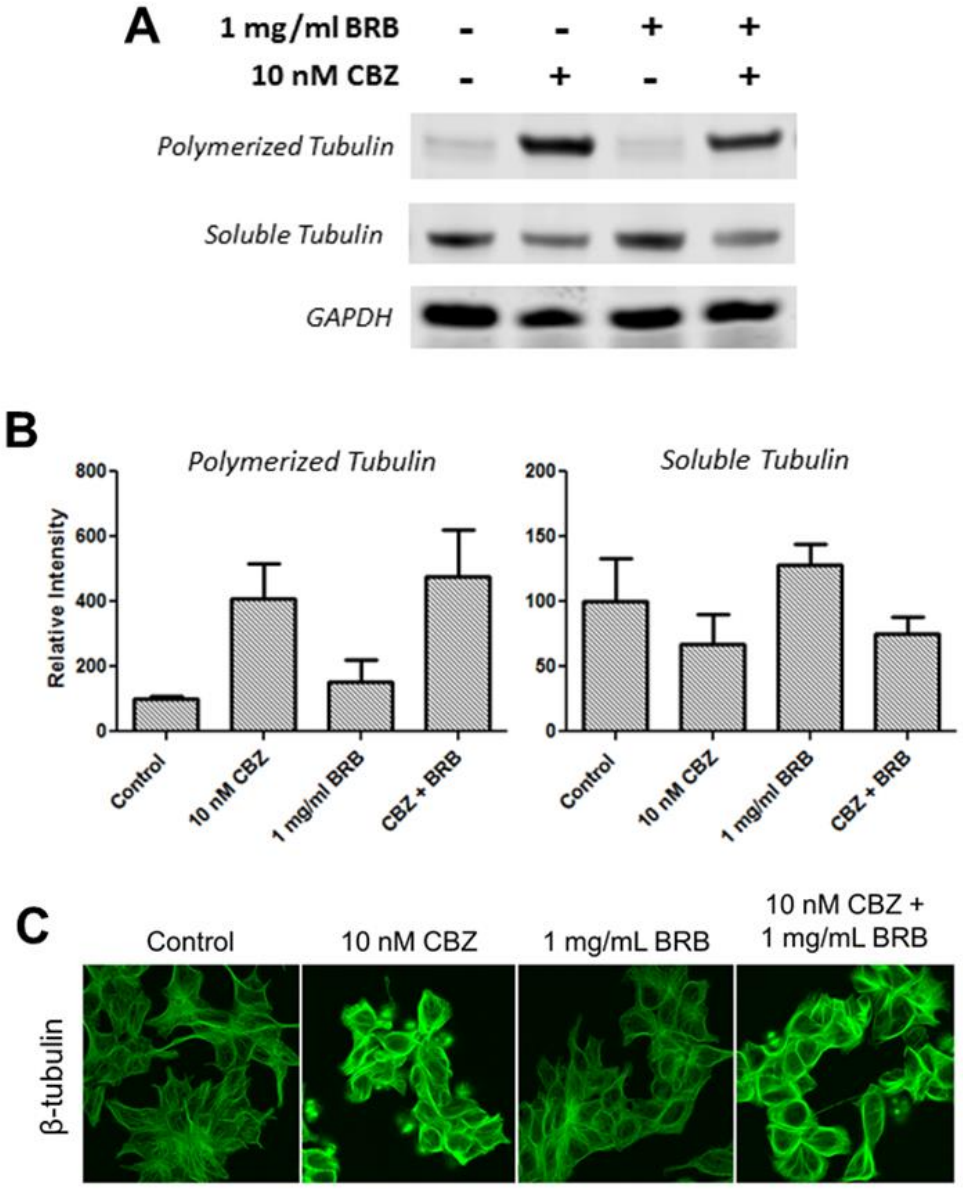


Figure 32. Combination effects of BRB extract (1 mg/mL) and CBZ (10 nM) in 22Rv1 cells. Levels of soluble and polymerized were determined by western blot (A), band intensity was quantified using Li-Cor Odyssey software (B). Microtubules (green) were visualized by confocal microscopy (C).

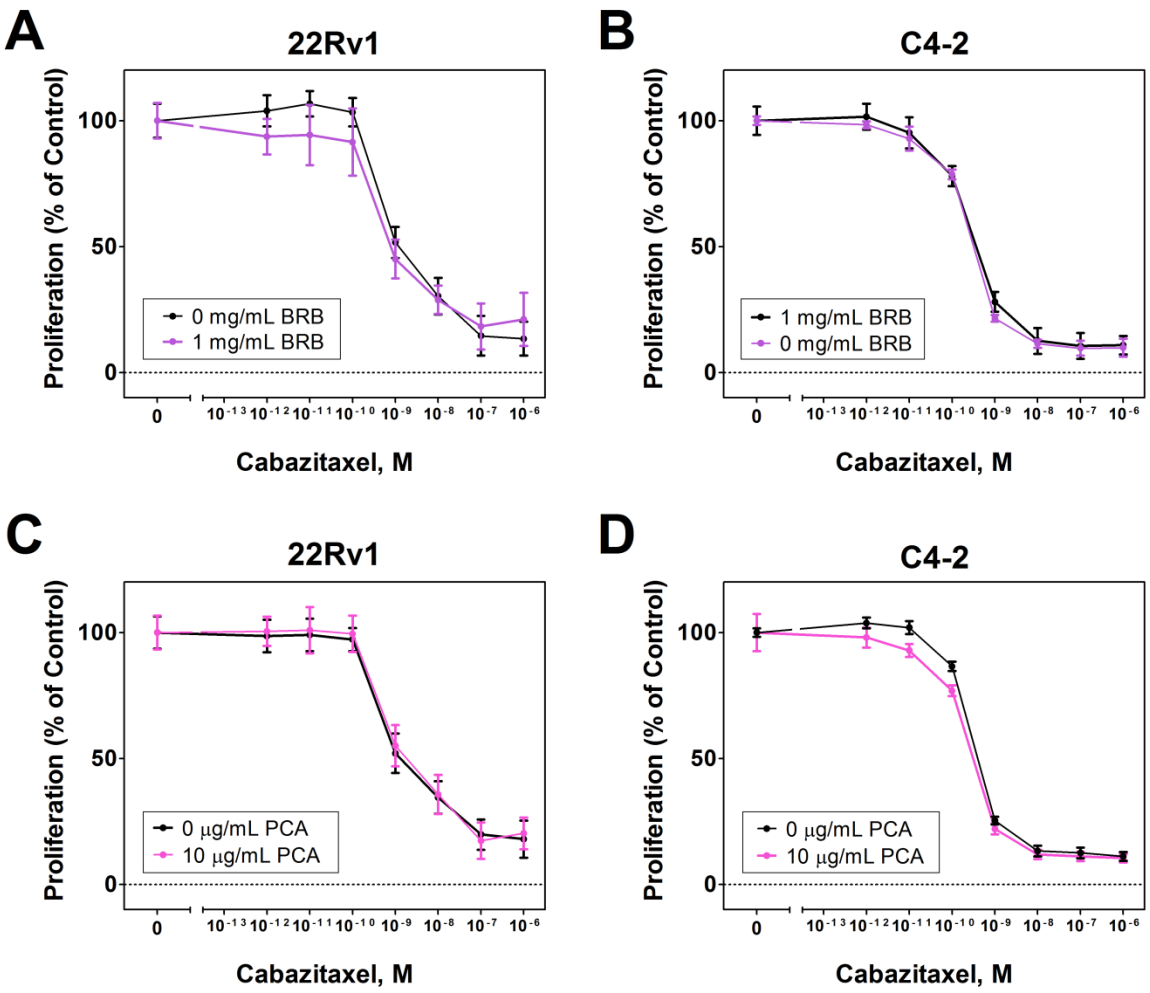


Figure 33. Combination effects of BRB extract, PCA and CBZ on proliferation of 22Rv1 and C4-2 cells. Proliferation was measured in 22Rv1 (A & C) and C4-2 (B & D) cells using the SRB assay after 72 hours treatment with BRB extract (1 mg/mL), PCA (10 µg/mL) and CBZ (0.001 – 1,000 nM). Results were normalized to vehicle control treated cells and are presented as mean ± SD.

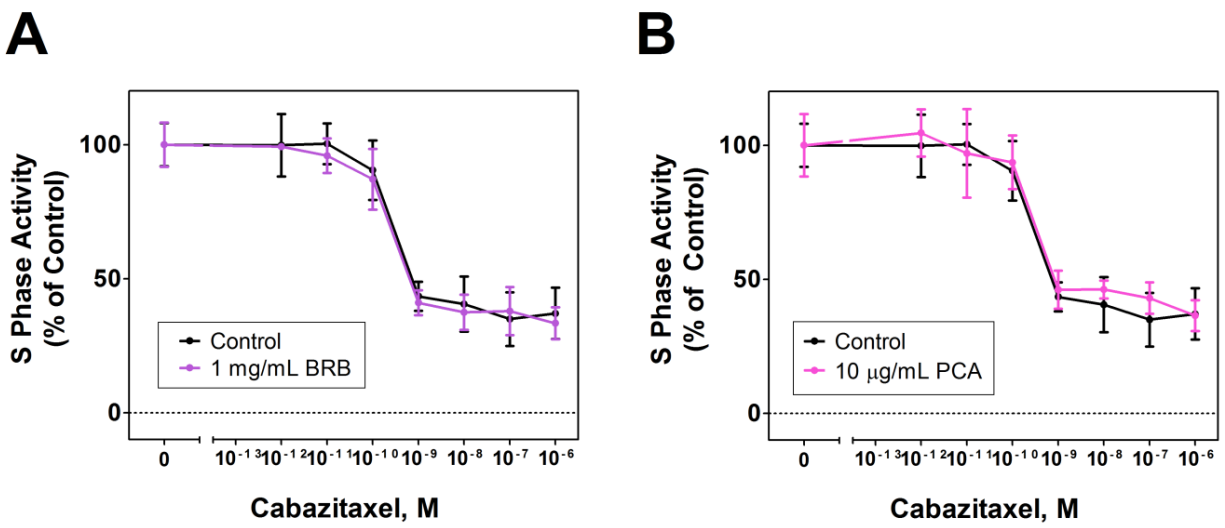


Figure 34. Effects of BRB extract and PCA in combination with CBZ on S phase activity in C4-2 cells. S phase activity was assessed by measuring incorporation of a thymidine analog into DNA of C4-2 cells after treatment with BRB extract (1 mg/mL), PCA (10 µg/mL) and CBZ (0.001 – 1,000 nM).

with BRB extract or PCA in combination with CBZ at the same concentrations that were used in the SRB assay. There were no differences in EdU incorporation for either BRB or PCA combination treated cells compared to CBZ (Figure 33 E & F).

iv. Black raspberry extract and protocatechuic acid do not inhibit efflux activity

Increased activity and expression of ATP binding cassette (ABC) transporters, including P-glycoprotein (P-gp; also known as MDR1 and ABCB1), has long been associated with multidrug resistance (MDR) to numerous drugs (55, 322-324). The efflux activity of these proteins, which reduces intracellular drug accumulation, has been implicated in taxane resistance in CRPC

patients (325, 326). Effects on BRBs on activity of efflux transporters has not be investigated, but MDR inhibition by numerous dietary agents has been reported (327). We evaluated efflux activity in C4-2 cells treated with BRB (1–1,000 mg/mL) and PCA (0.01–10 μg/mL) by measuring intracellular accumulation of fluorescent dye calcein AM. We observed no differences in intracellular calcein AM levels in cells treated with BRB or PCA compared to control (Figure 35), indicating neither BRB extract nor PCA affected efflux activity.

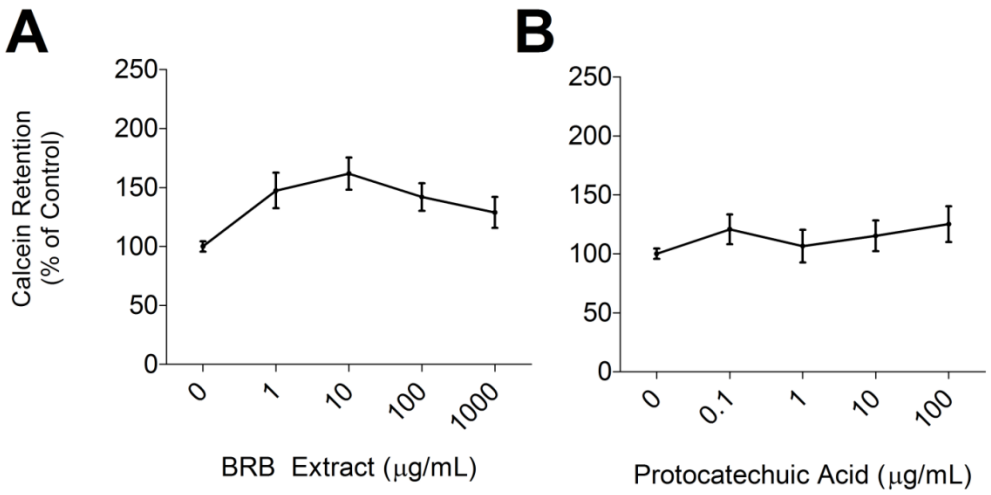


Figure 35. BRB extract and PCA do not alter efflux activity of 22Rv1 cells.

v. Black raspberry extract and protocatechuic acid do not alter CYP3A4 activity

CYP3A4 is the enzyme responsible for metabolizing taxanes (328). CYP3A4 activity can be modulated by pharmaceutical agents, certain foods and dietary supplements, and can have consequences on drug toxicity (329-331). We investigated the effects of BRB extract (0.1–1,000 mg/mL) and PCA (0.01–10 μg/mL) on CYP3A4 activity using CYP3A4 expression microsomes. We did not observe an effect of either BRBs or PCA on CYP3A4 activity (Figure 36).

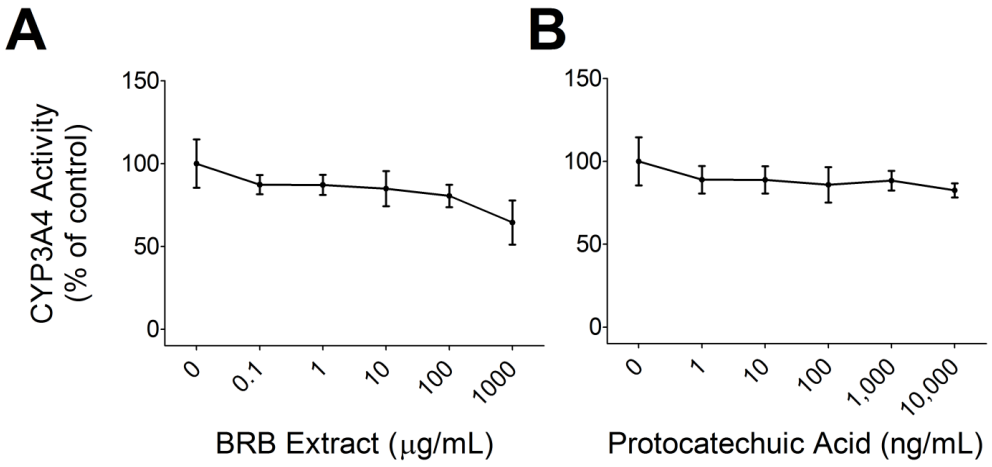


Figure 36. BRB extract and PCA do not alter CYP3A4 activity.

C. Discussion

Although we observed inhibition of microtubule assembly by BRBs *in vitro*, this result is likely an experimental artifact and not biologically relevant, since the same BRB extract did not elicit any effects on microtubules in cells. Microtubule formation relies on the hydrolysis of GTP to GDP to induce polymerization of tubulin subunits (332). Tubulin polymerization will occur spontaneously at 37ºC when tubulin is combined with GTP, but can be modulated by the presence of certain substances including GTPases, sucrose, and calcium ions (333, 334). In our experiment, the effects of BRB extract and PCA on tubulin polymerization were evaluated by combining them with a solution containing purified tubulin protein, GTP, and DAPI[5], then

[5] DAPI is used as a fluorescent reporter for microtubule formation due to its ability to bind tubulin polymers; when bound to microtubules, the fluorescence intensity of DAPI is enhanced (335).

measuring the increase in fluorescence intensity as an indicator of polymerization. The simplicity of this assay allows for straightforward identification of compounds that directly interact with tubulin, but lacks the complexity of the intracellular environment. Given that our BRB extract is a mixture of numerous constituents, it is possible that one or more of the components interferes with tubulin protein assembly or GTP hydrolysis in this assay, but do not normally affect microtubule dynamics under physiological conditions. The absence of effects by BRBs on the relative amount of polymerized tubulin or on morphology of microtubules observed in 22Rv1 cells supports this theory.

Circulating and intracellular levels of taxanes are regulated by several factors including metabolism by CYP3A4 and efflux activity of ABC transporters. The effects of berry anthocyanins on CYP3A4 and drug transporter activity have been reported by others. Cyanidin aglycones and glycosides had no effect on efflux activity of MDR1, whereas the cyanidin had a slight inhibitory effect on BCRP1 activity, but inhibition of BCRP1 by cyanidin glycosides was not as substantial (336). Similar results were reported in another study, in which cyanidin aglycones but not glycosides had an modest inhibitory effect on efflux activity (337). Additionally, anthocyanins inhibited CYP3A4 activity and expression hepatic cells, but only at high concentrations (50-100 μM). In the present study, we found no effects of BRB extract and PCA on efflux activity of 22Rv1 cells or CYP3A4 activity in microsomes. Although we did not study the effects of isolated anthocyanins in these assays, our findings appear to be consistent with findings of others demonstrating that efflux transporters and CYP3A4 activity are not substantially altered by anthocyanin glycosides.

We found no significant effects of PCA on mechanisms related to taxane toxicity, metabolism, or resistance. In the polymerization assay, we observed a slight enhancement in tubulin polymerization by PCA compared to control and by the combination of PCA and CBZ compared to CBZ alone, but these effects were not statistically significant. It is possible that

inability of PCA to elicit a response in these assays is due to the concentrations used in this study. We did not investigate concentrations of PCA higher than 10 µg/mL, thus it remains possible that higher pharmacological doses of PCA may have activity.

Dietary modifications and use of supplements, especially those with antioxidant activity, in combination with chemotherapy is an unresolved issue, as the beneficial or harmful effects of many agents on drug effectiveness are not known. Our findings suggest that intake of BRBs during taxane chemotherapy is safe and not likely to reduce or enhance drug efficacy. Although the results presented in this chapter were obtained through *in vitro* methodologies, we expect similar results would be observed *in vivo*. Considering the low bioavailability of BRB compounds, they will probably not reach sufficient circulating levels to have an effect on taxane mechanisms of action, uptake, or metabolism at target tissues. However, it is possible that BRB constituents could alter the efficacy of other chemotherapeutic drugs. The mechanism of action for taxanes is microtubule stabilization, which is not associated with oxidative stress, but many other agents – such as platinum-based chemotherapeutic drugs and alkylating agents – induce apoptosis primarily by generating reactive oxygen species and producing high levels of oxidative stress (338). BRB anthocyanins are known to exert potent antioxidant activity, and may is possible that even low levels could antagonize pro-oxidant activities of some drugs.

In conclusion, we found no evidence to suggest that BRBs or the anthocyanin metabolite PCA enhance effects of taxane chemotherapy. Likewise, we did not find any effect of BRB extract or PCA on taxane metabolism or mechanisms of resistance. These findings suggest that while consumption of BRBs during taxane chemotherapy will not provide a therapeutic benefit, it is safe and not likely to reduce drug effectiveness or increase toxicity.

V. MICROTUBULE POLYMERIZING EFFECTS OF ELLAGIC ACID AND IMPACT ON THE EFFECTIVENESS OF TAXANE CHEMOTHERAPY

A. Introduction

Ellagitannins are naturally occurring compounds found in pomegranates, berries, walnuts and numerous other foods that release ellagic acid (EA) upon hydrolysis, which is further metabolized by intestinal microflora to yield urolithins, such as urolithin A (UroA). Ellagitannins, EA, and urolithins exhibit potent antioxidant and anti-cancer activity in a variety of cell and animal models (195, 213, 244-247). In Chapter III, we reported that EA inhibited proliferation and anchorage independent growth in prostate cancer cell lines. Similar anti-proliferative effects of EA on prostate cancer cell lines have been reported by others (290, 339-341). Additionally, EA suppressed AR and PSA expression in LNCaP cells (342) and induced G2/M arrest in PC-3 and DU-145 cells (343). Moreover, LAPC-4 tumor xenograft growth was inhibited in mice fed an ellagitannin-enriched diet and EA metabolites could be detected in prostate tissue of these mice (290).

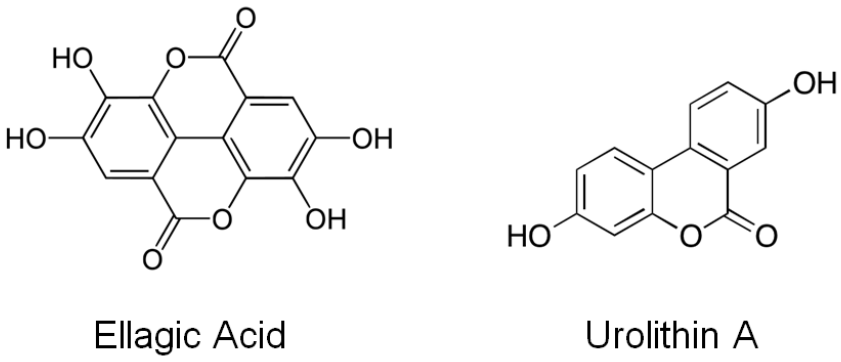


Figure 37. Chemical structures of ellagic acid and urolithin A

Little is known about the potential use of EA as an adjuvant to taxane chemotherapy for CRPC. Polyphenolic compounds with antioxidant activity have been suggested to antagonize drugs that exert cytotoxicity via induction of oxidative stress (344), but not much is known about their effects on other kinds of chemotherapeutic agents. There is some evidence to suggest that EA could interfere with the mechanisms of taxanes. It has been previously reported that EA sensitizes cancer cells to the effects of ionizing radiation (345) and inhibits ABCG2 transport, which is involved in drug resistance to chemotherapeutic agents (346). Only one clinical trial has reported the use of EA in CRPC patients receiving chemotherapy (347). In this study, men undergoing a regimen of vinorelbine and estramustine were co-treated with daily dose of EA administered orally for the duration of chemotherapy. EA combination treatment was associated with a reduction in serum PSA and overall survival, but these observations did not reach statistical significance. While, no studies have investigated EA in combinations with taxanes, a study evaluating the combination effects of pomegranate extract, a rich source of ellagitannins, and docetaxel reported an enhancement of drug efficacy on prostate cancer cell growth and inhibition of metastasis *in vivo* (348).

We investigated the effects of EA and its metabolite UroA in combination with taxane chemotherapeutic drugs, docetaxel (DX) and cabazitaxel (CBZ) in CRPC cell lines and in an animal model. We hypothesized that co-treatment of taxanes with EA could enhance drug effectiveness by increasing cytotoxic and apoptotic effects of taxanes, and inhibit mechanisms of taxane resistance.

B. Results

i. Ellagic acid and urolithin A inhibit growth of prostate cancer cells

We evaluated the growth inhibitory effects in androgen receptor expressing 22Rv1 and C4-2 cells, and in androgen receptor negative PC-3 cells. We previously reported anti-proliferative activity of EA on prostate cancer cells (Chapter III). In this study, we determined the effects of UroA (1 – 100 μM), the primary metabolite of EA, on cell proliferation using the SRB assay. UroA reduced proliferation (Figure 38B) at 10 μM in 22Rv1 (by 26.3 ± 8.2%) and PC-3 cells (by 26.1 ± 7.9%), and at 30 μM in C4-2 (by 38.7 ± 9.6%), 22Rv1 (by 83.7 ± 5.9%) and PC-3 cells (by 63.6 ± 3.0%).

ii. Ellagic acid induces microtubule assembly

As mentioned in previous chapters, taxanes act on microtubules by binding to β-tubulin, preventing disassembly of mitotic spindle structures, which leads to G2/M arrest and apoptosis (64, 320). Likewise, it has been previously shown that EA and UroA can induce apoptosis and cell cycle arrest in the G2/M phase of colon cancer cells (349, 350). Studies have demonstrated that polyphenolic compounds with structural similarity to EA can interact with tubulin and alter microtubule dynamics in endothelial cells (351). Therefore we investigated the effects of EA and UroA on tubulin polymerization using an *in vitro* (cell-free) assay. 10 μM EA enhanced tubulin polymerization by 61.0 ± 12.9% compared to control (Figure 39A). UroA also induced tubulin polymerization, but this effect was minimal and not significantly different from control.

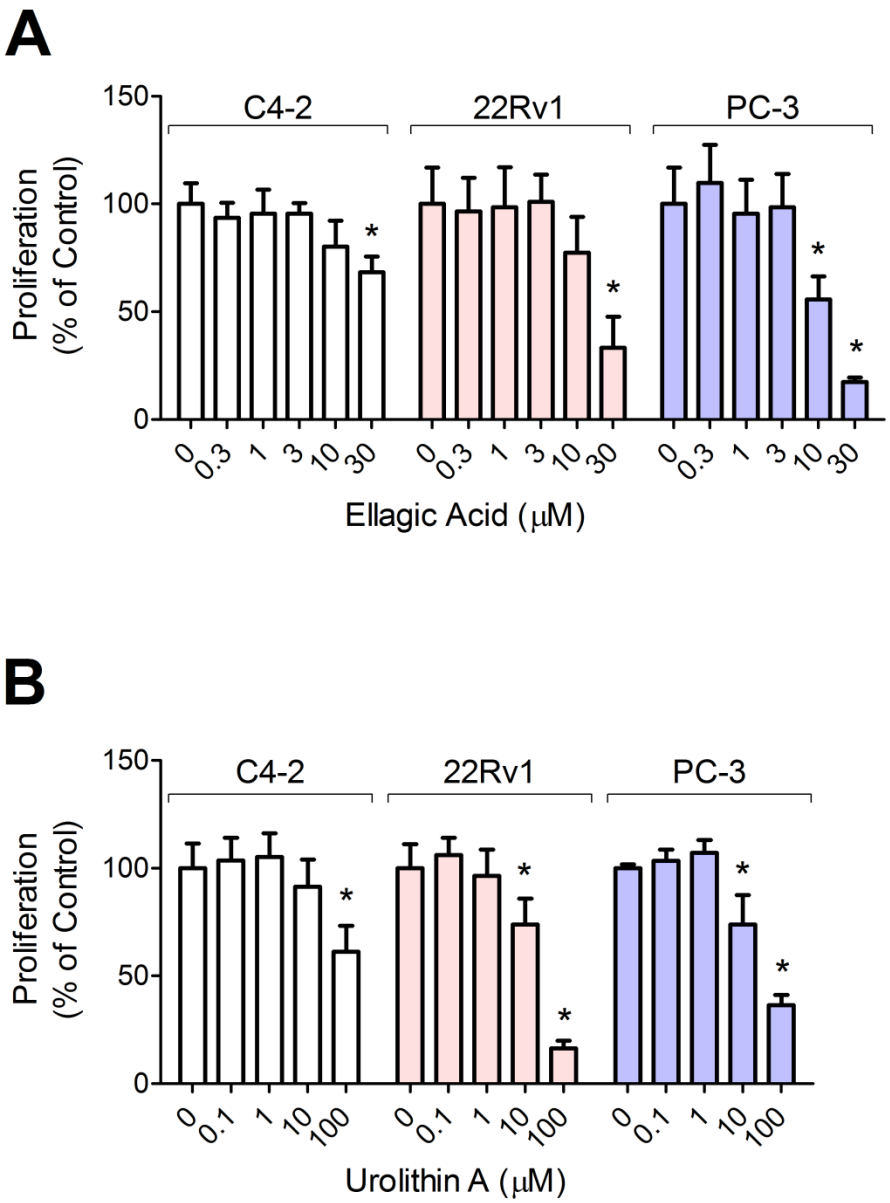


Figure 38. Effects of ellagic acid and urolithin A on proliferation of prostate cancer cells. Proliferation was measured in C4-2, 22Rv1 and PC-3 prostate cancer cell lines using the SRB assay after 72 hours treatment with 1-30 μM ellagic acid (A; figure previously shown in Chapter III) and 1-100 μM urolithin A (B) and normalized to vehicle control treated cells. Data represent mean ± SD; *significantly different from vehicle control $p < 0.05$.

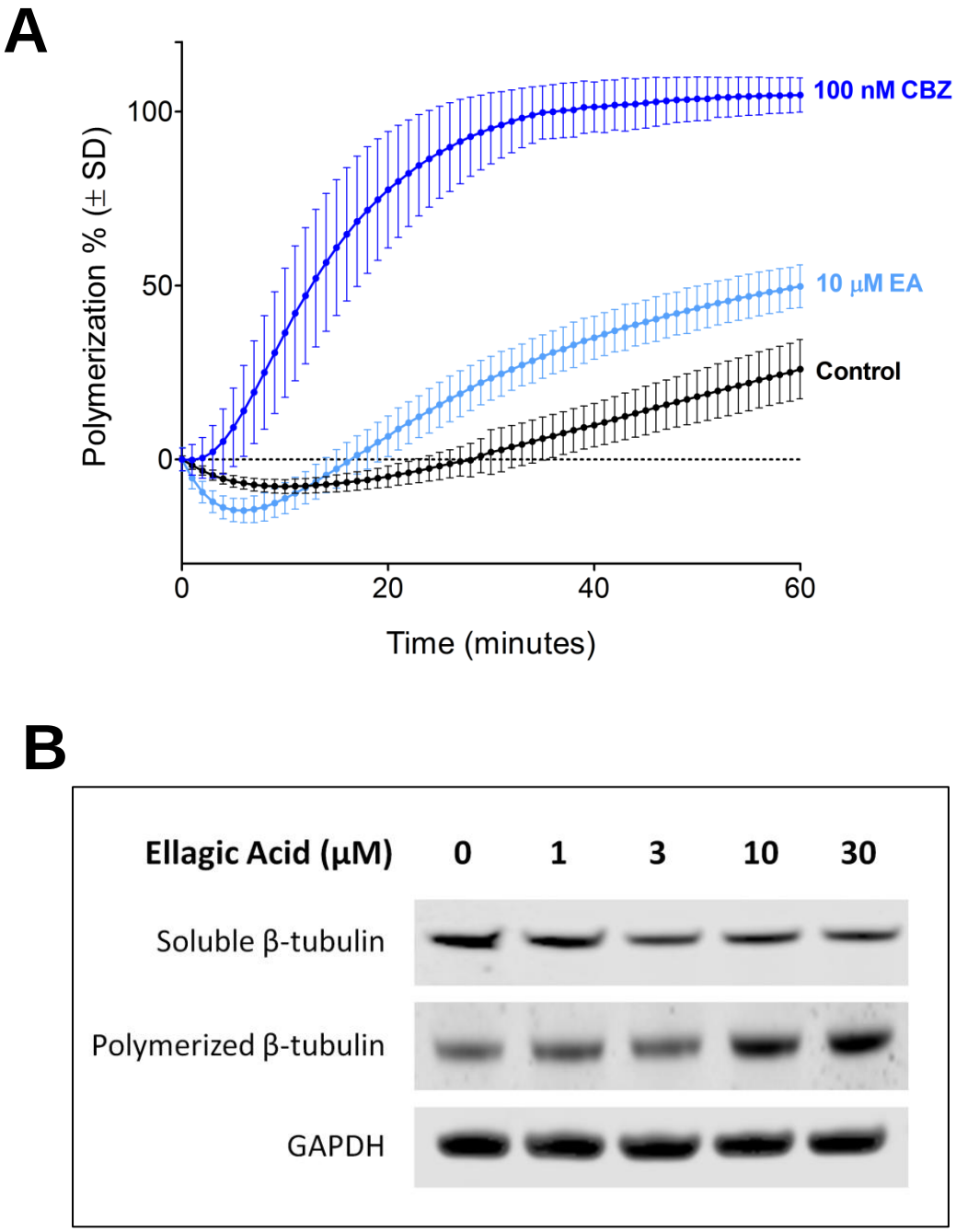


Figure 39. Ellagic acid induces tubulin polymerization. 10 μM EA induces tubulin polymerization *in vitro* using a cell-free assay (A). Levels of soluble and polymerized β-tubulin in 22Rv1 cells determined by western blotting after 24 hour treatment with 1 – 30 μM EA (B).

We next examined the effect of EA on microtubule assembly in 22Rv1 cells. The extent of microtubule polymerization was determined by the amount of soluble and polymerized β-tubulin. After treating 22Rv1 with EA (1 – 30 μM) for 24 hours, cells were lysed and tubulin present in supernatant (soluble) and pellets (polymerized) was analyzed by western blotting. Treatment of cells with EA resulted in a dose-dependent increase in polymerized β-tubulin (Figure 39B).

iii. Ellagic acid interferes with microtubule polymerizing effects of taxanes

Because EA induced tubulin microtubule assembly, we predicted that combination treatment with EA and CBZ would further enhance tubulin polymerizing activity of CBZ. We assessed tubulin polymerization effects of EA (10 μM) in combination with CBZ (100 nM) using the same cell-free assay as in the previous section. Contrary to our expectation, the combination of EA and CBZ inhibited the extent of tubulin polymerization compared to CBZ alone. As shown in Figure 40, CBZ induced tubulin polymerization as expected, but the maximal response was reduced with combination treatment of EA and CBZ.

We further investigated the effects of EA in combination with CBZ on microtubule assembly in 22Rv1 cells by western blotting and confocal microscopy following 24 hour incubation with vehicle, 10 μM EA, 10 nM CBZ, or 10 μM EA + 10 nM CBZ (Figure 41). Treatment with CBZ caused a profound change in microtubule appearance as visualized by an increase in microtubule density. Treatment with 10 μM EA alone increased tubulin polymerization slightly compared to control treated cells, but the combination of EA + CBZ decreased tubulin polymerization compared to CBZ alone.

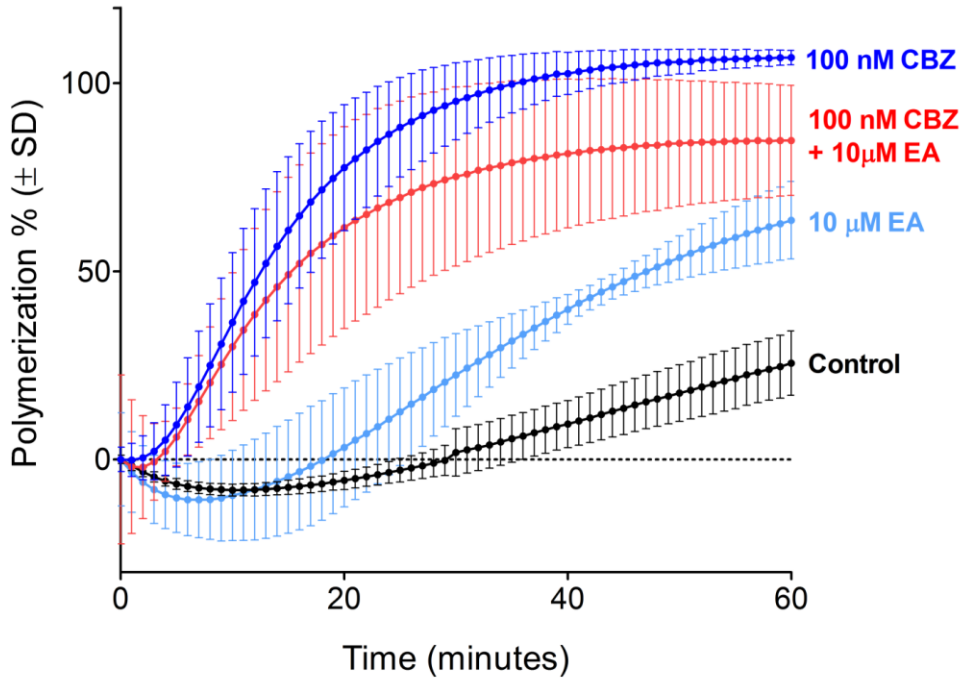


Figure 40. Combination effects of EA and CBZ on tubulin polymerization *in vitro*.

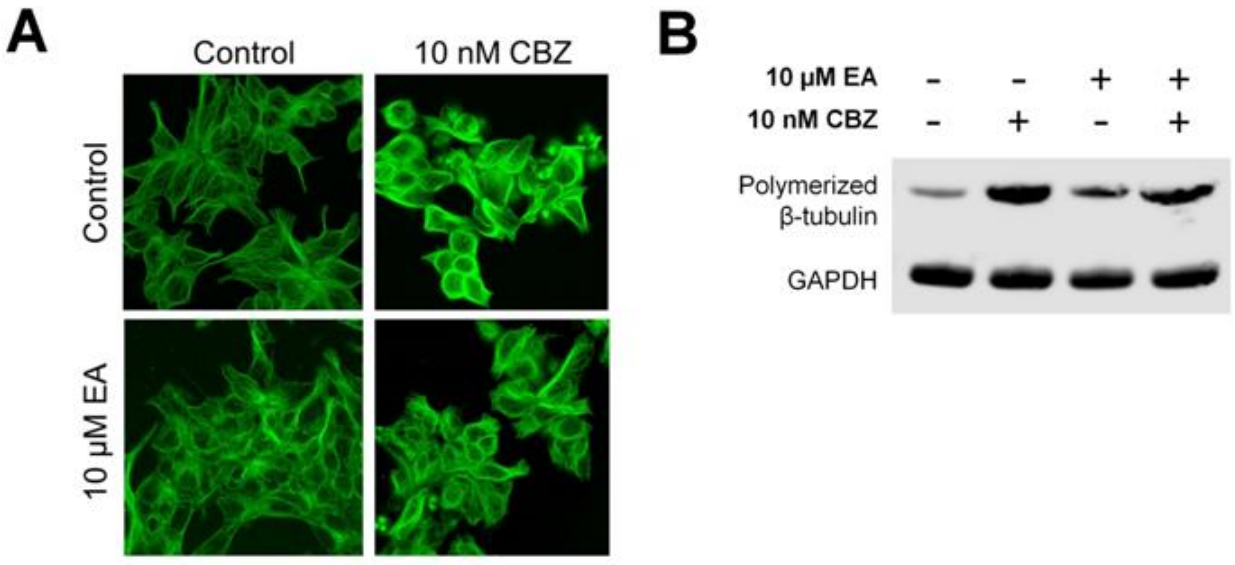


Figure 41. Combination effects of EA and CBZ on tubulin polymerization in 22Rv1 cells. Microtubules (green) were visualized by confocal microscopy (A), and levels of soluble and polymerized were determined by western blot (B).

iv. Ellagic acid does not alter the cytotoxicity of taxanes in CRPC cell lines

Based on previously reported growth inhibitory effects of EA and UroA on cancers cells, we hypothesized that the cytotoxicity of taxanes would be enhanced by co-treatment with EA and UroA. However, results from our tubulin polymerization assays suggest that EA may reduce taxane efficacy by interfering with ability to induce microtubule polymerization. To study combination effects on cell proliferation, we treated 22Rv1 cells with DX (0.1 – 10 nM) or CBZ (0.01 nM – 1 μM) alone and in combination with EA (3, 10 and 30 μM) or UroA (10 μM). Cells were treated for 72 hours and cell proliferation was measured using the SRB assay. As expected, both CBZ and DX exhibited potent cytotoxicity on 22Rv1 cells (Figure 58 & Figure 59, Appendix C). As shown in Figure 42, proliferation of cells treated with DX or CBZ in combination with EA did not differ significantly from cells treated with DX or CBZ alone (Figure 42 A & B). Likewise, combination treatment with UroA was not different than CBZ alone (Figure 42C). Additionally, we examined the effects of EA and CBZ on S-phase activity by performing an EdU (thymidine analog) incorporation assay as an indicator of DNA synthesis. We analyzed EdU incorporation in C4-2 cells treated with 10 μM EA in combination with CBZ (0.01 nM – 1 μM). Consistent with results from the SRB assay, no enhancement or inhibition of CBZ by EA was observed (Figure 42D). Together these results suggest that neither EA nor UroA treatment alters growth inhibitory effects of taxanes.

v. Ellagic acid binds to tubulin

The inhibition of microtubule polymerizing activity of taxanes by EA in a cell-free assay led us to speculate that EA may be acting directly on microtubules by binding to tubulin. We investigated the potential binding interactions of EA and tubulin using label-free, surface

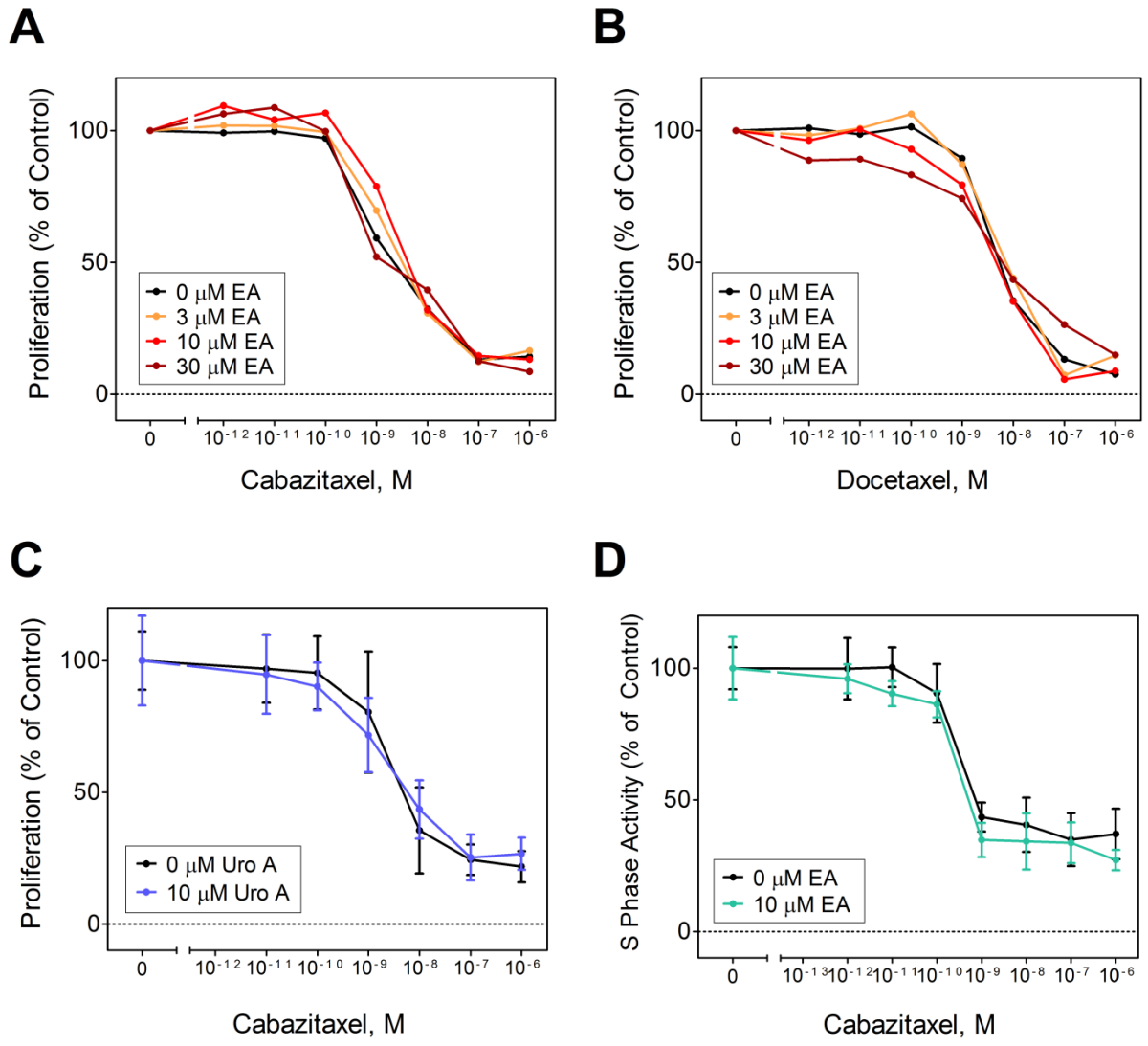
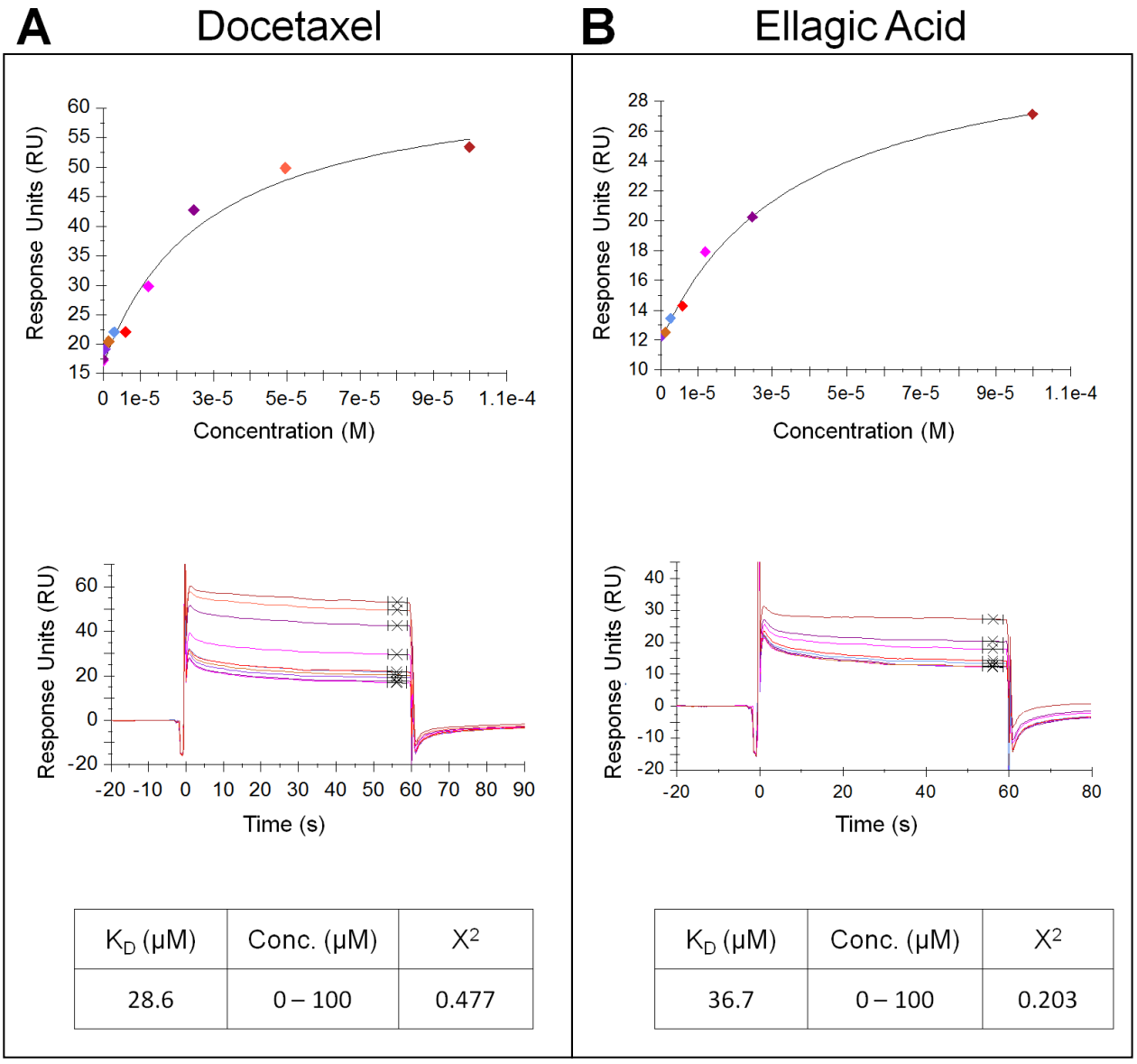


Figure 42. Ellagic acid and urolithin A do not alter cytotoxicity of taxanes. Proliferation was measured in 22Rv1 cells using the SRB assay after 72 hours treatment with EA (3, 10, 30 μM) in combination with cabazitaxel (A) or docetaxel (B). Combination of UroA (10 μM) and CBZ on proliferation of 22Rv1 cells (C). S phase activity in C4-2 cells after treatment with EA (10 μM) and CBZ (0.001 – 1,000 nM), results were normalized to vehicle control treated cells and are presented as mean ± SD (D).



K_D (μM)	Conc. (μM)	X^2
28.6	0 – 100	0.477

K_D (μM)	Conc. (μM)	X^2
36.7	0 – 100	0.203

Figure 43. Binding interaction of ellagic acid and tubulin. Binding affinity of docetaxel (A) and EA (B) for tubulin was determined by surface plasmon resonance. Response-concentration plots (top) and sensorgrams (bottom) are shown.

plasmon resonance (SPR) assay to test for direct interactions between EA and tubulin. EA displayed binding affinity for tubulin with an equilibrium dissociation constant (K_D) of 36.7 µM (Figure 43B). Docetaxel bound tubulin with a high affinity (K_D = 28.6 µM), as expected (Figure 43A).

vi. Ellagic acid inhibits efflux activity in 22Rv1 cells

Increased expression in P-gp and other efflux pumps can mediate resistance to taxanes (352). Ellagic acid has been shown to inhibit ABCG2 transport in ABCG2-overexpressing HEK293 cells, which is involved in drug resistance to chemotherapeutic agents (346). Therefore we examined the ability of EA to modulate efflux activity. We treated 22Rv1 cells with EA and measured intracellular accumulation of fluorescent dye calcein AM. We observed a dose dependent increase in intracellular calcein AM (Figure 44), indicating a reduction in efflux activity.

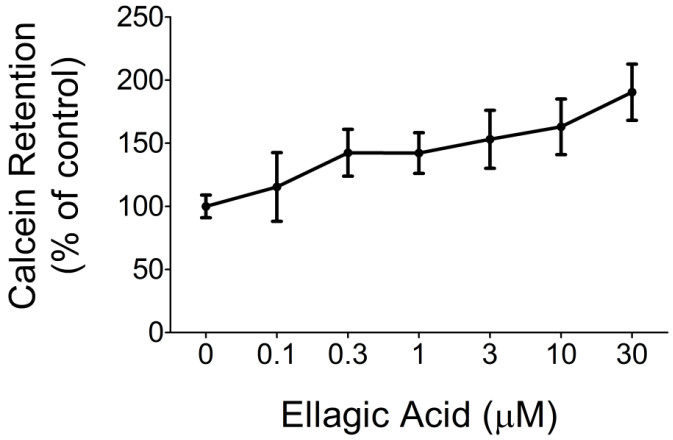


Figure 44. Ellagic acid inhibits efflux activity of 22Rv1 cells

vii. Ellagic acid alters AR and MAP2 expression in 22Rv1 cells

Others have demonstrated that EA can inhibit expression of AR and PSA in LNCaP cells (342), but it is not known if EA can alter AR expression in 22Rv1 cells, which express full length AR and two splice variants which lack the ligand binding domain of the AR and are constitutively active. We determined expression of AR by western blotting following treatment with EA (1 – 30 µM) for 24 hours. We observed a dose dependent decrease in expression of full length AR and smaller variants (Figure 45).

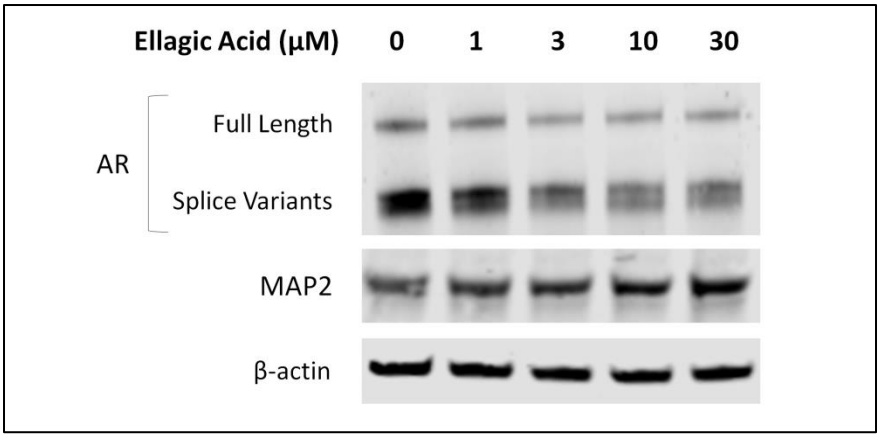


Figure 45. Ellagic acid reduces androgen receptor expression and increases MAP2 expression

viii. Ellagic acid does not modulate CYP3A4 activity

CYP3A4 activity can be modulated by pharmaceutical agents and dietary factors, including several polyphenolic compounds (328), which may lead to attenuation of drug efficacy or increased toxicity. To investigate effects of EA on the catalytic activity of CYP3A4, we treated CYP3A4 expressing microsomes with EA (1, 3, 10 and 30 µM) and quantified activity by

measuring the intensity of a fluorescence reporter metabolite of a CYP3A4 substrate. We did not observe an effect of EA at any concentration on CYP3A4 activity (Figure 46).

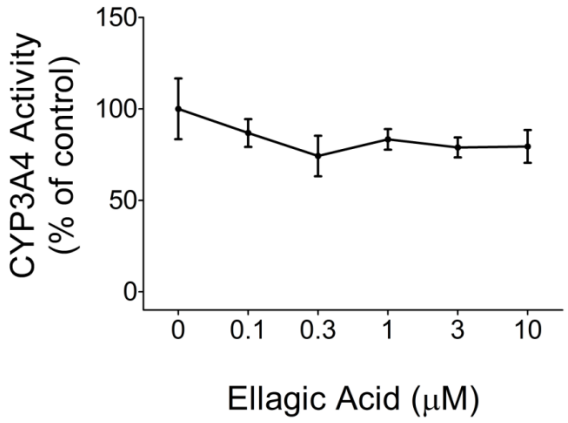


Figure 46. EA does not affect CYP3A4 activity

ix. Dietary ellagic acid does not alter efficacy of docetaxel on 22Rv1 tumor growth in mice

To examine the interaction of high and low dose EA with docetaxel on tumor growth *in vivo*, 22Rv1 cells were subcutaneously injected into mice. When tumors reached an approximate volume of 500 mm^3, animals were placed on either control diets or modified diets containing 2 or 4 g/kg EA for the duration of the study. After 1 week of dietary intervention, mice received 3 injections of DX (15 mg/kg) administered at 3 day intervals. Tumors were measured three times per week until tumor volume exceeded 3,000 mm^3.

We observed a high amount of variability in tumor volumes prior to chemotherapy and dietary interventions (growth curves for individual tumors can be found in Appendix C). Tumors that were not palpable or had not reached the exponential growth phase (volume of at least 350 mm^3) at the start of DX treatment were excluded from data analysis. Additionally, we observed 3

tumors (1 each in the DX + Ctrl, DX + Low EA and DX + High EA groups) with tumor volumes that exceeded 2,500 mm^3 at day 35 that were identified as outliers using Grubbs' test and removed from analysis.

There were no differences in average body weight at the start of the dietary interventions or at the start of chemotherapy. DX treatment resulted in a rapid decrease in body weight by an average of 13.8%, but weight loss did not differ across diet groups (Table VI). Body weight in all DX-treated groups had recovered by approximately day 60 (Figure 47A). As expected, tumor growth was delayed by DX treatment (Figure 47C). All tumors experienced a lag in growth rate following DX treatment that lasted approximately 10 days. None of the tumors exhibited a complete regression in volume in response to treatment. There was no growth delay in sham treated tumors, which reached an average volume of 3,000 mm^3 at 54.4 days, whereas DX treated tumors took 63.4 (control diet), 63.9 (low EA diet) and 61.9 (high EA diet) days to reach the same volume. Dietary EA had no impact on the effectiveness of DX as we did not observe significant differences in tumor growth rates, TGD or overall survival in DX + Low EA or DX + High EA groups compared to the DX + control diet group.

Table VI. Effects of dietary ellagic acid on efficacy of docetaxel in 22Rv1 xenografts

	Vehicle + Ctrl Diet	DX + Ctrl Diet	DX + Low EA	DX + High EA
Dietary EA (g/kg)	0	0	2	4
Treatment [a]	Vehicle	DX	DX	DX
Number of animals	6	10	6	6
No. of measureable tumors at day 35 (total 22Rv1 injections)	9 (12)	16 (20)	7 (12)	8 (12)
No. of tumors included in data analysis	6	10	5	6
No. of tumors excluded from data analysis				
Tumors < 350 mm^3 at day 35	3	5	1	1
Tumors > 2500 mm^3 at day 35	0	1	1	1
Average body weight (g ± SD)				
At start of dietary intervention (day 28)	31.0 ± 1.7	31.2 ± 3.5	28.3 ± 4.5	29.8 ± 0.8
At start of therapy (day 35)	31.2 ± 1.2	30.9 ± 2.4	29.3 ± 3.9	30.0 ± 0.9
One week after therapy (day 49)	32.2 ± 1.2	26.8 ± 3.6 [b]	24.5 ± 3.3 [b]	26.5 ± 2.1 [b]
Average tumor volume (mm^3 ± SD)				
At start of dietary intervention (day 28)	454.2 ± 231.3	477.2 ± 256.6	405.0 ± 136.0	408.8 ± 200.7
At start of therapy (day 35)	748.3 ± 262.9	1166.9 ± 489.2	762.0 ± 155.9	1301.8 ± 918.4
One week after therapy (day 49)	1972.0 ± 640.1	1730.8 ± 921.2	1584.2 ± 484.0	1591.8 ± 808.4
Time to reach 3,000 mm^3 (average number of days ± SD)	54.4 ± 5.7	63.4 ± 5.4 [b]	63.9 ± 5.5 [b]	61.9 ± 3.0 [b]
Tumor growth delay (days)	-	9.0	9.5	7.5

[a] Vehicle (saline) and DX (15 mg/kg body weight) treatments were administered by IP injection on days 35, 39, and 43; [b] $p < 0.05$ for difference with the vehicle + control diet group (ANOVA); SD = Standard Deviation

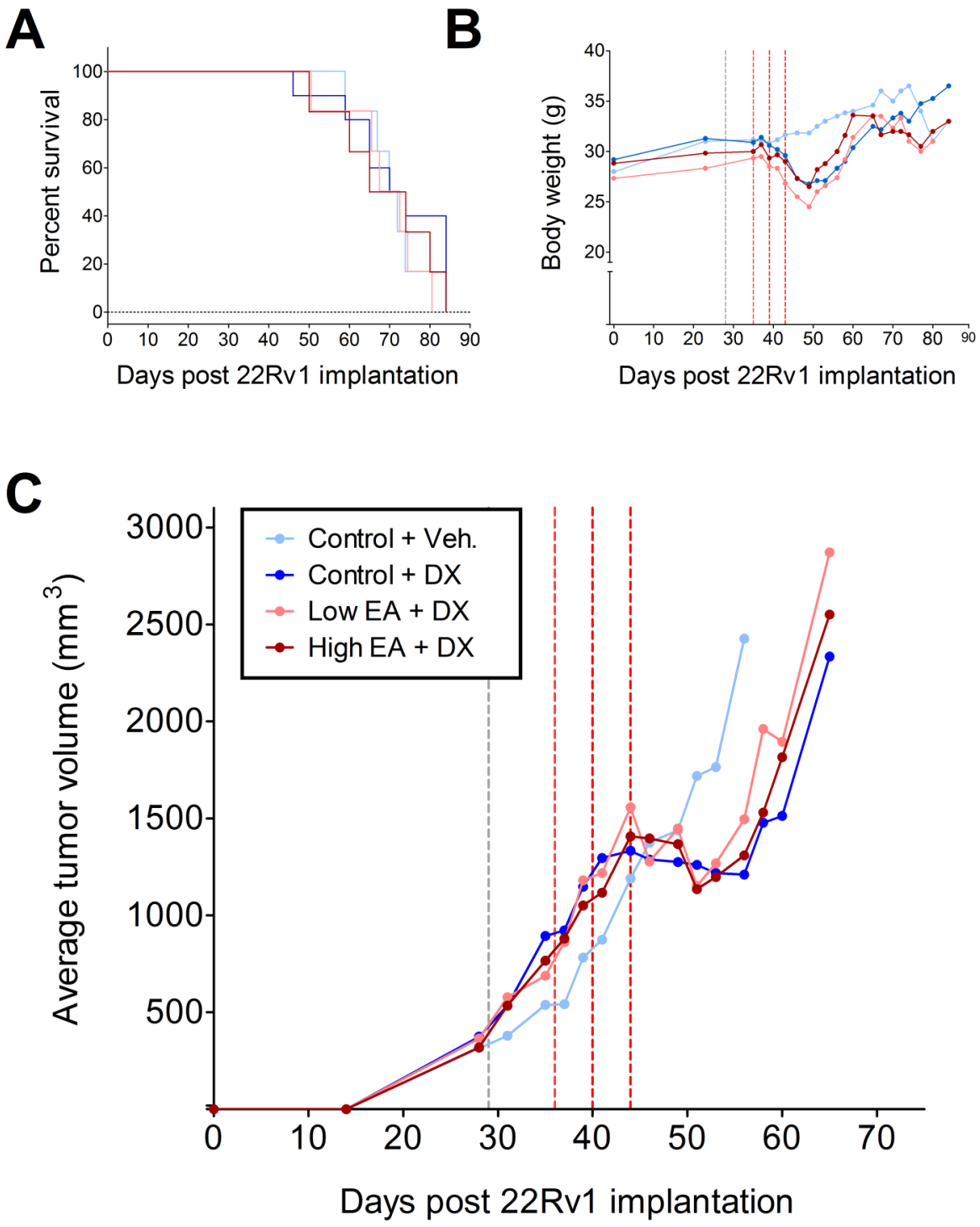


Figure 47. Ellagic acid does not enhance efficacy of docetaxel on 22Rv1 tumor growth in nude mice. Mice were implanted with 22Rv1 cells in matrigel on day 0. Mice were switched to modified diets on day 28 (gray dashed line) and received DX injections on days 35, 39 and 43 (red dashed lines). Survival curves (A); body weight (B); average tumor volume (C).

C. Discussion

Consistent with previous *in vitro* reports that EA and urolithins can inhibit proliferation of prostate cancer cells (343), we observed anti-proliferative effects of EA (presented in Chapter III) on CRPC cell lines and similar growth inhibitory effects by UroA, the primary metabolite of EA (presented in the current chapter). We also found that EA reduced the expression of full-length AR and AR splice variants in 22Rv1 cells, which is in-line with a study conducted by Naiki-Ito *et al* demonstrating that EA suppressed expression of PSA and AR in LNCaP cells (342), and downregulation of AR and PSA in LNCaP cells by urolithins has also been reported (353). Moreover, *in vitro* and *in vivo* studies have demonstrated that EA-rich pomegranate extracts inhibit proliferation, AR signaling, and metastasis of prostate cancer cells (248, 339-341, 348, 354-356).

EA can affect numerous cellular pathways and mechanisms associated with cancer development and progression, some of which include promoting apoptosis, reducing oxidative stress, inhibiting inflammation, preventing invasion and metastasis, and inhibiting angiogenesis (357). In this study, we report the first evidence that EA may target microtubules in prostate cancer cells. Microtubules are components of the cytoskeleton that play a major role in many cellular functions, facilitate mitosis, and are the target of several chemotherapeutic agents including taxanes. Microtubule targeting agents are a diverse class of agents with lots of structural variations, most of which are naturally occurring and produced by plants as broad-spectrum toxins against predators (358). Although there are no current reports of EA interactions with microtubules, several other natural polyphenolic compounds have been shown to interact with and disrupt microtubule dynamics (359-364).

We found that EA (10 and 30 μM) increased levels of polymerized β-tubulin in 22Rv1 cells. The effect of EA appears to be due to a direct interaction with tubulin subunits. We observed

that EA is able to bind tubulin and can increase the rate of tubulin polymerization in a cell-free tubulin assay. Although we also found that EA can increased expression of microtubule-associated protein 2 (MAP2), which is involved is involved in assembly and stability of microtubules (365), suggesting that cellular effects may be mediated by upregulation of proteins involved in microtubule dynamics.

Contrary to our hypothesis, results from our *in vitro* studies suggest possibility of antagonistic effects when used EA and taxanes are used in combination. Co-incubation of EA and CBZ caused a reduction in microtubule polymerization compared to CBZ alone in 22Rv1 cells and in the cell-free tubulin polymerization assay. Considering that EA is able to bind tubulin and reduce microtubule formation by taxanes in a cell-free assay, it is possible that EA and taxanes may competitively bind tubulin. The presence of EA may limit available taxane binding sites, leading to a reduction in taxane-tubulin interactions. The extent of EA and tubulin interactions warrants further investigation.

While these results suggest antagonism by EA on taxane activity, the effect is modest and does not appear to have a substantial impact on taxane efficacy. Although there was a visual reduction in microtubule density and bundling in 22Rv1 cells treated with EA + CBZ compared to CBZ treatment alone (Figure 41), there was not an apparent reduction in the number of apoptotic cells or number of cells with abnormal mitotic spindles. Also, differences in the levels of soluble and polymerized tubulin, as determined by western blotting, in 22Rv1 cells treated with EA in combination with CBZ compared to treatment with CBZ alone did not reach statistical significance. Additionally, the cytotoxicity of either CBZ or DX on CRPC cell lines was not affected by EA (3, 10 and 30 μM). Together these results suggest that effects of EA on tubulin polymerization in prostate cancer cells are not sufficient to abrogate mitotic arrest induced by CBZ.

Our xenograft mouse study provides further support for this conclusion. Dietary administration of EA (2 or 4 g/kg) did not have any effect on DX induced growth arrest of 22Rv1 tumors in nude mice. There was no significant difference in tumor growth rates, either during DX treatment (days 35-44) or following DX-induced (days 55-70). Likewise, dietary EA did not significantly reduce the duration (tumor growth delay) or magnitude (reduction in tumor volume) of DX response. Growth rates for all DX treated tumors (DX + control diet, DX + low EA, and DX + high EA) were significantly different compared to vehicle treatment; therefore it is not likely the antagonism of taxanes by EA was not observed due to lack of DX efficacy.

It is possible that we did not observe combination effects *in vivo* to due limited bioavailability of EA and metabolites in nude mice. Upon digestion EA is metabolized by the gut microflora to produce urolithin derivatives, which are more soluble and bioavailable than EA (246) and exert growth inhibitory effects on CRPC cells. Our xenograft study is limited in that we did not include an additional control group of animals that received dietary EA but not DX, and thus we do not know the effects of EA alone on the tumor growth and do not have an indication that EA and/or urolithin metabolites are bioavailable or bioactive in our model. Seeram *et al* reported that EA metabolites could not be detected in plasma or tissue following dietary administration of pomegranate extract, suggesting that nude mice may lack colonic microflora required to produce urolithins (290). The metabolism and bioavailability of EA in the present study was not evaluated, thus it remains unclear whether lack of combination effects of EA and CBZ is due to poor bioavailability or lack of efficacy. Future assessment of EA and urolithin levels in tumor tissue collected from nude mice may aid in the clarification of this issue. Nevertheless, our *in vitro* results suggest that lack of combination effects with EA and CBZ is attributed to the lack of significant biological effects rather than bioavailability issues.

In conclusion, although results of this study are consistent with previously reported anti-cancer effects of EA, we did not find evidence to support our hypothesis that EA can enhance

the efficacy of taxane chemotherapy. We found that EA is able to inhibit CRPC proliferation, reduce AR expression, and observed a novel microtubule polymerizing effect of EA *in vitro*, which may antagonize the activity of taxanes. However, the extent of this effect was small and does not appear to be hazardous when used in combination with taxanes, as we did not observe an inhibition of docetaxel efficacy by dietary EA on growth of 22Rv1 tumors in nude mice.

VI. COMBINATION EFFECTS OF SOY ISOFLAVONES AND TAXANE CHEMOTHERAPY

A. Introduction

The standard of care for castrate-resistant prostate cancer (CRPC) includes a regimen of taxane-based chemotherapy, with docetaxel (DX) as first line systemic treatment and cabazitaxel (CBZ) as a potential secondary option (299-302). These chemotherapeutic measures to control advanced-stage CRPC lack substantial efficacy and can severely impair the quality-of-life of the patient (41, 301, 303). The median survival for CRPC patients at this stage is approximately 10–12 months, with taxane chemotherapy only adding a survival benefit of 2–3 additional months (50, 366). Side effects of taxanes can be severe and include neutropenia, hypersensitivity reactions, neuropathy, nausea/vomiting, hair loss, and fluid retention (51, 367). Additionally, the clinically utility of DX and CBZ is often limited by the development of drug resistance (352). Thus, there is an urgent need to identify novel agents and strategies that can improve taxane-based therapy.

Numerous experimental studies have demonstrated chemopreventive activity of soy isoflavones in multiple cancer types. Soy isoflavones include genistein (Gen) and daidzein (Daid), which can be metabolized to produce equol. All three of these compounds have *in vitro* and *in vivo* anticancer activity against multiple types of cancers, particularly mammary cancer (368). Isoflavones have been shown to modulate expression of cell proliferation and apoptotic genes, inhibit cell migration and metastasis, and have antioxidant activity (369-371). Of the isoflavones found in soy, Gen is the most abundant and biologically active (3, 372), and therefore considerable effort has been spent elucidating mechanisms of Gen for chemoprevention strategies, but much less attention has been paid to potential interactions between Gen and chemotherapeutic drugs. Information regarding benefits or harm of Gen in combination with chemotherapy for CRPC is limited to only a few experimental studies (184, 189, 373-375), one of

which indicated the potential to enhance taxane efficacy (375). There are no human studies addressing this issue.

Consumption of soy foods, and thereby isoflavones, is typically low in a western diet, but concentrated soy supplements, such as Novasoy (Archer Daniels Midland Company), can provide high dietary levels of isoflavones. Plasma concentrations of isoflavones following intake of dietary soy supplements have been reported to range between 2–27 μM (376-378). Thus, it is possible that consumption of soy supplements can result in sufficient circulating quantities to exert biological effects. Considering the pleiotropic anticancer activities of soy isoflavones and lack of toxicity, dietary supplementation may provide a strategy for enhancing chemotherapeutic efficacy, reducing associated side effects, or preventing development of drug resistance.

The aims of this study were to 1) investigate whether physiologically attainable concentrations of soy isoflavones could act synergistically with taxanes to induce growth inhibition of prostate cancer cells *in vitro* and *in vivo*, and 2) explore effects of soy isoflavones on mechanisms of taxanes resistance and metabolism. Based on current literature (375), we expected that Gen would synergistically enhance the efficacy of taxanes, and we speculated that Daid and equol may also exhibit synergistic effects with taxanes due to similarities with Gen in their activity and structures.

B. Results

i. Soy isoflavones do not increase cytotoxicity of taxanes in CRPC cells

To determine if Gen, Daid, or equol alter cytotoxicity of taxanes, we measured combination effects on prostate cancer cell proliferation using the SRB assay. We first tested Gen, Daid and equol at a concentration of 10 μM (physiological relevant concentration) in combination with

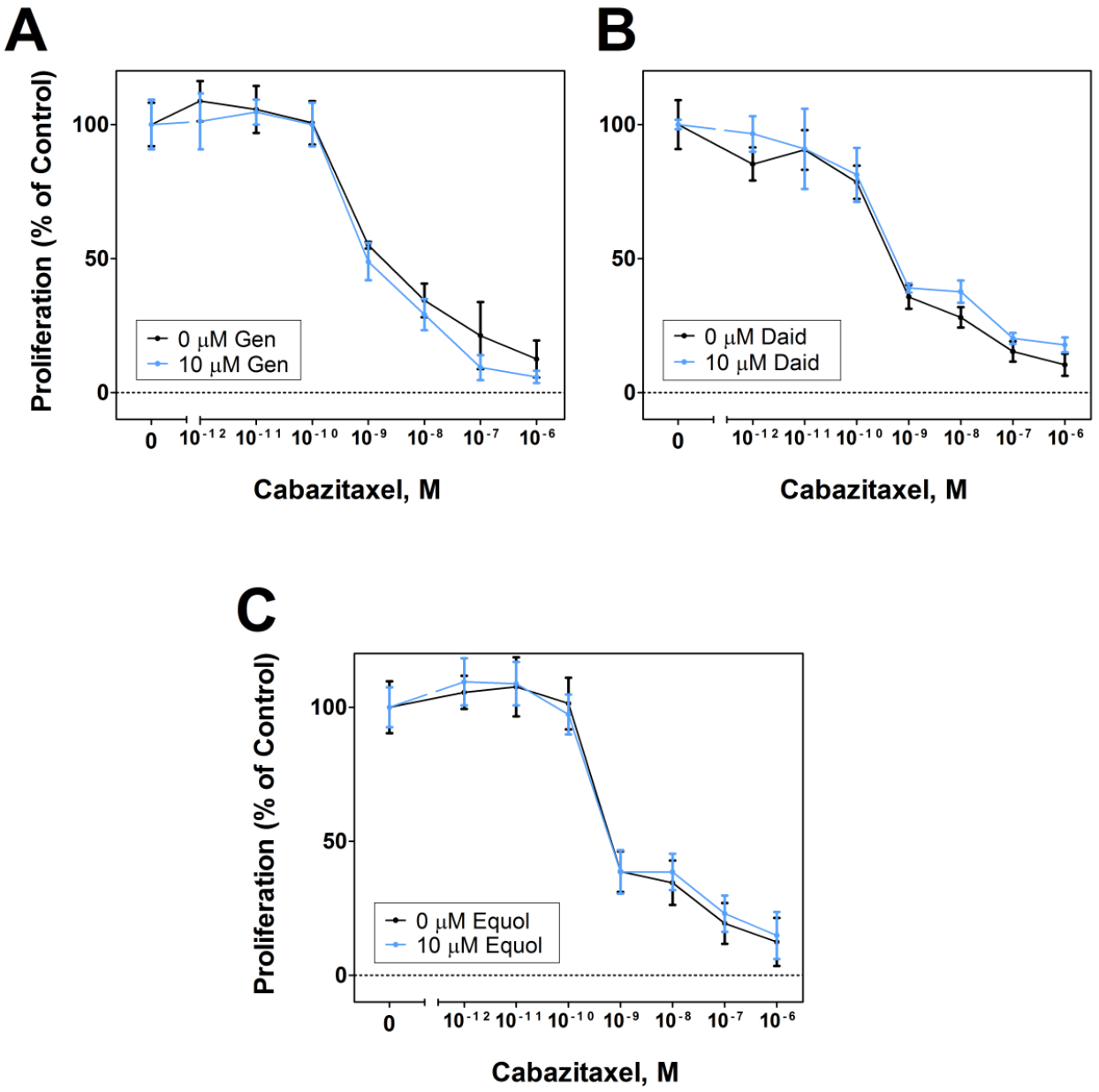


Figure 48. Soy isoflavones do not enhance cytotoxicity of cabazitaxel. Proliferation was measured in 22Rv1 cells using the SRB assay after 72 hours treatment with 10 µM Gen (A), 10 µM Daid (B), or 10 µM EQ (C) in combination with cabazitaxel (0.001 – 1,000 nM). Results were normalized to vehicle control treated cells and are presented as mean ± SD.

CBZ (0.01 nM – 1 µM) on 22Rv1 cells. We observed no enhancing or inhibiting effects by any of the isoflavones on cytotoxicity of CBZ (Figure 48).

Since we had expected to observe synergistic or additive effects of Gen in combination with taxanes based on previous reports (184, 374, 375), we further investigated combination effects of Gen + CBZ and Gen + DX using additional cell lines and dosing combinations. We used 22Rv1, C4-2, VCaP and PC-3 cell lines, which are all capable of androgen independent growth and resemble clinical phenotypes of CRPC, to examine effects of Gen (1, 10 and 100 µM) in combination with DX (0.1 – 10 nM). Co-treatment of 1 and 10 µM Gen with DX did not further reduce the growth of any of the prostate cancer cell lines compared to DX alone (Figure 49). There was slight antagonism at 100 µM Gen in combination with 10 nM DX in VCaP (CI = 2.75), PC-3 (CI = 1.85), and 22Rv1 cells (CI = 1.21) as determined by combination index analysis, but no synergistic effects were observed. Similarly, no combination effects were observed with Gen in combination with 0.1 and 1.0 nM CBZ, but antagonist activity was observed with the high dose of CBZ (10 nM) (Figure 50).

Additionally, we investigated combination effects of Gen (1, 10 and 100 µM) with CBZ on DNA synthesis activity by measuring incorporation of a thymidine analog (EdU) into DNA of C4-2 cells. There were no significant differences in EdU incorporation for Gen + CBZ combination treated cells compared to CBZ alone (Figure 52). Because these results were not consistent with previously reported synergistic activity of Gen with CBZ (375), we tested combination effects of CBZ and Gen using doses of similar to those used in that study (18 and 37 µM Gen) in 22Rv1 and C4-2 cells. Again, we did not find synergistic effects of Gen in combination with CBZ (Figure 51).

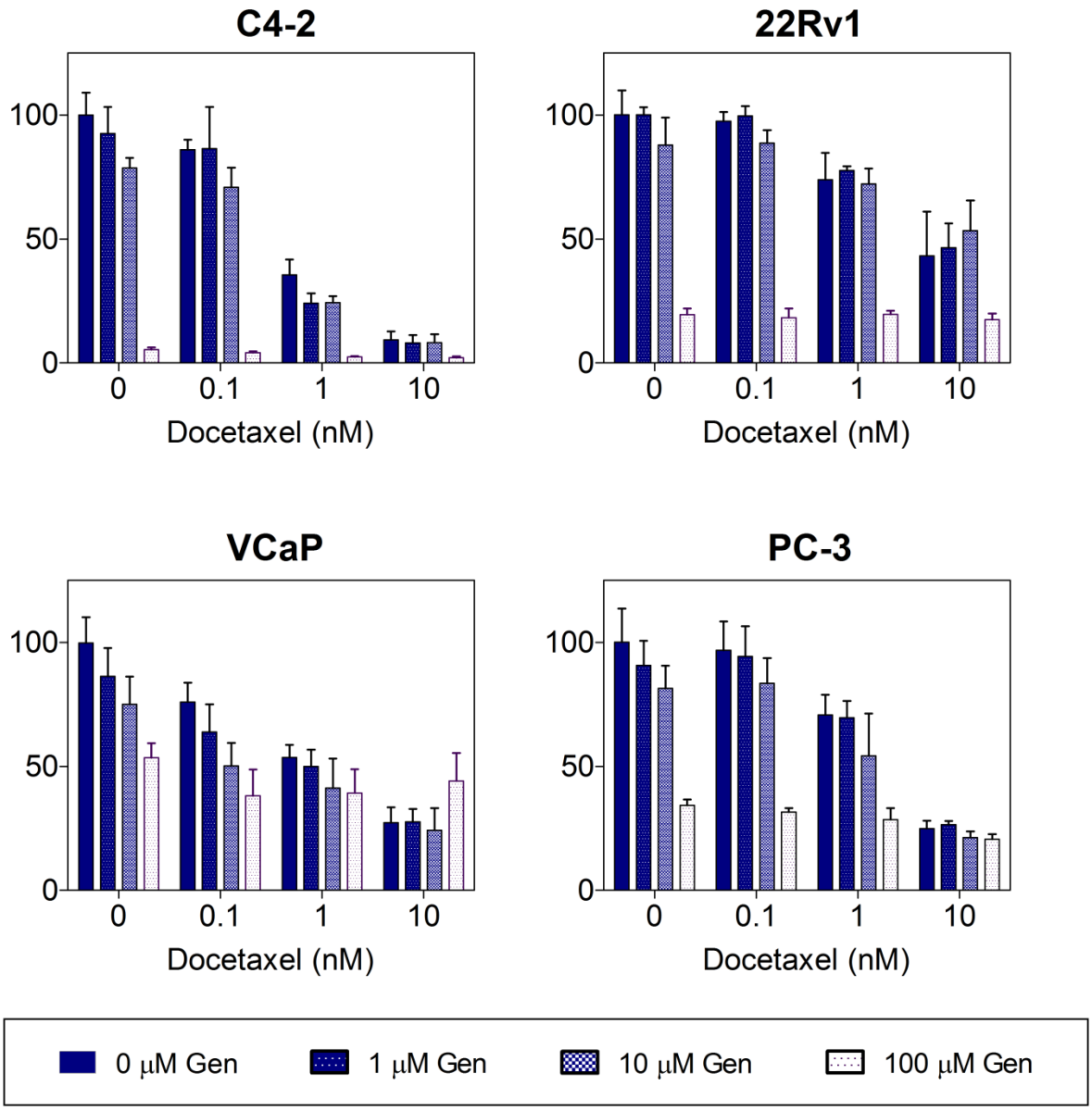


Figure 49. Combination effects of genistein and docetaxel on proliferation of prostate cancer cells. Proliferation was measured in CRPC cell lines using the SRB assay after 72 hours treatment with genistein (1-100 μM) and docetaxel (0.01–10 nM). Results were normalized to vehicle control treated cells and are presented as mean ± SD.

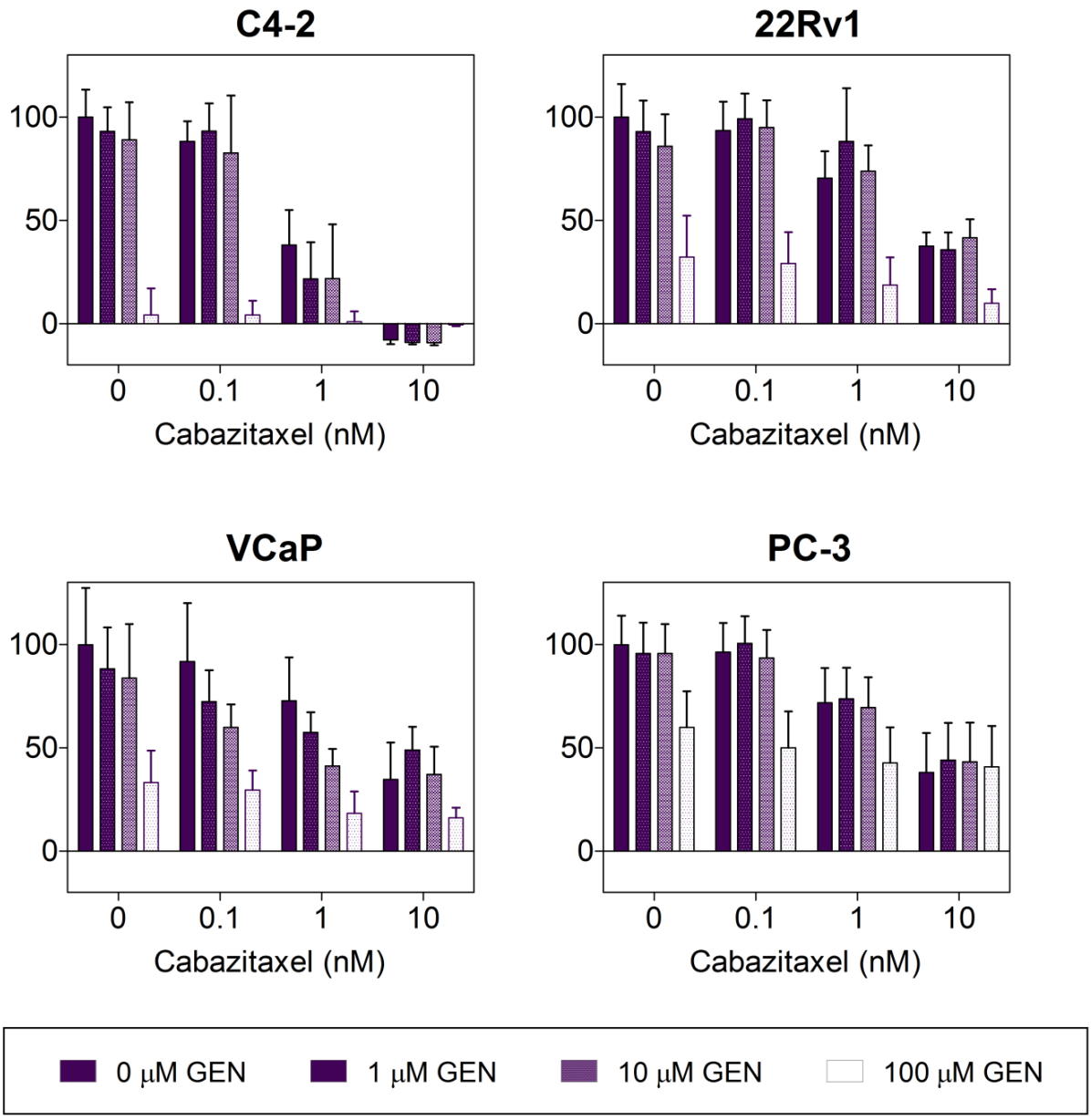


Figure 50. Combination effects of genistein and cabazitaxel on proliferation of prostate cancer cells. Proliferation was measured in CRPC cell lines using the SRB assay after 72 hours treatment with genistein (1-100 μM) and cabazitaxel (0.01–10 nM). Results were normalized to vehicle control treated cells and are presented as mean ± SD.

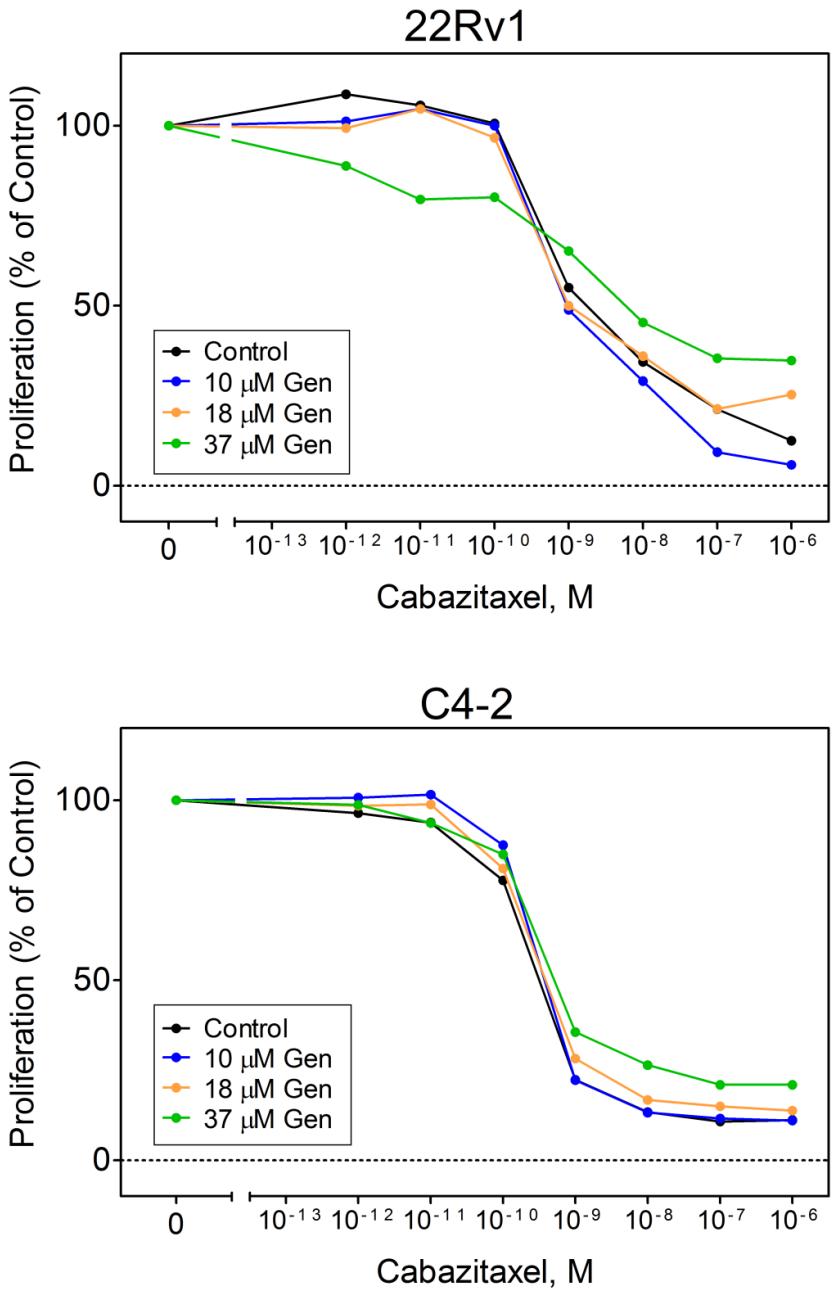


Figure 51. Combination effects of genistein and cabazitaxel on proliferation of 22Rv1 and C4-2 cells. Proliferation was measured in 22Rv1 and C4-2 cell lines using the SRB assay after 72 hours treatment with cabazitaxel (0.001 – 1,000 nM) and concentrations of genistein (18 & 37 µM) reported to have synergistic activity with taxanes (185).

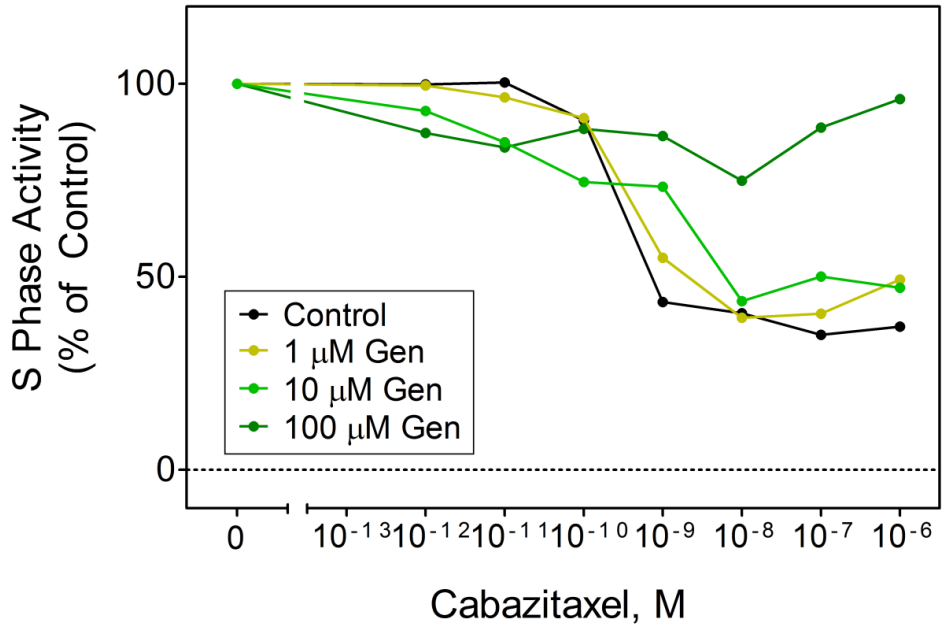


Figure 52. Combination effects of genistein and cabazitaxel on S phase activity of C4-2 cells. S phase activity was assessed by measuring incorporation of a thymidine analog into DNA of C4-2 cells after treatment with Gen (1-100 μM) and CBZ (0.001 – 1,000 nM).

ii. *Soy isoflavones do not inhibit microtubule assembly in vitro*

Taxanes exert cytotoxic effects by binding to and stabilizing microtubules preventing their disassembly, which leads to G2/M arrest and subsequent apoptosis (64, 320). It has been previously demonstrated that Gen can bind tubulin and act to inhibit microtubule polymerization (188), suggesting that genistein may interfere with the mechanism of action of taxanes. We investigated the effects of DX, CBZ, and Gen on tubulin polymerization using an *in vitro* (cell-free) assay. Because of the structural similarity of Gen with other soy isoflavones, we also tested effects of daidzein and equol in this assay. DX and CBZ increased the rate of tubulin polymerization in a dose-dependent manner, as expected (Figure 60, Appendix C). 10 and 100 μM Gen enhanced tubulin polymerization by 18.4% and 37.9% respectively, but this was not

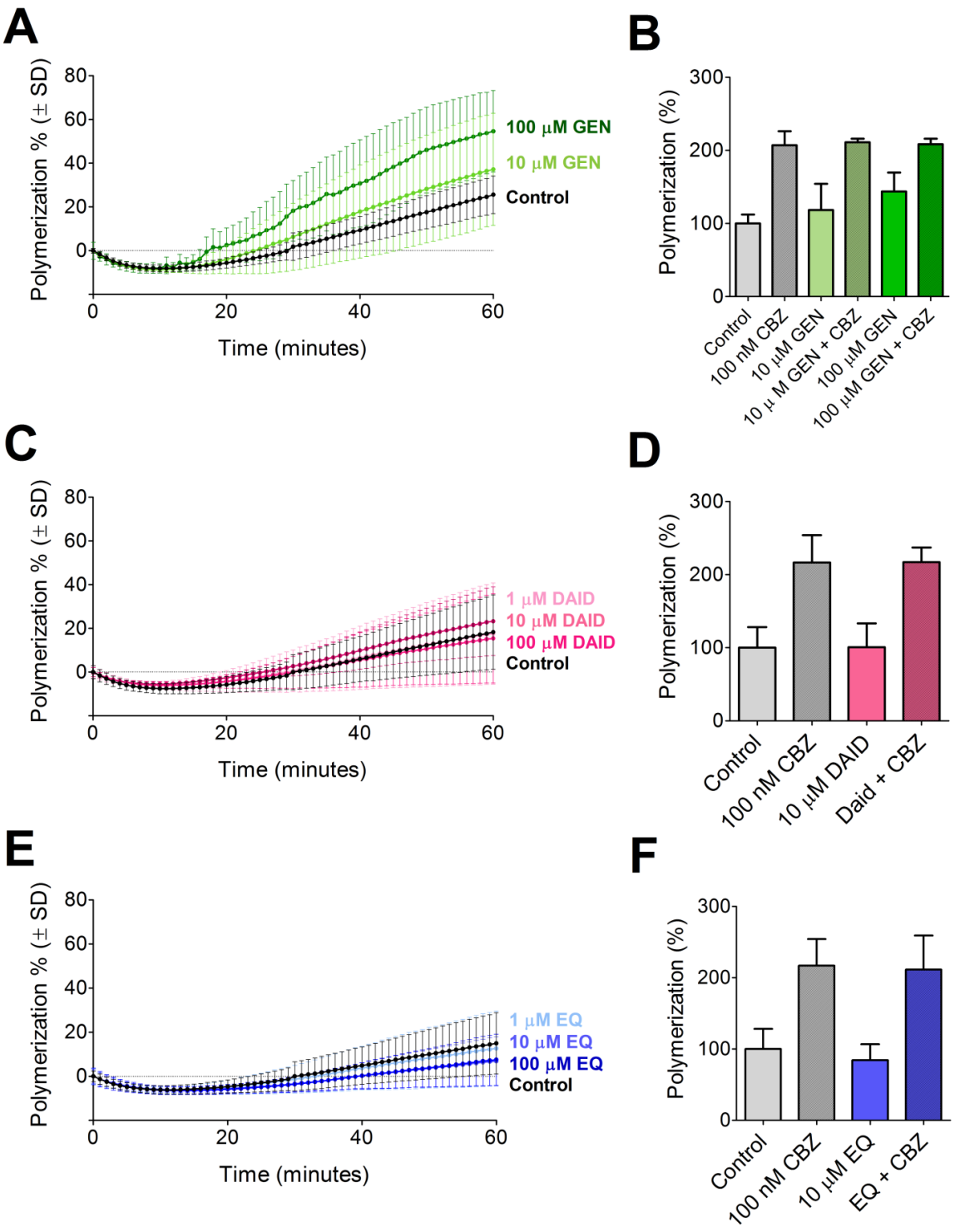


Figure 53. Effects of soy isoflavones on microtubule assembly *in vitro.* Tubulin polymerization in the presence of genistein (A) and daidzein (C) and equol (E) was measured by fluorescence intensity every minute for 1 hour. Combination effects were measured at 60 minutes of exposure to 100nM CBZ + 10 and 100 μM Gen (B), 100 nM CBZ + 10 μM Daid (D), and 100 nM CBZ + 10 μM equol (F).

statistically significant (Figure 53A). Similarly, neither Daid (1 – 100 μM) nor equol (1 – 100 μM) had an effect on microtubule assembly at any concentration (Figure 53C and Figure 53E). We also evaluated the combination effects of Gen (10 & 100 μM), Daid (10 μM) and equol (10 μM) with CBZ (100 nM). None of the CBZ + isoflavone combinations were significantly different compared to effects of CBZ alone (Figure 53B, Figure 53D and Figure 53F), suggesting that microtubule effects of CBZ are not affected by presence of soy isoflavones.

iii. Effect of soy isoflavones on efflux activity of 22Rv1 cells

Resistance of cancer cells to taxanes has been associated with increased drug efflux activity by ABC transporters (352). Previous studies have suggested that isoflavones may be able to reverse resistance through inhibition of P-gp, MRP and BCRP mediated efflux (379-382). Therefore we examined the ability of Gen, Daid and equol to modulate efflux activity. We treated 22Rv1 cells with Gen (1 nM – 100 μM), Daid (0.1 – 100 μM) and equol (0.1 – 100 μM) for 24 hours, then measured intracellular levels of the fluorescent dye calcein AM. Low doses of Gen (1 and 10 nM) had no statistically significant effect on intracellular accumulation of calcein AM, but 0.1 μM and 1 μM Gen increased intracellular calcein retention by 2.1-fold and 1.5-fold compared to controls, followed by a dose-dependent decrease at 10 and 100 μM (Figure 54). While a smaller dose range of Daid and equol were used, similar biphasic trends were observed for Daid (Figure 54B) and equol (Figure 54C).

iv. Genistein inhibits CYP3A4 activity

Metabolism of taxanes is catalyzed by CYP3A4, which also plays a role in the metabolism of Gen (328, 383). It has been demonstrated that Gen can inhibit several cytochrome P450

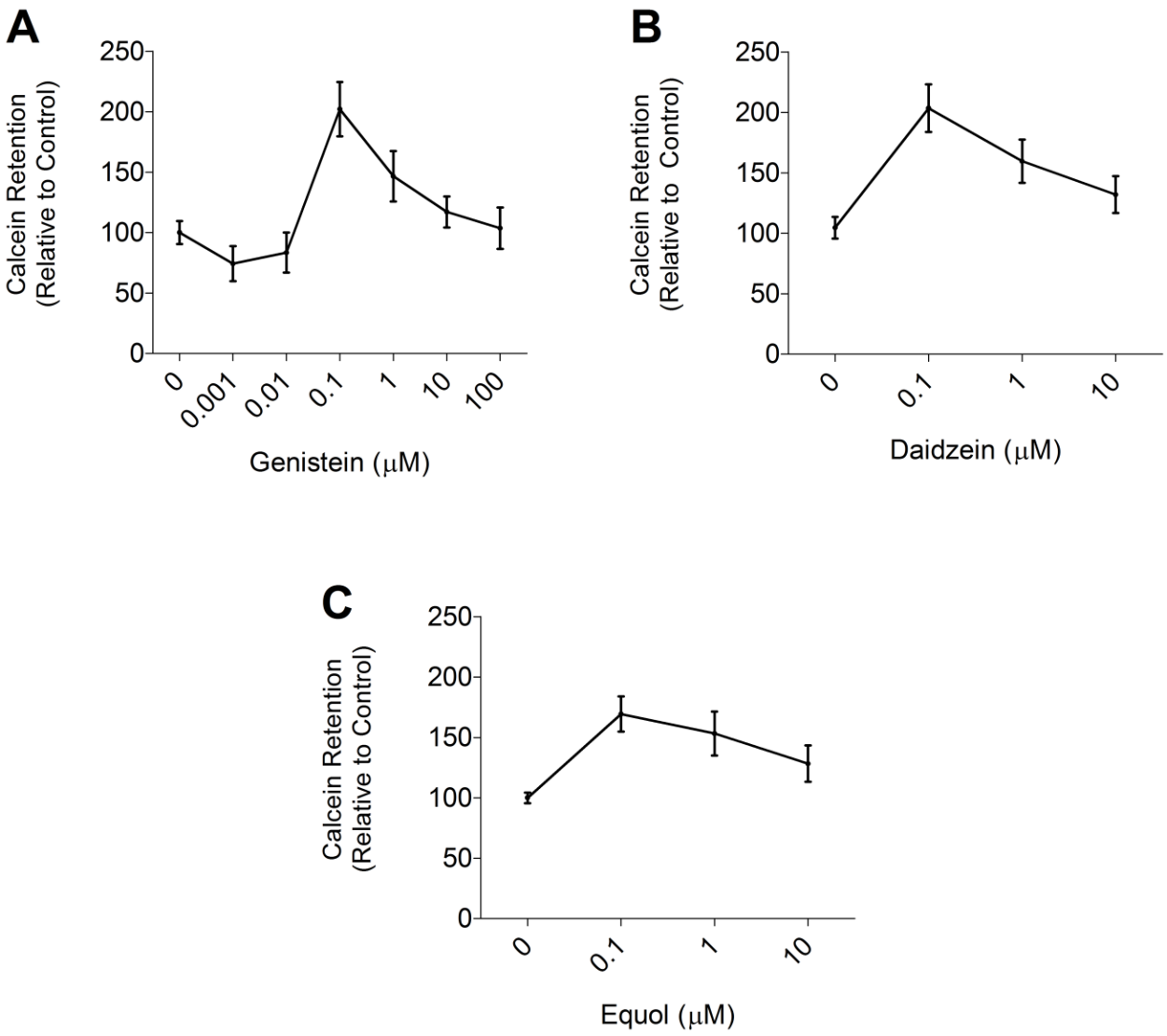


Figure 54. Effects of soy isoflavones on efflux activity of 22Rv1 cells.
Genistein 0.001 – 100 μM (A), 0.1 – 10 μM daidzein (B), and 0.1 – 10 μM equol (C).

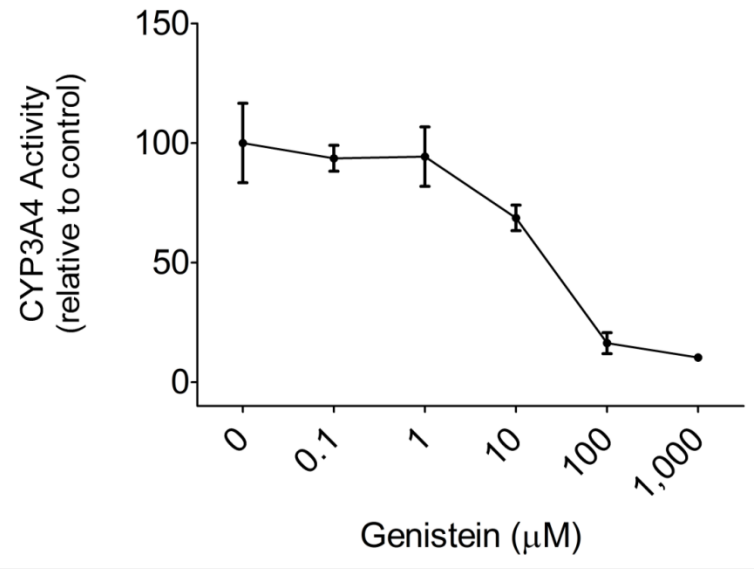
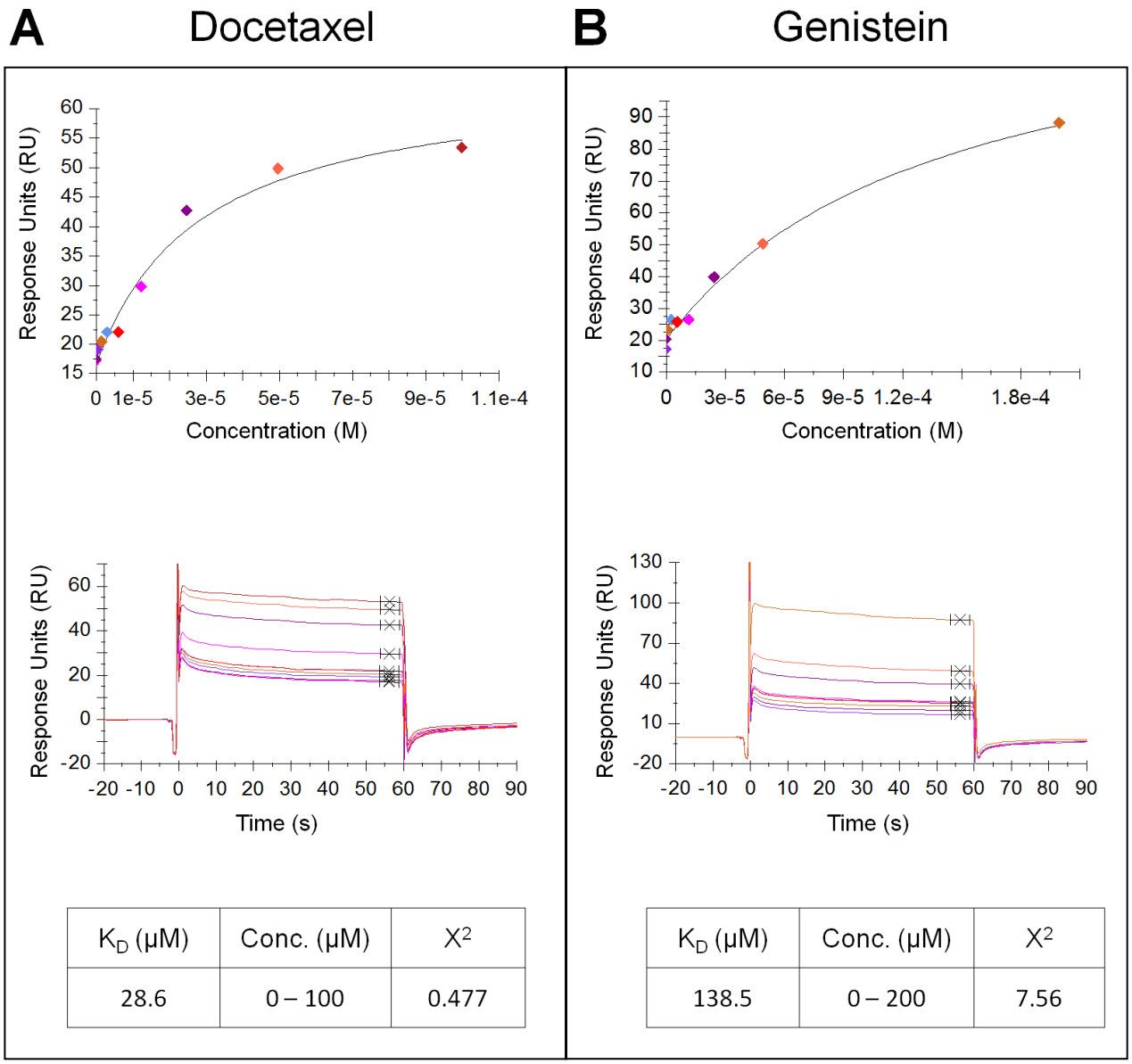


Figure 55. Genistein inhibits CYP3A4 activity.

enzymes, including CYP3A4 (384). To investigate effects of Gen on the catalytic activity of CYP3A4, we treated CYP3A4 expressing microsomes with Gen (0.1 – 1,000 μM), and quantified activity by measuring the intensity of a fluorescence reporter metabolite of a CYP3A4 substrate. Gen decreased CYP3A4 activity in a cell-free assay in a dose dependent manner: 1.7% reduction at 1 μM, 35.3% at 10 μM and 84.4% at 100 μM (Figure 55).

v. Genistein binding to tubulin

Others have reported that Gen is able to bind tubulin and depolymerize microtubules (188). We investigated the potential binding interactions of Gen and tubulin using label-free, surface



K_D (μM)	Conc. (μM)	X^2
28.6	0 – 100	0.477

K_D (μM)	Conc. (μM)	X^2
138.5	0 – 200	7.56

Figure 56. Binding interaction of genistein and tubulin. Binding affinity of docetaxel (A) and genistein (B) for tubulin was determined by surface plasmon resonance. Response-concentration plots (top) and sensorgrams (bottom) are shown.

plasmon resonance (SPR) to test for direct interactions between Gen and tubulin. Gen displayed low binding affinity for tubulin with an equilibrium dissociation constant (K_D) of 138.5 µM (Figure 56B). Docetaxel bound tubulin with a high affinity (K_D = 28.6 µM), as expected (Figure 56A).

vi. Dietary genistein does not alter the efficacy of docetaxel on 22Rv1 tumor growth in mice

To examine the effects of dietary genistein with docetaxel on tumor growth *in vivo*, 22Rv1 cells were subcutaneously injected into mice. Tumors reached an approximate volume of 500 mm^3 and animals were placed on either control diets or modified diets containing 250 or 500 mg/kg Gen for the duration of the study. After 1 week of dietary intervention, mice received 3 injections of DX (15 mg/kg) administered at 3 day intervals. Tumors were measured three times per week until tumor volume exceeded 3,000 mm^3.

We observed a high degree of variability in tumor volumes prior to chemotherapy and dietary interventions (tumor measurements for individual tumors can be found in Appendix C). Tumors that were not palpable or had not reached the exponential growth phase (volume of at least 350 mm^3) at the start of DX treatment were excluded from the data analysis. Additionally, we observed 1 tumor (DX + Ctrl group) with a volume that exceeded of 2,500 mm^3 at day 35, which was identified as an outlier using Grubbs' test and therefore removed from analysis.

There were no differences in average body weight at the start of the dietary interventions or at the start of chemotherapy. DX treatment resulted in a rapid decrease in body weight by an average of 16.7%, but weight loss did not differ across diet groups (Table VII). Body weight in all DX-treated groups had recovered by approximately day 60 (Figure 57).

As expected, tumor growth was delayed by DX treatment. All tumors experienced a lag in growth rate following DX treatment that lasted approximately 10 days, but we did not observe an overall decreased in tumor volume by DX, which is in contract to our pilot study with CBZ (data not shown), wherein 10 mg/kg CBZ treatment caused a reduction in tumor size. None of the DX treated tumors exhibited a substantial regression in volume in response to treatment. As expected, there was no growth delay of tumors in vehicle-treated animals, which reached an average volume of 3,000 mm^3 at 54.4 days, whereas DX treated tumors took 63.4 (control diet), 65.5 (low Gen diet) and 67.2 (high Gen diet) days to reach the same average volume. Dietary supplementation with either concentration of Gen failed to enhance effects of DX. No differences in tumor growth rates, tumor growth delay, or overall survival were observed in DX + Low Gen or DX + High Gen groups compared to the DX + control diet group.

Table VII. Effects of dietary genistein on efficacy of docetaxel in 22Rv1 xenografts

	Vehicle + Ctrl Diet	DX + Ctrl Diet	DX + Low Gen	DX + High Gen
Dietary genistein (mg/kg)	0	0	250	500
Treatment [a]	Vehicle	DX	DX	DX
Number of animals	6	10	6	6
No. of measureable tumors at day 35 (total 22Rv1 injections)	9 (12)	16 (20)	9 (12)	9 (12)
No. of tumors included in data analysis	6	10	6	7
No. of tumors excluded from data analysis				
Tumors < 350 mm^3 at day 35	3	5	3	2
Tumors > 2500 mm^3 at day 35	0	1	0	0
Average body weight (g ± SD)				
At start of dietary intervention (day 28)	31.0 ± 1.7	31.2 ± 3.5	30.5 ± 3.6	27.7 ± 2.8
At start of therapy (day 35)	31.2 ± 1.2	30.9 ± 2.4	30.2 ± 3.0	29.2 ± 2.0
One week after therapy (day 49)	32.2 ± 1.2	26.8 ± 3.6 [b]	24.8 ± 2.5 [b]	23.7 ± 1.9 [b]
Average tumor volume (mm^3 ± SD)				
At start of dietary intervention (day 28)	454.2 ± 231.3	477.2 ± 256.6	381.7 ± 286.4	473.4 ± 247.5
At start of therapy (day 35)	748.3 ± 262.9	1166.9 ± 489.2	850.7 ± 383.4	952.9 ± 496.3
One week after therapy (day 49)	1972.0 ± 640.1	1730.8 ± 921.2	1537.3 ± 648.8	1789.6 ± 1263.6
Time to reach 3,000 mm^3 (average number of days ± SD)	54.4 ± 5.7	63.4 ± 5.4 [b]	65.5 ± 6.7 [b]	67.2 ± 5.7 [b]
Tumor growth delay (days)	-	9.0	11.1	12.8

[a] Vehicle (saline) and DX (15 mg/kg body weight) treatments were administered by IP injection on days 35, 39, and 43; [b] $p < 0.05$ for difference with the vehicle + control diet group (ANOVA); SD = Standard Deviation

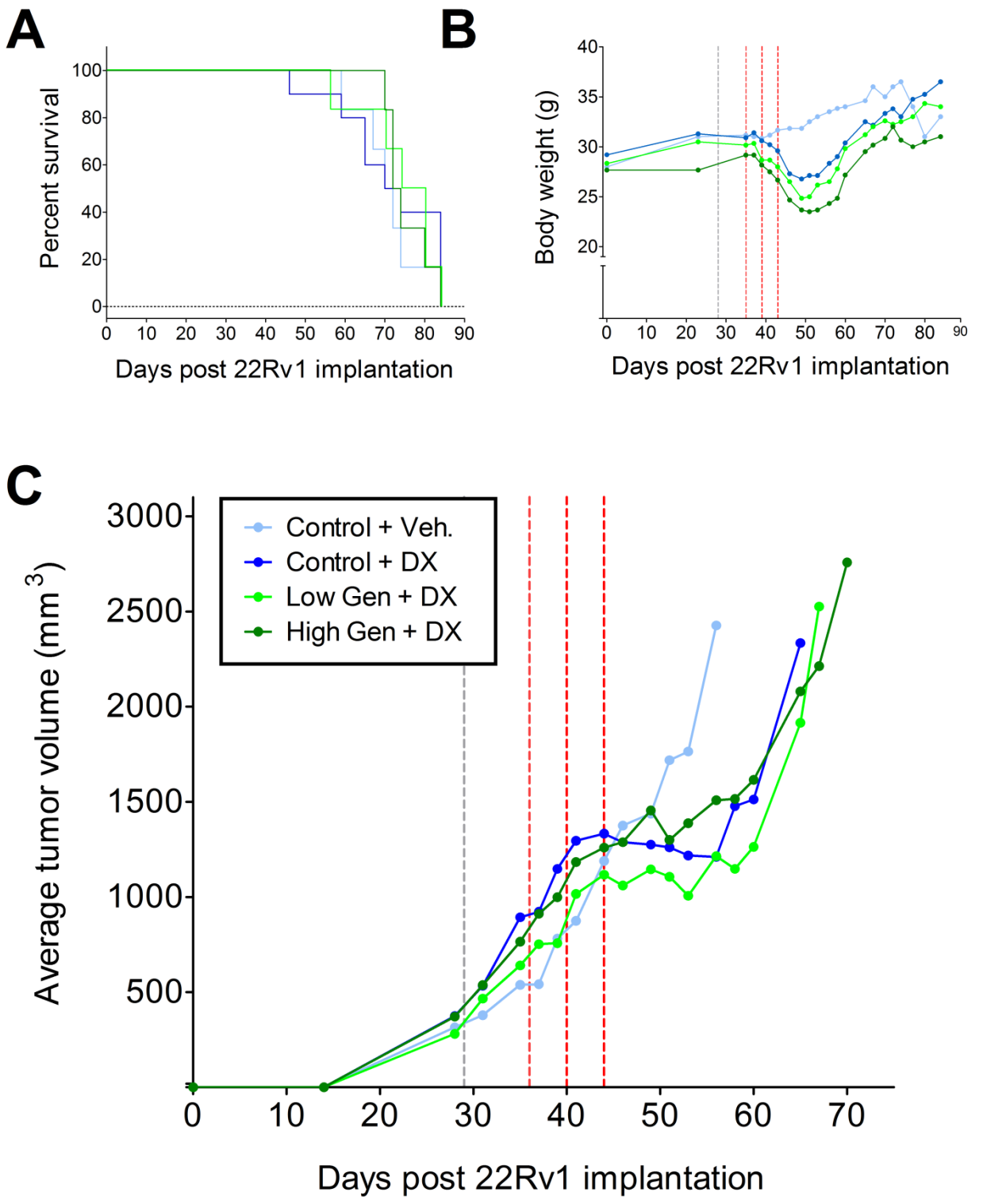


Figure 57. Genistein does not enhance efficacy of docetaxel on 22Rv1 tumor growth in nude mice. Mice were implanted with 22Rv1 cells in matrigel on day 0. Mice were switched to modified diets on day 28 (gray dashed line) and received DX injections on days 35, 39 and 43 (red dashed lines). Survival curves (A); body weight (B); average tumor volume (C).

C. Discussion

Soy isoflavones have a broad range of mechanisms that affect many cellular pathways and functions, several of which overlap with the mechanisms of chemotherapeutic drugs. In the present study, we evaluated the *in vitro* effects of Gen, Daid and equol on taxane cytotoxicity, metabolism and resistance, and also investigated *in vivo* effects of dietary Gen on efficacy of docetaxel in a xenograft model. We did not observe synergistic, additive or antagonistic effects of isoflavones on taxane cytotoxicity in our cell culture models, nor did Gen alter the growth inhibitory effects of DX in our prostate cancer xenograft animal model. Therefore, our findings do not support the hypothesis that soy isoflavones can enhance the efficacy of taxane chemotherapy for CRPC.

Previous *in vitro* studies have demonstrated synergistic effects with combination treatment of Gen with taxane chemotherapy in prostate cancer cells (184, 374, 375). We treated PC-3, 22Rv1, VCaP and C4-2 with combinations of Gen, CBZ, and DX similar to those reported by others, but could not confirm these results. We found no significant enhancement or inhibition of taxane cytotoxicity by Gen on prostate cancer cells. This finding was similar across all cell lines in our proliferation assay and in the EdU incorporation assay. In all of our *in vitro* studies, standard cell culture media was replaced with phenol red-free media containing 10% dextran-coated charcoal stripped FBS 24 hours prior to conducting assays. Others have reported performing *in vitro* experiments with serum-free media (184, 374, 375) or standard culture media supplemented with 5 or 10% FBS (184, 374, 375), which may account for differences between our studies.

Our results are consistent with findings from Mahoney *et al*, who reported results from an *in vitro* study on the combination effects of DX and phenoxodiol, a synthetic analogue of Gen, in prostate cancer cells (385). They found no combination effects of phenoxodiol (5, 10, 30 μM) on

DX (0.1, 1, 5, 10 nM) in DU145, LNCaP and PC-3 cell lines, but observed that the growth inhibitory actions of 100 nM DX were diminished by co-treatment with 5 or 10 μM phenoxodiol. We observed a similar trend in cells that were treated with Gen in combination with high doses of DX and CBZ, which suggests that Gen may confer protection to prostate cancer cells against exposure to high doses of taxanes. However, these observations are unlikely to be clinically meaningful, since effects were only observed at concentrations of taxanes much higher than relevant therapeutic doses, which are in the low nM range (386).

Dietary Gen failed to enhance efficacy of DX chemotherapy in our xenograft model. Neither the high nor low dose of Gen had a significant effect on tumor growth rates, either during DX treatment (days 35-44) or following DX-induced growth arrest (days 55-70). Likewise, dietary Gen did not significantly increase the duration (tumor growth delay) or magnitude (reduction in tumor volume) of DX response. Additionally, Gen did not alter weight loss during DX treatment, suggesting that Gen does not alleviate drug-induced systemic toxicities.

Taxanes are potent cytotoxic drugs and therefore it is possible that the robust effects of DX could mask any synergistic or additive effects by Gen. However, this is not likely to be the case in our experiment. The dosing schedule and concentration of DX used in our study induced a state of tumor growth arrest, rather than a regression in tumor volume. Therefore, if Gen enhanced effects of DX we would have been able to observe a decrease in tumor volume or a longer tumor growth delay.

Since we observed a substantial degree of variability in tumor volumes within groups, we investigated the possibly that Gen may differentially affect DX treatment based on tumor size. Following our initial data analysis, we stratified tumors into groups based on volume at the start of therapy. Results from this subgroup analysis were consistent with our previous analysis; we

found no significant differences in effect of Gen on DX related to tumor volume (data not shown).

Thus, the findings from our *in vivo* studies suggest that Gen does not improve taxane efficacy in CRPC, which is in contrast to a prior studies that showed co-treatment with Gen improved effects of taxanes in prostate cancer xenograft models (184, 374, 375). Gen mixed in the diet (at 0.1%) enhanced the inhibition by DX (I.V. injections of 5 mg/kg on three alternate days) on growth of PC-3 cells xenografted in bone of SCID mice (184). In a recently published paper, marked enhancement by Gen (100 mg/kg, I.P. injections three times per week) was reported of the inhibitory effect of CBZ (5 mg/kg, I.P. once weekly) on the growth of subcutaneously xenografted PC-3 cells (375); these investigators also found synergistic growth inhibition *in vitro* on PC-3 cells and LNCaP-C4-2 cells well as on the apoptotic effects of CBZ on PC-3 cells. Additionally, a fermented genistein product (genistein combined polysaccharide) also enhanced the *in vitro* apoptotic effect of DX on LNCaP cells and the inhibition of proliferation of 22Rv1 and PC-3 cells, but not inhibition of LNCaP cell proliferation (186). However, *in vitro* studies with leukemia and breast cancer cells have found that Gen can antagonize effects of taxanes. High concentrations of Gen (30 – 100 µM) reduced cytotoxicity and apoptosis induced by paclitaxel in 697 leukemia cells (191) and diminished paclitaxel-induced microtubule polymerization in MDA-MB-231 breast cancer cells (189).

Because of several limitations, previous studies on the combination of genistein and chemotherapy may not provide relevant information. In xenograft models and many of the *in vitro* experiments (184, 374, 387), results were obtained in PC-3 cells, a commonly used cell line for studying aggressive prostate cancer (388). PC-3 cells do not express androgen receptor or PSA, unlike most CRPC tumors in men following failure of ADT who are candidates for DX treatment. Additionally, route of administration and dosage are critical factors when investigating dietary agents in preclinical experiments. In the animal study by Zhang *et al,* Gen was

administered by I.P. injections and therefore was designed to assess the use of Gen as a pharmacological agent, rather than as a dietary intervention strategy. Finally, many of the *in vitro* studies used concentrations of Gen that are higher than what is achievable through consumption of a high soy diet or supplementation (>30 μM).

Although no clinical trials have evaluated effects of dietary Gen in CRPC patients while receiving taxane chemotherapy, there are some reports on combination effects of soy/genistein products in combination with chemotherapeutic agents. Results have been reported from clinical trials using the synthetic genistein analogue phenoxodiol in combination with chemotherapy in ovarian cancer patients (389, 390). Kelly *et al* reported safety and tolerability of phenoxodiol co-administered with paclitaxel, carboplatin, or cisplatin in a phase II clinical trial of 32 patients, and preliminary findings suggested efficacy (390). However, in the subsequent phase III trial of 142 ovarian cancer patients, the combination of carboplatin and oral phenoxodiol (400 mg daily, every 8 hours) failed to enhance effectiveness of carboplatin alone; no differences in overall survival or progression-free survival were observed (389). Consumption of Novasoy, a dietary isoflavone supplement, did not improve outcome of gemcitabine and erlotinib therapy in patients with advanced pancreatic cancer, and adversely affected tolerability of erlotinib (391). Another investigation on the combination effects of Novasoy and gemcitabine in breast cancer patients was terminated early due to lack of efficacy (392). Additionally, results from a recent phase I trial with pancreatic patients administered gemcitabine in combination with AXP107-11, sodium salt dehydrate form of genistein, demonstrated safety and tolerability (393). Although this study was not designed to address efficacy, the authors reported similar survival outcome to those reported in the previously mentioned study by El-Rayes *et al* (391), who concluded dietary isoflavones failed to provide any additional therapeutic benefit.

Taxanes induce apoptosis through a binding interaction with β-tubulin subunits of microtubules, which stabilizes the dynamics mitotic spindle structures, thereby preventing

proliferation cells from competing mitosis (64, 320). Several studies have report that Gen can bind to tubulin and also microtubule dynamics (187, 188), although other have not observed this (189, 190). We examined the effects of Gen, Daid, and equol, alone and in combination with CBZ, on tubulin polymerization and found no significant effect by any of these compounds on microtubule dynamics, in the presence or absence of CBZ. Additionally, Gen did not exhibit a high binding affinity to tubulin in our SPR experiments. These results suggest that soy isoflavones do not have microtubule inhibitory activity.

Cytochrome P450 enzymes (CYP) and ABC transport proteins regulate the pharmacokinetics and bioavailability xenobiotics, including taxanes which are metabolized by CYP3A4, and can be induced or induced by many compounds including drugs and dietary agents (394). Inhibition of metabolic enzymes and efflux transporters can increase systemic and intracellular levels of taxanes, which has implications for increasing bioavailability, but also drug toxicity and side effects. Inhibition of CYP3A4 activity by Gen has been demonstrated *in vitro* and in clinical trials (395, 396), and we observed similar effects in our *in vitro* experiments, as well as inhibition of efflux activity with Gen. Therefore, we reasoned that inhibition of CYP3A4 and drug transporters by co-treatment of taxanes with Gen could result in prolonged exposure to circulating drugs levels *in vivo*, possibly leading to increased cytotoxicity. In our animal study, we did not find any combination effects on tumor volume, nor did we observe any differences in body weight or signs of increased toxicity with combination treatment, suggesting there was not enhancement of drug toxicity mediated through inhibition of CYP3A4 or efflux activity by Gen. However, effects of Gen on hepatic CYP3A4 activity and expression levels of efflux transporters in mouse tissue from our study have not been evaluated yet.

In conclusion, our results with relevant cell models and clinically achievable concentrations of soy isoflavones and observations from the few clinical trials with soy products do not indicate that genistein or soy can enhance the effects of taxane chemotherapy.

VII. CONCLUSIONS AND DISCUSSION

The overall goal of this dissertation was to investigate whether constituents from dietary soy and black raspberries may be beneficial for the prevention and treatment of prostate cancer. Disappointingly, our results suggest that these dietary compounds have limited benefit for prostate cancer.

The use of bioactive dietary agents has long been considered an appealing approach to cancer prevention. Many bioactive compounds have pleiotropic cellular effects that target numerous pathways related to carcinogenesis and exhibit little to no toxicity (262, 397). The diverse biological effects of dietary compounds and phytochemicals can have an impact on cancer throughout the course of disease progression and therefore does not limit their role simply to prevention. Numerous dietary compounds and phytochemicals are being investigated as therapeutic agents, and there is a growing amount of evidence to suggest that dietary phytochemicals can be used as adjuvants to radiation, chemotherapy, and other conventional cancer treatments (398).

Despite advances in early detection and treatment modalities, prostate cancer – one of the most frequently diagnosed cancer types – remains a leading cause of cancer related death among men in United States (7). Considering the high prevalence of this disease, lack of preventive options and short-lived effectiveness of therapeutic agents, new strategies are needed to reduce incidence and disease progression, as well as improve existing treatment modalities and outcomes. Dietary constituents may provide such an approach for the management of prostate cancer, as compounds with anti-cancer activity could aid in prostate cancer prevention and delay disease progression, alleviate undesirable side effects of conventional therapies, prevent development of multidrug resistance, and/or enhance drug

effectiveness. Conversely, dietary compounds and phytochemicals with potent bioactivity also pose risk of adverse interactions with conventional cancer treatments.

Although much is not known concerning the biological effects of most dietary constituents, the use of dietary supplements, herbal remedies, and phytochemicals is very common in the general population and is especially high among cancer patients (307-310). A recent evaluation of supplement use by patients at a U.S. cancer center revealed that 61.2% of cancer patients reported using at least one dietary supplement (311), while other investigators have reported even higher proportions of supplement use among cancer patients (399, 400). Motivations for using dietary supplements or alternative therapies vary among patients, but common reasons include alleviating side effects of conventional treatment, desire to improve therapeutic response, and use as a last-resort option when other treatments have failed to provide benefit (304, 401). Moreover, the use of dietary supplements often occurs concurrently with other medications; 65.7% of adults ages 62-85 reported the concomitant use of dietary supplements with prescription medication (313). Despite the frequent use of supplements by cancer patients in combination with prescription medications, little information exists on potential beneficial or harmful interactions between most supplements and chemotherapy drugs.

The use of dietary antioxidants as potential adjuvants to chemotherapy has been of recent interest, but remains a controversial issue due to mixed reports of both beneficial and harmful effects (402-404). Antioxidants have been reported to enhance mechanisms of conventional therapy, alleviate toxic side effects, and reduce dosage of chemotherapeutic agents necessary to elicit a response (405, 406). However, antioxidants may interfere with drug activity, cause additional toxicity, or decrease efficacy, particularly if used in combination with chemotherapeutic agents that induce cytotoxicity via oxidative stress (344). Indeed, examples of adverse interactions between dietary antioxidants and conventional therapies have been

reported (407), highlighting the potential hazards of unregulated supplement use, extreme dietary modifications and increased antioxidant intake by patients receiving chemotherapy.

Given the complex nature of the digestive process and plethora of factors that influence absorption, metabolism, and excretion of ingested compounds, investigating dietary constituents as chemopreventive agents or as adjuvants to chemotherapy is challenging. *In vitro* and animals studies can provide valuable preclinical information on effects of dietary agents, but these experimental studies must be carefully designed with clinically relevant parameters. Often, selection of appropriate concentrations in cell culture experiments is overlooked and investigators report biological effects at levels that are not attainable through dietary consumption. For most dietary polyphenols and their metabolites, circulating and tissue levels are commonly observed in the nM range (408), but are commonly tested *in vitro* at μM and mM concentrations.

Additionally, the mode of administration when conducting *in vivo* preclinical studies is a fundamental factor. Many reports have demonstrated efficacy or bioavailability of dietary agents when administered intravenously or intraperitoneally. This can be a justified, perhaps preferred mode of delivery, for studies investigating pharmacological rather than dietary interventions, but oftentimes results may be misleading if investigators do not explicitly describe the intended future clinical purpose which typically is oral administration. The conflicting observations by our group (Chapter VI) compared to those of Zhang *et al* on the effects of Gen in combination with taxanes may attributed to differences in delivery of Gen to animals. Although there were numerous differences between our studies, their group administered Gen by I.P. injection, whereas animals in our study were exposed to Gen through the diet. Therefore, effects of dietary soy intake or isoflavone supplementation on taxane therapy cannot be predicated from their study, as it was not designed to investigate the use of Gen as a dietary intervention strategy.

A number of experimental studies have shown the ability of BRBs to prevent the development of cancer (1), including a rat model of mammary carcinogenesis (213-215). The effectiveness of dietary BRBs in prevention of mammary tumors demonstrates that BRB bioactive compounds can have systemic effects. Therefore, based on previous reports, the failure of BRBs to prevent rat prostate carcinogenesis in our study was surprising, but consistent with our *in vitro* observations. We were able to identify presence of a BRB metabolite, protocatechuic acid (PCA), in the rat prostate at levels between 1 and 10 ng/g following administration of a 5 or 10% BRB diet. When comparable concentrations of PCA were tested *in vitro,* we did not observe inhibition of growth, migration or anchorage independent growth of prostate cancer cells. Even much higher doses of PCA (up to 10 µg/mL) elicited very little response in our assays. As discussed in Chapter III, our results suggest that bioavailability of BRB compounds is low and concentrations of bioactive berry metabolites do not reach sufficiently high levels in the prostate to have an effect.

The two animal models of prostate carcinogenesis used in Chapter III both yield adenocarcinomas, but differ in several respects from each other. The MNU plus testosterone Wistar rat model consists of a single administration of the chemical carcinogen methylnitrosourea (MNU) followed by chronic low dose testosterone administration via slow release implants, which acts as a strong tumor promoter (409). In this model, adenocarcinomas develop mostly in the dorsolateral and anterior prostate lobes, but also appear in the seminal vesicles. These tumors develop in about 80% of animals after about one year, progressively grow and ultimately metastasize (410). The other model uses NBL rats treated with a combination with estradiol and testosterone via slow release implants. 90-100% of the animals in this model develop adenocarcinomas in the periurethral ducts of the dorsolateral and anterior prostate after 40-50 weeks (253, 254). Because these animals also develop large pituitary tumors, they often do not live long enough for the prostatic duct tumors to grow to a large size

and most are only detectable microscopically (254). The MNU plus testosterone model can detect cancer inhibitory compounds (411) and is predictive of the negative outcome of clinical trials with antioxidants (412). The NBL rat model has not been widely used to test cancer prevention, but appears to have a response to inhibitory compounds that is similar to that of the other rat model (413) and also predicted the negative outcome of clinical trials with antioxidants (253).

Concerning bioavailability of BRB compounds to the prostate, there are several questions left unanswered that should be addressed in future studies. First, although we were able to identify dose-dependent concentrations of PCA in the prostates of rats following BRB consumption, we were not able to measure the prostatic levels of other BRB compounds or metabolites of interest. Seeram *et al* reported levels of EA and UroA in the rat prostate that were higher than observed in other tissues (290). However, this was only observed following either a) I.P. injections of pomegranate extract or b) oral administration of isolated UroA, and therefore may not be representative of levels that can be achieved following dietary administration of whole-food products like BRBs.

Uptake of dietary anthocyanins in the gastrointestinal system is thought to occur by active transport of anthocyanin glycosides via sodium-glucose transporters or by passive diffusion of anthocyanidin aglycones following deglycosylation by β-glucosidase or LPH (Figure 29, page 65) (119, 123, 414). The process of anthocyanin absorption and uptake by intestinal cells has been well-characterized, but anthocyanin uptake by cells and tissues that are not typically involved in digestion is mostly unknown. We observed differential uptake of cyanidin-3-rutinoside in comparison to its aglycone by prostate cancer cells, which may explain the absence of inhibitory effects in our studies. While these studies were informative, they did not address the mechanisms of anthocyanin uptake. We speculate that prostate cells may lack appropriate transport proteins or enzymes necessary to facilitate uptake of anthocyanins across

the cell membrane. However, others have shown that prostate tumors express the sodium-glucose transporters that have been implicated in anthocyanin transport in GI tissue (122). Thus, other explanations are probable and further investigation is warranted. Knowledge of tissue levels and uptake of BRB compounds by prostate cells can improve our general understanding of flavonoid bioavailability. This information would not only be valuable for studies future studies involving the prostate gland, but may also be beneficial in evaluating potential dietary chemoprevention agents for other organ sites and would help to guide selection of appropriate concentrations for future *in vitro* studies.

Since beginning the work presented in this dissertation, a phase I clinical trial investigating BRBs for prostate cancer has begun (415). The primary purpose of this study is to assess safety and compliance of BRB confection in prostate cancer patients prior to undergoing prostatectomy, with secondary outcome measures of urinary identification of BRB metabolites, changes in PSA or PSA doubling time, and detection of BRB metabolites in prostate tissue. No reports of secondary outcomes have been published, but the investigators have reported the characterization of their compound and favorable sensory responses by patients (416). Although our *in vivo* results suggest limited bioavailable of BRB compounds and metabolites to the prostate, there is currently no clinical evidence demonstrating this. Therefore forthcoming results from this trial will be invaluable for directing future studies and may provide insight into the bioavailability of dietary polyphenols to the prostate.

Many bioactive dietary compounds display a broad range of cellular effects and therefore their interactions with chemotherapeutic agents is likely very complex and may include alterations in drug pharmacokinetics. Various dietary agents and phytochemicals have been shown to alter xenobiotic metabolism, either through inducing or inhibiting metabolic enzymes, which can have severe clinical consequences (417). Grapefruit juice and St. John's Wort are well-known examples of dietary factors that alter drug metabolism and systemic levels of some

drugs (418, 419). We found that genistein inhibited drug efflux transporters and CYP3A4 activity *in vitro* (Chapters VI). We did not evaluate effects of dietary Gen on metabolic enzymes *in vivo* as part of this study, but liver tissue collected from our xenograft animal studies could be evaluated for CYP3A4 activity and expression in future studies.

Both of our xenograft studies (Chapters V and VI) utilized 22Rv1 cells to generate tumors due their similarities to clinical characteristics of CRPC tumors. Notably, this cell line expresses androgen receptor, but is capable of androgen independent growth (420). 22Rv1 cells also express AR splice variants, which have been clinically implicated as drivers of disease progression and resistance to chemotherapy (112). Previous studies have demonstrated synergistic growth inhibition by combination of Gen with CBZ in xenograft models using PC-3 cells (185). Similarly, it has been reported that an EA-rich pomegranate extract enhanced the cytotoxicity of DX on C4-2 xenograft tumors (421). It is possible that the aggressiveness of 22Rv1 cells render them more resistant than other prostate cancer cell lines to polyphenolic intervention agents. Confirming the results of the current study in future xenograft studies using an alternate CRPC cell line, such as VCaP, would help elucidate any cell-type specific responses to BRB and soy constituents. Additionally, cell line specific outcomes could provide a more broad mechanistic insight on prostate cancer phenotypes and characteristics in relation to treatment response and effectiveness of prevention agents.

In conclusion, throughout this dissertation we attempted to use clinically and physiologically relevant *in vivo* and *in vitro* methods to characterize the effects of BRB and soy constituents for prostate cancer treatment and prevention. We demonstrated that dietary BRBs do not prevent prostate cancer development in rats or enhance taxane chemotherapy, likely because sufficient concentrations of bioactive BRB constituents in the prostate cannot be achieved through dietary consumption (Chapters III and IV). We have shown that EA can induce polymerization of tubulin, a novel mechanism for EA that appears to have minor, but likely inconsequential,

antagonist effect on taxane chemotherapy (Chapter V). And finally, relevant dietary levels of soy isoflavones do not enhance the efficacy of taxane chemotherapy (Chapter VI). In all aspects of this work, bioavailability was highlighted as a critical factor in the translational significance of our observations and these findings have general implications for the limited utility of dietary agents in disease treatment.

APPENDICES

APPENDIX A

UIC Animal Care Committee approval notice #14-079

UIC UNIVERSITY OF ILLINOIS AT CHICAGO

Office of Animal Care and Institutional
Biosafety Committee (OACIB) (M/C 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612

5/20/2015

Maarten C. Bosland
Pathology
M/C 847

Dear Dr. Bosland:

The protocol indicated below was reviewed in accordance with the Animal Care Policies and Procedures of the University of Illinois at Chicago and **renewed on 5/20/2015.**

Title of Application:	**Effects of Dietary Supplements on Chemotherapy for Castrate-resistant Prostate Cancer**
ACC NO:	**14-079**
Original Protocol Approval:	**5/30/2014 (3 year approval with annual continuation required).**
Current Approval Period:	**5/20/2015 to 5/20/2016**

Currently protocol NOT matched to specific funding source. Modification will need to be submitted prior to Just in time or acceptance of award to match protocol to external funding source. All animal work proposed in the funding application must be covered by an approved protocol.

UIC is the only performance site approved for this protocol.

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare, NIH. **This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the grant are matched to this ACC protocol.**

Thank you for complying with the Animal Care Policies and Procedures of the UIC.

Sincerely,

Bradley Merrill, PhD
Chair, Animal Care Committee

BM/*kg*
cc: BRL, ACC File

Phone (312) 996-1972 • Fax (312) 996-9088

APPENDIX A (continued)

UIC Animal Care Committee approval notice #10-065

UIC UNIVERSITY OF ILLINOIS AT CHICAGO

Office of Animal Care and Institutional
Biosafety Committee (OACIB) (M/C 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612

4/20/2012

Maarten C. Bosland
Pathology
M/C 847

Dear Dr. Bosland:

The protocol indicated below was reviewed in accordance with the Animal Care Policies and Procedures of the University of Illinois at Chicago and **renewed on 4/20/2012.**

Title of Application: **Preventive Activity of Black Raspberries Against Prostate Cancer**
ACC NO: **10-065**
Original Protocol Approval: **4/27/2010 (3 year approval with annual continuation required).**
Current Approval Period: **4/20/2012 to 4/20/2013**

Funding: ***Portions of this protocol are supported by the funding sources indicated in the table below.***
Number of funding sources: 1

Funding Agency	**Grant Title**			**Portion of Grant Matched**
NIH	*Preventive Activity Of Black Raspberries Against Prostate Cancer*			*Matched*
Grant Number	**Current Status**	**UIC PAF NO.**	**Performance Site**	**Grant PI**
R21 CA152870	*Funded*	*2010-02027*	*UIC*	*Maarten Bosland*

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare, NIH. **This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the grant are matched to this ACC protocol.**

Thank you for complying with the Animal Care Policies and Procedures of the UIC.

Sincerely,

Bradley Merrill, PhD
Chair, Animal Care Committee

BM/*kg*
cc: BRL, ACC File

Phone (312) 996-1972 • Fax (312) 996-9088

APPENDIX B

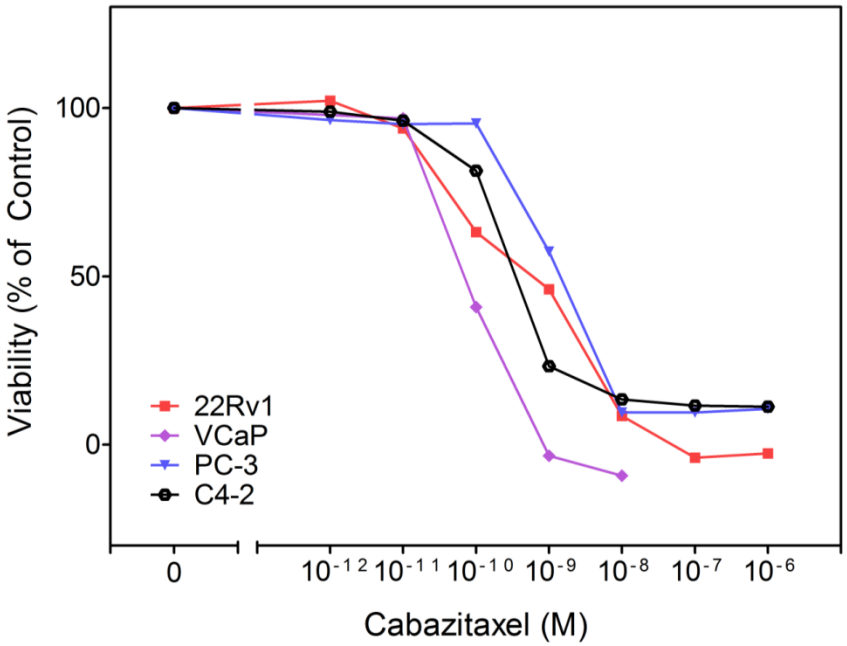
Table VIII. Characteristics of prostate cancer cell lines used in this dissertation

Cell Line	Doubling Time	Site of origin	AR	AR Mutation	Androgen Sensitivity[1]	PSA	Vimentin	Keratin				P53 Mutation
								5	14	8	18	
C4-2	48 hours	LNCaP cell xenograft	+	T877A	AI/S	+	-	-	-	+	+	WT
LAPC-4	72 hours	Lymph node metastases xenograft	+	WT	AD	+	-	+	-	+	+	A175H
LNCaP	60 hours	Lymph node metastases	+	T877A	AD	+	-	-	-	+	+	WT
22Rv1	35-40 hours	CWR22 cell xenograft	+	H874Y	AI/S	- / +	-	-	-	+	+	Q331R
PC-3	33 hours	Bone metastases	-	-	AI	-	+	+	-	+	+	138 frameshift, stop codon 169
VCaP	53 hours	Spinal cord metastases xenograft	+	WT	AI/S	+	-	-	-	+	+	A248W

[1] AD: androgen dependent, androgen are required for growth. AI/S: androgen independent/androgen responsive, androgens are not required for growth but show a growth response in their presence; AI: androgen-independent, do not need androgen to grow nor have their growth affected by androgens. Table references: (388, 422-428)

APPENDIX C

Figure 58. Growth inhibitory effects of cabazitaxel

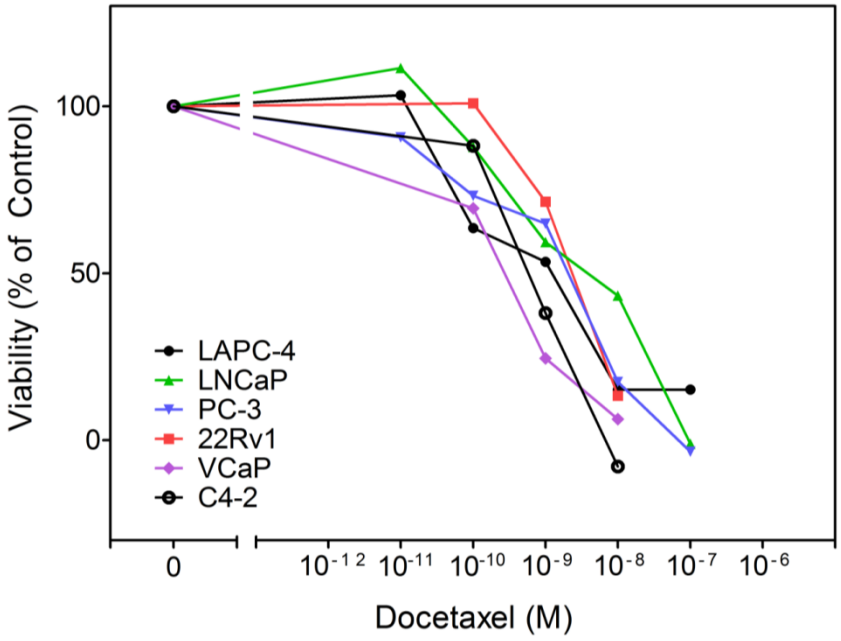


	LAPC-4			22Rv1			LNCaP		
Cabazitaxel (M)	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
0				100.00	0.85	3			
1 x 10^{-12}				102.17	1.10	3			
1 x 10^{-11}				93.97	12.98	3			
1 x 10^{-10}				63.11	4.41	3			
1 x 10^{-9}				46.16	2.68	3			
1 x 10^{-8}				8.53	1.62	3			
1 x 10^{-7}				-3.86	1.21	3			
1 x 10^{-6}				-2.61	0.48	3			

	PC-3			VCaP			C4-2		
Cabazitaxel (M)	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
0	100.00	7.44	3	100.00	2.33	6	100.00	3.71	4
1 x 10^{-12}	96.42	5.67	3				98.90	3.22	4
1 x 10^{-11}	95.21	4.51	3	96.94	3.72	6	96.23	2.86	4
1 x 10^{-10}	95.39	7.47	3	40.86	5.21	6	81.33	2.38	4
1 x 10^{-9}	57.33	1.08	3	-3.33	5.02	6	23.30	0.50	4
1 x 10^{-8}	9.55	2.14	3	-9.21	5.34	6	13.44	1.43	4
1 x 10^{-7}	9.53	8.29	3				11.60	3.35	4
1 x 10^{-6}	10.64	11.10	3				11.26	1.33	4

APPENDIX C (continued)

Figure 59. Growth inhibitory effects of docetaxel



Docetaxel (M)	LAPC-4			22Rv1			LNCaP		
	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
0	100.00	5.92	5	100.00	22.99	4	100.00	12.60	4
1 x 10^{-11}	103.32	5.7	5				111.44	8.01	4
1 x 10^{-10}	63.52	7.03	5	100.85	23.3	4	87.84	11.64	4
1 x 10^{-9}	53.38	6.95	5	71.42	24.16	4	59.35	10.39	4
1 x 10^{-8}	15.11	5.78	5	13.32	10.55	4	43.27	6.67	4
1 x 10^{-7}	15.13	3.92	5				-1.05	9.93	4

Docetaxel (M)	PC-3			VCaP			C4-2		
	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
0	100.00	7.73	4	100.00	22.41	3	100.00	13.14	3
1 x 10^{-11}	90.68	6.39	4						
1 x 10^{-10}	73.19	1.53	4	69.45	13.02	3	88.12	1.77	3
1 x 10^{-9}	64.83	4.42	4	24.54	2.46	3	38.06	8.49	3
1 x 10^{-8}	17.45	2.29	4	6.30	13.74	3	-7.90	1.95	3
1 x 10^{-7}	-3.40	3.13	4						

APPENDIX C (continued)

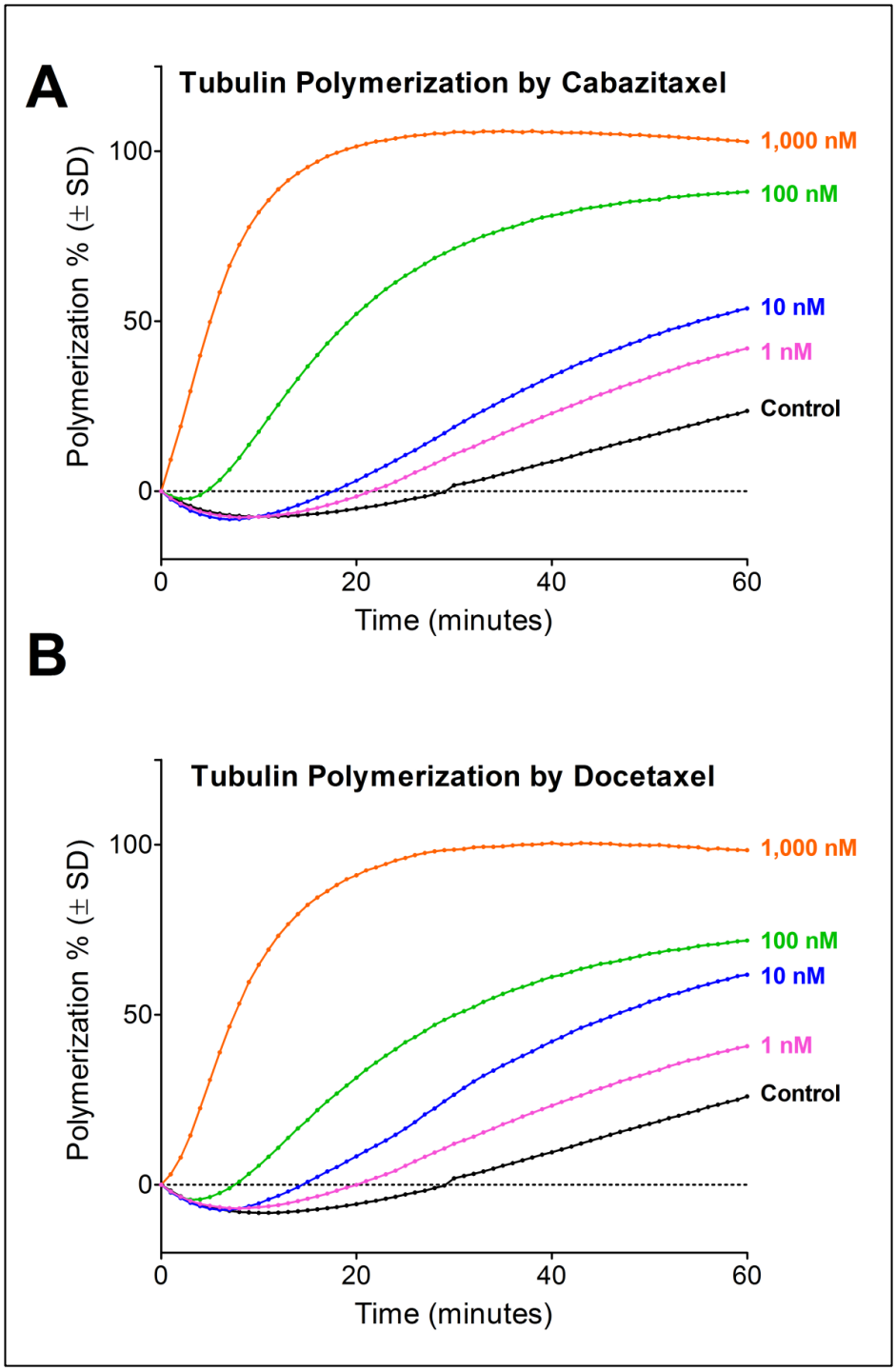


Figure 60. Taxanes dose-dependently induce microtubule assembly *in vitro*. Tubulin polymerization was measured every minute for 1 hour in the presence of cabazitaxel (A) and docetaxel (B).

APPENDIX C (continued)

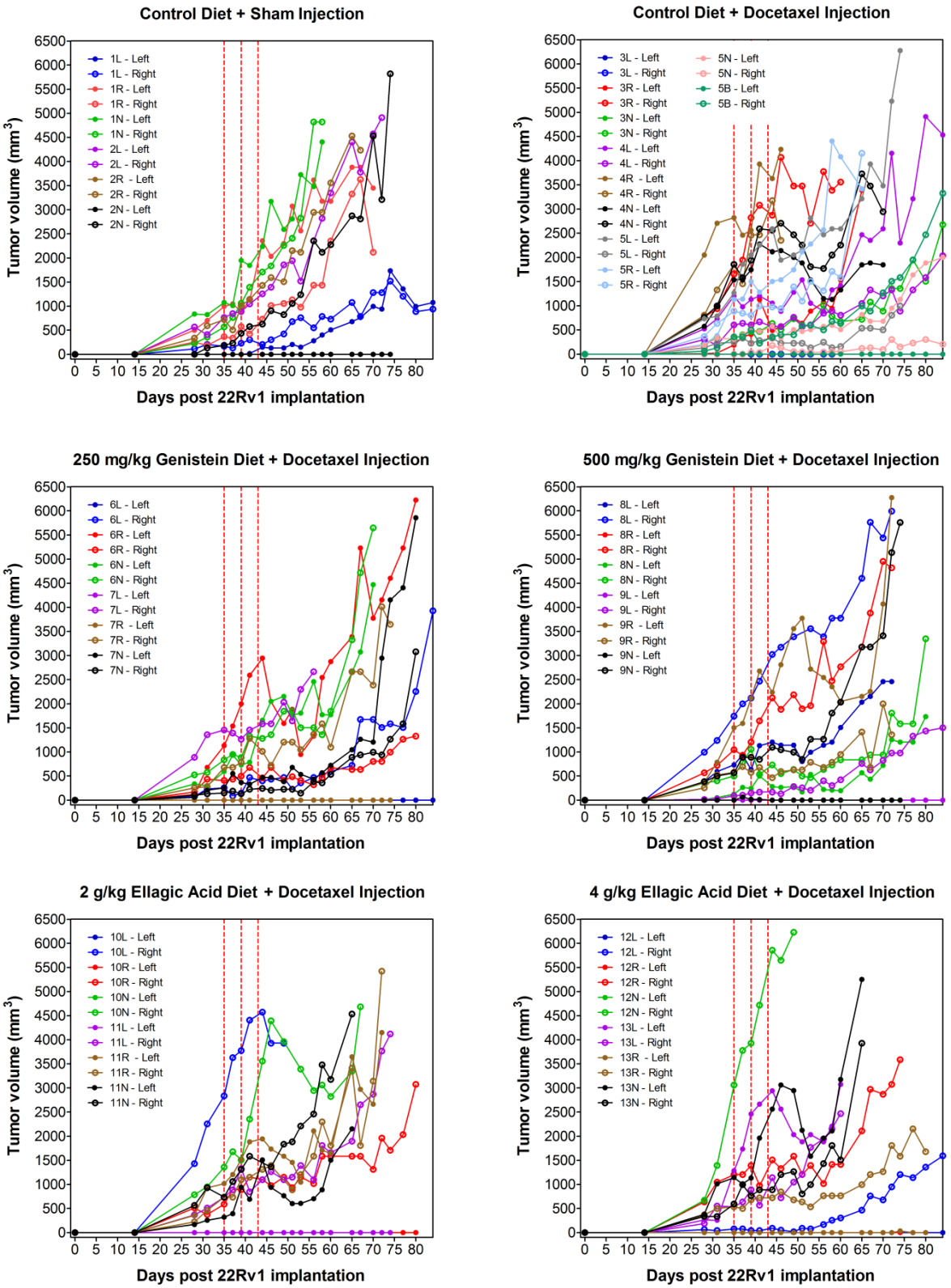


Figure 61. Tumor volume growth of individual 22Rv1 xenograft tumors

APPENDIX C (continued)

Table IX. IC_{50} values from proliferation assays with prostate cancer cells

	LAPC-4	LNCaP	VCaP	C4-2	22Rv1	PC-3
Docetaxel	0.48 nM	14.6 nM	0.24 nM	0.90 nM	1.2 nM	5.54 nM
Cabazitaxel	UND	UND	0.08 nM	0.37 nM	0.44 nM	1.34 nM
Genistein	93.19 µM	NA	128.80 µM	17.66 µM	62.61 µM	NA
BRB extract	NA	NA	NA	NA	NA	NA
Ellagic acid	NA	NA	NA	NA	10.87 µM	9.73 µM
Urolithin A	UND	UND	UND	NA	10.54 µM	24.88 µM
Cyanidin-3-rutinoside	NA	NA	NA	NA	NA	NA
Cyanidin	UND	UND	UND	20.02 µM	20.09 µM	149.00 µM
Protocatechuic acid	NA	NA	NA	NA	NA	NA

NA = Not applicable (cannot determine IC_{50} value; cell proliferation was not inhibited greater than 50%), UND = Undetermined (anti-proliferative effect of compound was not investigated with this cell line)

APPENDIX D

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International Agency for Research on Cancer

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Ref.: CN/75/1

20 July 2016

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1. Stoner GD. (2009) Foodstuffs for Preventing Cancer: The Preclinical and Clinical Development of Berries, *Cancer Prevention Research 2*, 187-194.

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3. Mahmoud AM, Yang W, and Bosland MC. (2014) Soy isoflavones and prostate cancer: a review of molecular mechanisms, *J Steroid Biochem Mol Biol 140*, 116-132.

4. Torre LA, Siegel RL, Ward EM, and Jemal A. (2016) Global Cancer Incidence and Mortality Rates and Trends--An Update, *Cancer Epidemiol Biomarkers Prev 25*, 16-27.

5. Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, and Bray, F. (2013) GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet], Lyon, France: International Agency for Research on Cancer.

6. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, and Bray F. (2012) International variation in prostate cancer incidence and mortality rates, *Eur Urol 61*, 1079-1092.

7. Siegel RL, Miller KD, and Jemal A. (2016) Cancer statistics, 2016, *CA Cancer J Clin 66*, 7-30.

8. Bell KJL, Del Mar C, Wright G, Dickinson J, and Glasziou P. (2015) Prevalence of incidental prostate cancer: A systematic review of autopsy studies, *International Journal of Cancer 137*, 1749-1757.

9. Cuzick J, Thorat MA, Andriole G, Brawley OW, Brown PH, Culig Z, Eeles RA, Ford LG, Hamdy FC, Holmberg L, Ilic D, Key TJ, La Vecchia C, Lilja H, Marberger M, Meyskens FL, Minasian LM, Parker C, Parnes HL, Perner S, Rittenhouse H, Schalken J, Schmid HP, Schmitz-Drager BJ, Schroder FH, Stenzl A, Tombal B, Wilt TJ, and Wolk A. (2014) Prevention and early detection of prostate cancer, *Lancet Oncol 15*, e484-492.

10. Kimura T. (2012) East meets West: ethnic differences in prostate cancer epidemiology between East Asians and Caucasians, *Chin J Cancer 31*, 421-429.

11. Dagnelie PC, Schuurman AG, Goldbohm RA, and Van den Brandt PA. (2004) Diet, anthropometric measures and prostate cancer risk: a review of prospective cohort and intervention studies, *BJU Int 93*, 1139-1150.

12. Alexander DD, Mink PJ, Cushing CA, and Sceurman B. (2010) A review and meta-analysis of prospective studies of red and processed meat intake and prostate cancer, *Nutr J 9*, 50.

13. Golabek T, Bukowczan J, Chlosta P, Powroznik J, Dobruch J, and Borowka A. (2014) Obesity and prostate cancer incidence and mortality: a systematic review of prospective cohort studies, *Urol Int 92*, 7-14.

14. Allott EH, Masko EM, and Freedland SJ. (2013) Obesity and prostate cancer: weighing the evidence, *Eur Urol 63*, 800-809.

15. Sutcliffe S, and Platz EA. (2007) Inflammation in the etiology of prostate cancer: an epidemiologic perspective, *Urol Oncol 25*, 242-249.

16. Bosland MC, and Mahmoud AM. (2011) Hormones and prostate carcinogenesis: Androgens and estrogens, *J Carcinog 10*, 33.

17. Moyer VA. (2012) Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement, *Ann Intern Med 157*, 120-134.

18. McNeal JE. (1988) Normal histology of the prostate, *Am J Surg Pathol 12*, 619-633.

19. Isaacs JT. (1983) Prostatic structure and function in relation to the etiology of prostatic cancer, *Prostate 4*, 351-366.

20. Bonkhoff H, and Remberger K. (1995) Morphogenetic aspects of normal and abnormal prostatic growth, *Pathol Res Pract 191*, 833-835.

21. Harik LR, and O'Toole KM. (2012) Nonneoplastic lesions of the prostate and bladder, *Arch Pathol Lab Med 136*, 721-734.

22. Bushman W. (2009) Etiology, epidemiology, and natural history of benign prostatic hyperplasia, *Urol Clin North Am 36*, 403-415, v.

23. Chang RT, Kirby R, and Challacombe BJ. (2012) Is there a link between BPH and prostate cancer?, *Practitioner 256*, 13-16, 12.

24. De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG, Nakai Y, Isaacs WB, and Nelson WG. (2007) Inflammation in prostate carcinogenesis, *Nat Rev Cancer 7*, 256-269.

25. Bostwick DG. (1989) The pathology of early prostate cancer, *CA: A Cancer Journal for Clinicians 39*, 376-393.

26. Nelson WG, De Marzo AM, and Isaacs WB. (2003) Prostate cancer, *New England Journal of Medicine 349*, 366-381.

27. Haggman MJ, Macoska JA, Wojno KJ, and Oesterling JE. (1997) The relationship between prostatic intraepithelial neoplasia and prostate cancer: critical issues, *J Urol 158*, 12-22.

28. Sakr WA, Haas GP, Cassin BF, Pontes JE, and Crissman JD. (1993) The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients, *Journal of Urology 150*, 379-385.

29. Albertsen PC, Hanley JA, and Fine J. (2005) 20-year outcomes following conservative management of clinically localized prostate cancer, *JAMA 293*, 2095-2101.

30. Lu-Yao GL, Albertsen PC, Moore DF, and et al. (2009) OUtcomes of localized prostate cancer following conservative management, *JAMA 302*, 1202-1209.

31. Johansson J, Andrén O, Andersson S, and et al. (2004) Natural history of early, localized prostate cancer, *JAMA 291*, 2713-2719.

32. Alva A, and Hussain M. (2014) Intermittent Androgen Deprivation Therapy in Advanced Prostate Cancer, *Current Treatment Options in Oncology 15*, 127-136.

33. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, Mason M, Matveev V, Wiegel T, Zattoni F, and Mottet N. (2014) EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer, *Eur Urol 65*, 467-479.

34. Fujimoto N. (2016) Role of the Androgen-Androgen Receptor Axis in the Treatment Resistance of Advanced Prostate Cancer: From Androgen-Dependent to Castration Resistant and Further, *J Uoeh 38*, 129-138.

35. Basch EM, Somerfield MR, Beer TM, Carducci MA, Higano CS, Hussain MHA, and Scher HI. (2007) American Society of Clinical Oncology Endorsement of the Cancer Care Ontario Practice Guideline on Nonhormonal Therapy for Men With Metastatic Hormone-Refractory (castration-resistant) Prostate Cancer, *Journal of Clinical Oncology 25*, 5313-5318.

36. Crawford ED, and Petrylak D. (2010) Castration-resistant prostate cancer: descriptive yet pejorative?, *J Clin Oncol 28*, e408.

37. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, Eisenberger MA, Higano C, Bubley GJ, Dreicer R, Petrylak D, Kantoff P, Basch E, Kelly WK, Figg WD, Small EJ, Beer TM, Wilding G, Martin A, and Hussain M. (2008) Design and End Points of Clinical Trials for Patients With Progressive Prostate Cancer and Castrate Levels of Testosterone: Recommendations of the Prostate Cancer Clinical Trials Working Group, *Journal of Clinical Oncology 26*, 1148-1159.

38. Katzenwadel A, and Wolf P. (2015) Androgen deprivation of prostate cancer: Leading to a therapeutic dead end, *Cancer Letters 367*, 12-17.

39. Harris WP, Mostaghel EA, Nelson PS, and Montgomery B. (2009) Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion, *Nat Clin Pract Urol 6*, 76-85.

40. Mukherji D, Omlin A, Pezaro C, Shamseddine A, and de Bono J. (2014) Metastatic castration-resistant prostate cancer (CRPC): preclinical and clinical evidence for the sequential use of novel therapeutics, *Cancer Metastasis Rev 33*, 555-566.

41. Schweizer MT, and Antonarakis ES. (2014) Chemotherapy and its evolving role in the management of advanced prostate cancer, *Asian J Androl 16*, 334-340.

42. Wu JN, Fish KM, Evans CP, deVere White RW, and Dall'Era MA. (2014) No improvement noted in overall or cause-specific survival for men presenting with metastatic prostate cancer over a 20-year period, *Cancer 120*, 818-823.

43. Huggins C, and Hodges CV. (1941) Studies on Prostatic Cancer. I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate, *Cancer Research 1*, 293-297.

44. Wong YNS, Ferraldeschi R, Attard G, and de Bono J. (2014) Evolution of androgen receptor targeted therapy for advanced prostate cancer, *Nat Rev Clin Oncol 11*, 365-376.

45. Schally AV, Comaru-Schally AM, Plonowski A, Nagy A, Halmos G, and Rekasi Z. (2000) Peptide analogs in the therapy of prostate cancer, *Prostate 45*, 158-166.

46. Rick FG, and Schally AV. (2015) Bench-to-bedside development of agonists and antagonists of luteinizing hormone-releasing hormone for treatment of advanced prostate cancer, *Urol Oncol 33*, 270-274.

47. Liao S, Howell DK, and Chang TM. (1974) Action of a nonsteroidal antiandrogen, flutamide, on the receptor binding and nuclear retention of 5 alpha-dihydrotestosterone in rat ventral prostate, *Endocrinology 94*, 1205-1209.

48. Saad F, and Hotte SJ. (2010) Guidelines for the management of castrate-resistant prostate cancer, *Can Urol Assoc J 4*, 380-384.

49. Cookson MS, Roth BJ, Dahm P, Engstrom C, Freedland SJ, Hussain M, Lin DW, Lowrance WT, Murad MH, Oh WK, Penson DF, and Kibel AS. (2013) Castration-resistant prostate cancer: AUA Guideline, *J Urol 190*, 429-438.

50. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, and Eisenberger MA. (2004) Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer, *N Engl J Med 351*, 1502-1512.

51. Schutz FA, Buzaid AC, and Sartor O. (2014) Taxanes in the management of metastatic castration-resistant prostate cancer: efficacy and management of toxicity, *Crit Rev Oncol Hematol 91*, 248-256.

52. Acar O, Esen T, and Lack NA. (2013) New therapeutics to treat castrate-resistant prostate cancer, *Scientific World Journal 2013*, 379641.

53. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels J-P, Kocak I, Gravis G, Bodrogi I, Mackenzie MJ, Shen L, Roessner M, Gupta S, and Sartor AO. (2010) Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial, *The Lancet 376*, 1147-1154.

54. Geethakumari PR, Cookson MS, and Kelly WK. (2016) The Evolving Biology of Castration-Resistant Prostate Cancer: Review of Recommendations From the Prostate Cancer Clinical Trials Working Group 3, *Oncology 30*, 187-195, 199.

55. Aggarwal BB, and Shishodia S. (2006) Molecular targets of dietary agents for prevention and therapy of cancer, *Biochem Pharmacol 71*, 1397-1421.

56. Patterson RE, Neuhouser ML, Hedderson MM, Schwartz SM, Standish LJ, and Bowen DJ. (2003) Changes in diet, physical activity, and supplement use among adults diagnosed with cancer, *J Am Diet Assoc 103*, 323-328.

57. Satia JA, Walsh JF, and Pruthi RS. (2009) Health behavior changes in white and African American prostate cancer survivors, *Cancer nursing 32*, 107.

58. Ponholzer A, Struhal G, and Madersbacher S. (2003) Frequent use of complementary medicine by prostate cancer patients, *European Urology 43*, 604-608.

59. Saxe GA, Major JM, Nguyen JY, Freeman KM, Downs TM, and Salem CE. (2006) Potential attenuation of disease progression in recurrent prostate cancer with plant-based diet and stress reduction, *Integr Cancer Ther 5*, 206-213.

60. Frattaroli J, Weidner G, Dnistrian AM, Kemp C, Daubenmier JJ, Marlin RO, Crutchfield L, Yglecias L, Carroll PR, and Ornish D. (2008) Clinical events in prostate cancer lifestyle trial: results from two years of follow-up, *Urology 72*, 1319-1323.

61. Ornish D, Magbanua MJM, Weidner G, Weinberg V, Kemp C, Green C, Mattie MD, Marlin R, Simko J, Shinohara K, Haqq CM, and Carroll PR. (2008) Changes in prostate gene expression in men undergoing an intensive nutrition and lifestyle intervention, *Proceedings of the National Academy of Sciences 105*, 8369-8374.

62. Yang M, Kenfield SA, Van Blarigan EL, Batista JL, Sesso HD, Ma J, Stampfer MJ, and Chavarro JE. (2015) Dietary patterns after prostate cancer diagnosis in relation to disease-specific and total mortality, *Cancer Prev Res (Phila) 8*, 545-551.

63. Dumontet C, and Jordan MA. (2010) Microtubule-binding agents: a dynamic field of cancer therapeutics, *Nat Rev Drug Discov 9*, 790-803.

64. Weaver BA. (2014) How Taxol/paclitaxel kills cancer cells, *Mol Biol Cell 25*, 2677-2681.

65. Wani MC, Taylor HL, Wall ME, Coggon P, and McPhail AT. (1971) Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia, *J Am Chem Soc 93*, 2325-2327.

66. Fuchs DA, and Johnson RK. (1978) Cytologic evidence that taxol, an antineoplastic agent from Taxus brevifolia, acts as a mitotic spindle poison, *Cancer Treat Rep 62*, 1219-1222.

67. Walsh V, and Goodman J. (2002) The billion dollar molecule: Taxol in historical and theoretical perspective, *Clio Medica 66*, 245-267.

68. Mollinedo F, and Gajate C. (2003) Microtubules, microtubule-interfering agents and apoptosis, *Apoptosis 8*, 413-450.

69. Appendino G. (1995) Naturally occurring taxoids, In *Pharmacochemistry Library* (Vittorio F, Ed.), pp 7-53, Elsevier.

70. Honore S, Pasquier E, and Braguer D. (2005) Understanding microtubule dynamics for improved cancer therapy, *Cellular and Molecular Life Sciences CMLS 62*, 3039-3056.

71. Downing KH, and Nogales E. (1998) Tubulin structure: insights into microtubule properties and functions, *Current opinion in structural biology 8*, 785-791.

72. Yvon AM, Wadsworth P, and Jordan MA. (1999) Taxol suppresses dynamics of individual microtubules in living human tumor cells, *Mol Biol Cell 10*, 947-959.

73. Jordan MA, and Wilson L. (2004) Microtubules as a target for anticancer drugs, *Nat Rev Cancer 4*, 253-265.

74. Derry WB, Wilson L, and Jordan MA. (1995) Substoichiometric binding of taxol suppresses microtubule dynamics, *Biochemistry 34*, 2203-2211.

75. Nogales E, Wolf SG, Khan IA, Luduena RF, and Downing KA. (1995) Structure of tubulin at 6.5A and location of the taxol-binding site, *Nature 375*, 424-427.

76. Musacchio A, and Salmon ED. (2007) The spindle-assembly checkpoint in space and time, *Nat Rev Mol Cell Biol 8*, 379-393.

77. Tao W. (2005) The mitotic checkpoint in cancer therapy, *Cell Cycle 4*, 1495-1499.

78. Jordan MA. (1996) Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death, *Cancer Res. 56*, 816-825.

79. Weaver BAA, and Cleveland DW. (2005) Decoding the links between mitosis, cancer, and chemotherapy: The mitotic checkpoint, adaptation, and cell death, *Cancer Cell 8*, 7-12.

80. Jordan MA, Toso RJ, Thrower D, and Wilson L. (1993) Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations, *Proc Natl Acad Sci U S A 90*, 9552-9556.

81. Cheetham P, and Petrylak DP. (2013) Tubulin-targeted agents including docetaxel and cabazitaxel, *Cancer J 19*, 59-65.

82. Kuroda K, Liu H, Kim S, Guo M, Navarro V, and Bander NH. (2009) Docetaxel down-regulates the expression of androgen receptor and prostate-specific antigen but not prostate-specific membrane antigen in prostate cancer cell lines: implications for PSA surrogacy, *Prostate 69*, 1579-1585.

83. Mistry SJ, and Oh WK. (2013) New paradigms in microtubule-mediated endocrine signaling in prostate cancer, *Mol Cancer Ther 12*, 555-566.

84. Gan L, Chen S, Wang Y, Watahiki A, Bohrer L, Sun Z, Wang Y, and Huang H. (2009) Inhibition of the androgen receptor as a novel mechanism of taxol chemotherapy in prostate cancer, *Cancer Res 69*, 8386-8394.

85. Haldar S, Chintapalli J, and Croce CM. (1996) Taxol induces bcl-2 phosphorylation and death of prostate cancer cells, *Cancer Res 56*, 1253-1255.

86. Hernández-Vargas H, Palacios J, and Moreno-Bueno G. (2007) Telling Cells How to Die: Docetaxel Therapy in Cancer Cell Lines, *Cell Cycle 6*, 780-783.

87. Jordan MA, Toso RJ, Thrower D, and Wilson L. (1993) Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations, *Proc. Natl Acad. Sci. USA 90*, 9552-9556.

88. Chen JG, and Horwitz SB. (2002) Differential mitotic responses to microtubule-stabilizing and -destabilizing drugs, *Cancer Res 62*, 1935-1938.

89. Hornick JE, Bader JR, Tribble EK, Trimble K, Breunig JS, Halpin ES, Vaughan KT, and Hinchcliffe EH. (2008) Live-cell analysis of mitotic spindle formation in taxol-treated cells, *Cell Motil Cytoskeleton 65*, 595-613.

90. Azarenko O, Smiyun G, Mah J, Wilson L, and Jordan MA. (2014) Antiproliferative Mechanism of Action of the Novel Taxane Cabazitaxel as Compared with the Parent Compound Docetaxel in MCF7 Breast Cancer Cells, *American Association for Cancer Research 13*, 2092-2103.

91. Ogden A, Rida PC, Reid MD, and Aneja R. (2014) Interphase microtubules: chief casualties in the war on cancer?, *Drug Discov Today 19*, 824-829.

92. Park SB, Lin CSY, Krishnan AV, Friedlander ML, Lewis CR, and Kiernan MC. (2011) Early, progressive, and sustained dysfunction of sensory axons underlies paclitaxel-induced neuropathy, *Muscle & Nerve 43*, 367-374.

93. Gornstein E, and Schwarz TL. (2014) The paradox of paclitaxel neurotoxicity: Mechanisms and unanswered questions, *Neuropharmacology 76, Part A*, 175-183.

94. Field JJ, Kanakkanthara A, and Miller JH. (2014) Microtubule-targeting agents are clinically successful due to both mitotic and interphase impairment of microtubule function, *Bioorganic & Medicinal Chemistry 22*, 5050-5059.

95. Zhu ML, Horbinski CM, Garzotto M, Qian DZ, Beer TM, and Kyprianou N. (2010) Tubulin-targeting chemotherapy impairs androgen receptor activity in prostate cancer, *Cancer Res 70*, 7992-8002.

96. Darshan MS, Loftus MS, Thadani-Mulero M, Levy BP, Escuin D, Zhou XK, Gjyrezi A, Chanel-Vos C, Shen R, Tagawa ST, Bander NH, Nanus DM, and Giannakakou P. (2011) Taxane-induced blockade to nuclear accumulation of the androgen receptor predicts clinical responses in metastatic prostate cancer, *Cancer Res 71*, 6019-6029.

97. Mackler NJ, and Pienta KJ. (2005) Drug insight: Use of docetaxel in prostate and urothelial cancers, *Nat Clin Pract Urol 2*, 92-100.

98. Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, and Green DR. (2010) The BCL-2 family reunion, *Molecular cell 37*, 299-310.

99. Youle RJ, and Strasser A. (2008) The BCL-2 protein family: opposing activities that mediate cell death, *Nat Rev Mol Cell Biol 9*, 47-59.

100. Seruga B, Ocana A, and Tannock IF. (2011) Drug resistance in metastatic castration-resistant prostate cancer, *Nat Rev Clin Oncol 8*, 12-23.

101. Derry WB, Wilson L, Khan IA, Luduena RF, and Jordan MA. (1997) Taxol differentially modulates the dynamics of microtubules assembled from unfractionated and purified beta-tubulin isotypes, *Biochemistry 36*, 3554-3562.

102. Panda D, Miller HP, Banerjee A, Luduena RF, and Wilson L. (1994) Microtubule dynamics in vitro are regulated by the tubulin isotype composition, *Proc Natl Acad Sci U S A 91*, 11358-11362.

103. Xiao H, Verdier-Pinard P, Fernandez-Fuentes N, Burd B, Angeletti R, Fiser A, Horwitz SB, and Orr GA. (2006) Insights into the mechanism of microtubule stabilization by Taxol, *Proceedings of the National Academy of Sciences 103*, 10166-10173.

104. Mozzetti S, Ferlini C, Concolino P, Filippetti F, Raspaglio G, Prislei S, Gallo D, Martinelli E, Ranelletti FO, Ferrandina G, and Scambia G. (2005) Class III beta-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients, *Clin Cancer Res 11*, 298-305.

105. Sanchez C, Mendoza P, Contreras HR, Vergara J, McCubrey JA, Huidobro C, and Castellon EA. (2009) Expression of multidrug resistance proteins in prostate cancer is related with cell sensitivity to chemotherapeutic drugs, *Prostate 69*, 1448-1459.

106. O'Neill AJ, Prencipe M, Dowling C, Fan Y, Mulrane L, Gallagher WM, O'Connor D, O'Connor R, Devery A, Corcoran C, Rani S, O'Driscoll L, Fitzpatrick JM, and Watson RW. (2011) Characterisation and manipulation of docetaxel resistant prostate cancer cell lines, *Mol Cancer 10*, 126.

107. Gottesman MM, Fojo T, and Bates SE. (2002) Multidrug resistance in cancer: role of ATP-dependent transporters, *Nat. Rev. Cancer 2*, 48-58.

108. Bhalla KN. (2003) Microtubule-targeted anticancer agents and apoptosis, *Oncogene 22*, 9075-9086.

109. Tamaki H, Harashima N, Hiraki M, Arichi N, Nishimura N, Shiina H, Naora K, and Harada M. (2014) Bcl-2 family inhibition sensitizes human prostate cancer cells to docetaxel and promotes unexpected apoptosis under caspase-9 inhibition, *Oncotarget 5*, 11399-11412.

110. Thadani-Mulero M, Portella L, Sun S, Sung M, Matov A, Vessella RL, Corey E, Nanus DM, Plymate SR, and Giannakakou P. (2014) Androgen receptor splice variants determine taxane sensitivity in prostate cancer, *Cancer Res 74*, 2270-2282.

111. Onstenk W, Sieuwerts AM, Kraan J, Van M, Nieuweboer AJ, Mathijssen RH, Hamberg P, Meulenbeld HJ, De Laere B, Dirix LY, van Soest RJ, Lolkema MP, Martens JW, van Weerden WM, Jenster GW, Foekens JA, de Wit R, and Sleijfer S. (2015) Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells, *Eur Urol 68*, 939-945.

112. Antonarakis ES, Lu C, Luber B, Wang H, Chen Y, Nakazawa M, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, and Luo J. (2015) Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer, *JAMA Oncol 1*, 582-591.

113. Zhang G, Liu X, Li J, Ledet E, Alvarez X, Qi Y, Fu X, Sartor O, Dong Y, and Zhang H. (2015) Androgen receptor splice variants circumvent AR blockade by microtubule-targeting agents, *Oncotarget 6*, 23358-23371.

114. Berrieman HK, Lind MJ, and Cawkwell L. (2004) Do beta-tubulin mutations have a role in resistance to chemotherapy?, *Lancet Oncol. 5*, 158-164.

115. Seve P, and Dumontet C. (2008) Is class III beta-tubulin a predictive factor in patients receiving tubulin-binding agents?, *Lancet Oncol. 9*, 168-175.

116. Verrills NM. (2006) Alterations in gamma-actin and tubulin-targeted drug resistance in childhood leukemia, *J. Natl Cancer Inst. 98*, 1363-1374.

117. Cimino S, Sortino G, Favilla V, Castelli T, Madonia M, Sansalone S, Russo GI, and Morgia G. (2012) Polyphenols: Key Issues Involved in Chemoprevention of Prostate Cancer, *Oxidative Medicine and Cellular Longevity 2012*, 632959.

118. D'Archivio M, Filesi C, Vari R, Scazzocchio B, and Masella R. (2010) Bioavailability of the polyphenols: status and controversies, *Int J Mol Sci 11*, 1321-1342.

119. Nemeth K, Plumb GW, Berrin JG, Juge N, Jacob R, Naim HY, Williamson G, Swallow DM, and Kroon PA. (2003) Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans, *Eur J Nutr 42*, 29-42.

120. Day AJ, Cañada FJ, Díaz JC, Kroon PA, McLauchlan R, Faulds CB, Plumb GW, Morgan MRA, and Williamson G. (2000) Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase, *FEBS Letters 468*, 166-170.

121. Faria A, Pestana D, Azevedo J, Martel F, de Freitas V, Azevedo I, Mateus N, and Calhau C. (2009) Absorption of anthocyanins through intestinal epithelial cells - Putative involvement of GLUT2, *Mol Nutr Food Res 53*, 1430-1437.

122. Scafoglio C, Hirayama BA, Kepe V, Liu J, Ghezzi C, Satyamurthy N, Moatamed NA, Huang J, Koepsell H, Barrio JR, and Wright EM. (2015) Functional expression of sodium-glucose transporters in cancer, *Proc Natl Acad Sci 112*, E4111-4119.

123. Zou TB, Feng D, Song G, Li HW, Tang HW, and Ling WH. (2014) The role of sodium-dependent glucose transporter 1 and glucose transporter 2 in the absorption of cyanidin-3-o-beta-glucoside in Caco-2 cells, *Nutrients 6*, 4165-4177.

124. Forman D, Bray, F., Brewster, D.H., Gombe Mbalawa, C., Kohler, B., Piñeros, M., Steliarova-Foucher, E., Swaminathan, R (2014) *Cancer Incidence in Five Continents. Vol X*.

125. Narod S. (1999) Genetic epidemiology of prostate cancer, *Biochim Biophys Acta 1423*, F1-13.

126. Shibata A, and Whittemore AS. (1997) Genetic predisposition to prostate cancer: Possible explanations for ethnic differences in risk, *The Prostate 32*, 65-72.

127. Minamoto T, Mai M, and Ronai Ze. (1999) Environmental factors as regulators and effectors of multistep carcinogenesis, *Carcinogenesis 20*, 519-527.

128. Watanabe M, Nakayama T, Shiraishi T, Stemmermann GN, and Yatani R. (2000) Comparative studies of prostate cancer in Japan versus the United States: A review, *Urologic Oncology: Seminars and Original Investigations 5*, 274-283.

129. Hwang YW, Kim SY, Jee SH, Kim YN, and Nam CM. (2009) Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies, *Nutr Cancer 61*, 598-606.

130. Yan L, and Spitznagel EL. (2009) Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis, *Am J Clin Nutr 89*, 1155-1163.

131. Setchell KD, and Cole SJ. (2003) Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling, *J Agric Food Chem 51*, 4146-4155.

132. Messina M, Nagata C, and Wu AH. (2006) Estimated Asian adult soy protein and isoflavone intakes, *Nutr Cancer 55*, 1-12.

133. Travis RC, Allen NE, Appleby PN, Price A, Kaaks R, Chang-Claude J, Boeing H, Aleksandrova K, Tjønneland A, Johnsen NF, Overvad K, Ramón Quirós J, González CA, Molina-Montes E, Sánchez MJ, Larrañaga N, Castaño JM, Ardanaz E, Khaw KT, Wareham N, Trichopoulou A, Karapetyan T, Rafnsson SB, Palli D, Krogh V, Tumino R, Vineis P, Bueno-de-Mesquita HB, Stattin P, and Johansson M. (2012) Prediagnostic concentrations of plasma genistein and prostate cancer risk in 1,605 men with prostate cancer and 1,697 matched control participants in EPIC, *Cancer Causes Control 23*.

134. Morton MS, Arisaka O, Miyake N, Morgan LD, and Evans BA. (2002) Phytoestrogen concentrations in serum from Japanese men and women over forty years of age, *J Nutr 132*, 3168-3171.

135. Adlercreutz H, Markkanen H, and Watanabe S. (1993) Plasma concentrations of phyto-oestrogens in Japanese men, *Lancet 342*, 1209-1210.

136. Ozasa K, Nakao M, Watanabe Y, Hayashi K, Miki T, Mikami K, Mori M, Sakauchi F, Washio M, Ito Y, Suzuki K, Wakai K, and Tamakoshi A. (2004) Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men, *Cancer Sci 95*, 65-71.

137. Wu Y, Zhang L, Na R, Xu J, Xiong Z, Zhang N, Dai W, Jiang H, and Ding Q. (2015) Plasma genistein and risk of prostate cancer in Chinese population, *Int Urol Nephrol 47*, 965-970.

138. Marks LS, Kojima M, Demarzo A, Heber D, Bostwick DG, Qian J, Dorey FJ, Veltri RW, Mohler JL, and Partin AW. (2004) Prostate cancer in native Japanese and Japanese-American men: Effects of dietary differences on prostatic tissue, *Urology 64*, 765-771.

139. Whittemore AS, Wu AH, Kolonel LN, John EM, Gallagher RP, Howe GR, West DW, Teh CZ, and Stamey T. (1995) Family history and prostate cancer risk in black, white, and Asian men in the United States and Canada, *Am J Epidemiol 141*, 732-740.

140. Cook LS, Goldoft M, Schwartz SM, and Weiss NS. (1999) Incidence of Adenocarcinoma of the Prostate in Asian Immigrants to the United States, *The Journal of Urology 161*, 152-155.

141. Gardner CD, Oelrich B, Liu JP, Feldman D, Franke AA, and Brooks JD. (2009) Prostatic soy isoflavone concentrations exceed serum levels after dietary supplementation, *The Prostate 69*, 719-726.

142. Ornish D, Weidner G, Fair WR, Marlin R, Pettengill EB, Raisin CJ, Dunn-Emke S, Crutchfield L, Jacobs FN, Barnard RJ, Aronson WJ, McCormac P, McKnight DJ, Fein JD, Dnistrian AM, Weinstein J, Ngo TH, Mendell NR, and Carroll PR. (2005) Intensive Lifestyle Changes May Affect the Progression of Prostate Cancer, *The Journal of Urology 174*, 1065-1070.

143. Dalais FS, Meliala A, Wattanapenpaiboon N, Frydenberg M, Suter DAI, Thomson WK, and Wahlqvist ML. (2004) Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer, *Urology 64*, 510-515.

144. Lazarevic B, Hammarström C, Yang J, Ramberg H, Diep LM, Karlsen SJ, Kucuk O, Saatcioglu F, Taskèn KA, and Svindland A. (2012) The effects of short-term genistein intervention on prostate biomarker expression in patients with localised prostate cancer before radical prostatectomy, *Br J Nutr 108*.

145. deVere White RW, Tsodikov A, Stapp EC, Soares SE, Fujii H, and Hackman RM. (2010) Effects of a high dose, aglycone-rich soy extract on prostate-specific antigen and serum isoflavone concentrations in men with localized prostate cancer, *Nutr Cancer 62*, 1036-1043.

146. Hamilton-Reeves JM, Rebello SA, Thomas W, Kurzer MS, and Slaton JW. (2008) Effects of soy protein isolate consumption on prostate cancer biomarkers in men with HGPIN, ASAP, and low-grade prostate cancer, *Nutr Cancer 60*, 7-13.

147. Hamilton-Reeves JM, Banerjee S, Banerjee SK, Holzbeierlein JM, Thrasher JB, Kambhampati S, Keighley J, and Veldhuizen P. (2013) Short-term soy isoflavone intervention in patients with localized prostate cancer: a randomized, double-blind, placebo-controlled trial, *PLoS One 8*.

148. Bosland MC, Kato I, Zeleniuch-Jacquotte A, Schmoll J, Enk-Rueter E, Melamed J, Kong M, Macias V, Balla A, Lumey L, Xie H, Gao W, Walden P, Lepor H, Taneja S, Randolph C, Schlicht M, Meserve-Watanabe H, Deaton R, and Davies J. (2013) Effect of soy protein isolate supplementation on biochemical recurrence of prostate cancer after radical prostatectomy: A randomized trial, *JAMA 310*, 170-178.

149. van Die MD, Bone KM, Williams SG, and Pirotta MV. (2014) Soy and soy isoflavones in prostate cancer: a systematic review and meta-analysis of randomized controlled trials, *BJU Int 113*, E119-130.

150. Chun J, Kim GM, Lee KW, Choi ID, Kwon GH, Park JY, Jeong SJ, Kim JS, and Kim JH. (2007) Conversion of isoflavone glucosides to aglycones in soymilk by fermentation with lactic acid bacteria, *J Food Sci 72*, M39-44.

151. Chang TS, Ding HY, Tai SS, and Wu CY. (2007) Metabolism of the soy isoflavones daidzein and genistein by fungi used in the preparation of various fermented soybean foods, *Biosci Biotechnol Biochem 71*, 1330-1333.

152. Zaheer K, and Akhtar MH. (2015) An Updated Review of Dietary Isoflavones: Nutrition, Processing, Bioavailability and Impacts on Human Health, *Crit Rev Food Sci Nutr*, 0.

153. Yuan JP, Wang JH, and Liu X. (2007) Metabolism of dietary soy isoflavones to equol by human intestinal microflora--implications for health, *Mol Nutr Food Res 51*, 765-781.

154. Setchell KD, and Clerici C. (2010) Equol: pharmacokinetics and biological actions, *J Nutr 140*, 1363s-1368s.

155. Rowland I, Faughnan M, Hoey L, Wahala K, Williamson G, and Cassidy A. (2003) Bioavailability of phyto-oestrogens, *Br J Nutr 89 Suppl 1*, S45-58.

156. An J, Tzagarakis-Foster C, Scharschmidt TC, Lomri N, and Leitman DC. (2001) Estrogen receptor beta-selective transcriptional activity and recruitment of coregulators by phytoestrogens, *J Biol Chem 276*, 17808-17814.

157. Jiang Y, Gong P, Madak-Erdogan Z, Martin T, Jeyakumar M, Carlson K, Khan I, Smillie TJ, Chittiboyina AG, Rotte SC, Helferich WG, Katzenellenbogen JA, and Katzenellenbogen BS. (2013) Mechanisms enforcing the estrogen receptor beta selectivity of botanical estrogens, *Faseb j 27*, 4406-4418.

158. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, and Fukami Y. (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases, *J Biol Chem 262*, 5592-5595.

159. Russo M, Russo GL, Daglia M, Kasi PD, Ravi S, Nabavi SF, and Nabavi SM. (2016) Understanding genistein in cancer: The “good” and the “bad” effects: A review, *Food Chemistry 196*, 589-600.

160. Kazi A, Daniel KG, Smith DM, Kumar NB, and Dou QP. (2003) Inhibition of the proteasome activity, a novel mechanism associated with the tumor cell apoptosis-inducing ability of genistein, *Biochem Pharmacol 66*, 965-976.

161. Li Y, Bhuiyan M, and Sarkar FH. (1999) Induction of apoptosis and inhibition of c-erbB-2 in MDA-MB-435 cells by genistein, *Int J Oncol 15*, 525-533.

162. Lian F, Bhuiyan M, Li YW, Wall N, Kraut M, and Sarkar FH. (1998) Genistein-induced G2-M arrest, p21WAF1 upregulation, and apoptosis in a non-small-cell lung cancer cell line, *Nutr Cancer 31*, 184-191.

163. Nakamura Y, Yogosawa S, Izutani Y, Watanabe H, Otsuji E, and Sakai T. (2009) A combination of indol-3-carbinol and genistein synergistically induces apoptosis in human colon cancer HT-29 cells by inhibiting Akt phosphorylation and progression of autophagy, *Mol Cancer 8*, 100.

164. Ambra R, Rimbach G, de Pascual Teresa S, Fuchs D, Wenzel U, Daniel H, and Virgili F. (2006) Genistein affects the expression of genes involved in blood pressure regulation and angiogenesis in primary human endothelial cells, *Nutr Metab Cardiovasc Dis 16*, 35-43.

165. Pugalendhi P, Manoharan S, Panjamurthy K, Balakrishnan S, and Nirmal MR. (2009) Antigenotoxic effect of genistein against 7,12-dimethylbenz[a]anthracene induced genotoxicity in bone marrow cells of female Wistar rats, *Pharmacol Rep 61*, 296-303.

166. Foti P, Erba D, Riso P, Spadafranca A, Criscuoli F, and Testolin G. (2005) Comparison between daidzein and genistein antioxidant activity in primary and cancer lymphocytes, *Arch Biochem Biophys 433*, 421-427.

167. Xiao X, Liu Z, Wang R, Wang J, Zhang S, Cai X, Wu K, Bergan RC, Xu L, and Fan D. (2015) Genistein suppresses FLT4 and inhibits human colorectal cancer metastasis, *Oncotarget 6*, 3225-3239.

168. Zhang LL, Li L, Wu DP, Fan JH, Li X, Wu KJ, Wang XY, and He DL. (2008) A novel anti-cancer effect of genistein: reversal of epithelial mesenchymal transition in prostate cancer cells, *Acta Pharmacol Sin 29*, 1060-1068.

169. Zhou JR, Yu L, Zhong Y, Nassr RL, Franke AA, Gaston SM, and Blackburn GL. (2002) Inhibition of orthotopic growth and metastasis of androgen-sensitive human prostate tumors in mice by bioactive soybean components, *Prostate 53*, 143-153.

170. Miekus K, and Madeja Z. (2007) Genistein inhibits the contact-stimulated migration of prostate cancer cells, *Cell Mol Biol Lett 12*, 348-361.

171. Kumi-Diaka JK, Hassanhi M, Merchant K, and Horman V. (2006) Influence of genistein isoflavone on matrix metalloproteinase-2 expression in prostate cancer cells, *J Med Food 9*, 491-497.

172. Lamartiniere CA, Cotroneo MS, Fritz WA, Wang J, Mentor-Marcel R, and Elgavish A. (2002) Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate, *J Nutr 132*, 552s-558s.

173. Davis JN, Kucuk O, and Sarkar FH. (2002) Expression of prostate-specific antigen is transcriptionally regulated by genistein in prostate cancer cells, *Mol Carcinog 34*, 91-101.

174. Shenouda NS, Zhou C, Browning JD, Ansell PJ, Sakla MS, Lubahn DB, and Macdonald RS. (2004) Phytoestrogens in common herbs regulate prostate cancer cell growth in vitro, *Nutr Cancer 49*, 200-208.

175. Hsieh T-C, and Wu JM. (2009) Targeting CWR22Rv1 Prostate Cancer Cell Proliferation and Gene Expression by Combinations of the Phytochemicals EGCG, Genistein and Quercetin, *Anticancer Res 29*, 4025-4032.

176. Shen JC, Klein RD, Wei Q, Guan Y, Contois JH, Wang TT, Chang S, and Hursting SD. (2000) Low-dose genistein induces cyclin-dependent kinase inhibitors and G(1) cell-cycle arrest in human prostate cancer cells, *Mol Carcinog 29*, 92-102.

177. Wang BF, Wang JS, Lu JF, Kao TH, and Chen BH. (2009) Antiproliferation effect and mechanism of prostate cancer cell lines as affected by isoflavones from soybean cake, *J Agric Food Chem 57*, 2221-2232.

178. Davis JN, Kucuk O, and Sarkar FH. (1999) Genistein inhibits NF-kappa B activation in prostate cancer cells, *Nutr Cancer 35*, 167-174.

179. Kumi-Diaka J, Sanderson NA, and Hall A. (2000) The mediating role of caspase-3 protease in the intracellular mechanism of genistein-induced apoptosis in human prostatic carcinoma cell lines, DU145 and LNCaP, *Biol Cell 92*, 595-604.

180. Davis JN, Muqim N, Bhuiyan M, Kucuk O, Pienta KJ, and Sarkar FH. (2000) Inhibition of prostate specific antigen expression by genistein in prostate cancer cells, *Int J Oncol 16*, 1091-1097.

181. El Touny LH, and Banerjee PP. (2009) Identification of a biphasic role for genistein in the regulation of prostate cancer growth and metastasis, *Cancer Res 69*, 3695-3703.

182. Wang J, Eltoum IE, and Lamartiniere CA. (2007) Genistein chemoprevention of prostate cancer in TRAMP mice, *Journal of Carcinogenesis 6*, 3.

183. Li Y, Hong X, Hussain M, Sarkar SH, Li R, and Sarkar FH. (2005) Gene expression profiling revealed novel molecular targets of docetaxel and estramustine combination treatment in prostate cancer cells, *Mol Cancer Ther 4*, 389-398.

184. Li Y, Kucuk O, Hussain M, Abrams J, Cher ML, and Sarkar FH. (2006) Antitumor and antimetastatic activities of docetaxel are enhanced by genistein through regulation of osteoprotegerin/receptor activator of nuclear factor-kappaB (RANK)/RANK ligand/MMP-9 signaling in prostate cancer, *Cancer Res 66*, 4816-4825.

185. Zhang S, Wang Y, Chen Z, Kim S, Iqbal S, Chi A, Ritenour C, Wang YA, Kucuk O, and Wu D. (2013) Genistein enhances the efficacy of cabazitaxel chemotherapy in metastatic castration-resistant prostate cancer cells, *Prostate*.

186. Burich RA, Holland WS, Vinall RL, Tepper C, White RW, and Mack PC. (2008) Genistein combined polysaccharide enhances activity of docetaxel, bicalutamide and Src kinase inhibition in androgen-dependent and independent prostate cancer cell lines, *BJU Int 102*, 1458-1466.

187. Ahmed AA, Goldsmith J, Fokt I, Le XF, Krzysko KA, Lesyng B, Bast RC, Jr., and Priebe W. (2011) A genistein derivative, ITB-301, induces microtubule depolymerization and mitotic arrest in multidrug-resistant ovarian cancer, *Cancer Chemother Pharmacol 68*, 1033-1044.

188. Mukherjee S, Acharya BR, Bhattacharyya B, and Chakrabarti G. (2010) Genistein arrests cell cycle progression of A549 cells at the G(2)/M phase and depolymerizes interphase microtubules through binding to a unique site of tubulin, *Biochemistry 49*, 1702-1712.

189. Liao CH, Pan SL, Guh JH, and Teng CM. (2004) Genistein inversely affects tubulin-binding agent-induced apoptosis in human breast cancer cells, *Biochem Pharmacol 67*, 2031-2038.

190. Rusin A, Gogler A, Glowala-Kosinska M, Bochenek D, Gruca A, Grynkiewicz G, Zawisza J, Szeja W, and Krawczyk Z. (2009) Unsaturated genistein disaccharide glycoside as a novel agent affecting microtubules, *Bioorg Med Chem Lett 19*, 4939-4943.

191. Ponnathpur V, Ibrado AM, Reed JC, Ray S, Huang Y, Self S, Bullock G, Nawabi A, and Bhalla K. (1995) Effects of modulators of protein kinases on taxol-induced apoptosis of human leukemic cells possessing disparate levels of p26BCL-2 protein, *Clin Cancer Res 1*, 1399-1406.

192. Paredes-López O, Cervantes-Ceja M, Vigna-Pérez M, and Hernández-Pérez T. (2010) Berries: Improving Human Health and Healthy Aging, and Promoting Quality Life—A Review, *Plant Foods for Human Nutrition 65*, 299-308.

193. Wang L-S, Hecht SS, Carmella SG, Yu N, Larue B, Henry C, McIntyre C, Rocha C, Lechner JF, and Stoner GD. (2009) Anthocyanins in Black Raspberries Prevent Esophageal Tumors in Rats, *Cancer Prevention Research 2*, 84-93.

194. Wang L-S, Hecht S, Carmella S, Seguin C, Rocha C, Yu N, Stoner K, Chiu S, and Stoner G. (2010) Berry Ellagitannins May Not Be Sufficient for Prevention of Tumors in the Rodent Esophagus, *Journal of Agricultural Food Chemistry 58*, 3992-3995.

195. Ross HA, McDougall GJ, and Stewart D. (2007) Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts, *Phytochemistry 68*, 218-228.

196. Lu H, Li J, Zhang D, Stoner GD, and Huang C. (2006) Molecular Mechanisms Involved in Chemoprevention of Black Raspberry Extracts: From Transcription Factors to Their Target Genes, *Nutr Cancer 54*, 69-78.

197. Stan SD, Kar S, Stoner GD, and Singh SV. (2008) Bioactive food components and cancer risk reduction, *J Cell Biochem 104*, 339-356.

198. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, and Prior RL. (2006) Concentrations of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption, *J Agric Food Chem 54*, 4069-4075.

199. Aiyer HS, Kichambare S, and Gupta RC. (2008) Prevention of oxidative DNA damage by bioactive berry components, *Nutr Cancer 60 Suppl 1*, 36-42.

200. Seeram NP, Adams LS, Zhang Y, Lee R, Sand D, Scheuller HS, and Heber D. (2006) Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry, and Strawberry Extracts Inhibit Growth and Stimulate Apoptosis of Human Cancer Cells In Vitro, *J Agric Food Chem 54*, 9329-9339.

201. Zikri NN, Riedl KM, Wang L-S, Lechner J, Schwartz SJ, and Stoner GD. (2009) Black Raspberry Components Inhibit Proliferation, Induce Apoptosis, and Modulate Gene Expression in Rat Esophageal Epithelial Cells, *Nutrition and Cancer 61*, 816-826.

202. Medda R, Lyros O, Schmidt JL, Jovanovic N, Nie L, Link BJ, Otterson MF, Stoner GD, Shaker R, and Rafiee P. (2015) Anti inflammatory and anti angiogenic effect of black raspberry extract on human esophageal and intestinal microvascular endothelial cells, *Microvasc Res 97*, 167-180.

203. Mace TA, King SA, Ameen Z, Elnaggar O, Young G, Riedl KM, Schwartz SJ, Clinton SK, Knobloch TJ, Weghorst CM, and Lesinski GB. (2014) Bioactive compounds or metabolites from black raspberries modulate T lymphocyte proliferation, myeloid cell differentiation and Jak/STAT signaling, *Cancer Immunol Immunother 63*, 889-900.

204. Zhang Z, Knobloch TJ, Seamon LG, Stoner GD, Cohn DE, Paskett ED, Fowler JM, and Weghorst CM. (2011) A black raspberry extract inhibits proliferation and regulates apoptosis in cervical cancer cells, *Gynecologic Oncology 123*, 401-406.

205. Chen T, Rose ME, Hwang H, Nines RG, and Stoner GD. (2006) Black raspberries inhibit N-nitrosomethylbenzylamine (NMBA)-induced angiogenesis in rat esophagus parallel to the suppression of COX-2 and iNOS, *Carcinogenesis 27*, 2301-2307.

206. Peiffer DS, Zimmerman NP, Wang LS, Ransom BW, Carmella SG, Kuo CT, Siddiqui J, Chen JH, Oshima K, Huang YW, Hecht SS, and Stoner GD. (2014) Chemoprevention of esophageal cancer with black raspberries, their component anthocyanins, and a major anthocyanin metabolite, protocatechuic acid, *Cancer Prevention Research 7*, 574-584.

207. Chen T, Hwang H, Rose ME, Nines RG, and Stoner GD. (2006) Chemopreventive properties of black raspberries in N-nitrosomethylbenzylamine-induced rat esophageal tumorigenesis: down-regulation of cyclooxygenase-2, inducible nitric oxide synthase, and c-Jun, *Cancer Res 66*, 2853-2859.

208. Stoner GD, Chen T, Kresty LA, Aziz RM, Reinemann T, and Nines R. (2006) Protection Against Esophageal Cancer in Rodents With Lyophilized Berries: Potential Mechanisms, *Nutr Cancer 54*, 33-46.

209. Wang L-S, Dombkowski AA, Seguin C, Rocha C, Cukovic D, Mukundan A, Henry C, and Stoner GD. (2011) Mechanistic basis for the chemopreventive effects of black raspberries at a late stage of rat esophageal carcinogenesis, *Molecular Carcinogenesis 50*, 291-300.

210. Kresty LA, Morse MA, Morgan C, Carlton PS, Lu J, Gupta A, Blackwood M, and Stoner GD. (2001) Chemoprevention of Esophageal Tumorigenesis by Dietary Administration of Lyophilized Black Raspberries, *Cancer Res 61*, 6112-6119.

211. Harris GK, Gupta A, Nines RG, Kresty LA, Habib SG, Frankel WL, LaPerle K, Gallaher DD, Schwartz SJ, and Stoner GD. (2001) Effects of Lyophilized Black Raspberries on Azoxymethane-Induced Colon Cancer and 8-Hydroxy-2′-Deoxyguanosine Levels in the Fischer 344 Rat, *Nutr Cancer 40*, 125-133.

212. Duncan FJ, Martin JR, Wulff BC, Stoner GD, Tober KL, Oberyszyn TM, Kusewitt DF, and Van Buskirk AM. (2009) Topical Treatment with Black Raspberry Extract Reduces Cutaneous UVB-Induced Carcinogenesis and Inflammation, *Cancer Prevention Research 2*, 665-672.

213. Aiyer HS, and Gupta RC. (2010) Berries and Ellagic Acid Prevent Estrogen-Induced Mammary Tumorigenesis by Modulating Enzymes of Estrogen Metabolism, *Cancer Prevention Research 3*, 727-737.

214. Aiyer HS, Srinivasan C, and Gupta RC. (2008) Dietary Berries and Ellagic Acid Diminish Estrogen-Mediated Mammary Tumorigenesis ∈ ACI rats, *Nutr Cancer 60*, 227-234.

215. Ravoori S, Vadhanam MV, Aqil F, and Gupta RC. (2012) Inhibition of Estrogen-Mediated Mammary Tumorigenesis by Blueberry and Black Raspberry, *J Agric Food Chem 60*, 5547-5555.

216. Wang L-S, Burke CA, Hasson H, Kuo C-T, Molmenti CLS, Seguin C, Liu P, Huang TH-M, Frankel WL, and Stoner GD. (2014) A Phase Ib Study of the Effects of Black Raspberries on Rectal Polyps in Patients with Familial Adenomatous Polyposis, *Cancer Prevention Research 7*, 666-674.

217. Wang L-S, Arnold M, Huang Y-W, Sardo C, Seguin C, Martin E, Huang THM, Riedl K, Schwartz S, Frankel W, Pearl D, Xu Y, Winston J, Yang G-Y, and Stoner G. (2011) Modulation of Genetic and Epigenetic Biomarkers of Colorectal Cancer in Humans by Black Raspberries: A Phase I Pilot Study, *Clinical cancer research : an official journal of the American Association for Cancer Research 17*, 598-610.

218. Kresty LA, Frankel WL, Hammond CD, Baird ME, Mele JM, Stoner GD, and Fromkes JJ. (2006) Transitioning From Preclinical to Clinical Chemopreventive Assessments of Lyophilized Black Raspberries: Interim Results Show Berries Modulate Markers of Oxidative Stress in Barrett's Esophagus Patients, *Nutr Cancer 54*, 148-156.

219. Mallery SR, Zwick JC, Pei P, Tong M, Larsen PE, Shumway BS, Lu B, Fields HW, Mumper RJ, and Stoner GD. (2008) Topical application of a bioadhesive black raspberry gel modulates gene expression and reduces cyclooxygenase 2 protein in human premalignant oral lesions, *Cancer Res 68*, 4945-4957.

220. Mallery SR, Budendorf DE, Larsen MP, Pei P, Tong M, Holpuch AS, Larsen PE, Stoner GD, Fields HW, Chan KK, Ling Y, and Liu Z. (2011) Effects of Human Oral Mucosal Tissue, Saliva, and Oral Microflora on Intraoral Metabolism and Bioactivation of Black Raspberry Anthocyanins, *Cancer Prevention Research 4*, 1209-1221.

221. Mallery SR, Tong M, Shumway BS, Curran AE, Larsen PE, Ness GM, Kennedy KS, Blakey GH, Kushner GM, Vickers AM, Han B, Pei P, and Stoner GD. (2014) Topical Application of a Mucoadhesive Freeze-Dried Black Raspberry Gel Induces Clinical and Histologic Regression and Reduces Loss of Heterozygosity Events in Premalignant Oral Intraepithelial Lesions: Results from a Multicentered, Placebo-Controlled Clinical Trial, *Clinical Cancer Research 20*, 1910-1924.

222. Shumway BS, Kresty LA, Larsen PE, Zwick JC, Lu B, Fields HW, Mumper RJ, Stoner GD, and Mallery SR. (2008) Effects of a Topically Applied Bioadhesive Berry Gel on Loss of Heterozygosity Indices in Premalignant Oral Lesions, *Clinical Cancer Research 14*, 2421-2430.

223. Mallery SR, Stoner GD, Larsen PE, Fields HW, Rodrigo KA, Schwartz SJ, Tian Q, Dai J, and Mumper RJ. (2007) Formulation and in-vitro and in-vivo evaluation of a mucoadhesive gel containing freeze dried black raspberries: implications for oral cancer chemoprevention, *Pharm Res 24*, 728-737.

224. Kresty LA, Fromkes JJ, Frankel WL, Hammond CD, Seeram NP, Baird M, and Stoner GD (2016) *A phase I pilot study evaluating the beneficial effects of black raspberries in patients with Barrett's esophagus*.

225. Pan P, Skaer CW, Stirdivant SM, Young MR, Stoner GD, Lechner JF, Huang Y-W, and Wang L-S. (2015) Beneficial Regulation of Metabolic Profiles by Black Raspberries in Human Colorectal Cancer Patients, *Cancer Prevention Research 8*, 743-750.

226. Mentor-Marcel RA, Bobe G, Sardo C, Wang L-S, Kuo C-T, Stoner G, and Colburn NH. (2012) Plasma Cytokines as Potential Response Indicators to Dietary Freeze-Dried Black Raspberries in Colorectal Cancer Patients, *Nutrition and Cancer 64*, 820-825.

227. Galvano F, La Fauci L, Lazzarino G, Fogliano V, Ritieni A, Ciappellano S, Battistini NC, Tavazzi B, and Galvano G. (2004) Cyanidins: metabolism and biological properties, *J Nutr Biochem 15*, 2-11.

228. Andersen ØM, and Jordheim M. (2001) Anthocyanins, In *eLS*, John Wiley & Sons, Ltd.

229. Paredes-López FD-VaO. (2002) Pigments as Food Colorants, In *Natural Colorants for Food and Nutraceutical Uses*, CRC Press.

230. Castañeda-Ovando A, Pacheco-Hernández MdL, Páez-Hernández ME, Rodríguez JA, and Galán-Vidal CA. (2009) Chemical studies of anthocyanins: A review, *Food Chem 113*, 859-871.

231. Borkowski T, Szymusiak H, Gliszczyńska-Swigło A, and Tyrakowska B. (2005) The effect of 3-O-β-glucosylation on structural transformations of anthocyanidins, *Food Research International 38*, 1031-1037.

232. Khandelwal N, and Abraham SK. (2014) Intake of anthocyanidins pelargonidin and cyanidin reduces genotoxic stress in mice induced by diepoxybutane, urethane and endogenous nitrosation, *Environmental Toxicology and Pharmacology 37*, 837-843.

233. Gasiorowski K, Szyba K, Brokos B, Kolaczynska B, Jankowiak-Wlodarczyk M, and Oszmianski J. (1997) Antimutagenic activity of anthocyanins isolated from Aronia melanocarpa fruits, *Cancer Lett 119*, 37-46.

234. Giampieri F, Alvarez-Suarez JM, Mazzoni L, Forbes-Hernandez TY, Gasparrini M, Gonzalez-Paramas AM, Santos-Buelga C, Quiles JL, Bompadre S, Mezzetti B, and Battino M. (2014) An anthocyanin-rich strawberry extract protects against oxidative stress damage and improves mitochondrial functionality in human dermal fibroblasts exposed to an oxidizing agent, *Food & Function 5*, 1939-1948.

235. Pratheeshkumar P, Son Y-O, Wang X, Divya SP, Joseph B, Hitron JA, Wang L, Kim D, Yin Y, Roy RV, Lu J, Zhang Z, Wang Y, and Shi X. (2014) Cyanidin-3-Glucoside inhibits UVB-induced oxidative damage and inflammation by regulating MAP kinase and NF-κB signalling pathways in SKH-1 hairless mice skin, *Toxicol Appl Pharmacol 280*, 127-137.

236. Shih P-H, Yeh C-T, and Yen G-C. (2007) Anthocyanins Induce the Activation of Phase II Enzymes through the Antioxidant Response Element Pathway against Oxidative Stress-Induced Apoptosis, *Journal of Agricultural Food Chemistry 55*, 9427-9435.

237. Bartikova H, Skalova L, Drsata J, and Bousova I. (2013) Interaction of anthocyanins with drug-metabolizing and antioxidant enzymes, *Curr Med Chem 20*, 4665-4679.

238. Dvorak Z, Srovnalova A, Svecarova M, and Vrzal R. (2014) The effect of anthocyans on the expression of selected phase II xenobiotic-metabolizing enzymes in primary cultures of human hepatocytes, *Food Funct 5*, 2145-2151.

239. Chang YC, Huang HP, Hsu JD, Yang SF, and Wang CJ. (2005) Hibiscus anthocyanins rich extract-induced apoptotic cell death in human promyelocytic leukemia cells, *Toxicol Appl Pharmacol 205*, 201-212.

240. Chen P-N, Chu S-C, Chiou H-L, Chiang C-L, Yang S-F, and Hsieh Y-S. (2005) Cyanidin 3-Glucoside and Peonidin 3-Glucoside Inhibit Tumor Cell Growth and Induce Apoptosis In Vitro and Suppress Tumor Growth In Vivo, *Nutr Cancer 53*, 232-243.

241. Hui C, Bin Y, Xiaoping Y, Long Y, Chunye C, Mantian M, and Wenhua L. (2010) Anticancer Activities of an Anthocyanin-Rich Extract From Black Rice Against Breast Cancer Cells In Vitro and In Vivo, *Nutrition and Cancer 62*, 1128-1136.

242. Malik M, Zhao C, Schoene N, Guisti MM, Moyer MP, and Magnuson BA. (2003) Anthocyanin-Rich Extract From Aronia meloncarpa E. Induces a Cell Cycle Block in Colon Cancer but Not Normal Colonic Cells, *Nutrition and Cancer 46*, 186-196.

243. Bagchi D, Sen CK, Bagchi M, and Atalay M. (2004) Anti-angiogenic, Antioxidant, and Anti-carcinogenic Properties of a Novel Anthocyanin-Rich Berry Extract Formula, *Biochemistry (Moscow) 69*, 75-80.

244. Heber D. (2008) Multitargeted therapy of cancer by ellagitannins, *Cancer Lett 269*, 262-268.

245. Labrecque L, Lamy S, Chapus A, Mihoubi S, Durocher Y, Cass B, Bojanowski MW, Gingras D, and Béliveau R. (2005) Combined inhibition of PDGF and VEGF receptors by ellagic acid, a dietary-derived phenolic compound, *Carcinogenesis 26*, 821-826.

246. Landete JM. (2011) Ellagitannins, ellagic acid and their derived metabolites: a review about source, metabolism, functions and health, *Food Research International 44*, 1150-1160.

247. Vanella L, Di Giacomo C, Acquaviva R, Barbagallo I, Li Volti G, Cardile V, Abraham NG, and Sorrenti V. (2013) Effects of ellagic Acid on angiogenic factors in prostate cancer cells, *Cancers (Basel) 5*, 726-738.

248. Wang L, Ho J, Glackin C, and Martins-Green M. (2012) Specific pomegranate juice components as potential inhibitors of prostate cancer metastasis, *Transl Oncol 5*, 344-355.

249. Bala I, Bhardwaj V, Hariharan S, and Kumar MNVR. (2006) Analytical methods for assay of ellagic acid and its solubility studies, *Journal of Pharmaceutical and Biomedical Analysis 40*, 206-210.

250. Karakaya S. (2004) Bioavailability of phenolic compounds, *Crit Rev Food Sci Nutr 44*, 453-464.

251. Zafrilla P, Ferreres F, and Tomás-Barberán FA. (2001) Effect of Processing and Storage on the Antioxidant Ellagic Acid Derivatives and Flavonoids of Red Raspberry (Rubus idaeus) Jams, *Journal of Agricultural and Food Chemistry 49*, 3651-3655.

252. Vichai V, and Kirtikara K. (2006) Sulforhodamine B colorimetric assay for cytotoxicity screening, *Nat Protoc 1*, 1112-1116.

253. Özten N, Horton L, Lasano S, and Bosland MC. (2010) Selenomethionine and α-Tocopherol do not Inhibit Prostate Carcinogenesis in the Testosterone plus Estradiol-Treated NBL Rat Model, *Cancer Prevention Research 3*, 371-380.

254. McCormick DL, Rao KV, Dooley L, Steele VE, Lubet RA, Kelloff GJ, and Bosland MC. (1998) Influence of N-methyl-N-nitrosourea, testosterone, and N-(4-hydroxyphenyl)-all-trans-retinamide on prostate cancer induction in Wistar-Unilever rats, *Cancer Res 58*, 3282-3288.

255. Bosland MC, Ford H, and Horton L. (1995) Induction at high incidence of ductal prostate adenocarcinomas in NBL/Cr and Sprague-Dawley Hsd:SD rats treated with a combination of testosterone and estradiol-17 beta or diethylstilbestrol, *Carcinogenesis 16*, 1311-1317.

256. Ofner P, Bosland MC, and Vena RL. (1992) Differential effects of diethylstilbestrol and estradiol-17 beta in combination with testosterone on rat prostate lobes, *Toxicol Appl Pharmacol 112*, 300-309.

257. Chen W, Wang D, Wang L-s, Bei D, Wang J, See WA, Mallery SR, Stoner GD, and Liu Z. (2012) Pharmacokinetics of protocatechuic acid in mouse and its quantification in human plasma using LC–tandem mass spectrometry, *Journal of Chromatography B 908*, 39-44.

258. Chou T-C. (2010) Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method, *Cancer Res 70*, 440-446.

259. Teicher BA. (2006) Tumor models for efficacy determination, *Molecular Cancer Therapeutics 5*, 2435-2443.

260. Vrignaud P, Sémiond D, Lejeune P, Bouchard H, Calvet L, Combeau C, Riou J-F, Commerçon A, Lavelle F, and Bissery M-C. (2013) Preclinical Antitumor Activity of Cabazitaxel, a Semisynthetic Taxane Active in Taxane-Resistant Tumors, *Clinical Cancer Research 19*, 2973-2983.

261. Grubbs FE. (1969) Procedures for Detecting Outlying Observations in Samples, *Technometrics 11*, 1-21.

262. Stan SD, Kar S, Stoner GD, and Singh SV. (2008) Bioactive food components and cancer risk reduction, *Journal of Cellular Biochemistry 104*, 339-356.

263. Wang L-S, and Stoner GD. (2008) Anthocyanins and their role in cancer prevention, *Cancer Lett 269*, 281-290.

264. Meiers S, Kemény M, Weyand U, Gastpar R, von Angerer E, and Marko D. (2001) The Anthocyanidins Cyanidin and Delphinidin Are Potent Inhibitors of the Epidermal Growth-Factor Receptor, *J Agric Food Chem 49*, 958-962.

265. Rodrigo KA, Rawal Y, Renner RJ, Schwartz SJ, Tian Q, Larsen PE, and Mallery SR. (2006) Suppression of the Tumorigenic Phenotype in Human Oral Squamous Cell Carcinoma Cells by an Ethanol Extract Derived From Freeze-Dried Black Raspberries, *Nutr Cancer 54*, 58-68.

266. Guadamillas MC, Cerezo A, and Del Pozo MA. (2011) Overcoming anoikis--pathways to anchorage-independent growth in cancer, *J Cell Sci 124*, 3189-3197.

267. Taddei ML, Giannoni E, Fiaschi T, and Chiarugi P. (2012) Anoikis: an emerging hallmark in health and diseases, *J Pathol 226*, 380-393.

268. Bi X, Fang W, Wang L-S, Stoner GD, and Yang W. (2010) Black Raspberries Inhibit Intestinal Tumorigenesis in Apc1638+/- and Muc2-/- Mouse Models of Colorectal Cancer, *Cancer Prevention Research 3*, 1443-1450.

269. Yamaguchi H, Wyckoff J, and Condeelis J. (2005) Cell migration in tumors, *Curr Opin Cell Biol 17*, 559-564.

270. Friedl P, and Wolf K. (2003) Tumour-cell invasion and migration: diversity and escape mechanisms, *Nat Rev Cancer 3*, 362-374.

271. Medda R, Lyros O, Schmidt JL, Jovanovic N, Nie L, Link BJ, Otterson MF, Stoner GD, Shaker R, and Rafiee P. (2015) Anti inflammatory and anti angiogenic effect of black raspberry extract on human esophageal and intestinal microvascular endothelial cells, *Microvascular Research 97*, 167-180.

272. Youdim KA, Martin A, and Joseph JA. (2000) Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress, *Free Radic Biol Med 29*, 51-60.

273. Lazzé MC, Pizzala R, Savio M, Stivala LA, Prosperi E, and Bianchi L. (2003) Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis 535*, 103-115.

274. Huang ST, Yang RC, Wu HT, Wang CN, and Pang JH. (2011) Zinc-chelation contributes to the anti-angiogenic effect of ellagic acid on inhibiting MMP-2 activity, cell migration and tube formation, *PLoS One 6*, e18986.

275. Pitchakarn P, Chewonarin T, Ogawa K, Suzuki S, Asamoto M, Takahashi S, Shirai T, and Limtrakul P. (2013) Ellagic acid inhibits migration and invasion by prostate cancer cell lines, *Asian Pac J Cancer Prev 14*, 2859-2863.

276. Zhao M, Tang SN, Marsh JL, Shankar S, and Srivastava RK. (2013) Ellagic acid inhibits human pancreatic cancer growth in Balb c nude mice, *Cancer Lett 337*, 210-217.

277. He J, and Giusti MM. (2010) Anthocyanins: Natural Colorants with Health-Promoting Properties, *Annual Review of Food Science and Technology 1*, 163-187.

278. Faria A, Fernandes I, Mateus N, and Calhau C. (2013) Bioavailability of Anthocyanins, In *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes* (Ramawat GK, and Mérillon J-M, Eds.), pp 2465-2487, Springer Berlin Heidelberg, Berlin, Heidelberg.

279. Faria A, Fernandes I, Norberto S, Mateus N, and Calhau C. (2014) Interplay between Anthocyanins and Gut Microbiota, *J Agric Food Chem 62*, 6898-6902.

280. Keppler K, and Humpf H-U. (2005) Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora, *Bioorganic & Medicinal Chemistry 13*, 5195-5205.

281. Kamiloglu S, Capanoglu E, Grootaert C, and Van Camp J. (2015) Anthocyanin Absorption and Metabolism by Human Intestinal Caco-2 Cells--A Review, *Int J Mol Sci 16*, 21555-21574.

282. Ludwig IA, Mena P, Calani L, Borges G, Pereira-Caro G, Bresciani L, Del Rio D, Lean ME, and Crozier A. (2015) New insights into the bioavailability of red raspberry anthocyanins and ellagitannins, *Free Radic Biol Med 89*, 758-769.

283. Ichiyanagi T, Shida Y, Rahman MM, Hatano Y, and Konishi T. (2005) Extended glucuronidation is another major path of cyanidin 3-O-beta-D-glucopyranoside metabolism in rats, *J Agric Food Chem 53*, 7312-7319.

284. Ichiyanagi T, Shida Y, Rahman MM, Hatano Y, Matsumoto H, Hirayama M, and Konishi T. (2005) Metabolic pathway of cyanidin 3-O-beta-D-glucopyranoside in rats, *J Agric Food Chem 53*, 145-150.

285. Woodward GM, Needs PW, and Kay CD. (2011) Anthocyanin-derived phenolic acids form glucuronides following simulated gastrointestinal digestion and microsomal glucuronidation, *Mol Nutr Food Res 55*, 378-386.

286. He J, Wallace TC, Keatley KE, Failla ML, and Giusti MM. (2009) Stability of black raspberry anthocyanins in the digestive tract lumen and transport efficiency into gastric and small intestinal tissues in the rat, *J Agric Food Chem 57*, 3141-3148.

287. Felgines C, Texier O, Garcin P, Besson C, Lamaison JL, and Scalbert A. (2009) Tissue distribution of anthocyanins in rats fed a blackberry anthocyanin-enriched diet, *Mol Nutr Food Res 53*, 1098-1103.

288. Marczylo TH, Cooke D, Brown K, Steward WP, and Gescher AJ. (2009) Pharmacokinetics and metabolism of the putative cancer chemopreventive agent cyanidin-3-glucoside in mice, *Cancer Chemother Pharmacol 64*, 1261-1268.

289. Kakkar S, and Bais S. (2014) A review on protocatechuic Acid and its pharmacological potential, *ISRN Pharmacol 2014*, 952943.

290. Seeram NP, Aronson WJ, Zhang Y, Henning SM, Moro A, Lee RP, Sartippour M, Harris DM, Rettig M, Suchard MA, Pantuck AJ, Belldegrun A, and Heber D. (2007) Pomegranate ellagitannin-derived metabolites inhibit prostate cancer growth and localize to the mouse prostate gland, *J Agric Food Chem 55*, 7732-7737.

291. González-Sarrías A, Giménez-Bastida JA, García-Conesa MT, Gómez-Sánchez MB, García-Talavera NV, Gil-Izquierdo A, Sánchez-Álvarez C, Fontana-Compiano LO, Morga-Egea JP, Pastor-Quirante FA, Martínez-Díaz F, Tomás-Barberán FA, and Espín JC. (2010) Occurrence of urolithins, gut microbiota ellagic acid metabolites and proliferation markers expression response in the human prostate gland upon consumption of walnuts and pomegranate juice, *Molecular Nutrition & Food Research 54*, 311-322.

292. Singletary K, and Liao CH. (1989) Ellagic acid effects on the carcinogenicity, DNA-binding and metabolism of 7,12-dimethylbenz(a)anthracene (DMBA), *In Vivo 3*, 173-175.

293. Larrosa M, Gonzalez-Sarrias A, Garcia-Conesa MT, Tomas-Barberan FA, and Espin JC. (2006) Urolithins, ellagic acid-derived metabolites produced by human colonic microflora, exhibit estrogenic and antiestrogenic activities, *J Agric Food Chem 54*, 1611-1620.

294. Schmitt E, and Stopper H. (2001) Estrogenic activity of naturally occurring anthocyanidins, *Nutr Cancer 41*, 145-149.

295. Ravoori S, Vadhanam MV, Sahoo S, Srinivasan C, and Gupta RC. (2007) Mammary tumor induction in ACI rats exposed to low levels of 17beta-estradiol, *Int J Oncol 31*, 113-120.

296. Tam NNC, Leav I, and Ho S-M. (2007) Sex Hormones Induce Direct Epithelial and Inflammation-Mediated Oxidative/Nitrosative Stress That Favors Prostatic Carcinogenesis in the Noble Rat, *The American Journal of Pathology 171*, 1334-1341.

297. Kirby M, Hirst C, and Crawford ED. (2011) Characterising the castration-resistant prostate cancer population: a systematic review, *Int J Clin Pract 65*, 1180-1192.

298. Rubin MA. (2008) Targeted therapy of cancer: new roles for pathologists--prostate cancer, *Mod Pathol 21 Suppl 2*, S44-55.

299. Galsky MD, and Vogelzang NJ. (2010) Docetaxel-based combination therapy for castration-resistant prostate cancer, *Ann Oncol 21*, 2135-2144.

300. MacVicar GR, and Hussain MH. (2013) Emerging therapies in metastatic castration-sensitive and castration-resistant prostate cancer, *Curr Opin Oncol 25*, 252-260.

301. Seruga B, and Tannock IF. (2011) Chemotherapy-based treatment for castration-resistant prostate cancer, *J Clin Oncol 29*, 3686-3694.

302. Zhao S, and Yu EY. (2013) Castrate-resistant prostate cancer: postdocetaxel management, *Curr Opin Urol 23*, 201-207.

303. Wei JT, Dunn RL, Sandler HM, McLaughlin PW, Montie JE, Litwin MS, Nyquist L, and Sanda MG. (2002) Comprehensive comparison of health-related quality of life after contemporary therapies for localized prostate cancer, *J Clin Oncol 20*, 557-566.

304. Singh H, Maskarinec G, and Shumay DM. (2005) Understanding the Motivation for Conventional and Complementary/Alternative Medicine Use Among Men With Prostate Cancer, *Integr Cancer Ther 4*, 187-194.

305. Eng J, Ramsum D, Verhoef M, Guns E, Davison J, and Gallagher R. (2003) A Population-Based Survey of Complementary and Alternative Medicine Use in Men Recently Diagnosed with Prostate Cancer, *Integr Cancer Ther 2*, 212-216.

306. Bishop FL, Rea A, Lewith H, Chan YK, Saville J, Prescott P, Elm Ev, and Lewith GT. (2011) Complementary medicine use by men with prostate cancer: a systematic review of prevalence studies, *Prostate Cancer Prostatic Dis 14*, 1-13.

307. Luo Q, and Asher GN. (2016) Complementary and Alternative Medicine Use at a Comprehensive Cancer Center, *Integr Cancer Ther*.

308. Mani J, Juengel E, Arslan I, Bartsch G, Filmann N, Ackermann H, Nelson K, Haferkamp A, Engl T, and Blaheta RA. (2015) Use of complementary and alternative medicine before and after organ removal due to urologic cancer, *Patient preference and adherence 9*, 1407-1412.

309. Bailey RL, Gahche JJ, Lentino CV, Dwyer JT, Engel JS, Thomas PR, Betz JM, Sempos CT, and Picciano MF. (2011) Dietary Supplement Use in the United States, 2003–2006, *J Nutr 141*, 261-266.

310. Greenlee H, White E, Patterson RE, and Kristal AR. (2004) Supplement use among cancer survivors in the Vitamins and Lifestyle (VITAL) study cohort, *J Altern Complement Med 10*, 660-666.

311. Luc L, Baumgart C, Weiss E, Georger L, Ambrosone CB, Zirpoli G, and McCann SE. (2015) Dietary supplement use among participants of a databank and biorepository at a comprehensive cancer centre, *Public Health Nutr 18*, 916-926.

312. Westerlund A, Steineck G, Balter K, Stattin P, Gronberg H, and Hedelin M. (2011) Dietary supplement use patterns in men with prostate cancer: the Cancer Prostate Sweden study, *Annals of Oncology 22*, 967-972.

313. Qato DM, Wilder J, Schumm LP, Gillet V, and Alexander GC. (2016) Changes in Prescription and Over-the-Counter Medication and Dietary Supplement Use Among Older Adults in the United States, 2005 vs 2011, *JAMA Intern Med 176*, 473-482.

314. Stoner GD, Wang LS, Zikri N, Chen T, Hecht SS, Huang C, Sardo C, and Lechner JF. (2007) Cancer prevention with freeze-dried berries and berry components, *Semin Cancer Biol 17*, 403-410.

315. Yin MC, Lin CC, Wu HC, Tsao SM, and Hsu CK. (2009) Apoptotic effects of protocatechuic acid in human breast, lung, liver, cervix, and prostate cancer cells: potential mechanisms of action, *J Agric Food Chem 57*, 6468-6473.

316. Serafim TL, Carvalho FS, Marques MP, Calheiros R, Silva T, Garrido J, Milhazes N, Borges F, Roleira F, Silva ET, Holy J, and Oliveira PJ. (2011) Lipophilic caffeic and ferulic acid derivatives presenting cytotoxicity against human breast cancer cells, *Chem Res Toxicol 24*, 763-774.

317. Sehitoglu MH, Farooqi AA, Qureshi MZ, Butt G, and Aras A. (2014) Anthocyanins: targeting of signaling networks in cancer cells, *Asian Pac J Cancer Prev 15*, 2379-2381.

318. Vanella L, Di Giacomo C, Acquaviva R, Barbagallo I, Cardile V, Kim DH, Abraham NG, and Sorrenti V. (2013) Apoptotic markers in a prostate cancer cell line: effect of ellagic acid, *Oncol Rep 30*, 2804-2810.

319. Sudan S, and Rupasinghe HP. (2014) Quercetin-3-O-glucoside Induces Human DNA Topoisomerase II Inhibition, Cell Cycle Arrest and Apoptosis in Hepatocellular Carcinoma Cells, *Anticancer Research 34*, 1691-1699.

320. Abal M, Andreu JM, and Barasoain I. (2003) Taxanes: microtubule and centrosome targets, and cell cycle dependent mechanisms of action, *Curr Cancer Drug Targets 3*, 193-203.

321. Nguyen V, Tang J, Oroudjev E, Lee CJ, Marasigan C, Wilson L, and Ayoub G. (2010) Cytotoxic effects of bilberry extract on MCF7-GFP-tubulin breast cancer cells, *J Med Food 13*, 278-285.

322. Kartner N, Riordan, and Ling V. (1983) Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines, *Science 221*, 1285-1288.

323. Stark GR. (1986) Cancer chemotherapy. Progress in understanding multidrug resistance, *Nature 324*, 407-408.

324. Bradshaw DM, and Arceci RJ. (1998) Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance, *Journal of Clinical Oncology 16*, 3674-3690.

325. Zhu Y, Liu C, Nadiminty N, Lou W, Tummala R, Evans CP, and Gao AC. (2013) Inhibition of ABCB1 expression overcomes acquired docetaxel resistance in prostate cancer, *Mol Cancer Ther 12*, 1829-1836.

326. Mahon KL, Henshall SM, Sutherland RL, and Horvath LG. (2011) Pathways of chemotherapy resistance in castration-resistant prostate cancer, *Endocrine-Related Cancer 18*, R103-R123.

327. Wink M, Ashour ML, and El-Readi MZ. (2012) Secondary Metabolites from Plants Inhibiting ABC Transporters and Reversing Resistance of Cancer Cells and Microbes to Cytotoxic and Antimicrobial Agents, *Front Microbiol 3*, 130.

328. Monsarrat B, Royer I, Wright M, and Cresteil T. (1997) Biotransformation of taxoids by human cytochromes P450: structure-activity relationship, *Bull Cancer 84*, 125-133.

329. Gomez-Lechon MJ, Rodeiro I, Donato MT, Lahoz A, Garrido G, and Delgado R. (2008) Interactions of Polyphenols with the P450 System: Possible Implications on Human Therapeutics, *Mini-Reviews in Medicinal Chemistry 8*, 97-106.

330. Wanwimolruk S, and Prachayasittikul V. (2014) Cytochrome P450 enzyme mediated herbal drug interactions (Part 1), *EXCLI Journal 13*, 347-391.

331. Rodríguez-Fragoso LaR-E, Jorge. (2013) Fruit/Vegetable-Drug Interactions: Effects on Drug Metabolizing Enzymes and Drug Transporters, In *Drug Discovery* (El-Shemy H, Ed.), InTech.

332. Akhmanova A, and Steinmetz MO. (2015) Control of microtubule organization and dynamics: two ends in the limelight, *Nat Rev Mol Cell Biol 16*, 711-726.

333. Shelanski ML, Gaskin F, and Cantor CR. (1973) Microtubule assembly in the absence of added nucleotides, *Proc Natl Acad Sci U S A 70*, 765-768.

334. Weisenberg RC. (1972) Microtubule formation in vitro in solutions containing low calcium concentrations, *Science 177*, 1104-1105.

335. Bonne D, Heusele C, Simon C, and Pantaloni D. (1985) 4',6-Diamidino-2-phenylindole, a fluorescent probe for tubulin and microtubules, *J Biol Chem 260*, 2819-2825.

336. Dreiseitel A, Oosterhuis B, Vukman KV, Schreier P, Oehme A, Locher S, Hajak G, and Sand PG. (2009) Berry anthocyanins and anthocyanidins exhibit distinct affinities for the efflux transporters BCRP and MDR1, *Br J Pharmacol 158*, 1942-1950.

337. Vrzal R. (2016) Anthocyanidins but not anthocyanins inhibit P-glycoprotein-mediated calcein extrusion - possible implication for orally administered drugs, *Fundam Clin Pharmacol 30*, 248-252.

338. Conklin KA. (2004) Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness, *Integrative Cancer Therapies 3*, 294-300.

339. Hong MY, Seeram NP, and Heber D. (2008) Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor, *J Nutr Biochem 19*, 848-855.

340. Koyama S, Cobb LJ, Mehta HH, Seeram NP, Heber D, Pantuck AJ, and Cohen P. (2010) Pomegranate extract induces apoptosis in human prostate cancer cells by modulation of the IGF-IGFBP axis, *Growth Horm IGF Res 20*, 55-62.

341. Rettig MB, Heber D, An J, Seeram NP, Rao JY, Liu H, Klatte T, Belldegrun A, Moro A, Henning SM, Mo D, Aronson WJ, and Pantuck A. (2008) Pomegranate extract inhibits androgen-independent prostate cancer growth through a nuclear factor-kappaB-dependent mechanism, *Mol Cancer Ther 7*, 2662-2671.

342. Naiki-Ito A, Chewonarin T, Tang M, Pitchakarn P, Kuno T, Ogawa K, Asamoto M, Shirai T, and Takahashi S. (2015) Ellagic acid, a component of pomegranate fruit juice, suppresses androgen-dependent prostate carcinogenesis via induction of apoptosis, *Prostate 75*, 151-160.

343. Vicinanza R, Zhang Y, Henning SM, and Heber D. (2013) Pomegranate Juice Metabolites, Ellagic Acid and Urolithin A, Synergistically Inhibit Androgen-Independent Prostate Cancer Cell Growth via Distinct Effects on Cell Cycle Control and Apoptosis, *Evid Based Complement Alternat Med 2013*, 247504.

344. Tong L, Chuang C-C, Wu S, and Zuo L. (2015) Reactive oxygen species in redox cancer therapy, *Cancer Lett 367*, 18-25.

345. Nambiar D, Rajamani P, and Singh RP. (2011) Effects of phytochemicals on ionization radiation-mediated carcinogenesis and cancer therapy, *Mutat Res 728*, 139-157.

346. Tan KW, Li Y, Paxton JW, Birch NP, and Scheepens A. (2013) Identification of novel dietary phytochemicals inhibiting the efflux transporter breast cancer resistance protein (BCRP/ABCG2), *Food Chem 138*, 2267-2274.

347. Falsaperla M, Morgia G, Tartarone A, Ardito R, and Romano G. (2005) Support Ellagic Acid Therapy in Patients with Hormone Refractory Prostate Cancer (HRPC) on Standard Chemotherapy Using Vinorelbine and Estramustine Phosphate, *European Urology 47*, 449-455.

348. Wang Y, Zhang S, Iqbal S, Chen Z, Wang X, Wang YA, Liu D, Bai K, Ritenour C, Kucuk O, and Wu D. (2013) Pomegranate extract inhibits the bone metastatic growth of human prostate cancer cells and enhances the in vivo efficacy of docetaxel chemotherapy, *Prostate*.

349. Gonzalez-Sarrias A, Espin JC, Tomas-Barberan FA, and Garcia-Conesa MT. (2009) Gene expression, cell cycle arrest and MAPK signalling regulation in Caco-2 cells exposed to ellagic acid and its metabolites, urolithins, *Mol Nutr Food Res 53*, 686-698.

350. Gonzalez-Sarrias A, Tome-Carneiro J, Bellesia A, Tomas-Barberan FA, and Espin JC. (2015) The ellagic acid-derived gut microbiota metabolite, urolithin A, potentiates the anticancer effects of 5-fluorouracil chemotherapy on human colon cancer cells, *Food Funct 6*, 1460-1469.

351. Touil YS, Fellous A, Scherman D, and Chabot GG. (2009) Flavonoid-induced morphological modifications of endothelial cells through microtubule stabilization, *Nutr Cancer 61*, 310-321.

352. Kroon J, Kooijman S, Cho N-J, Storm G, and van der Pluijm G. (2016) Improving Taxane-Based Chemotherapy in Castration-Resistant Prostate Cancer, *Trends in Pharmacological Sciences 37*, 451-462.

353. Sanchez-Gonzalez C, Ciudad CJ, Noe V, and Izquierdo-Pulido M. (2014) Walnut polyphenol metabolites, urolithins A and B, inhibit the expression of the prostate-specific antigen and the androgen receptor in prostate cancer cells, *Food Funct 5*, 2922-2930.

354. Albrecht M, Jiang W, Kumi-Diaka J, Lansky EP, Gommersall LM, Patel A, Mansel RE, Neeman I, Geldof AA, and Campbell MJ. (2004) Pomegranate extracts potently suppress proliferation, xenograft growth, and invasion of human prostate cancer cells, *J Med Food 7*, 274-283.

355. Malik A, Afaq F, Sarfaraz S, Adhami VM, Syed DN, and Mukhtar H. (2005) Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer, *Proc Natl Acad Sci U S A 102*, 14813-14818.

356. Malik A, and Mukhtar H. (2006) Prostate Cancer Prevention Through Pomegranate Fruit, *Cell Cycle 5*, 371-373.

357. Bell C, and Hawthorne S. (2008) Ellagic acid, pomegranate and prostate cancer — a mini review, *Journal of Pharmacy and Pharmacology 60*, 139-144.

358. Amos LA. (2011) What tubulin drugs tell us about microtubule structure and dynamics, *Seminars in Cell & Developmental Biology 22*, 916-926.

359. Mukhtar E, Adhami VM, Sechi M, and Mukhtar H. (2015) Dietary flavonoid fisetin binds to β-tubulin and disrupts microtubule dynamics in prostate cancer cells, *Cancer Letters 367*, 173-183.

360. Azarenko O, Jordan MA, and Wilson L. (2014) Erucin, the Major Isothiocyanate in Arugula *Eruca sativa*, Inhibits Proliferation of MCF7 Tumor Cells by Suppressing Microtubule Dynamics, *PLoS One 9*, e100599.

361. Sinha S, Amin H, Nayak D, Bhatnagar M, Kacker P, Chakraborty S, Kitchlu S, Vishwakarma R, Goswami A, and Ghosal S. (2015) Assessment of microtubule depolymerization property of flavonoids isolated from Tanacetum gracile in breast cancer cells by biochemical and molecular docking approach, *Chem Biol Interact 239*, 1-11.

362. Juhem A, Boumendjel A, Touquet B, Guillot A, Popov A, Ronot X, and Martel-Frachet V. (2013) AG11, a novel dichloroflavanone derivative with anti-mitotic activity towards human bladder cancer cells, *Anticancer Res 33*, 4445-4452.

363. Mira A, and Shimizu K. (2015) In vitro Cytotoxic Activities and Molecular Mechanisms of Angelica shikokiana Extract and its Isolated Compounds, *Pharmacogn Mag 11*, S564-569.

364. Azarenko O, Okouneva T, Singletary KW, Jordan MA, and Wilson L. (2008) Suppression of microtubule dynamic instability and turnover in MCF7 breast cancer cells by sulforaphane, *Carcinogenesis 29*, 2360-2368.

365. Illenberger S, Drewes G, Trinczek B, Biernat J, Meyer HE, Olmsted JB, Mandelkow EM, and Mandelkow E. (1996) Phosphorylation of microtubule-associated proteins MAP2 and MAP4 by the protein kinase p110mark. Phosphorylation sites and regulation of microtubule dynamics, *J Biol Chem 271*, 10834-10843.

366. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, Gravis G, Bodrogi I, Mackenzie MJ, Shen L, Roessner M, Gupta S, and Sartor AO. (2010) Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial, *Lancet 376*, 1147-1154.

367. de Weger VA, Beijnen JH, and Schellens JH. (2014) Cellular and clinical pharmacology of the taxanes docetaxel and paclitaxel--a review, *Anticancer Drugs 25*, 488-494.

368. Hwang KA, and Choi KC. (2015) Anticarcinogenic Effects of Dietary Phytoestrogens and Their Chemopreventive Mechanisms, *Nutr Cancer 67*, 796-803.

369. Vitale DC, Piazza C, Melilli B, Drago F, and Salomone S. (2013) Isoflavones: estrogenic activity, biological effect and bioavailability, *Eur J Drug Metab Pharmacokinet 38*, 15-25.

370. Adjakly M, Ngollo M, Boiteux JP, Bignon YJ, Guy L, and Bernard-Gallon D. (2013) Genistein and daidzein: different molecular effects on prostate cancer, *Anticancer Res 33*, 39-44.

371. Lund TD, Blake C, Bu L, Hamaker AN, and Lephart ED. (2011) Equol an isoflavonoid: potential for improved prostate health, in vitro and in vivo evidence, *Reprod Biol Endocrinol 9*, 4.

372. Li HQ, Luo Y, and Qiao CH. (2012) The mechanisms of anticancer agents by genistein and synthetic derivatives of isoflavone, *Mini Rev Med Chem 12*, 350-362.

373. Li X, and Choi JS. (2007) Effect of genistein on the pharmacokinetics of paclitaxel administered orally or intravenously in rats, *Int J Pharm 337*, 188-193.

374. Li Y, Ahmed F, Ali S, Philip PA, Kucuk O, and Sarkar FH. (2005) Inactivation of nuclear factor kappaB by soy isoflavone genistein contributes to increased apoptosis induced by chemotherapeutic agents in human cancer cells, *Cancer Res 65*, 6934-6942.

375. Zhang S, Wang Y, Chen Z, Kim S, Iqbal S, Chi A, Ritenour C, Wang YA, Kucuk O, and Wu D. (2013) Genistein enhances the efficacy of cabazitaxel chemotherapy in metastatic castration-resistant prostate cancer cells, *Prostate 73*.

376. Miltyk W, Craciunescu CN, Fischer L, Jeffcoat RA, Koch MA, Lopaczynski W, Mahoney C, Jeffcoat RA, Crowell J, Paglieri J, and Zeisel SH. (2003) Lack of significant genotoxicity of purified soy isoflavones (genistein, daidzein, and glycitein) in 20 patients with prostate cancer, *The American journal of clinical nutrition 77*, 875-882.

377. Busby MG, Jeffcoat AR, Bloedon LT, Koch MA, Black T, Dix KJ, Heizer WD, Thomas BF, Hill JM, Crowell JA, and Zeisel SH. (2002) Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men, *The American journal of clinical nutrition 75*, 126-136.

378. King RA, and Bursill DB. (1998) Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans, *The American journal of clinical nutrition 67*, 867-872.

379. Jager W, Winter O, Halper B, Salamon A, Sartori M, Gajdzik L, Hamilton G, Theyer G, Graf J, and Thalhammer T. (1997) Modulation of liver canalicular transport processes by the tyrosine-kinase inhibitor genistein: implications of genistein metabolism in the rat, *Hepatology 26*, 1467-1476.

380. Imai Y, Tsukahara S, Asada S, and Sugimoto Y. (2004) Phytoestrogens/flavonoids reverse breast cancer resistance protein/ABCG2-mediated multidrug resistance, *Cancer Res 64*, 4346-4352.

381. Zhang S, Yang X, and Morris ME. (2004) Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport, *Mol Pharmacol 65*, 1208-1216.

382. Castro AF, and Altenberg GA. (1997) Inhibition of drug transport by genistein in multidrug-resistant cells expressing P-glycoprotein, *Biochem Pharmacol 53*, 89-93.

383. Moon YJ, Wang X, and Morris ME. (2006) Dietary flavonoids: effects on xenobiotic and carcinogen metabolism, *Toxicol In Vitro 20*, 187-210.

384. Kopecna-Zapletalova M, Krasulova K, Anzenbacher P, Hodek P, and Anzenbacherova E. (2016) Interaction of isoflavonoids with human liver microsomal cytochromes P450: inhibition of CYP enzyme activities, *Xenobiotica*, 1-8.

385. Mahoney S, Arfuso F, Rogers P, Hisheh S, Brown D, Millward M, and Dharmarajan A. (2012) Cytotoxic effects of the novel isoflavone, phenoxodiol, on prostate cancer cell lines, *Journal of Biosciences 37*, 73-84.

386. Zasadil LM, Andersen KA, Yeum D, Rocque GB, Wilke LG, Tevaarwerk AJ, Raines RT, Burkard ME, and Weaver BA. (2014) Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles, *Science translational medicine 6*, 243-229.

387. Kucuk O, Sarkar FH, Djuric Z, Sakr W, Pollak MN, Khachik F, Banerjee M, Bertram JS, and Wood DP. (2002) Effects of lycopene supplementation in patients with localized prostate cancer, *Exp Biol Med (Maywood) 227*.

388. Sobel RE, and Sadar MD. (2005) Cell lines used in prostate cancer research: a compendium of old and new lines-part 1, *J Urol 173*, 342-359.

389. Fotopoulou C, Vergote I, Mainwaring P, Bidzinski M, Vermorken JB, Ghamande SA, Harnett P, Del Prete SA, Green JA, Spaczynski M, Blagden S, Gore M, Ledermann J, Kaye S, and Gabra H. (2014) Weekly AUC2 carboplatin in acquired platinum-resistant ovarian cancer with or without oral phenoxodiol, a sensitizer of platinum cytotoxicity: the phase III OVATURE multicenter randomized study, *Annals of Oncology 25*, 160-165.

390. Kelly MG, Mor G, Husband A, O'Malley DM, Baker L, Azodi M, Schwartz PE, and Rutherford TJ. (2011) Phase II Evaluation of Phenoxodiol in Combination With Cisplatin or Paclitaxel in Women With Platinum/Taxane-Refractory/Resistant Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancers, *International Journal of Gynecological Cancer 21*, 633-639.

391. El-Rayes BF, Philip PA, Sarkar FH, Shields AF, Ferris AM, Hess K, Kaseb AO, Javle MM, Varadhachary GR, Wolff RA, and Abbruzzese JL. (2011) A phase II study of isoflavones, erlotinib, and gemcitabine in advanced pancreatic cancer, *Investigational New Drugs 29*, 694-699.

392. Barbara Ann Karmanos Cancer Institute. Gemcitabine Hydrochloride and Genistein in Treating Women With Stage IV Breast Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2005- [cited 2016 June 23]. Available from: https://clinicaltrials.gov/ct2/show/NCT00244933 NLM Identifier: NCT00244933.

393. Lohr JM, Karimi M, Omazic B, Kartalis N, Verbeke CS, Berkenstam A, and Frodin JE. (2016) A phase I dose escalation trial of AXP107-11, a novel multi-component crystalline form of genistein, in combination with gemcitabine in chemotherapy-naive patients with unresectable pancreatic cancer, *Pancreatology*.

394. Oshiro C, Marsh S, McLeod H, Carrillo M, Klein T, and Altman R. (2009) Taxane Pathway, *Pharmacogenetics and genomics 19*, 979-983.

395. Foster BC, Vandenhoek S, Hana J, Krantis A, Akhtar MH, Bryan M, Budzinski JW, Ramputh A, and Arnason JT. (2003) In vitro inhibition of human cytochrome P450-mediated metabolism of marker substrates by natural products, *Phytomedicine 10*, 334-342.

396. Xiao CQ, Chen R, Lin J, Wang G, Chen Y, Tan ZR, and Zhou HH. (2012) Effect of genistein on the activities of cytochrome P450 3A and P-glycoprotein in Chinese healthy participants, *Xenobiotica 42*, 173-178.

397. Osiecki H, Schloss J, Domagala C, and Johnson B (2002) *Cancer: A Nutritional, Biochemical Approach*, Bio Concepts Publishing.

398. Banudevi S, Swaminathan S, and Maheswari KU. (2015) Pleiotropic Role of Dietary Phytochemicals in Cancer: Emerging Perspectives for Combinational Therapy, *Nutr Cancer 67*, 1021-1048.

399. Kumar NB, Hopkins K, Allen K, Riccardi D, Besterman-Dahan K, and Moyers S. (2002) Use of complementary/integrative nutritional therapies during cancer treatment: implications in clinical practice, *Cancer Control 9*, 236-243.

400. Gupta D, Lis CG, Birdsall TC, and Grutsch JF. (2005) The use of dietary supplements in a community hospital comprehensive cancer center: implications for conventional cancer care, *Support Care Cancer 13*, 912-919.

401. Verhoef MJ, Balneaves LG, Boon HS, and Vroegindewey A. (2005) Reasons for and characteristics associated with complementary and alternative medicine use among adult cancer patients: a systematic review, *Integr Cancer Ther 4*, 274-286.

402. Block KI, Koch AC, Mead MN, Tothy PK, Newman RA, and Gyllenhaal C. (2008) Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of the evidence from randomized controlled trials, *Int J Cancer 123*, 1227-1239.

403. Moss RW. (2006) Should Patients Undergoing Chemotherapy and Radiotherapy Be Prescribed Antioxidants?, *Integr Cancer Ther 5*, 63-82.

404. Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, and Blumberg JB. (2008) Should Supplemental Antioxidant Administration Be Avoided During Chemotherapy and Radiation Therapy?, *J Natl Cancer Inst 100*, 773-783.

405. Prasad KN. (2004) Multiple Dietary Antioxidants Enhance the Efficacy of Standard and Experimental Cancer Therapies and Decrease Their Toxicity, *Integr Cancer Ther 3*, 310-322.

406. Simone CB, 2nd, Simone NL, Simone V, and Simone CB. (2007) Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival, part 1, *Altern Ther Health Med 13*, 22-28.

407. Yasueda A, Urushima H, and Ito T. (2016) Efficacy and Interaction of Antioxidant Supplements as Adjuvant Therapy in Cancer Treatment: A Systematic Review, *Integr Cancer Ther 15*, 17-39.

408. Manach C, Williamson G, Morand C, Scalbert A, and Rémésy C. (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, *The American Journal of Clinical Nutrition 81*, 230S-242S.

409. Bosland MC. (2014) Testosterone Treatment Is a Potent Tumor Promoter for the Rat Prostate, *Endocrinology 155*, 4629-4633.

410. Bosland MC. (1999) Use of animal models in defining efficacy of chemoprevention agents against prostate cancer, *Eur Urol 35*, 459-463.

411. McCormick DL, Johnson WD, Bosland MC, Lubet RA, and Steele VE. (2007) Chemoprevention of rat prostate carcinogenesis by soy isoflavones and by Bowman-Birk inhibitor, *Nutr Cancer 57*, 184-193.

412. McCormick DL, Rao KV, Johnson WD, Bosland MC, Lubet RA, and Steele VE. (2010) Null activity of selenium and vitamin e as cancer chemopreventive agents in the rat prostate, *Cancer Prev Res (Phila) 3*, 381-392.

413. Christov KT, Moon RC, Lantvit DD, Boone CW, Steele VE, Lubet RA, Kelloff GJ, and Pezzuto JM. (2002) 9-cis-retinoic acid but not 4-(hydroxyphenyl)retinamide inhibits prostate intraepithelial neoplasia in Noble rats, *Cancer Res 62*, 5178-5182.

414. Fleschhut J, Kratzer F, Rechkemmer G, and Kulling SE. (2006) Stability and biotransformation of various dietary anthocyanins in vitro, *Eur J Nutr 45*, 7-18.

415. Ohio State University Comprehensive Cancer Center. Aborption and Metabolism of Lyophilized Black Raspberry Food Products in Men With Prostate Cancer Undergoing Surgery. In: Clinicaltrials.Gov [internet]. Bethesda, MD: National library of medicine (US). 2013 - [cited 2016 June 25]. Available from: https://clinicaltrials.gov/ct2/show/ NCT01823562 NLM Indentifier: NCT01823562.

416. Gu J, Ahn-Jarvis JH, Riedl KM, Schwartz SJ, Clinton SK, and Vodovotz Y. (2014) Characterization of black raspberry functional food products for cancer prevention human clinical trials, *J Agric Food Chem 62*, 3997-4006.

417. Nowack R. (2008) Review article: cytochrome P450 enzyme, and transport protein mediated herb-drug interactions in renal transplant patients: grapefruit juice, St John's Wort - and beyond!, *Nephrology 13*, 337-347.

418. Di YM, Li CG, Xue CC, and Zhou SF. (2008) Clinical drugs that interact with St. John's wort and implication in drug development, *Curr Pharm Des 14*, 1723-1742.

419. Bressler R. (2006) Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs, *Geriatrics 61*, 12-18.

420. Sobel RE, and Sadar MD. (2005) Cell Lines Used in Prostate Cancer Research: A Compendium of Old and New Lines - Part 1, *Journal of Urology 173*, 342-359.

421. Wang Y, Zhang S, Iqbal S, Chen Z, Wang X, Wang YA, Liu D, Bai K, Ritenour C, Kucuk O, and Wu D. (2014) Pomegranate extract inhibits the bone metastatic growth of human prostate cancer cells and enhances the in vivo efficacy of docetaxel chemotherapy, *Prostate 74*, 497-508.

422. Sobel RE, and Sadar MD. (2005) Cell lines used in prostate cancer research: a compendium of old and new lines-part 2, *The Journal of Urology 173*, 360-372.

423. van Bokhoven A, Varella-Garcia M, Korch C, Johannes WU, Smith EE, Miller HL, Nordeen SK, Miller GJ, and Lucia MS. (2003) Molecular characterization of human prostate carcinoma cell lines, *The Prostate 57*, 205-225.

424. Korenchuk S, Lehr JE, L MC, Lee YG, Whitney S, Vessella R, Lin DL, and Pienta KJ. (2001) VCaP, a cell-based model system of human prostate cancer, *In Vivo 15*, 163-168.

425. Sramkoski RM, Pretlow TG, Giaconia JM, Pretlow TP, Schwartz S, Sy MS, Marengo SR, Rhim JS, Zhang D, and Jacobberger JW. (1999) A new human prostate carcinoma cell line, 22Rv1, *In Vitro Cell Dev Biol Anim 35*, 403-409.

426. Thalmann GN, Anezinis PE, Chang SM, Zhau HE, Kim EE, Hopwood VL, Pathak S, von Eschenbach AC, and Chung LW. (1994) Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer, *Cancer Res 54*, 2577-2581.

427. Wu HC, Hsieh JT, Gleave ME, Brown NM, Pathak S, and Chung LW. (1994) Derivation of androgen-independent human LNCaP prostatic cancer cell sublines: role of bone stromal cells, *International Journal of Cancer 57*, 406-412.

428. Tepper CG, Boucher DL, Ryan PE, Ma A-H, Xia L, Lee L-F, Pretlow TG, and Kung H-J. (2002) Characterization of a Novel Androgen Receptor Mutation in a Relapsed CWR22 Prostate Cancer Xenograft and Cell Line, *Cancer Res 62*, 6606-6614.

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PUBLICATIONS

Peer reviewed journal articles:

Jillian N Eskra, Jan Willem Kuiper, Paul Walden, Maarten C Bosland, Nur Ozten. (2016) Interactive effects of 9-*cis*-Retinoic Acid and Androgens on Proliferation, Differentiation, and Apoptosis of LNCaP Prostate Cancer Cells. **European Journal of Cancer Prevention.** In press.

BM Dietz, GK Hagos, JN Eskra, GT Wijewickrama, JR Anderson, D Nikolic, J Guo, B Wright, S Chen, GF Pauli, RB van Breeman, JL Bolton. (2013) Differential Regulation of Detoxification Enzymes in Hepatic and Mammary Tissue by Hops (Humulus lupulus) in vitro and in vivo. **Molecular Nutrition and Food Research** 57, 1055-1066.

R Tell, CA Rivera, J Eskra, LN Taglia, A Blunier, Q Tian Wang, and RV Benya. (2011) Gastrin-Releasing Peptide Signaling Alters Colon Cancer Invasiveness via Heterochromatin Protein 1Hsβ, **The American Journal of Pathology** 178, 672-678.

Review articles:

Maarten C. Bosland, Nur Ozten, Jillian N. Eskra and Abeer M. Mahmoud. (2015) A perspective on prostate carcinogenesis and chemoprevention. **Current Pharmacology Reports**1,1-8.

Abstracts:

JN Eskra, A Dodge, MC Bosland. (2015) Genistein does not alter the effectiveness of taxane chemotherapeutics on human prostate cancer cells. **Cancer Research** 75, 910.

J Eskra, A Dodge, M Bosland. (2015) Black raspberry constituents and genistein do not alter the effectiveness of taxane chemotherapy in human prostate cancer cells. **The FASEB Journal** 29.

JN Eskra, M Schlicht, MC Bosland. (2014) Effects of black raspberries and their constituents on rat prostate carcinogenesis and human prostate cancer cell growth, **Cancer Research** 74, 2131.

BM Dietz, J Eskra, M Darji, S Chen, F Pauli, and JL Bolton. (2013) Red Clover Exhibits Multifaceted Activity on Breast Cancer Cells. Abstract for poster presentation, **Society of Toxicology Annual Meeting**, San Antonio, Texas.

Maarten C Bosland, Michael Schlicht, Jillian Eskra, Zongfa Liu. (2013) Inhibitory effects of black raspberry consumption on prostate carcinogenesis in rats, **Cancer Research** 73, LB-185.

Jillian Eskra, Maarten Bosland, Nur Ozten. (2013) Effects of 9-cis-retinoic acid on proliferation, differentiation, and apoptosis of LNCaP prostate cancer cells, **Cancer Research** 73, 2585.

B Dietz, G Hagos, J Eskra, L Hemachandra, S Chen, D Nikolic, R van Breemen, G Pauli, N Farnsworth, and J Bolton. (2011) Cancer Preventive Properties of Botanical Dietary Supplements used in Women's Health with an Example of Hops and Black Cohosh, **Planta Medica** 77, S10.

R DeFlorio, J Eskra, D Stone. (2008) Gβ Phosphorylation Plays A Role in Chemotropic Growth. **Yeast Genetics & Molecular Biology Meeting**, Toronto, Canada.

Other publications:

Jillian Eskra. Culture methods for VCaP prostate cancer cells (2015) **Protocol Exchange** DOI:10.1038/protex.2015.077.

PRESENTATIONS

Invited talks:

Anticancer effects of black raspberry constituents and interactions with taxane chemotherapy. **Johns Hopkins University, Department of Urology**. March 2016.

Combination effects of soy and black raspberry polyphenols with taxane chemotherapy. **International Summer School: Plant Derived Polyphenols as Drug Leads.** Technische Universität Dresden, Germany. October 2015.

Conference presentations:

Microtubule polymerizing effects of ellagic acid reduce efficacy of taxane chemotherapy. Poster Presentation. **American Association for Cancer Research Annual Meeting**. New Orleans, Louisiana, 2016.

Genistein does not alter the effectiveness of taxane chemotherapeutics on human prostate cancer cells. Poster Presentation. **American Association for Cancer Research Annual Meeting**. Philadelphia, Pennsylvania, 2015.

Black raspberry constituents and genistein do not alter the effectiveness of taxane chemotherapy in human prostate cancer cells. Poster Presentation. **Experimental Biology Meeting**. Boston, Massachusetts, 2015.

Effects of Black Raspberries and Their Constituents on Rat Prostate Carcinogenesis and Human Prostate Cancer Cell Growth. Poster Presentation. **UIC Cancer Center Research Forum**. Chicago, IL, 2014.

Effects of Black Raspberries and Their Constituents on Rat Prostate Carcinogenesis and Human Prostate Cancer Cell Growth. Poster Presentation. **American Association for Cancer Research Annual Meeting**, San Diego, California, 2014.

Growth inhibitory effects of 9-*cis*-Retinoic Acid on Prostate Cancer Cells. Poster Presentation. **UIC Research Forum**, Chicago, IL, 2013.

Effects of 9-*cis*-Retinoic Acid on Proliferation, Differentiation, and Apoptosis of LNCaP Prostate Cancer Cells. Poster Presentation. **American Association for Cancer Research Annual Meeting**, Washington DC, 2013.

Departmental presentations:

Microtubule polymerizing effects of ellagic acid reduce efficacy of taxane chemotherapy. Pathology Department Works in Progress Seminar, UIC. March 31, 2016.

Effects of soy and black raspberries on the efficacy of docetaxel in prostate cancer cells. Pathology Department Works in Progress Seminar, UIC. September 18, 2014.

Thesis Proposal: Effects of soy and black raspberries on the efficacy of chemotherapy for castrate-resistant prostate cancer. June 12, 2014.

Black raspberry compounds for the prevention and treatment of prostate cancer. Pathology Department Works in Progress Seminar, UIC. June 20, 2013.

TRAINING & RESEARCH SUPPORT

2014 - 2015 **CCTS Pre-Doctoral Education for Clinical and Translational Scientists Fellowship ($30,000)**
Combination effects of genistein and taxane chemotherapy for castrate-resistant prostate cancer
UIC Center for Clinical and Translational Science

2015 **International Summer School Travel Award (€950)**
Technische Universität Dresden

2013 - 2015 **Chancellor's Research Fellowship ($8,000)**
Bioactive constituents of soy and black raspberries as therapeutic agents for prostate cancer
Graduate College, University of Illinois at Chicago

2015, 2014 & 2013 **Graduate Student Presenter Award ($250)**
Graduate College, University of Illinois at Chicago

2015, 2014 & 2013 **Pre-Doctoral Student Travel Grant ($275)**
Graduate Student Council, University of Illinois at Chicago

AWARDS & HONORS

2015 **Graduate Research Award, Honorable Mention**
Women in Science and Engineering (WISE), UIC

2015 **Chancellor's Student Service & Leadership Award**
University of Illinois at Chicago

2008 **Departmental Distinction in Biological Sciences**
College of Liberal Arts and Sciences, University of Illinois at Chicago

INSTITUTIONAL SERVICE & PROFESSIONAL ACTIVITIES

2014 – 2015	**President**, Graduate Education in Medical Sciences Student Association (GEMSSA), College of Medicine, University of Illinois at Chicago
2015	**Event Coordinator***,* Pint of Science International Festival, Chicago, IL
2014 – 2016	**Regional Science Fair Judge**, Chicago Public Schools, Chicago, IL
2014 – 2015	**Senator**, University of Illinois at Chicago Senate
2014 – 2016	**Peer Reviewer***,* UIC Interdisciplinary Undergraduate Research Journal
2013 – 2014	**Treasurer,** Graduate Education in Medical Sciences Student Association (GEMSSA), College of Medicine, University of Illinois at Chicago
2013 – 2016	**Founder & Member,** Graduate Education in Medical Sciences Student Association (GEMSSA), College of Medicine, University of Illinois at Chicago
2013 – 2015	**Departmental Representative,** Graduate Student Council, Graduate College, University of Illinois at Chicago

TEACHING & MENTORSHIP

Teaching experience:

April 2016	**Guest Lecturer "Dietary Supplements & Chemoprevention"** Pathobiology of Cancer, University of Illinois at Chicago

Student mentorship:

2014 – 2015	Alaina Dodge, undergraduate student
2010 – 2011	Monika Darji, undergraduate student
2010	Cody Vitek, high school student

PROFESSIONAL AFFILIATIONS

American Association for Cancer Research (AACR)

2012 – Present	Associate Member
2012 – Present	Women in Cancer Research Member

American Society for Nutrition (ASN)

2014 – 2016	Student Member