

## Adjuvant Prostate Cancer Treatment Strategies

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The following table categorizes suggested adjuvant measures for prostate cancer control with respect to their likely utility as retardants of cancer growth and spread, as adjuvants to chemotherapy, and recommended priority of use. Priority 1 therapies are suggested as first-line measures, feasible for continuing use after diagnosis; some of these have potential for boosting the efficacy of chemotherapy if this ultimately becomes necessary. Suggested dose schedules are provided for these agents; these are provisional and may change in light of future research. Priority 2 measures can be considered for future use if chemotherapy is required – particularly if chemoresistance develops. I.v. ascorbate can be used alone or as an adjuvant to chemotherapy in progressive cancer. Most priority 2 agents, as well as metformin and salsalate, require a prescription, and doctor supervision is mandatory; your doctor could determine dosing after reviewing the pertinent literature. Doctor approval of low-dose aspirin is also wise, and use of spirulina at the same time as chemotherapy or i.v. ascorbate is not recommended. Importantly, these measures should be considered as adjuvants to, *not substitutes for*, clinically proven strategies such as surgery/radiotherapy, androgen antagonism, docetaxel chemotherapy, bisphosphonates, and approved immunotherapies. Abstracts of pertinent research are appended below. This list does not pretend to be exhaustive.

	Growth Control	Chemo Adjuvant	Priority	Suggested Dose
Metformin	X	X	1	500 mg 3 times daily
Pomegranate Juice	X	?	1	1-2 cups daily
Lycopene	X	X	1	15 mg twice daily
Fish Omega-3	X		1	2-4 g daily
Green Tea Catechins	X	X	1	250 mg twice daily
Melatonin	X	X	1	10-20 mg at bedtime
Low-Dose Aspirin	X		1	81 mg daily
Whole-Food Vegan Diet	X		1	
Vitamin D	X		1	5,000-10,000 IU daily
Spirulina	X	--	1	15-30 g daily
Grape Seed Extract	X		1	200 mg twice daily
NF-kappaB Antagonists	X	X	1	Salsalate – 1500 mg twice daily

Soy Isoflavones	X		1	100 mg daily
DIM	X	X	1	300 mg twice daily
Tocotrienols	X	X	1	125 mg twice daily
Modified Citrus Pectin	X		1	Pecta-SolC 5 g, 3 times daily
GcMAF	X		1	100 ng i.m.once weekly
I.V. Ascorbate	X	X	2	
Valproate	X	X	2	
Nelfinavir		X	2	
Hydroxychloroquine		X	2	
Itraconazole	X	X	2	
Met Cyclophosphamide	X		2	
Selenate	X		2	

## Metformin

Nobes JP, Langley SE, Klopper T, Russell-Jones D, Laing RW. A prospective, randomized pilot study evaluating the effects of metformin and lifestyle intervention on patients with prostate cancer receiving androgen deprivation therapy. *BJU Int* 2012 May;109(10):1495-502.

Abstract: Study Type - Therapy (RCT) Level of Evidence 1b What's known on the subject? and What does the study add? Men with prostate cancer have higher rates of non-cancer mortality and CV morbidity and some of that excess risk has been attributed to the treatment they receive. ADT is an established treatment option for men with locally-advanced and metastatic prostate cancer and, although it has been shown to confer a disease-free survival advantage, it has also been associated with an increased incidence of CV disease and the metabolic syndrome (characterized by a cluster of CV risk factors, including insulin resistance). The benefits of the insulin sensitizer metformin and lifestyle intervention for reducing the incidence of metabolic syndrome have been shown in patients with impaired glucose tolerance. At the time of writing, the present study is the first to use metformin and lifestyle intervention in men with prostate cancer with the aim of reducing the risk of developing ADT-related CV morbidity and the metabolic syndrome. The study shows that lifestyle changes and metformin may indeed reduce the complications of androgen suppression in these men. Although further investigations are needed to establish which of the two interventions may be most beneficial, the favourable effects of a combination of these interventions on patients' quality of life and the potential for improved overall survival are of clinical significance. OBJECTIVE: To investigate the effects of metformin and lifestyle changes on the development of androgen deprivation therapy (ADT)-related metabolic syndrome. PATIENTS AND METHODS: Men with prostate cancer due to receive ADT were recruited and randomized. Controls received ADT alone. Men in the intervention arm received ADT with 6 months of metformin, a low glycaemic index diet and an exercise

programme. All patients were investigated pretreatment and at 6 months for the metabolic syndrome, as well as for related biochemical and physical parameters. RESULTS: In total, 40 men were recruited and randomized (20 to each arm). After 6 months, significant improvements in abdominal girth ( $P=0.05$ ), weight ( $P < 0.001$ ), body mass index ( $P < 0.001$ ) and systolic blood pressure ( $P=0.01$ ) were seen in the intervention arm compared to controls. Biochemical markers of insulin resistance did not differ significantly. CONCLUSIONS: The present study shows the potential benefits of metformin and lifestyle changes in ADT-treated men. Further studies will aim to determine which intervention is most important, and may show that overall survival can be improved

Clements A, Gao B, Yeap SH, Wong MK, Ali SS, Gurney H. Metformin in prostate cancer: two for the price of one. *Ann Oncol* 2011 December;22(12):2556-60.

Abstract: BACKGROUND: Androgen deprivation therapy (ADT) for prostate cancer treatment induces a metabolic syndrome, which may contribute to non-cancer-related morbidity and mortality. Metformin may abrogate these effects. Additionally, metformin has potential antineoplastic activity in various malignancies including prostate cancer. MATERIALS AND METHODS: A literature review using PubMed with the keywords: AMPK, androgen deprivation therapy, insulin resistance, metabolic syndrome, metformin and prostate cancer was undertaken. RESULTS: This overview will look at the current evidence linking ADT and metabolic syndrome while discussing ongoing clinical trials under way assessing the effectiveness of metformin in abrogating these effects. The potential antineoplastic activity of metformin, mediated by multiple proposed mechanisms based on evidence from preclinical and clinical studies, will also be elucidated in this review. CONCLUSIONS: Overall available data support the potential dual benefit of metformin on ADT-induced metabolic syndrome and in its antineoplastic activity in prostate cancer, justifying the need for ongoing clinical trials to confirm these effects as the evidence currently available for standard practice is lacking

Iliopoulos D, Hirsch HA, Struhl K. Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. *Cancer Res* 2011 May 1;71(9):3196-201.

Abstract: Metformin, the first-line drug for treating diabetes, selectively kills the chemotherapy resistant subpopulation of cancer stem cells (CSC) in genetically distinct types of breast cancer cell lines. In mouse xenografts, injection of metformin and the chemotherapeutic drug doxorubicin near the tumor is more effective than either drug alone in blocking tumor growth and preventing relapse. Here, we show that metformin is equally effective when given orally together with paclitaxel, carboplatin, and doxorubicin, indicating that metformin works together with a variety of standard chemotherapeutic agents. In addition, metformin has comparable effects on tumor regression and preventing relapse when combined with a four-fold reduced dose of doxorubicin that is not effective as a monotherapy. Finally, the combination of metformin and doxorubicin prevents relapse in xenografts generated with prostate and lung cancer cell lines. These observations provide further evidence for the CSC hypothesis for cancer relapse, an experimental rationale for using metformin as part of combinatorial therapy in a variety of clinical settings, and for reducing the chemotherapy dose in cancer patients

Colquhoun AJ, Venier NA, Vandersluis AD et al. Metformin enhances the antiproliferative and apoptotic effect of bicalutamide in prostate cancer. *Prostate Cancer Prostatic Dis* 2012 May 22.

Abstract: Background: Prostate cancer incidence and mortality vary dramatically by geographical location. Both are higher in developed countries. Some attribute this to westernized lifestyles of high-energy diets and limited physical activity with consequent obesity. Obesity and obesity-related diseases like diabetes cause hyperinsulinaemia, which upregulates pro-survival cell signalling.

Previous work revealed diet-induced hyperinsulinaemia enhances prostate cancer xenograft growth in vivo. Metformin, an antidiabetic medication, reduces hyperinsulinaemia and also exhibits antineoplastic properties. Herein, we assess the potential additive benefit of combining bicalutamide antiandrogen therapy with metformin, in vitro and in vivo. Methods: Using clonogenic assays, we assessed the effect of bicalutamide and/or metformin on clonogenicity in prostate cancer cell lines. Western blot and cell cycle analyses were used to elucidate mechanisms of interaction between the drugs in androgen receptor (AR)-positive (LNCaP) and AR-negative (PC3) cell lines. The combination treatment regimen was assessed in vivo using an LNCaP murine xenograft model. Results: Micromolar bicalutamide or millimolar metformin caused a significant dose-dependent reduction in clonogenicity ( $P < 0.001$ ). Combination treatment further significantly reduced clonogenicity ( $P < 0.005$ ) with greater effects in AR-positive cells. Western blot and cell cycle analyses suggested differing mechanisms of interaction in AR-positive and -negative cell lines. Following combination treatment, LNCaP cells exhibited an altered cell proliferation (decreased phospho mammalian target of rapamycin expression) and perturbed cell cycle kinetics (G1/S cell cycle arrest). PC3 cells showed evidence of enhanced apoptosis (increased Bcl-2-associated X protein and decreased total caspase 3 expression). Markedly diminished tumour growth occurred following combination treatment in vivo ( $P < 0.001$ ). Conclusion: Combining bicalutamide and metformin significantly reduces prostate cancer cell growth further than either monotherapy. In AR-positive cells, this effect appeared to be mediated by reducing proliferation rates, whereas in AR-negative cells the combination treatment appeared to promote apoptosis. This combination drug regimen may improve prostate-cancer-specific survival by the direct antineoplastic properties outlined. Prostate Cancer and Prostatic Diseases advance online publication, 22 May 2012; doi:10.1038/pcan.2012.16

## Pomegranate Juice

Paller CJ, Ye X, Wozniak PJ et al. A randomized phase II study of pomegranate extract for men with rising PSA following initial therapy for localized prostate cancer. *Prostate Cancer Prostatic Dis* 2012 June 12.

Abstract: Background: Pomegranate juice has been associated with PSA doubling time (PSADT) elongation in a single-arm phase II trial. This study assesses biological activity of two doses of pomegranate extract (POMx) in men with recurrent prostate cancer, using changes in PSADT as the primary outcome. Methods: This randomized, multi-center, double-blind phase II, dose-exploring trial randomized men with a rising PSA and without metastases to receive 1 or 3 g of POMx, stratified by baseline PSADT and Gleason score. Patients (104) were enrolled and treated for up to 18 months. The intent-to-treat (ITT) population was 96% white, with median age 74.5 years and median Gleason score 7. This study was designed to detect a 6-month on-study increase in PSADT from baseline in each arm. Results: Overall, median PSADT in the ITT population lengthened from 11.9 months at baseline to 18.5 months after treatment ( $P < 0.001$ ). PSADT lengthened in the low-dose group from 11.9 to 18.8 months and 12.2 to 17.5 months in the high-dose group, with no significant difference between dose groups ( $P = 0.554$ ). PSADT increases  $> 100\%$  of baseline were observed in 43% of patients. Declining PSA levels were observed in 13 patients (13%). In all, 42% of patients discontinued treatment before meeting the protocol-definition of PSA progression, or 18 months, primarily due to a rising PSA. No significant changes occurred in testosterone. Although no clinically significant toxicities were seen, diarrhea was seen in 1.9% and 13.5% of patients in the 1- and 3-g dose groups, respectively. CONCLUSIONS: POMx treatment was associated with  $\geq 6$  month increases in PSADT in both treatment arms without adverse effects. The significance of this on-study slowing of PSADT remains unclear, reinforcing the need for placebo-controlled studies in this patient population. Prostate Cancer and Prostatic Diseases advance online publication, 12 June 2012; doi:10.1038/pcan.2012.20

Wang L, Alcon A, Yuan H, Ho J, Li QJ, Martins-Green M. Cellular and molecular mechanisms of pomegranate juice-induced anti-metastatic effect on prostate cancer cells. *Integr Biol (Camb)* 2011 July;3(7):742-54.

Abstract: Prostate cancer is the second leading cause of cancer-related deaths among US males. Pomegranate juice (PJ), a natural product, was shown in a clinical trial to inhibit progression of this disease. However, the underlying mechanisms involved in the anti-progression effects of PJ on prostate cancer remain unclear. Here we show that, in addition to causing cell death of hormone-refractory prostate cancer cells, PJ also increases cell adhesion and decreases cell migration of the cells that do not die. We hypothesized that PJ does so by stimulating the expression and/or activation of molecules that alter the cytoskeleton and the adhesion machinery of prostate cancer cells, resulting in enhanced cell adhesion and reduced cell migration. We took an integrative approach to these studies by using Affimetrix gene arrays to study gene expression, microRNA arrays to study the non-coding RNAs, molecules known to be dysregulated in cancer cells, and Luminex Multiplex array assays to study the level of secreted pro-inflammatory cytokines/chemokines. PJ up-regulates genes involved in cell adhesion such as E-cadherin, intercellular adhesion molecule 1 (ICAM-1) and down-regulates genes involved in cell migration such as hyaluronan-mediated motility receptor (HMMR) and type I collagen. In addition, anti-invasive microRNAs such as miR-335, miR-205, miR-200, and miR-126, were up-regulated, whereas pro-invasive microRNA such as miR-21 and miR-373, were down-regulated. Moreover, PJ significantly reduced the level of secreted pro-inflammatory cytokines/chemokines such as IL-6, IL-12p40, IL-1beta and RANTES, thereby having the potential to decrease inflammation and its impact on cancer progression. PJ also inhibits the ability of the chemokine SDF1alpha to chemoattract these cancer cells. SDF1alpha and its receptor CXCR4 are important in metastasis of cancer cells to the bone. Discovery of the mechanisms by which this enhanced adhesion and reduced migration are accomplished can lead to sophisticated and effective prevention of metastasis in prostate and potentially other cancers

Jiang J, Eliaz I, Sliva D. Suppression of growth and invasive behavior of human prostate cancer cells by ProstaCaid: mechanism of activity. *Int J Oncol* 2011 June;38(6):1675-82.

Abstract: Since the use of dietary supplements as alternative treatments or adjuvant therapies in cancer treatment is growing, a scientific verification of their biological activity and the detailed mechanisms of their action are necessary for the acceptance of dietary supplements in conventional cancer treatments. In the present study we have evaluated the anti-cancer effects of dietary supplement ProstaCaid (PC) which contains mycelium from medicinal mushrooms (*Ganoderma lucidum*, *Coriolus versicolor*, *Phellinus linteus*), saw palmetto berry, pomegranate, pumpkin seed, green tea [40% epigallocatechin-3-gallate (EGCG)], Japanese knotweed (50% resveratrol), extracts of turmeric root (BCM-95(R)), grape skin, pygeum bark, sarsaparilla root, *Scutellaria barbata*, eleuthero root, Job's tears, astragalus root, skullcap, dandelion, coptis root, broccoli, and stinging nettle, with purified vitamin C, vitamin D3, selenium, quercetin, citrus bioflavonoid complex, beta sitosterol, zinc, lycopene, alpha lipoic acid, boron, berberine and 3,3'-diindolylmethane (DIM). We show that PC treatment resulted in the inhibition of cell proliferation of the highly invasive human hormone refractory (independent) PC-3 prostate cancer cells in a dose- and time-dependent manner with IC50 56.0, 45.6 and 39.0 microg/ml for 24, 48 and 72 h, respectively. DNA-microarray analysis demonstrated that PC inhibits proliferation through the modulation of expression of *CCND1*, *CDK4*, *CDKN1A*, *E2F1*, *MAPK6* and *PCNA* genes. In addition, PC also suppresses metastatic behavior of PC-3 by the inhibition of cell adhesion, cell migration and cell invasion, which was associated with the down-regulation of expression of *CAV1*, *IGF2*, *NR2F1*, and *PLAU* genes and suppressed secretion of the urokinase plasminogen activator (uPA) from PC-3 cells. In conclusion, the dietary supplement PC is a promising natural complex with the potency to inhibit invasive human prostate cancer

Gasmi J, Sanderson JT. Growth Inhibitory, Antiandrogenic, and Pro-apoptotic Effects of Punicic Acid in LNCaP Human Prostate Cancer Cells. *J Agric Food Chem* 2010 November 10.

Abstract: Prostate cancer is a commonly diagnosed cancer in men, and dietary chemoprevention by pomegranate (*Punica granatum*) extracts has shown noticeable benefits. In this study, we investigated the growth inhibitory, antiandrogenic, and pro-apoptotic effects of 13 pure compounds found in the pomegranate in androgen-dependent LNCaP human prostate cancer cells. Cells deprived of steroid hormones were exposed to increasing concentrations (1-100  $\mu\text{M}$ ) of pomegranate compounds in the presence of 0.1 nM dihydrotestosterone (DHT), and the inhibition of cell growth was measured by WST-1 colorimetric assay after a 4 day exposure. Four compounds, epigallocatechin gallate (EGCG), delphinidin chloride, kaempferol, and puniceic acid, were found to inhibit DHT-stimulated cell growth at concentrations of 10  $\mu\text{M}$  and above. These four pomegranate compounds inhibited DHT-stimulated androgen receptor nuclear accumulation and the expression of the androgen receptor-dependent genes prostate specific antigen and steroid 5 $\alpha$ -reductase type 1 at concentrations  $\geq 10$   $\mu\text{M}$ . We determined the possible contribution of apoptosis to the observed decrease in cell growth and found that three compounds, EGCG, kaempferol, and, in particular, puniceic acid, induced DNA fragmentation after a 24 h treatment, at concentrations in the 10-100  $\mu\text{M}$  range. Puniceic acid, an important fatty acid in pomegranate seeds, was further found to induce intrinsic apoptosis via a caspase-dependent pathway. In conclusion, puniceic acid, the main constituent of pomegranate seed (70-80%), exhibited potent growth inhibitory activities in androgen-dependent LNCaP cells, which appear to be mediated by both antiandrogenic and pro-apoptotic mechanisms

Koyama S, Cobb LJ, Mehta HH et al. Pomegranate extract induces apoptosis in human prostate cancer cells by modulation of the IGF-IGFBP axis. *Growth Horm IGF Res* 2010 February;20(1):55-62.

Abstract: The IGF axis is critical for the regulation of apoptosis in many human cancer cell lines. Recently, potent anti-tumorigenic effects of pomegranate juice and extracts have been reported. Consequently, pomegranate has potential not only as a treatment but also as a preventative measure against certain types of cancer, including prostate. In this study, we investigated the relationship between pomegranate-induced apoptosis in human prostate cancer cells and the IGF/IGFBP system. Treatment of LAPC4 prostate cancer cells with 10  $\mu\text{g}/\text{ml}$  POMx, a highly potent pomegranate extract prepared from skin and arils minus seeds and standardized to ellagitannin content (37% punicalagins by HPLC), resulted in inhibition of cell proliferation and induction of apoptosis. Interestingly, co-treatment with POMx and IGFBP-3 revealed synergistic stimulation of apoptosis and additive inhibition of cell growth. Western blot analysis revealed that treatment with POMx or POMx/IGFBP-3 combination resulted in increased JNK phosphorylation, and decreased Akt and mTOR activation, consistent with a growth inhibitory, pro-apoptotic function. We also investigated the relationship between IGF-1 and pomegranate-induced apoptosis in 22RV1 prostate cancer cells. Co-treatment with 100  $\text{ng}/\text{ml}$  IGF-1 completely blocked apoptosis induction by POMx. In contrast, IGF-1 failed to inhibit POMx-induced apoptosis in R(-) cells, suggesting the importance of IGF-IR. POMx-treatment decreased Igf1 mRNA expression in a dose-dependent manner indicating that its actions also involve tumor-specific suppression of IGF-1. These studies revealed novel interactions between the IGF system and pomegranate-induced apoptosis

Rettig MB, Heber D, An J et al. Pomegranate extract inhibits androgen-independent prostate cancer growth through a nuclear factor-kappaB-dependent mechanism. *Mol Cancer Ther* 2008 September;7(9):2662-71.

Abstract: Constitutive nuclear factor-kappaB (NF-kappaB) activation is observed in androgen-independent prostate cancer and represents a predictor for biochemical recurrence after radical

prostatectomy. Dietary agents such as pomegranate extract (PE) have received increasing attention as potential agents to prevent the onset or progression of many malignancies, including prostate cancer. Here, we show that PE inhibited NF-kappaB and cell viability of prostate cancer cell lines in a dose-dependent fashion in vitro. Importantly, maximal PE-induced apoptosis was dependent on PE-mediated NF-kappaB blockade. In the LAPC4 xenograft model, PE delayed the emergence of LAPC4 androgen-independent xenografts in castrated mice through an inhibition of proliferation and induction of apoptosis. Moreover, the observed increase in NF-kappaB activity during the transition from androgen dependence to androgen independence in the LAPC4 xenograft model was abrogated by PE. Our study represents the first description of PE as a promising dietary agent for the prevention of the emergence of androgen independence that is driven in part by heightened NF-kappaB activity

Heber D. Multitargeted therapy of cancer by ellagitannins. *Cancer Lett* 2008 October 8;269(2):262-8.

Abstract: Ellagitannins are bioactive polyphenols that have antioxidant and anti-inflammatory bioactivities. Pomegranate juice has the highest concentration of ellagitannins of any commonly consumed juice and contains the unique ellagitannin, punicalagin. Punicalagin is the largest molecular weight polyphenol known. Ellagitannins are not absorbed intact into the blood stream but are hydrolyzed to ellagic acid. They are also metabolized by gut flora into urolithins which are conjugated in the liver and excreted in the urine. These urolithins are also bioactive and inhibit prostate cancer cell growth. Inhibition of Nuclear Factor Kappa-B activation has been shown in prostate cancer cells and in human prostate cancer xenografts in mice. In clinical studies, pomegranate juice administration led to a decrease in the rate of rise of Prostate Specific Antigen after primary treatment with surgery or radiation. Continued translational research on the chemopreventive potential of pomegranate ellagitannins is ongoing

Sartippour MR, Seeram NP, Rao JY et al. Ellagitannin-rich pomegranate extract inhibits angiogenesis in prostate cancer in vitro and in vivo. *Int J Oncol* 2008 February;32(2):475-80.

Abstract: Angiogenesis is critical to tumor growth and is stimulated by tissue hypoxia due to poor oxygen delivery. In turn, cellular hypoxia leads to angiogenesis via the induction of hypoxia-inducible factor-1alpha (HIF-1alpha) and vascular endothelial growth factor (VEGF) at a cellular level. Pomegranate juice and extracts, which are rich sources of ellagitannins, have been shown to have chemopreventive potential against prostate cancer, but there have been no studies on the effects of an ellagitannin-rich pomegranate extract on angiogenesis. Human prostate cancer cells (LNCaP) and human umbilical vein endothelial cells (HUVEC) were incubated with a pomegranate extract standardized to ellagitannin content (POMx), under normoxic and hypoxic conditions in vitro. Human prostate cancer cells (LAPC4) were injected subcutaneously into severe combined immunodeficient (SCID) mice and the effects of oral administration of POMx on tumor growth, microvessel density, and HIF-1alpha and VEGF expression were determined after 4 weeks of treatment. POMx inhibited the proliferation of LNCaP and HUVEC cells significantly under both normoxic and hypoxic conditions. HIF-1alpha and VEGF protein levels were also reduced by POMx under hypoxic conditions. POMx decreased prostate cancer xenograft size, tumor vessel density, VEGF peptide levels and HIF-1alpha expression after 4 weeks of treatment in SCID mice. These results demonstrate that an ellagitannin-rich pomegranate extract can inhibit tumor-associated angiogenesis as one of several potential mechanisms for slowing the growth of prostate cancer in chemopreventive applications. Further studies in humans are needed to confirm that angiogenesis can be inhibited by an ellagitannin-rich pomegranate extract administered orally as a dietary supplement

Bell C, Hawthorne S. Ellagic acid, pomegranate and prostate cancer -- a mini review. *J Pharm Pharmacol* 2008 February;60(2):139-44.

Abstract: There is currently a shifting focus towards finding natural compounds that may prevent or treat cancer, due to the problems that exist with current chemotherapeutic regimens. The fruit of the *Punica granatum* (pomegranate) contains hundreds of phytochemicals and pomegranate extracts have recently been shown to exhibit antioxidant properties, thought to be due to the action of ellagic acid, the main polyphenol in pomegranate. In this mini review the effects of pomegranate extracts and ellagic acid on the proliferation of prostate cancer cells and their future potential are discussed

Seeram NP, Aronson WJ, Zhang Y et al. Pomegranate ellagitannin-derived metabolites inhibit prostate cancer growth and localize to the mouse prostate gland. *J Agric Food Chem* 2007 September 19;55(19):7732-7.

Abstract: Our group has shown in a phase II clinical trial that pomegranate juice (PJ) increases prostate specific antigen (PSA) doubling time in prostate cancer (CaP) patients with a rising PSA. Ellagitannins (ETs) are the most abundant polyphenols present in PJ and contribute greatly towards its reported biological properties. On consumption, ETs hydrolyze to release ellagic acid (EA), which is then converted by gut microflora to 3,8-dihydroxy-6H-dibenzo[b, d]pyran-6-one (uroolithin A, UA) derivatives. Despite the accumulating knowledge of ET metabolism in animals and humans, there is no available data on the pharmacokinetics and tissue disposition of urolithins. Using a standardized ET-enriched pomegranate extract (PE), we sought to further define the metabolism and tissue distribution of ET metabolites. PE and UA (synthesized in our laboratory) were administered to C57BL/6 wild-type male mice, and metabolite levels in plasma and tissues were determined over 24 h. ET metabolites were concentrated at higher levels in mouse prostate, colon, and intestinal tissues as compared to other tissues after administration of PE or UA. We also evaluated the effects of PE on CaP growth in severe combined immunodeficient (SCID) mice injected subcutaneously with human CaP cells (LAPC-4). PE significantly inhibited LAPC-4 xenograft growth in SCID mice as compared to vehicle control. Finally, EA and several synthesized urolithins were shown to inhibit the growth of human CaP cells in vitro. The chemopreventive potential of pomegranate ETs and localization of their bioactive metabolites in mouse prostate tissue suggest that pomegranate may play a role in CaP treatment and chemoprevention. This warrants future human tissue bioavailability studies and further clinical studies in men with CaP

Pantuck AJ, Leppert JT, Zomorodian N et al. Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer. *Clin Cancer Res* 2006 July 1;12(13):4018-26.

Abstract: PURPOSE: Phytochemicals in plants may have cancer preventive benefits through antioxidation and via gene-nutrient interactions. We sought to determine the effects of pomegranate juice (a major source of antioxidants) consumption on prostate-specific antigen (PSA) progression in men with a rising PSA following primary therapy. EXPERIMENTAL DESIGN: A phase II, Simon two-stage clinical trial for men with rising PSA after surgery or radiotherapy was conducted. Eligible patients had a detectable PSA > 0.2 and < 5 ng/mL and Gleason score < or = 7. Patients were treated with 8 ounces of pomegranate juice daily (Wonderful variety, 570 mg total polyphenol gallic acid equivalents) until disease progression. Clinical end points included safety and effect on serum PSA, serum-induced proliferation and apoptosis of LNCaP cells, serum lipid peroxidation, and serum nitric oxide levels. RESULTS: The study was fully accrued after efficacy criteria were met. There were no serious adverse events reported and the treatment was well tolerated. Mean PSA doubling time significantly increased with treatment from a mean of 15 months at baseline to 54 months posttreatment ( $P < 0.001$ ). In vitro assays comparing pretreatment and posttreatment patient serum on the growth of LNCaP showed a 12% decrease in cell proliferation and a 17% increase in apoptosis ( $P = 0.0048$  and  $0.0004$ , respectively), a 23% increase in serum nitric oxide ( $P = 0.0085$ ), and significant ( $P < 0.02$ ) reductions in oxidative state and sensitivity to oxidation of serum lipids after versus before

pomegranate juice consumption. CONCLUSIONS: We report the first clinical trial of pomegranate juice in patients with prostate cancer. The statistically significant prolongation of PSA doubling time, coupled with corresponding laboratory effects on prostate cancer in vitro cell proliferation and apoptosis as well as oxidative stress, warrant further testing in a placebo-controlled study

Pantuck AJ, Zomorodian N, Belldegrun AS. Phase-II Study of pomegranate juice for men with prostate cancer and increasing PSA. *Curr Urol Rep* 2006 January;7(1):7.

Malik A, Afaq F, Sarfaraz S, Adhami VM, Syed DN, Mukhtar H. Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proc Natl Acad Sci U S A* 2005 October 11;102(41):14813-8.

Abstract: Prostate cancer is the most common invasive malignancy and the second leading cause of cancer-related deaths among U.S. males, with a similar trend in many Western countries. One approach to control this malignancy is its prevention through the use of agents present in diet consumed by humans. Pomegranate from the tree *Punica granatum* possesses strong antioxidant and antiinflammatory properties. We recently showed that pomegranate fruit extract (PFE) possesses remarkable antitumor-promoting effects in mouse skin. In this study, employing human prostate cancer cells, we evaluated the antiproliferative and proapoptotic properties of PFE. PFE (10-100 microg/ml; 48 h) treatment of highly aggressive human prostate cancer PC3 cells resulted in a dose-dependent inhibition of cell growth/cell viability and induction of apoptosis. Immunoblot analysis revealed that PFE treatment of PC3 cells resulted in (i) induction of Bax and Bak (proapoptotic); (ii) down-regulation of Bcl-X(L) and Bcl-2 (antiapoptotic); (iii) induction of WAF1/p21 and KIP1/p27; (iv) a decrease in cyclins D1, D2, and E; and (v) a decrease in cyclin-dependent kinase (cdk) 2, cdk4, and cdk6 expression. These data establish the involvement of the cyclin kinase inhibitor-cyclin-cdk network during the antiproliferative effects of PFE. Oral administration of PFE (0.1% and 0.2%, wt/vol) to athymic nude mice implanted with androgen-sensitive CWR22Rnu1 cells resulted in a significant inhibition in tumor growth concomitant with a significant decrease in serum prostate-specific antigen levels. We suggest that pomegranate juice may have cancer-chemopreventive as well as cancer-chemotherapeutic effects against prostate cancer in humans

Seeram NP, Adams LS, Henning SM et al. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J Nutr Biochem* 2005 June;16(6):360-7.

Abstract: Pomegranate (*Punica granatum* L.) fruits are widely consumed as juice (PJ). The potent antioxidant and anti-atherosclerotic activities of PJ are attributed to its polyphenols including punicalagin, the major fruit ellagitannin, and ellagic acid (EA). Punicalagin is the major antioxidant polyphenol ingredient in PJ. Punicalagin, EA, a standardized total pomegranate tannin (TPT) extract and PJ were evaluated for in vitro antiproliferative, apoptotic and antioxidant activities. Punicalagin, EA and TPT were evaluated for antiproliferative activity at 12.5-100 microg/ml on human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620) and prostate (RWPE-1, 22Rv1) tumor cells. Punicalagin, EA and TPT were evaluated at 100 microg/ml concentrations for apoptotic effects and at 10 microg/ml concentrations for antioxidant properties. However, to evaluate the synergistic and/or additive contributions from other PJ phytochemicals, PJ was tested at concentrations normalized to deliver equivalent amounts of punicalagin (w/w). Apoptotic effects were evaluated against the HT-29 and HCT116 colon cancer cell lines. Antioxidant effects were evaluated using inhibition of lipid peroxidation and Trolox equivalent antioxidant capacity (TEAC) assays. Pomegranate juice showed greatest antiproliferative activity against all cell lines by inhibiting proliferation from 30% to 100%.

At 100 microg/ml, PJ, EA, punicalagin and TPT induced apoptosis in HT-29 colon cells. However, in the HCT116 colon cells, EA, punicalagin and TPT but not PJ induced apoptosis. The trend in antioxidant activity was PJ>TPT>punicalagin>EA. The superior bioactivity of PJ compared to its purified polyphenols illustrated the multifactorial effects and chemical synergy of the action of multiple compounds compared to single purified active ingredients

Lansky EP, Harrison G, Froom P, Jiang WG. Pomegranate (*Punica granatum*) pure chemicals show possible synergistic inhibition of human PC-3 prostate cancer cell invasion across Matrigel. *Invest New Drugs* 2005 March;23(2):121-2.

Abstract: Four pure chemicals, ellagic acid (E), caffeic acid (C), luteolin (L) and punicalagin (P), all important components of the aqueous compartments or oily compartment of pomegranate fruit (*Punica granatum*), and each belonging to different representative chemical classes and showing known anticancer activities, were tested as potential inhibitors of in vitro invasion of human PC-3 prostate cancer cells in an assay employing Matrigel artificial membranes. All compounds significantly inhibited invasion when employed individually. When C, P, and L were equally combined at the same gross dosage (4 microg/ml) as when the compounds were tested individually, a supraditive inhibition of invasion was observed, measured by the Kruskal-Wallis non-parametric test

Albrecht M, Jiang W, Kumi-Diaka J et al. Pomegranate extracts potently suppress proliferation, xenograft growth, and invasion of human prostate cancer cells. *J Med Food* 2004;7(3):274-83.

Abstract: We completed a multicenter study of the effects of pomegranate cold-pressed (Oil) or supercritical CO<sub>2</sub>-extracted (S) seed oil, fermented juice polyphenols (W), and pericarp polyphenols (P) on human prostate cancer cell xenograft growth in vivo, and/or proliferation, cell cycle distribution, apoptosis, gene expression, and invasion across Matrigel, in vitro. Oil, W, and P each acutely inhibited in vitro proliferation of LNCaP, PC-3, and DU 145 human cancer cell lines. The dose of P required to inhibit cell proliferation of the prostate cancer cell line LNCaP by 50% (ED<sub>50</sub>) was 70 microg/mL, whereas normal prostate epithelial cells (hPrEC) were significantly less affected (ED<sub>50</sub> = 250 g/mL). These effects were mediated by changes in both cell cycle distribution and induction of apoptosis. For example, the androgen-independent cell line DU 145 showed a significant increase from 11% to 22% in G<sub>2</sub>/M cells (P <.05) by treatment with Oil (35 microg/mL) with a modest induction of apoptosis. In other cell lines/treatments, the apoptotic response predominated, for example, in PC-3 cells treated with P, at least partially through a caspase 3-mediated pathway. These cellular effects coincided with rapid changes in mRNA levels of gene targets. Thus, 4-hour treatment of DU 145 cells with Oil (35 microg/mL) resulted in significant 2.3 +/- 0.001-fold (mean +/- SEM) up-regulation of the cyclin-dependent kinase inhibitor p21((waf1/cip1)) (P <.01) and 0.6 +/- 0.14-fold down-regulation of c-myc (P <.05). In parallel, all agents potently suppressed PC-3 invasion through Matrigel, and furthermore P and S demonstrated potent inhibition of PC-3 xenograft growth in athymic mice. Overall, this study demonstrates significant antitumor activity of pomegranate-derived materials against human prostate cancer

## Lycopene

Tang Y, Parmakhtiar B, Simoneau AR et al. Lycopene enhances docetaxel's effect in castration-resistant prostate cancer associated with insulin-like growth factor I receptor levels. *Neoplasia* 2011 February;13(2):108-19.

Abstract: Docetaxel is currently the most effective drug for the treatment of castration-resistant prostate cancer (CRPC), but it only extends life by an average of 2 months. Lycopene, an antioxidant phytochemical, has antitumor activity against prostate cancer (PCa) in several models and is generally

safe. We present data on the interaction between docetaxel and lycopene in CRPC models. The growth-inhibitory effect of lycopene on PCa cell lines was positively associated with insulin-like growth factor I receptor (IGF-IR) levels. In addition, lycopene treatment enhanced the growth-inhibitory effect of docetaxel more effectively on DU145 cells with IGF-IR high expression than on those PCa cell lines with IGF-IR low expression. In a DU145 xenograft tumor model, docetaxel plus lycopene caused tumor regression, with a 38% increase in antitumor efficacy ( $P = .047$ ) when compared with docetaxel alone. Lycopene inhibited IGF-IR activation through inhibiting IGF-I stimulation and by increasing the expression and secretion of IGF-BP3. Downstream effects include inhibition of AKT kinase activity and survivin expression, followed by apoptosis. Together, the enhancement of docetaxel's antitumor efficacy by lycopene supplementation justifies further clinical investigation of lycopene and docetaxel combination for CRPC patients. CRPC patients with IGF-IR-overexpressing tumors may be most likely to benefit from this combination

Schwenke C, Ubrig B, Thurmann P, Eggersmann C, Roth S. Lycopene for advanced hormone refractory prostate cancer: a prospective, open phase II pilot study. *J Urol* 2009 March;181(3):1098-103.

Abstract: **PURPOSE:** We investigated the influence of lycopene on the clinical and laboratory course in men with hormone refractory prostate cancer. To our knowledge this study represents the first time that subjective assessments of the course of therapy have been recorded. **MATERIAL AND METHODS:** We performed a prospective, open phase II pilot study, in which patients with progressive hormone refractory prostate cancer were included. Lycopene supplementation (15 mg) was given daily for 6 months. Followup laboratory tests and clinical examinations were done monthly. Changes to analgesic use and quality of life (European Organisation for Research and Treatment of Cancer QLQ-C30) were measured. The study end point was a significant change in serum prostate specific antigen, clinical progression or the end of the 6-month observation period. **RESULTS:** A total of 18 patients 64 to 85 years old (median age 73) were enrolled in the study during a 20-month period, of whom 17 could be analyzed. Five of the 17 patients (29%) withdrew from the study prematurely, including 4 of 5 because of prostate specific antigen progression and/or tumor associated complications, and 1 due to an allergic reaction to lycopene. Median prostate specific antigen doubled in 6 months from 42.7 ng/ml (range 13.8 to 521.6) in 17 patients to 96.4 ng/ml (range 13.5 to 1,240) in 12. Stable prostate specific antigen was observed in 5 of 17 patients (29%). None of the patients had a greater than 50% decrease in prostate specific antigen. Patients experienced a slight deterioration in mean health status at the end of the study compared to the outset. However, two-thirds of the patients experienced an improved or unchanged situation regardless of the clinical and biochemical course. **CONCLUSIONS:** No clinically relevant benefits were shown for patients with advanced stages of the disease

Kumar NB, Besterman-Dahan K, Kang L et al. Results of a Randomized Clinical Trial of the Action of Several Doses of Lycopene in Localized Prostate Cancer: Administration Prior to Radical Prostatectomy. *Clin Med Urol* 2008 April 16;1:1-14.

Abstract: **PURPOSE:** The purpose of this Phase II randomized-controlled trial was to evaluate the safety and effect of administering several doses of lycopene to men with clinically localized prostate cancer, on intermediate endpoint biomarkers implicated in prostate carcinogenesis. **METHODS:** Forty-five eligible men with clinically localized prostate cancer were supplemented with 15, 30 or 45 mg of lycopene or no supplement from biopsy to prostatectomy. Compliance to study agent, toxicity, changes in plasma lycopene, serum steroid hormones, PSA and tissue Ki-67 were analyzed from baseline to completion of intervention. **RESULTS:** Forty-two of forty-five five subjects completed the intervention for approximately 30 days from the time of biopsy until prostatectomy. Plasma lycopene increased from baseline to post treatment in all treatment groups with greatest increase observed in

the 45 mg lycopene-supplemented arm compared to the control arm without producing any toxicity. Overall, subjects with prostate cancer had lower baseline levels of plasma lycopene similar to those observed in previous studies in men with prostate cancer. Serum free testosterone decreased with 30 mg lycopene supplementation and total estradiol increased significantly with 30 mg and 45 mg supplementation from baseline to end of treatment, with no significant increases in serum PSA or tissue Ki-67. These changes were not significant compared to the control arm for this sample size and duration of intervention. CONCLUSIONS: Although antioxidant properties of lycopene have been hypothesized to be primarily responsible for its beneficial effects, our study suggests that other mechanisms mediated by steroid hormones may also be involved

Liu X, Allen JD, Arnold JT, Blackman MR. Lycopene inhibits IGF-I signal transduction and growth in normal prostate epithelial cells by decreasing DHT-modulated IGF-I production in co-cultured reactive stromal cells. *Carcinogenesis* 2008 April;29(4):816-23.

Abstract: Prostate stromal and epithelial cell communication is important in prostate functioning and cancer development. Primary human stromal cells from normal prostate stromal cells (PRSC) maintain a smooth muscle phenotype, whereas those from prostate cancer (6S) display reactive and fibroblastic characteristics. Dihydrotestosterone (DHT) stimulates insulin-like growth factor-I (IGF-I) production by 6S but not PRSC cells. Effects of reactive versus normal stroma on normal human prostate epithelial (NPE or PREC) cells are poorly understood. We co-cultured NPE plus 6S or PRSC cells to compare influences of different stromal cells on normal epithelium. Because NPE and PREC cells lose androgen receptor (AR) expression in culture, DHT effects must be modulated by associated stromal cells. When treated with camptothecin (CM), NPE cells, alone and in stromal co-cultures, displayed a dose-dependent increase in DNA fragmentation. NPE/6S co-cultures exhibited reduced CM-induced cell death with exposure to DHT, whereas NPE/PRSC co-cultures exhibited CM-induced cell death regardless of DHT treatment. DHT blocked CM-induced, IGF-I-mediated, NPE death in co-cultured NPE/6S cells without, but not with, added anti-IGF-I and anti-IGF-R antibodies. Lycopene consumption is inversely related to human prostate cancer risk and inhibits IGF-I and androgen signaling in rat prostate cancer. In this study, lycopene, in dietary concentrations, reversed DHT effects of 6S cells on NPE cell death, decreased 6S cell IGF-I production by reducing AR and beta-catenin nuclear localization and inhibited IGF-I-stimulated NPE and PREC growth, perhaps by attenuating IGF-I's effects on serine phosphorylation of Akt and GSK3beta and tyrosine phosphorylation of GSK3. This study expands the understanding of the preventive mechanisms of lycopene in prostate cancer

Vaishampayan U, Hussain M, Banerjee M et al. Lycopene and soy isoflavones in the treatment of prostate cancer. *Nutr Cancer* 2007;59(1):1-7.

Abstract: Dietary intake of lycopene and soy has been associated with a lower risk of prostate cancer. In vitro studies with lycopene and genistein, a soy isoflavone, have shown induction of apoptosis and inhibition of cell growth in androgen-sensitive (LNCaP) and androgen-independent (PC3 and VeCaP) prostate cancer cell lines. In a previous Phase II clinical trial in prostate cancer patients, we observed prostate-specific antigen (PSA) stabilization with soy isoflavone intake. In this Phase II clinical trial, we investigated the efficacy of lycopene alone or in combination with soy isoflavones on serum PSA levels in men with prostate cancer. To be eligible for the study, men with prostate cancer had to have rising serum PSA following local therapy or while on hormone therapy. Study population included 71 eligible patients who had 3 successive rising PSA levels or a minimum PSA of 10 ng/ml at 2 successive evaluations prior to starting therapy. Subjects were randomly assigned to receive a tomato extract capsule containing 15 mg of lycopene alone (n = 38) or together with a capsule containing 40 mg of a soy isoflavone mixture (n = 33) twice daily orally for a maximum of 6 mo. One patient on the lycopene arm did not receive therapy due to his inability to ingest the study pill. There was no decline

in serum PSA in either group qualifying for a partial or complete response. However, 35 of 37 (95%) evaluable patients in the lycopene group and 22 of 33 (67%) evaluable patients in the lycopene plus soy isoflavone group achieved stable disease described as stabilization in serum PSA level. The data suggest that lycopene and soy isoflavones have activity in prostate cancer patients with PSA relapse disease and may delay progression of both hormone-refractory and hormone-sensitive prostate cancer. However, there may not be an additive effect between the 2 compounds when taken together. Future studies are warranted to further investigate the efficacy of lycopene and soy isoflavones in prostate cancer as well as the mechanism of potential negative interaction between them

Ellinger S, Ellinger J, Stehle P. Tomatoes, tomato products and lycopene in the prevention and treatment of prostate cancer: do we have the evidence from intervention studies? *Curr Opin Clin Nutr Metab Care* 2006 November;9(6):722-7.

Abstract: PURPOSE OF REVIEW: Lycopene-rich foods such as fresh tomatoes and tomato products are discussed as potential effectors in the prevention and therapy of prostate cancer. This review provides an overview on the efficacy of supplementation with tomatoes, tomato products and lycopene on appropriate surrogate endpoint biomarkers such as DNA damage and metabolites of the insulin-like growth factor pathway in healthy individuals and prostate cancer patients. RECENT FINDINGS: Intervention studies show that the daily consumption of one serving of tomatoes or tomato products, but not supplementation with lycopene alone, increases the resistance of mononuclear leukocytes against DNA strand breaks induced by reactive oxygen species in healthy volunteers. Data from clinical trials with prostate cancer patients are scarce and contradictory. There is a paucity of reliable data on DNA damage in prostate tissue. SUMMARY: Increasing evidence suggests that a single serving of tomatoes or tomato products ingested daily may contribute to protect from DNA damage. As DNA damage seems to be involved in the pathogenesis of prostate cancer, the regular ingestion of tomatoes or tomato products might prevent the disease. Further well-designed studies are necessary to establish the role of tomatoes and tomato products in the prevention and therapy of prostate cancer

Barber NJ, Zhang X, Zhu G et al. Lycopene inhibits DNA synthesis in primary prostate epithelial cells in vitro and its administration is associated with a reduced prostate-specific antigen velocity in a phase II clinical study. *Prostate Cancer Prostatic Dis* 2006;9(4):407-13.

Abstract: Interest in lycopene has focused primarily on its use in the chemoprevention of prostate cancer (CaP); there are few clinical trials involving men with established disease. In addition, most data examining its mechanism of action have been obtained from experiments using immortal cell lines. We report the inhibitory effect(s) of lycopene in primary prostate epithelial cell (PEC) cultures, and the results of a pilot phase II clinical study investigating whole-tomato lycopene supplementation on the behavior of established CaP, demonstrating a significant and maintained effect on prostate-specific antigen velocity over 1 year. These data reinforce the justification for a large, randomized, placebo-controlled study

Clark PE, Hall MC, Borden LS, Jr. et al. Phase I-II prospective dose-escalating trial of lycopene in patients with biochemical relapse of prostate cancer after definitive local therapy. *Urology* 2006 June;67(6):1257-61.

Abstract: OBJECTIVES: To report a prospective trial of lycopene supplementation in biochemically relapsed prostate cancer. METHODS: A total of 36 men with biochemically relapsed prostate cancer were enrolled in a dose-escalating, Phase I-II trial of lycopene supplementation. Six consecutive cohorts of 6 patients each received daily supplementation with 15, 30, 45, 60, 90, and 120 mg/day for 1 year. The serum levels of prostate-specific antigen (PSA) and plasma levels of lycopene were

measured at baseline and every 3 months. The primary endpoints were PSA response (defined as a 50% decrease in serum PSA from baseline), pharmacokinetics, and the toxicity/tolerability of this regimen. RESULTS: A total of 36 patients were enrolled. The median age was 74 years (range 56 to 83), with a median serum PSA at entry of 4.4 ng/mL (range 0.8 to 24.9). No serum PSA responses were observed, and 37% of patients had PSA progression. The median time to progression was not reached. Toxicity was mild, with 1 patient discontinuing therapy because of diarrhea. Significant elevations of plasma lycopene were noted at 3 months and then appeared to plateau for all six dose levels. The plasma levels for doses between 15 and 90 mg/day were similar, with additional elevation only at 120 mg/day. CONCLUSIONS: Lycopene supplementation in men with biochemically relapsed prostate cancer is safe and well tolerated. The plasma levels of lycopene were similar for a wide dose range (15 to 90 mg/day) and plateaued by 3 months. Lycopene supplementation at the doses used in this study did not result in any discernible response in serum PSA

Ansari MS, Gupta NP. A comparison of lycopene and orchidectomy vs orchidectomy alone in the management of advanced prostate cancer. *BJU Int* 2003 September;92(4):375-8.

Abstract: OBJECTIVE: To compare the efficacy of lycopene plus orchidectomy with orchidectomy alone in the management of advanced prostate cancer. PATIENTS AND METHODS: Fifty-four patients with histologically confirmed metastatic prostatic cancer (M1b or D2) and a performance status of 0-2 (World Health Organization) were entered into the trial between March 2000 and June 2002. The trial comprised two treatment arms, i.e. patients were randomized to orchidectomy alone or orchidectomy plus lycopene (OL), each of 27 patients. Lycopene was started on the day of orchidectomy at 2 mg twice daily. Patients were evaluated clinically before and every 3 months after the intervention, with measurements of prostate-specific antigen (PSA), a bone scan and uroflowmetry, with the clinical response assessed as the change in these variables. RESULTS: At 6 months there was a significant reduction in PSA level in both treatments, but more marked in the OL group (mean 9.1 and 26.4 ng/mL,  $P = 0.9$ ). After 2 years these changes were more consistent in the OL group (mean 3.01 and 9.02 ng/mL;  $P < 0.001$ ). Eleven (40%) patients in orchidectomy and 21 (78%) in the OL group had a complete PSA response ( $P < 0.05$ ), with a partial response in nine (33%) and four (15%), and progression in seven (25%) and two (7%), respectively ( $P < 0.05$ ). Bone scans showed that in the orchidectomy arm only four (15%) patients had a complete response, vs eight (30%) in the OL group ( $P < 0.02$ ), with a partial response in 19 (70%) and 17 (63%), and progression in four (15%) and two (7%), respectively ( $P < 0.02$ ). There was a significant improvement in peak flow rate in the OL group, with a mean difference of +1.17 mL/s ( $P < 0.04$ ). Of the 54 patients who entered the trial, 19 (35%) died, 12 (22%) in orchidectomy and seven (13%) in OL group ( $P < 0.001$ ). CONCLUSION: Adding lycopene to orchidectomy produced a more reliable and consistent decrease in serum PSA level; it not only shrinks the primary tumour but also diminishes the secondary tumours, providing better relief from bone pain and lower urinary tract symptoms, and improving survival compared with orchidectomy alone

Kucuk O, Sarkar FH, Djuric Z et al. Effects of lycopene supplementation in patients with localized prostate cancer. *Exp Biol Med (Maywood)* 2002 November;227(10):881-5.

Abstract: Epidemiological studies have shown an inverse association between dietary intake of lycopene and prostate cancer risk. We conducted a clinical trial to investigate the biological and clinical effects of lycopene supplementation in patients with localized prostate cancer. Twenty-six men with newly diagnosed prostate cancer were randomly assigned to receive a tomato oleoresin extract containing 30 mg of lycopene ( $n = 15$ ) or no supplementation ( $n = 11$ ) for 3 weeks before radical prostatectomy. Biomarkers of cell proliferation and apoptosis were assessed by Western blot analysis in benign and cancerous prostate tissues. Oxidative stress was assessed by measuring the peripheral blood lymphocyte DNA oxidation product 5-hydroxymethyl-deoxyuridine (5-OH-mdU).

Usual dietary intake of nutrients was assessed by a food frequency questionnaire at baseline. Prostatectomy specimens were evaluated for pathologic stage, Gleason score, volume of cancer, and extent of high-grade prostatic intraepithelial neoplasia. Plasma levels of lycopene, insulin-like growth factor-1, insulin-like growth factor binding protein-3, and prostate-specific antigen were measured at baseline and after 3 weeks of supplementation or observation. After intervention, subjects in the intervention group had smaller tumors (80% vs 45%, less than 4 ml), less involvement of surgical margins and/or extra-prostatic tissues with cancer (73% vs 18%, organ-confined disease), and less diffuse involvement of the prostate by high-grade prostatic intraepithelial neoplasia (33% vs 0%, focal involvement) compared with subjects in the control group. Mean plasma prostate-specific antigen levels were lower in the intervention group compared with the control group. This pilot study suggests that lycopene may have beneficial effects in prostate cancer. Larger clinical trials are warranted to investigate the potential preventive and/or therapeutic role of lycopene in prostate cancer

Kucuk O, Sarkar FH, Sakr W et al. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidemiol Biomarkers Prev* 2001 August;10(8):861-8.

Abstract: An inverse association has been observed between dietary intake of lycopene and the risk of prostate cancer. We investigated the effects of lycopene supplementation in patients with prostate cancer. Twenty-six men with newly diagnosed, clinically localized (14 T(1) and 12 T(2)) prostate cancer were randomly assigned to receive 15 mg of lycopene (n = 15) twice daily or no supplementation (n = 11) for 3 weeks before radical prostatectomy. Biomarkers of differentiation and apoptosis were assessed by Western blot analysis on benign and malignant parts of the prostate gland. Prostatectomy specimens were entirely embedded, step-sectioned, and evaluated for pathological stage, Gleason score, volume of cancer, and extent of high-grade prostatic intraepithelial neoplasia. Plasma levels of lycopene, insulin-like growth factor-1 (IGF-1), IGF binding protein-3, and prostate-specific antigen were measured at baseline and after 3 weeks of supplementation or observation. Eleven (73%) subjects in the intervention group and two (18%) subjects in the control group had no involvement of surgical margins and/or extra-prostatic tissues with cancer (P = 0.02). Twelve (84%) subjects in the lycopene group and five (45%) subjects in the control group had tumors <4 ml in size (P = 0.22). Diffuse involvement of the prostate by high-grade prostatic intraepithelial neoplasia was present in 10 (67%) subjects in the intervention group and in 11 (100%) subjects in the control group (P = 0.05). Plasma prostate-specific antigen levels decreased by 18% in the intervention group, whereas they increased by 14% in the control group (P = 0.25). Expression of connexin 43 in cancerous prostate tissue was 0.63 +/- 0.19 absorbance in the lycopene group compared with 0.25 +/- 0.08 in the control group (P = 0.13). Expression of bcl-2 and bax did not differ significantly between the two study groups. IGF-1 levels decreased in both groups (P = 0.0002 and P = 0.0003, respectively). The results suggest that lycopene supplementation may decrease the growth of prostate cancer. However, no firm conclusions can be drawn at this time because of the small sample size

### **Fish Omega-3s**

Epstein MM, Kasperzyk JL, Mucci LA et al. Dietary Fatty Acid intake and prostate cancer survival in orebro county, sweden. *Am J Epidemiol* 2012 August 1;176(3):240-52.

Abstract: Although dietary fat has been associated with prostate cancer risk, the association between specific fatty acids and prostate cancer survival remains unclear. Dietary intake of 14 fatty acids was analyzed in a population-based cohort of 525 Swedish men with prostate cancer in Orebro County (1989-1994). Multivariable hazard ratios and 95% confidence intervals for time to prostate cancer death by quartile and per standard deviation increase in intake were estimated by Cox proportional hazards regression. Additional models examined the association by stage at diagnosis (localized: T0-T2/M0; advanced: T0-T4/M1, T3-T4/M0). Among all men, those with the highest omega-3

docosahexaenoic acid and total marine fatty acid intakes were 40% less likely to die from prostate cancer (P(trend) = 0.05 and 0.04, respectively). Among men with localized prostate cancer, hazard ratios of 2.07 (95% confidence interval: 0.93, 4.59; P(trend) = 0.03) for elevated total fat, 2.39 (95% confidence interval: 1.06, 5.38) for saturated myristic acid, and 2.88 (95% confidence interval: 1.24, 6.67) for shorter chain (C4-C10) fatty acid intakes demonstrated increased risk for disease-specific mortality for the highest quartile compared with the lowest quartile. This study suggests that high intake of total fat and certain saturated fatty acids may worsen prostate cancer survival, particularly among men with localized disease. In contrast, high marine omega-3 fatty acid intake may improve disease-specific survival for all men

Wang S, Wu J, Suburu J et al. Effect of dietary polyunsaturated fatty acids on castration-resistant Pten-null prostate cancer. *Carcinogenesis* 2012 February;33(2):404-12.

Abstract: A common treatment of advanced prostate cancer involves the deprivation of androgens. Despite the initial response to hormonal therapy, eventually all the patients relapse. In the present study, we sought to determine whether dietary polyunsaturated fatty acid (PUFA) affects the development of castration-resistant prostate cancer. Cell culture, patient tissue microarray, allograft, xenograft, prostate-specific Pten knockout and omega-3 desaturase transgenic mouse models in conjunction with dietary manipulation, gene knockdown and knockout approaches were used to determine the effect of dietary PUFA on castration-resistant Pten-null prostate cancer. We found that deletion of Pten increased androgen receptor (AR) expression and Pten-null prostate cells were castration resistant. Omega-3 PUFA slowed down the growth of castration-resistant tumors as compared with omega-6 PUFA. Omega-3 PUFA decreased AR protein to a similar extent in tumor cell cytosolic and nuclear fractions but had no effect on AR messenger RNA level. Omega-3 PUFA treatment appeared to accelerate AR protein degradation, which could be blocked by proteasome inhibitor MG132. Knockdown of AR significantly slowed down prostate cancer cell proliferation in the absence of androgens. Our data suggest that omega-3 PUFA inhibits castration-resistant prostate cancer in part by accelerating proteasome-dependent degradation of the AR protein. Dietary omega-3 PUFA supplementation in conjunction with androgen ablation may significantly delay the development of castration-resistant prostate cancer in patients compared with androgen ablation alone

Aronson WJ, Kobayashi N, Barnard RJ et al. Phase II prospective randomized trial of a low-fat diet with fish oil supplementation in men undergoing radical prostatectomy. *Cancer Prev Res (Phila)* 2011 December;4(12):2062-71.

Abstract: Preclinical studies suggest lowering dietary fat and decreasing the ratio of omega-6 to omega-3 polyunsaturated fatty acids decreases the risk of prostate cancer development and progression. We conducted a phase II randomized trial to test the effect of decreasing dietary fat combined with decreasing the dietary omega-6:omega-3 ratio on biomarkers related to prostate cancer development and progression. Patients undergoing radical prostatectomy were randomly assigned to receive a low-fat diet with 5 grams of fish oil daily (dietary omega-6:omega-3 ratio of 2:1) or a control Western diet (omega-6:omega-3 ratio of 15:1) for four to six weeks prior to surgery. The primary endpoint was change in serum insulin-like growth factor I (IGF-1) between arms. Secondary endpoints were serum IGFBP-1, prostate prostaglandin E2 levels, omega-6:omega-3 fatty acid ratios, COX-2, and markers of proliferation and apoptosis. Fifty-five patients were randomized and 48 completed the trial. There was no treatment difference in the primary outcome. Positive secondary outcomes in the low-fat fish oil versus Western group were reduced benign and malignant prostate tissue omega-6:omega-3 ratios, reduced proliferation (Ki-67 index), and reduced proliferation in an ex vivo bioassay when patient sera was applied to prostate cancer cells in vitro. In summary, four to six weeks of a low-fat diet and fish oil capsules to achieve an omega-6:omega-3 fatty acid ratio of 2:1 had no effect on serum IGF-1 levels, though in secondary analyses, the intervention resulted in

decreased prostate cancer proliferation and decreased prostate tissue omega-6:omega-3 ratios. These results support further studies evaluating reduction of dietary fat with fish oil supplementation on modulating prostate cancer biology

Friedrichs W, Ruparel SB, Marciniak RA, DeGraffenried L. Omega-3 fatty acid inhibition of prostate cancer progression to hormone independence is associated with suppression of mTOR signaling and androgen receptor expression. *Nutr Cancer* 2011;63(5):771-7.

Abstract: Currently, progression of prostate cancer to androgen independence remains the primary obstacle to improved survival. In order to improve overall survival, novel treatment strategies that are based upon specific molecular mechanisms that prolong the androgen-dependent state and that are useful for androgen-independent disease need to be identified. Both epidemiological as well as preclinical data suggest that omega-3 fatty acids are effective primary tumor prevention agents; however, their efficacy at preventing and treating refractory prostate cancer has not been as thoroughly investigated. We used an in vitro model of androgen ablation to determine the effect of treatment with omega-3 fatty acids on the progression to an androgen-independent state. The omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were able to prevent progression of LNCaP cells while the omega-6 fatty acid arachidonic acid (AA) actually promoted cell growth under conditions of hormone depletion. These results correlated with a decrease in the expression of the androgen receptor as well as suppression of the Akt/mTOR signaling pathway. Connecting the mechanisms by which omega-3 fatty acids affect phenotypic outcome is important for effective exploitation of these nutrient agents as a therapeutic approach. Understanding these processes is critical for the development of effective dietary intervention strategies that improve overall survival

Cavazos DA, Price RS, Apte SS, deGraffenried LA. Docosahexaenoic acid selectively induces human prostate cancer cell sensitivity to oxidative stress through modulation of NF-kappaB. *Prostate* 2011 September 15;71(13):1420-8.

Abstract: BACKGROUND: Oxidative burden is strongly implicated in the pathogenesis of age-related diseases, including prostate cancer tumor formation. As omega-3 fatty acids possess known antioxidant properties, we investigated the effects of docosahexaenoic acid (DHA-22:6n-3), one component of fish oil, in modulating the effects of oxidative DNA damage in LNCaP and PacMetUT1 human prostate adenocarcinoma cells and in a normal human prostate cell line, PrEC. METHODS: Cell survival was determined by an inhibition of colony formation assay. DNA double-strand breaks, NF-kappaB subcellular localization and relative survivin expression levels were determined by immunofluorescence and survivin expression levels confirmed by immunoblot assay. Measurement of NF-kappaB transcriptional activity was investigated by dual luciferase assay. RESULTS: LNCaP and PacMetUT1 cells pretreated with DHA and pulsed with 32 microM H(2)O(2) exhibit decreased survival compared to PrEC. gamma-H2AX foci, indicating DNA double-strand breaks, were associated with translocation of NF-kappaB into the nucleus, whereas exposure to DHA prior to H(2)O(2) treatment prevented NF-kappaB translocation. Further, DHA attenuated H(2)O(2)-induced NF-kappaB transcriptional activity and diminished expression of the downstream target, survivin. CONCLUSIONS: NF-kappaB is heavily implicated in promoting prosurvival signaling and may be critical for resistance to the chronic oxidative stress observed in the pathogenesis of prostate cancer. Our studies indicate that exposure of cells to physiologically achievable levels of DHA prior to treatment with H(2)O(2) results in decreased cancer cell survival which is associated with nuclear exclusion of NF-kappaB. We therefore propose that DHA selectively sensitizes prostate cancer cells to growth arrest through attenuation of the NF-kappaB survival pathway

Szymanski KM, Wheeler DC, Mucci LA. Fish consumption and prostate cancer risk: a review and meta-analysis. *Am J Clin Nutr* 2010 November;92(5):1223-33.

Abstract: **BACKGROUND:** Prostate cancer incidence varies 60-fold globally, which suggests the roles of lifestyle and dietary factors in its cause. To our knowledge, a comprehensive assessment of the association between fish consumption and prostate cancer incidence and mortality has not been reported. **OBJECTIVE:** We conducted a meta-analysis of fish intake and prostate cancer by focusing on the incidence of prostate cancer and prostate cancer-specific mortality and included subgroup analyses based on race, fish type, method of fish preparation, and high-grade and high-stage cancer. **DESIGN:** We searched MEDLINE and EMBASE databases (May 2009) for case-control and cohort studies that assessed fish intake and prostate cancer risk. Two authors independently assessed eligibility and extracted data. **RESULTS:** There was no association between fish consumption and a significant reduction in prostate cancer incidence [12 case-control studies (n = 5777 cases and 9805 control subjects), odds ratio (OR): 0.85; 95% CI: 0.72, 1.00; and 12 cohort studies (n = 445,820), relative risk (RR): 1.01; 95% CI: 0.90, 1.14]. It was not possible to perform a meta-analysis for high-grade disease (one case-control study, OR: 1.44; 95% CI: 0.58, 3.03), locally advanced disease (one cohort study, RR: 0.80; 95% CI: 0.61, 1.13), or metastatic disease (one cohort study, RR: 0.56; 95% CI: 0.37, 0.86). There was an association between fish consumption and a significant 63% reduction in prostate cancer-specific mortality [4 cohort studies (n = 49,661), RR: 0.37; 95% CI: 0.18, 0.74]. **CONCLUSION:** Our analyses provide no strong evidence of a protective association of fish consumption with prostate cancer incidence but showed a significant 63% reduction in prostate cancer-specific mortality

Fradet V, Cheng I, Casey G, Witte JS. Dietary omega-3 fatty acids, cyclooxygenase-2 genetic variation, and aggressive prostate cancer risk. *Clin Cancer Res* 2009 April 1;15(7):2559-66.

Abstract: **PURPOSE:** Dietary intake of long-chain omega-3 (LC n-3) polyunsaturated fatty acids may reduce inflammation and in turn decrease risk of prostate cancer development and progression. This potential effect may be modified by genetic variation in cyclooxygenase-2 (COX-2), a key enzyme in fatty acid metabolism and inflammation. **EXPERIMENTAL DESIGN:** We used a case-control study of 466 men diagnosed with aggressive prostate cancer and 478 age- and ethnicity-matched controls. Diet was assessed with a semiquantitative food frequency questionnaire, and nine COX-2 tag single nucleotide polymorphisms (SNP) were genotyped. We used logistic regression models to estimate odds ratios (OR) for association and interaction. **RESULTS:** Increasing intake of LC n-3 was strongly associated with a decreased risk of aggressive prostate cancer (P(trend) <or= 0.0001). The OR (95% confidence interval) for prostate cancer comparing the highest with the lowest quartile of n-3 intake was of 0.37 (0.25-0.54). The LC n-3 association was modified by SNP rs4648310 (+8897 A/G), flanking the 3' region of COX-2 (P(interaction) = 0.02). In particular, the inverse association was even stronger among men with this variant SNP. This reflected the observation that men with low LC n-3 intake and the variant rs4648310 SNP had an increased risk of disease (OR, 5.49; 95% confidence interval, 1.80-16.7), which was reversed by increasing intake of LC n-3. **CONCLUSIONS:** Dietary LC n-3 polyunsaturated fatty acids appear protective for aggressive prostate cancer, and this effect is modified by the COX-2 SNP rs4648310. Our findings support the hypothesis that LC n-3 may impact prostate inflammation and carcinogenesis through the COX-2 enzymatic pathway

Chavarro JE, Stampfer MJ, Hall MN, Sesso HD, Ma J. A 22-y prospective study of fish intake in relation to prostate cancer incidence and mortality. *Am J Clin Nutr* 2008 November;88(5):1297-303.

Abstract: **BACKGROUND:** Fish and seafood n-3 fatty acids may prevent or delay the progression of prostate cancer, but epidemiologic studies do not uniformly support this hypothesis. **OBJECTIVE:** We examined the relation of fish and seafood n-3 fatty acid intakes with prostate cancer incidence

and mortality. DESIGN: We conducted a prospective cohort study among 20,167 men participating in the Physician's Health Study who were free of cancer in 1983. RESULTS: During 382 144 person-years of follow-up, 2161 men were diagnosed with prostate cancer and 230 died of prostate cancer. Fish intake was unrelated to prostate cancer incidence. Survival analysis among the men diagnosed with prostate cancer revealed that those consuming fish  $\geq 5$  times/wk had a 48% lower risk of prostate cancer death than did men consuming fish less than once weekly [relative risk (RR) = 0.52; 95% CI: 0.30, 0.91; P for trend = 0.05]. A similar association was found between seafood n-3 fatty acid intake and prostate cancer mortality (RR(Q5 versus Q1) = 0.64; 95% CI: 0.42, 0.99; P for trend = 0.02). These associations became stronger when the analyses were restricted to clinically detected cases. CONCLUSION: These results suggest that fish intake is unrelated to prostate cancer incidence but may improve prostate cancer survival

McEntee MF, Ziegler C, Reel D et al. Dietary n-3 polyunsaturated fatty acids enhance hormone ablation therapy in androgen-dependent prostate cancer. *Am J Pathol* 2008 July;173(1):229-41.

Abstract: Hormone ablation therapy typically causes regression of prostate cancer and represents an important means of treating this disease, particularly after metastasis. However, hormone therapy inevitably loses its effectiveness as tumors become androgen-independent, and this conversion often leads to death because of subsequent poor responses to other forms of treatment. Because environmental factors, such as diet, have been strongly linked to prostate cancer, we examined the effects of dietary polyunsaturated fatty acids (PUFAs; at 1.5 wt%) on growth of androgen-dependent (CWR22) and androgen-independent (CWR22R) human prostatic cancer xenografts, the acute response of CWR22 tumors to ablation therapy, and their progression to androgen independence. Significant diet-induced changes in tumor n-3 or n-6 PUFA content had no effect on CWR22 or CWR22R tumors growing with or without androgen support, respectively. However, dietary changes that increased tumor eicosapentaenoic acid and linoleic acid content enhanced responses to ablation therapy, measured by cancer cell apoptosis and mitosis. In addition, relapse to androgen-independent growth (measured by renewed increases in tumor volume and serum prostate-specific antigen after ablation) positively correlated with tumor arachidonic acid content. There was no correlation between expression of 15-lipoxygenase isozymes or their products and tumor growth or responses to ablation. In conclusion, dietary n-3 PUFA may enhance the response of prostate cancer to ablation therapy and retard progression to androgen-independent growth by altering tumor PUFA content

Kelavkar UP, Hutzley J, Dhir R, Kim P, Allen KG, McHugh K. Prostate tumor growth and recurrence can be modulated by the omega-6:omega-3 ratio in diet: athymic mouse xenograft model simulating radical prostatectomy. *Neoplasia* 2006 February;8(2):112-24.

Abstract: Evidence indicates that a diet rich in omega (omega)-6 polyunsaturated fatty acids (PUFAs) [e.g., linoleic acid (LA)] increases prostate cancer (PCa) risk, whereas a diet rich in omega-3 decreases risk. Precisely how these PUFAs affect disease development remains unclear. So we examined the roles that PUFAs play in PCa, and we determined if increased omega-3 consumption can impede tumor growth. We previously demonstrated an increased expression of an omega-6 LA-metabolizing enzyme, 15-lipoxygenase-1 (15-LO-1, ALOX15), in prostate tumor tissue compared with normal adjacent prostate tissue, and that elevated 15-LO-1 activity in PCa cells has a protumorigenic effect. A PCa cell line, Los Angeles Prostate Cancer-4 (LAPC-4), expresses prostate-specific antigen (PSA) as well as an active 15-LO-1 enzyme. Therefore, to study whether or not the protumorigenic role of 15-LO-1 and dietary omega-6 LA can be modulated by altering omega-3 levels through diet, we surgically removed tumors caused by LAPC-4 cells (mouse model to simulate radical prostatectomy). Mice were then randomly divided into three different diet groups-namely, high omega-6 LA, high omega-3 stearidonic acid (SDA), and no fat-and examined the effects of omega-6 and omega-3 fatty acids in diet on LAPC-4 tumor recurrence by monitoring for PSA. Mice

in these diet groups were monitored for food consumption, body weight, and serum PSA indicative of the presence of LAPC-4 cells. Fatty acid methyl esters from erythrocyte membranes were examined for omega-6 and omega-3 levels to reflect long-term dietary intake. Our results provide evidence that prostate tumors can be modulated by the manipulation of omega-6:omega-3 ratios through diet and that the omega-3 fatty acid SDA [precursor of eicosapentaenoic acid (EPA)] promotes apoptosis and decreases proliferation in cancer cells, causing decreased PSA doubling time, compared to omega-6 LA fatty acid, likely by competing with the enzymes of LA and AA pathways, namely, 15-LO-1 and cyclooxygenases (COXs). Thus, EPA and DHA (major components of fish oil) could potentially be promising dietary intervention agents in PCa prevention aimed at 15-LO-1 and COX-2 as molecular targets. These observations also provide clues as to its mechanisms of action

Dewailly E, Mulvad G, Sloth PH, Hansen JC, Behrendt N, Hart Hansen JP. Inuit are protected against prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2003 September;12(9):926-7.

Abstract: Incidence and mortality rates for prostate cancer are reported to be low among Inuit, but this finding must be additionally supported given the difficulty of obtaining a precise medical diagnosis in the Arctic. We conducted an autopsy study in 1990-1994 among 61 deceased males representative of all deaths occurring in Greenland and found only one invasive prostate cancer. Histological data were available for 27 autopsies and revealed no latent carcinoma. Our results suggest that in situ carcinoma is rare among Inuit and that their traditional diet, which is rich in omega-3 polyunsaturated fatty acids and selenium, may be an important protective factor

Terry P, Lichtenstein P, Feychting M, Ahlbom A, Wolk A. Fatty fish consumption and risk of prostate cancer. *Lancet* 2001 June 2;357(9270):1764-6.

Abstract: Consumption of fatty fish might reduce the risk of prostate cancer, although epidemiological studies of fish consumption are rare. We studied the association between fish consumption and prostate cancer in a population-based prospective cohort of 6272 Swedish men. During 30 years of follow-up, men who ate no fish had a two-fold to three-fold higher frequency of prostate cancer than those who ate moderate or high amounts did. Our results suggest that fish consumption could be associated with decreased risk of prostate cancer

## **Green Tea Polyphenols**

Henning SM, Wang P, Said J et al. Polyphenols in brewed green tea inhibit prostate tumor xenograft growth by localizing to the tumor and decreasing oxidative stress and angiogenesis. *J Nutr Biochem* 2012 March 7.

Abstract: It has been demonstrated in various animal models that the oral administration of green tea (GT) extracts in drinking water can inhibit tumor growth, but the effects of brewed GT on factors promoting tumor growth, including oxidant damage of DNA and protein, angiogenesis and DNA methylation, have not been tested in an animal model. To explore these potential mechanisms, brewed GT was administered instead of drinking water to male severe combined immunodeficiency (SCID) mice with androgen-dependent human LAPC4 prostate cancer cell subcutaneous xenografts. Tumor volume was decreased significantly in mice consuming GT, and tumor size was significantly correlated with GT polyphenol (GTP) content in tumor tissue. There was a significant reduction in hypoxia-inducible factor 1-alpha and vascular endothelial growth factor protein expression. GT consumption significantly reduced oxidative DNA and protein damage in tumor tissue as determined by 8-hydroxydeoxyguanosine/deoxyguanosine ratio and protein carbonyl assay, respectively. Methylation is known to inhibit antioxidative enzymes such as glutathione S-transferase pi to permit reactive oxygen species promotion of tumor growth. GT inhibited tumor 5-cytosine DNA

methyltransferase 1 mRNA and protein expression significantly, which may contribute to the inhibition of tumor growth by reactivation of antioxidative enzymes. This study advances our understanding of tumor growth inhibition by brewed GT in an animal model by demonstrating tissue localization of GTPs in correlation with inhibition of tumor growth. Our results suggest that the inhibition of tumor growth is due to GTP-mediated inhibition of oxidative stress and angiogenesis in the LAPC4 xenograft prostate tumor in SCID mice

Stearns ME, Wang M. Synergistic Effects of the Green Tea Extract Epigallocatechin-3-gallate and Taxane in Eradication of Malignant Human Prostate Tumors. *Transl Oncol* 2011 June;4(3):147-56.

Abstract: We have examined whether epigallocatechin-3-gallate (EGCG), and extract of green tea, in combination with taxane (i.e., paclitaxel and docetaxel), exerts a synergistic activity in blocking human prostate PC-3ML tumor cell growth in vitro and in vivo. Growth assays in vitro revealed that the IC(50) values were approximately 30 microM, approximately 3 nM, and approximately 6 nM, for EGCG, paclitaxel and docetaxel, respectively. Isobolograms generated from the data clearly indicated that EGCG in combination with paclitaxel or docetaxel had an additive effect in blocking tumor cell growth. EGCG combined with taxane also had an additive effect to increase the expression of apoptotic genes, (p53, p73, p21, and caspase 3) and the percent apoptosis observed in vitro and in tumor modeling studies in severe combined immunodeficient mice. The tumor modeling studies clearly showed that EGCG plus taxane injected intraperitoneally (i.p.) induced a significant increase in apoptosis rates (TUNEL assays) and eliminated preexisting tumors generated from PC-3ML cells implanted i.p., increasing disease-free survival rates to greater than 90%. More importantly, the combination therapy (i.p. biweekly) blocked metastases after intravenous injection of PC-3ML cells through the tail vein. In mice treated with EGCG plus taxane, the disease-free survival rates increased from 0% (in untreated mice) to more than 70% to 80% in treated mice. Taken together, these data demonstrate for the first time that EGCG in combination with taxane may provide a novel therapeutic treatment of advanced prostate cancer

Lee SC, Chan WK, Lee TW et al. Effect of a prodrug of the green tea polyphenol (-)-epigallocatechin-3-gallate on the growth of androgen-independent prostate cancer in vivo. *Nutr Cancer* 2008;60(4):483-91.

Abstract: Epigallocatechin-3-gallate (EGCG) is the major and most potent polyphenol compound of green tea that has been shown to have anticancer effects against various types of cancers. In this study, in addition to the EGCG compound, a synthetic derivative, the peracetate of EGCG (EGCG-P), was used to investigate the inhibitory effects on growth of androgen-independent prostate cancer in vivo. The advantage of EGCG-P is that it may act as a prodrug, leading to higher bioavailability than EGCG itself. The aim of our study was to compare the differences between EGCG and EGCG-P on their inhibitory effect on androgen-independent prostate cancer, CWR22R, xenograft model in nude mice. The mice were administered daily with solvent dimethyl sulfoxide, EGCG, and EGCG-P separately through intraperitoneal injection for 20 days. Tumor volume and body weight of nude mice were recorded daily. Serum prostate-specific antigen (PSA) levels were also measured before and after the treatment. The effects of both EGCG and EGCG-P on tumor cell proliferation were assessed by immunohistochemical (IHC) method using antibodies against Ki-67 and proliferating cell nuclear antigen. The apoptotic effect was evaluated by IHC against B-cell non-Hodgkin lymphoma-2 and terminal deoxynucleotidyl transferase dUTP nick-end labeling assay by in situ apoptosis detection kit. Moreover, the potential suppression of angiogenesis by EGCG and EGCG-P on prostate cancer was examined by IHC against CD31. Our results revealed that treatment of EGCG and EGCG-P compounds suppressed the growth of CWR22R xenografts without causing any detectable side effects in nude mice. The suppression of growth of the tumor was correlated with the decrease of serum PSA level together with the reduction in tumor angiogenesis and an increase in apoptosis on

prostate cancer cells. The results showed that treatment of EGCG and EGCG-P inhibited tumor growth and angiogenesis while promoting apoptosis of the prostate cancer cells in vivo. Our results suggest that EGCG-P may be a more stable and useful compound for increasing the therapeutic anticancer effects in androgen-independent prostate cancer

Liao S, Umekita Y, Guo J, Kokontis JM, Hiipakka RA. Growth inhibition and regression of human prostate and breast tumors in athymic mice by tea epigallocatechin gallate. *Cancer Lett* 1995 September 25;96(2):239-43.

Abstract: The human prostate cancer cell lines, PC-3 (androgen-insensitive) and LNCaP 104-R (androgen-repressed) were inoculated subcutaneously into nude mice to produce prostate tumors. Intraperitoneal injection of green tea (-)epigallocatechin-3-gallate but not structurally related catechins, such as (-)epicatechin-3-gallate, inhibited the growth and rapidly reduced the size of human prostate tumors in nude mice. (-)Epigallocatechin-3-gallate also rapidly inhibited the growth of tumor growth formed by the human mammary cancer cell line MCF-7 in nude mice. It is possible that there is a relationship between the high consumption of green tea and the low incidence of prostate and breast cancers in some Asian countries

## Melatonin

Lissoni P, Brivio F, Fumagalli L et al. Neuroimmunomodulation in medical oncology: application of psychoneuroimmunology with subcutaneous low-dose IL-2 and the pineal hormone melatonin in patients with untreatable metastatic solid tumors. *Anticancer Res* 2008 March;28(2B):1377-81.

Abstract: BACKGROUND: Anticancer immunity is under psychoneuroendocrine regulation, mainly via the pineal gland and brain opioid system, which may stimulate and inhibit antitumor immunity respectively. Cancer-related immuno-suppression does not depend only on functional damage of immune cells, but also on alterations of systems responsible for the neuroimmunomodulation, the most frequent of which is a decline in blood levels of the pineal hormone melatonin (MLT). PATIENTS AND METHODS: A study was performed to evaluate the influence of an exogenous administration of MLT alone or MLT plus subcutaneous (SC) low-dose interleukin-2 on tumor progression and survival time in patients with untreatable metastatic solid tumors. The study included 846 patients with metastatic solid tumor (non-small cell lung cancer or gastrointestinal tract tumors) randomized to receive the best supportive care only, supportive care plus MLT (20 mg/day, orally in the evening), or MLT plus SC low-dose IL-2 (3 MIU/day for 5 days/week, for 4 consecutive weeks). RESULTS: The MLT alone was able to induce a significant increase of disease stabilization and survival time with respect to supportive care alone. The association of IL-2 with MLT provided a further improvement in the percentage of tumor regressions and of 3-year survival with respect to MLT alone. CONCLUSION: The administration of IL-2 and the pineal hormone MLT may induce control of neoplastic growth and a prolonged survival time in patients with metastatic solid tumors, for whom no other conventional anticancer therapy is available

Lissoni P, Tancini G, Barni S et al. Treatment of cancer chemotherapy-induced toxicity with the pineal hormone melatonin. *Support Care Cancer* 1997 March;5(2):126-9.

Abstract: Experimental data have suggested that the pineal hormone melatonin (MLT) may counteract chemotherapy-induced myelosuppression and immunosuppression. In addition, MLT has been shown to inhibit the production of free radicals, which play a part in mediating the toxicity of chemotherapy. A study was therefore performed in an attempt to evaluate the influence of MLT on chemotherapy toxicity. The study involved 80 patients with metastatic solid tumors who were in poor clinical condition (lung cancer: 35; breast cancer: 31; gastrointestinal tract tumors: 14). Lung cancer patients

were treated with cisplatin and etoposide, breast cancer patients with mitoxantrone, and gastrointestinal tract tumor patients with 5-fluorouracil plus folates. Patients were randomised to receive chemotherapy alone or chemotherapy plus MLT (20 mg/day p.o. in the evening). Thrombocytopenia was significantly less frequent in patients concomitantly treated with MLT. Malaise and asthenia were also significantly less frequent in patients receiving MLT. Finally, stomatitis and neuropathy were less frequent in the MLT group, albeit without statistically significant differences. Alopecia and vomiting were not influenced by MLT. This pilot study seems to suggest that the concomitant administration of the pineal hormone MLT during chemotherapy may prevent some chemotherapy-induced side-effects, particularly myelosuppression and neuropathy. Evaluation of the impact of MLT on chemotherapy efficacy will be the aim of future clinical investigations

Shiu SY, Leung WY, Tam CW, Liu VW, Yao KM. Melatonin MT(1) receptor-induced transcriptional up-regulation of p27(Kip1) in prostate cancer antiproliferation is mediated via inhibition of constitutively active nuclear factor kappa B (NF-kappaB): potential implications on prostate cancer chemoprevention and therapy. *J Pineal Res* 2012 June 20.

Abstract: Our laboratory has recently demonstrated a melatonin MT(1) receptor-mediated antiproliferative signaling mechanism in androgen receptor (AR)-positive prostate epithelial cells which involves up-regulation of p27(Kip1) through dual activation of Galpha(s) /protein kinase A (PKA) and Galpha(q) /protein kinase C (PKC) in parallel, and down-regulation of activated AR signaling via PKC stimulation. The aim of the present investigation was to identify the transcription factor that mediates melatonin's up-regulatory effect on p27(Kip1) in LNCaP and 22Rv1 prostate cancer cells. Deletion mapping and reporter assays of the p27(Kip1) promoter revealed that the putative melatonin-responsive transcription factor binds to a 116 base-pair region of the promoter sequence, which contains a potential nuclear factor kappa B (NF-kappaB) binding site. When the NF-kappaB binding site was abolished by site-directed mutagenesis, the stimulatory effect of melatonin on p27(Kip1) promoter activity was mitigated. Notably, melatonin inhibited the DNA binding of activated NF-kappaB via MT(1) receptor-induced PKA and PKC stimulation. Furthermore, melatonin's up-regulatory effect on p27(Kip1) transcription and consequent cell antiproliferation were abrogated by NF-kappaB activator but mimicked by NF-kappaB inhibitor. The results indicate that inhibition of constitutively active NF-kappaB via melatonin MT(1) receptor-induced dual activation of (Galpha(s) ) PKA and (Galpha(q) ) PKC can de-repress the p27(Kip1) promoter leading to transcriptional up-regulation of p27(Kip1) . MT(1) receptor-mediated inhibition of activated NF-kappaB signaling provides a novel mechanism supporting the use of melatonin in prostate cancer chemoprevention and therapy

Rodriguez-Garcia A, Mayo JC, Hevia D, Quiros-Gonzalez I, Navarro M, Sainz RM. Phenotypic changes caused by melatonin increased sensitivity of prostate cancer cells to cytokine-induced apoptosis. *J Pineal Res* 2012 May 31.

Abstract: Melatonin has antiproliferative properties in prostate cancer cells. Melatonin reduces proliferation without increasing apoptosis, and it promotes cell differentiation into a neuroendocrine phenotype. Because neuroendocrine cells displayed an androgen-independent growth and high resistance to radiotherapy and chemotherapy, the role of molecules that induce neuroendocrine differentiation was questioned in terms of their usefulness as oncostatic agents. By using human epithelial androgen-dependent and androgen-independent prostate cancer cells, the role of melatonin in drug-induced apoptosis was studied after acute treatments. In addition to cytokines such as hrTNF-alpha and TRAIL, chemotherapeutic compounds, including doxorubicin, docetaxel, or etoposide, were employed in combination with melatonin to promote cell death. Melatonin promotes cell toxicity caused by cytokines without influencing the actions of chemotherapeutic agents. In addition, antioxidant properties of melatonin were confirmed in prostate cancer cells. However, its ability to

increase cell death caused by cytokines was independent of the redox changes. Finally, phenotypic changes caused by chronic treatment with the indole, that is, neuroendocrine differentiation, make cells significantly more sensitive to cytokines and slightly more sensitive to some chemotherapeutic compounds. Thus, melatonin is a good inhibitor of the proliferation of prostate cancer cells, promoting phenotypic changes that do not increase survival mechanisms and make cells more sensitive to cytokines such as TNF-alpha or TRAIL

Kabasakal L, Sener G, Balkan J, Dogru-Abbasoglu S, Keyer-Uysal M, Uysal M. Melatonin and beta-glucan alone or in combination inhibit the growth of dunning prostatic adenocarcinoma. *Oncol Res* 2011;19(6):259-63.

Abstract: In this study, the effects of melatonin or beta-glucan treatments on tumor growth, pro-oxidant, and antioxidant status in tumor tissue were investigated in Dunning 3327 MatLyLu prostatic adenocarcinoma model. Prostate cancer (PCa) was induced by single intradermal injection of  $2 \times 10^4$  MatLyLu cells into the right hind leg of Copenhagen rats. Melatonin (10 mg/kg/daily; IP) or beta-glucan (50 mg/kg/daily; orally) treatments applied alone and together continued for 39 days. Melatonin or beta-glucan treatments alone or together inhibited tumor growth and decreased malondialdehyde (MDA) levels in tumor tissues of Dunning rats. However, there were no significant differences in tumor volumes and MDA levels among treatment groups. Melatonin and melatonin + beta-glucan treatments elevated glutathione (GSH) levels and superoxide dismutase, glutathione peroxidase, and glutathione transferase activities in tumor tissues. However, beta-glucan treatment did not influence GSH levels and antioxidant enzyme activities in tumor tissue of Dunning rats. These results indicate that melatonin and beta-glucan treatments alone or together inhibit tumor progression and oxidative stress in tumor tissues of rats with Dunning PCa

Park JW, Hwang MS, Suh SI, Baek WK. Melatonin down-regulates HIF-1 alpha expression through inhibition of protein translation in prostate cancer cells. *J Pineal Res* 2009 May;46(4):415-21.

Abstract: Melatonin, the main secretory product of the pineal gland, has been shown to exert an oncostatic activity in cancer cells. Recently, several studies have shown that melatonin has antiangiogenic properties. However, the mechanism by which melatonin exerts antiangiogenic effects is not understood. Hypoxia inducible factor (HIF)-1 is a transcription factor which mediates adaptive response to changes in tissue oxygenation. HIF-1 is a heterodimer formed by the association of a constitutively expressed HIF-1 beta subunit and a HIF-1 alpha subunit, the expression of which is highly regulated. In this study, pharmacologic concentrations of melatonin was found to inhibit expression of HIF-1 alpha protein under both normoxic and hypoxic conditions in DU145, PC-3, and LNCaP prostate cancer cells without affecting HIF-1 alpha mRNA levels. Consistent with the reduction in HIF-1 alpha protein levels, melatonin inhibited HIF-1 transcriptional activity and the release of vascular endothelial growth factor. We found that the suppression of HIF-1 alpha expression by melatonin correlated with dephosphorylation of p70S6K and its direct target RPS6, a pathway known to regulate HIF-1 alpha expression at the translational level. Metabolic labeling assays indicated that melatonin inhibits de novo synthesis of HIF-1 alpha protein. Taken together, these results suggest that the pharmacologic concentration of melatonin inhibits HIF-1 alpha expression through the suppression of protein translation in prostate cancer cells

Joo SS, Yoo YM. Melatonin induces apoptotic death in LNCaP cells via p38 and JNK pathways: therapeutic implications for prostate cancer. *J Pineal Res* 2009 August;47(1):8-14.

Abstract: Apoptosis, a form of cell death, is a fundamental process for the development and maintenance of multicellular organisms that promotes the removal of damaged, senescent or unwanted cells. Induction of cancer cell apoptosis is an important strategy of anticancer therapy. In

this study, we examined if melatonin, the main secretory product of the pineal gland, inhibited the growth of prostate cancer cells (LNCaP) and promoted apoptosis via mitogen-activated protein kinases (MAPKs), which are closely associated with apoptosis and survival. Melatonin treatment significantly inhibited the growth of LNCaP cells in a dose- and time-dependent manner. It clearly induced both an early stage of apoptosis (propidium iodide(-), FITC Annexin-V(+)) and a late apoptosis/secondary necrosis (propidium iodide(+) and FITC Annexin-V(+)), which indicated induction of serial stages of apoptosis in cells. Moreover, melatonin markedly activated c-JUN N-terminal kinase (JNK) and p38 kinase, whereas extracellular signal-regulated kinase (ERK) was not responsive to melatonin. Treatment with MAPK inhibitors, PD98059 (ERK inhibitor), SP600125 (JNK inhibitor) and SB202190 (p38 inhibitor), confirmed that melatonin-induced apoptosis was JNK- and p38-dependent, but ERK-independent. In the presence of PD98059, caspase-3 activity increased, while levels of Bax/cytochrome c (Cyt c) and Bcl-2 decreased. These effects were opposite to those observed with SP600125 and SB202190 treatments. Together, these results strongly suggest that JNK and p38 activation directly participate in apoptosis induced by melatonin. Thus, melatonin may be of promise for anti-prostate cancer strategies

Connor TP. Melatonin as an adjuvant to therapeutic prostate cancer vaccines. *J Pineal Res* 2008 September;45(2):224.

Tam CW, Mo CW, Yao KM, Shiu SY. Signaling mechanisms of melatonin in antiproliferation of hormone-refractory 22Rv1 human prostate cancer cells: implications for prostate cancer chemoprevention. *J Pineal Res* 2007 March;42(2):191-202.

Abstract: There is an unmet clinical demand for safe and effective pharmaceuticals/nutraceuticals for prostate cancer prevention and hormone-refractory prostate cancer treatment. Previous laboratory and human studies of our laboratory demonstrated an association between the antiproliferative action of melatonin and melatonin MT(1) receptor expression in prostate cancer. The aim of this study was to determine, using a pharmacological approach, the signaling mechanisms of melatonin in hormone-refractory 22Rv1 human prostate cancer cell antiproliferation. Both immunoreactive MT(1) and MT(2) subtypes of G protein-coupled melatonin receptor were expressed in 22Rv1 cells. Melatonin inhibited, concentration dependently, cell proliferation, upregulated p27(Kip1) gene transcription and protein expression, and downregulated activated androgen signaling in 22Rv1 cells. While the effects of melatonin were mimicked by 2-iodomelatonin, a high-affinity nonselective MT(1) and MT(2) receptor agonist, melatonin effects were blocked by luzindole, a nonselective MT(1) and MT(2) receptor antagonist, but were unaffected by 4-phenyl-2-propionamidotetraline, a selective MT(2) receptor antagonist. Importantly, we discovered that the antiproliferative effect of melatonin exerted via MT(1) receptor on p27(Kip1) gene and protein upregulation is mediated by a novel signaling mechanism involving co-activation of protein kinase C (PKC) and PKA in parallel. Moreover, we also showed that a melatonin/MT(1)/PKC mechanism is involved in melatonin-induced downregulation of activated androgen signal transduction in 22Rv1 cells. Taken together with the known molecular mechanisms of prostate cancer progression and transition to androgen independence, our data provide strong support for melatonin to be a promising small-molecule useful for prostate cancer primary prevention and secondary prevention of the development and progression of hormone refractoriness

Sainz RM, Mayo JC, Tan DX, Leon J, Manchester L, Reiter RJ. Melatonin reduces prostate cancer cell growth leading to neuroendocrine differentiation via a receptor and PKA independent mechanism. *Prostate* 2005 April 1;63(1):29-43.

Abstract: BACKGROUND: Melatonin, the main secretory product of the pineal gland, inhibits the

growth of several types of cancer cells. Melatonin limits human prostate cancer cell growth by a mechanism which involves the regulation of androgen receptor function but it is not clear whether other mechanisms may also be involved. METHODS: Time-course and dose-dependent studies were performed using androgen-dependent (LNCaP) and independent (PC3) prostate cancer cells. Cell number, cell viability, and cell cycle progression were studied. Neuroendocrine differentiation of these cells was evaluated by studying morphological and biochemical markers. Finally, molecular mechanisms including the participation of melatonin membrane receptors, intracellular cAMP levels, and the PKA signal transduction pathway were also analyzed. RESULTS: Melatonin treatment dramatically reduced the number of prostate cancer cells and stopped cell cycle progression in both LNCaP and PC3 cells. In addition, it induced cellular differentiation as indicated by obvious morphological changes and neuroendocrine biochemical parameters. The role of melatonin in cellular proliferation and differentiation of prostate cancer cells is not mediated by its membrane receptors nor related to PKA activation. CONCLUSIONS: The treatment of prostate cancer cells with pharmacological concentrations of melatonin influences not only androgen-sensitive but also androgen-insensitive epithelial prostate cancer cells. Cell differentiation promoted by melatonin is not mediated by PKA activation although it increases, in a transitory manner, intracellular cAMP levels. Melatonin markedly influences the proliferative status of prostate cancer cells. These effects should be evaluated thoroughly since melatonin levels are diminished in aged individuals when prostate cancer typically occurs

Shiu SY, Law IC, Lau KW, Tam PC, Yip AW, Ng WT. Melatonin slowed the early biochemical progression of hormone-refractory prostate cancer in a patient whose prostate tumor tissue expressed MT1 receptor subtype. *J Pineal Res* 2003 October;35(3):177-82.

Abstract: Melatonin inhibited the proliferation of hormone-independent LNCaP prostate cancer cells partly via MT1 receptor activation both in vitro and in nude mice xenograft model. In this study, the melatonin receptor expression in the prostate cancer tissue of a patient with bone metastases and the effect of melatonin on the biochemical progression of hormone-refractory prostate tumor which later developed in the same patient were reported. Saturation and competition 2-[125I]iodomelatonin binding assays were conducted on prostate tumor tissue obtained by transurethral resection of the prostate from the index patient. The receptor subtype identity of melatonin receptor expressed in the cancer tissue was determined by comparison of the rank order of inhibition constants ( $K_i$ ) of various melatonergic ligands and the affinity of 4-phenyl-2-propionamidotetraline relative to melatonin in inhibiting 2-[125I]iodomelatonin binding to the tumor sample and to human cell lines stably transfected with MT1 or MT2 melatonin receptor subtype. MT1 receptor expression in the cancer tissue was also examined by immunohistochemistry. The surgically castrated patient later developed biochemical relapse of his disease. His serum total prostate-specific antigen (PSA) level was monitored before and during treatment with 5 mg/day oral melatonin at 20:00 hr. High-affinity ( $K_d = 103.7$  pm) MT1 melatonin receptor subtype was expressed by the patient's prostate cancer. As indicated by his PSA levels, melatonin induced stabilization of his hormone-refractory disease for 6 wk. This report validates melatonin's oncostatic action on prostate cancer and the potential involvement of MT1 receptor subtype in the attendant antiproliferative signal transduction as suggested by recent preclinical laboratory findings in a human

Marelli MM, Limonta P, Maggi R, Motta M, Moretti RM. Growth-inhibitory activity of melatonin on human androgen-independent DU 145 prostate cancer cells. *Prostate* 2000 November 1;45(3):238-44.

Abstract: BACKGROUND: The pineal hormone melatonin has been shown to exert a direct oncostatic activity on neoplastic cells, particularly from breast cancer. In the present study, we evaluated the effects of melatonin on the proliferation and on the cell cycle distribution of human androgen-independent DU 145 prostate cancer cells. Experiments were also performed to gain

insights into the possible mechanism of action of the hormone. **METHODS:** The effects of melatonin on DU 145 cell proliferation was analyzed by counting the cells by hemocytometer at the end of treatment. The effects of the pineal hormone on cell cycle distribution were evaluated by FACS analysis. RT-PCR studies were performed to detect Mel(1a) and Mel(1b) expression in DU 145 cells. The cellular localization of (125)I-melatonin binding sites was investigated by radioreceptor assay. A commercially available binding-protein assay kit was utilized to evaluate intracellular cAMP levels. **RESULTS:** Melatonin, in physiological doses, significantly inhibited DU 145 cell proliferation and induced cell cycle withdrawal by accumulating cells in G0/G1 phase. The mRNA for Mel(1a) receptors was found to be expressed in DU 145 cells; however, by radioreceptor assay, no binding sites for (125)I-melatonin could be detected in membrane preparations, suggesting that, in these cells, the level of translation of this mRNA is too low to possibly mediate the antiproliferative action of the hormone. In agreement with this hypothesis, melatonin did not affect forskolin-induced intracellular cAMP accumulation. Binding sites for (125)I-melatonin were found in nuclear extracts of DU 145 cells. **CONCLUSIONS:** Melatonin exerts a direct oncostatic activity on human androgen-independent prostate cancer cells, by affecting cell cycle progression. This activity seems to be mediated by nuclear, but not by membrane, receptors

Rimler A, Culig Z, Levy-Rimler G et al. Melatonin elicits nuclear exclusion of the human androgen receptor and attenuates its activity. *Prostate* 2001 October 1;49(2):145-54.

**Abstract: BACKGROUND:** The androgen receptor (AR) promotes growth and functionality of androgen sensitive benign and cancer tissues. The pineal hormone melatonin is an androgen protagonist in vivo and in vitro. The interference of melatonin in the AR cascade was explored. **METHODS:** The effects of melatonin on AR expression, level, agonist and androgen-response element (ARE) binding, reporter gene activity and intracellular localization were explored in prostate cancer LNCaP cell line. **RESULTS:** Melatonin increased immunoreactive AR cells in the absence and presence of dihydrotestosterone. Despite this increase and maintenance of AR agonist binding capacity, the androgen-induced reporter gene activity and suppression of AR-mRNA were attenuated. Immunocytochemical analysis and subcellular fractionation studies revealed nuclear exclusion of AR by melatonin. **CONCLUSIONS:** The melatonin-mediated nuclear exclusion of the AR may explain the attenuation of AR activity in the prostate cancer cells. This is the first demonstration of a hormone-induced mislocalization of the AR in prostate epithelial cells and may represent a novel route for regulating AR activity

Lissoni P, Cazzaniga M, Tancini G et al. Reversal of clinical resistance to LHRH analogue in metastatic prostate cancer by the pineal hormone melatonin: efficacy of LHRH analogue plus melatonin in patients progressing on LHRH analogue alone. *Eur Urol* 1997;31(2):178-81.

**Abstract: OBJECTIVE:** Experimental and preliminary clinical studies have suggested that the pineal hormone melatonin (MLT) may stimulate hormone receptor expression on both normal and cancer cells. Moreover, MLT has appeared to inhibit the growth of some cancer cell lines, including prostate cancer, either by exerting a direct cytostatic action, or by decreasing the endogenous production of some tumor growth factors, such as prolactin (PRL) and insulin-like growth factor-1 (IGF-1). On this basis, a study was carried out to evaluate the clinical efficacy of a neuroendocrine combination consisting of the LHRH analogue triptorelin plus MLT in metastatic prostate cancer progressing on triptorelin alone. **MATERIAL AND METHODS:** The study including 14 consecutive metastatic prostate cancer patients with poor clinical conditions (median age: 70.5 years; median PS: 50%), refractory or resistant to a previous therapy with the LHRH analogue triptorelin alone. Triptorelin was injected i.m. at 3.75 mg every 28 days, and MLT was given orally at 20 mg/day in the evening every day until progression, starting 7 days prior to triptorelin. **RESULTS AND CONCLUSIONS:** A decrease in PSA serum levels greater than 50% was obtained in 8/14 (57%) patients. Moreover, PSA

mean concentrations significantly decreased on therapy of triptorelin plus MLT. In addition, a normalization of platelet number was obtained in 3/5 patients with persistent thrombocytopenia prior to study. Mean serum levels of both PRL and IGF-1 significantly decreased on therapy. Finally, a survival longer than 1 year was achieved in 9/14 (64%) patients. This preliminary study would suggest that the concomitant administration of the pineal hormone MLT may overcome the clinical resistance to LHRH analogues and improve the clinical conditions in metastatic prostatic cancer patients

## Low-Dose Aspirin

Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* 2012 April 28;379(9826):1591-601.

Abstract: BACKGROUND: Daily aspirin reduces the long-term incidence of some adenocarcinomas, but effects on mortality due to some cancers appear after only a few years, suggesting that it might also reduce growth or metastasis. We established the frequency of distant metastasis in patients who developed cancer during trials of daily aspirin versus control. METHODS: Our analysis included all five large randomised trials of daily aspirin ( $\geq 75$  mg daily) versus control for the prevention of vascular events in the UK. Electronic and paper records were reviewed for all patients with incident cancer. The effect of aspirin on risk of metastases at presentation or on subsequent follow-up (including post-trial follow-up of in-trial cancers) was stratified by tumour histology (adenocarcinoma vs other) and clinical characteristics. FINDINGS: Of 17,285 trial participants, 987 had a new solid cancer diagnosed during mean in-trial follow-up of 6.5 years (SD 2.0). Allocation to aspirin reduced risk of cancer with distant metastasis (all cancers, hazard ratio [HR] 0.64, 95% CI 0.48-0.84,  $p=0.001$ ; adenocarcinoma, HR 0.54, 95% CI 0.38-0.77,  $p=0.0007$ ; other solid cancers, HR 0.82, 95% CI 0.53-1.28,  $p=0.39$ ), due mainly to a reduction in proportion of adenocarcinomas that had metastatic versus local disease (odds ratio 0.52, 95% CI 0.35-0.75,  $p=0.0006$ ). Aspirin reduced risk of adenocarcinoma with metastasis at initial diagnosis (HR 0.69, 95% CI 0.50-0.95,  $p=0.02$ ) and risk of metastasis on subsequent follow-up in patients without metastasis initially (HR 0.45, 95% CI 0.28-0.72,  $p=0.0009$ ), particularly in patients with colorectal cancer (HR 0.26, 95% CI 0.11-0.57,  $p=0.0008$ ) and in patients who remained on trial treatment up to or after diagnosis (HR 0.31, 95% CI 0.15-0.62,  $p=0.0009$ ). Allocation to aspirin reduced death due to cancer in patients who developed adenocarcinoma, particularly in those without metastasis at diagnosis (HR 0.50, 95% CI 0.34-0.74,  $p=0.0006$ ). Consequently, aspirin reduced the overall risk of fatal adenocarcinoma in the trial populations (HR 0.65, 95% CI 0.53-0.82,  $p=0.0002$ ), but not the risk of other fatal cancers (HR 1.06, 95% CI 0.84-1.32,  $p=0.64$ ; difference,  $p=0.003$ ). Effects were independent of age and sex, but absolute benefit was greatest in smokers. A low-dose, slow-release formulation of aspirin designed to inhibit platelets but to have little systemic bioavailability was as effective as higher doses. INTERPRETATION: That aspirin prevents distant metastasis could account for the early reduction in cancer deaths in trials of daily aspirin versus control. This finding suggests that aspirin might help in treatment of some cancers and provides proof of principle for pharmacological intervention specifically to prevent distant metastasis. FUNDING: None

## Whole-Food Vegan Diet

Frattaroli J, Weidner G, Dnistrian AM et al. Clinical events in prostate cancer lifestyle trial: results from two years of follow-up. *Urology* 2008 December;72(6):1319-23.

Abstract: OBJECTIVES: Previous research has demonstrated that patients with prostate cancer participating in the Prostate Cancer Lifestyle Trial had a reduction in prostate-specific antigen (PSA)

levels, inhibition of LNCaP cell growth, and fewer prostate cancer-related clinical events at the end of 1 year compared with controls. The aim of this study was to examine the clinical events in this trial during a 2-year period. **METHODS:** The Prostate Cancer Lifestyle Trial was a 1-year randomized controlled clinical trial of 93 patients with early-stage prostate cancer (Gleason score <7, PSA 4-10 ng/mL) undergoing active surveillance. The patients in the experimental arm were encouraged to adopt a low-fat, plant-based diet, to exercise and practice stress management, and to attend group support sessions. The control patients received the usual care. **RESULTS:** By 2 years of follow-up, 13 of 49 (27%) control patients and 2 of 43 (5%) experimental patients had undergone conventional prostate cancer treatment (radical prostatectomy, radiotherapy, or androgen deprivation,  $P < .05$ ). No differences were found between the groups in other clinical events (eg, cardiac), and no deaths occurred. Three of the treated control patients but none of the treated experimental patients had a PSA level of  $\geq 10$  ng/mL, and 1 treated control patient but no treated experimental patients had a PSA velocity of  $>2$  ng/mL/y before treatment. No significant differences were found between the untreated experimental and untreated control patients in PSA change or velocity at the end of 2 years. **CONCLUSIONS:** Patients with early-stage prostate cancer choosing active surveillance might be able to avoid or delay conventional treatment for at least 2 years by making changes in their diet and lifestyle

Ornish D, Weidner G, Fair WR et al. Intensive lifestyle changes may affect the progression of prostate cancer. *J Urol* 2005 September;174(3):1065-9.

**Abstract:** **PURPOSE:** Men with prostate cancer are often advised to make changes in diet and lifestyle, although the impact of these changes has not been well documented. Therefore, we evaluated the effects of comprehensive lifestyle changes on prostate specific antigen (PSA), treatment trends and serum stimulated LNCaP cell growth in men with early, biopsy proven prostate cancer after 1 year. **MATERIALS AND METHODS:** Patient recruitment was limited to men who had chosen not to undergo any conventional treatment, which provided an unusual opportunity to have a nonintervention randomized control group to avoid the confounding effects of interventions such as radiation, surgery or androgen deprivation therapy. A total of 93 volunteers with serum PSA 4 to 10 ng/ml and cancer Gleason scores less than 7 were randomly assigned to an experimental group that was asked to make comprehensive lifestyle changes or to a usual care control group. **RESULTS:** None of the experimental group patients but 6 control patients underwent conventional treatment due to an increase in PSA and/or progression of disease on magnetic resonance imaging. PSA decreased 4% in the experimental group but increased 6% in the control group ( $p = 0.016$ ). The growth of LNCaP prostate cancer cells (American Type Culture Collection, Manassas, Virginia) was inhibited almost 8 times more by serum from the experimental than from the control group (70% vs 9%,  $p < 0.001$ ). Changes in serum PSA and also in LNCaP cell growth were significantly associated with the degree of change in diet and lifestyle. **CONCLUSIONS:** Intensive lifestyle changes may affect the progression of early, low grade prostate cancer in men. Further studies and longer term followup are warranted

Ngo TH, Barnard RJ, Anton T et al. Effect of isocaloric low-fat diet on prostate cancer xenograft progression to androgen independence. *Cancer Res* 2004 February 15;64(4):1252-4.

**Abstract:** An isocaloric low-fat diet has been shown to slow androgen-sensitive Los Angeles Prostate Cancer-4 (LAPC-4) tumor growth in a mouse xenograft model. LAPC-4 cells were injected into male severe combined immunodeficient mice. After palpable tumors developed, the mice were divided into three groups, high-fat intact, high-fat castration, and low-fat castration. Tumor latency (18 versus 9 weeks;  $P < 0.001$ ) and mouse survival (20.8  $\pm$  1.3 versus 13  $\pm$  0.7 weeks;  $P < 0.01$ ) were significantly longer in the low-fat castration versus high-fat castration group. Reduced dietary fat intake

delayed conversion from androgen-sensitive to -insensitive prostate cancer and significantly prolonged survival of severe combined immunodeficient mice bearing LAPC-4 xenografts

Barnard RJ, Ngo TH, Leung PS, Aronson WJ, Golding LA. A low-fat diet and/or strenuous exercise alters the IGF axis in vivo and reduces prostate tumor cell growth in vitro. *Prostate* 2003 August 1;56(3):201-6.

Abstract: **BACKGROUND:** Prostate cancer is the most common solid-tumor cancer in US males but is rare in Asian males. When Asian men adopt the US lifestyle, clinical prostate cancer increases greatly. Epidemiological data from men in the US indicate that regular activity may reduce the risk for prostate cancer. **METHODS:** Serum was obtained from three groups of similar-aged men, Control, Diet and Exercise, and Exercise alone were used to stimulate LNCaP cells in culture. Growth and apoptosis of tumor cells were measured. Serum samples were also used to measure insulin, IGF-1, IGFBP-1. **RESULTS:** The Diet and Exercise and the Exercise alone groups had lower serum insulin and IGF-1 but higher IGFBP-1 compared to Controls. LNCaP cell growth was reduced in both groups compared to Control and there was a major increase in apoptosis of tumor cells. **CONCLUSIONS:** A low-fat diet and/or intensive exercise results in change in serum hormones and growth factors in vivo that can reduce growth and induce apoptosis of LNCaP prostate tumor cells in vitro

Ngo TH, Barnard RJ, Tymchuk CN, Cohen P, Aronson WJ. Effect of diet and exercise on serum insulin, IGF-I, and IGFBP-1 levels and growth of LNCaP cells in vitro (United States). *Cancer Causes Control* 2002 December;13(10):929-35.

Abstract: **OBJECTIVE:** Accumulating evidence indicates that prostate cancer is associated with high levels of serum IGF-I. This study was conducted to determine whether a low-fat diet and exercise (DE) intervention may modulate the IGF axis and reduce prostate cancer cell growth in vitro. **METHODS:** Fasting serum was obtained from 14 men (age 60 +/- 3 years) participating in an 11-day DE program and from eight similarly aged men who had followed the DE program for 14.2 +/- 1.7 years (long-term). Insulin, IGF-I, IGFBP-1, and IGFBP-3 were measured by ELISA, and serum was used to stimulate LNCaP cell growth in vitro. **RESULTS:** Serum IGF-I levels decreased by 20% while IGFBP-1 increased by 53% after 11-day DE. In the long-term group, IGF-I was 55% lower, while IGFBP-1 was 150% higher relative to baseline. Serum insulin decreased by 25% after 11-day DE and was 68% lower in the long-term group, relative to baseline. No changes in serum IGFBP-3 were observed. Serum-stimulated LNCaP cell growth was reduced by 30% in post-11-day serum and by 44% in long-term serum relative to baseline. LNCaP cells incubated with post-DE serum showed increased apoptosis/ necrosis, compared to baseline. **CONCLUSIONS:** A low-fat diet and exercise intervention induces in-vivo changes in the circulating IGF axis and is associated with reduced growth and enhanced apoptosis/necrosis of LNCaP tumor cells in vitro

## Vitamin D

Swami S, Krishnan AV, Wang JY et al. Dietary vitamin D(3) and 1,25-dihydroxyvitamin D(3) (calcitriol) exhibit equivalent anticancer activity in mouse xenograft models of breast and prostate cancer. *Endocrinology* 2012 June;153(6):2576-87.

Abstract: 1,25-dihydroxyvitamin D(3) [1,25(OH)(2)D(3) or calcitriol], the hormonally active vitamin D metabolite, exhibits anticancer actions in models of breast cancer and prostate cancer. Because CYP27B1 (1 $\alpha$ -hydroxylase), the enzyme catalyzing 1,25(OH)(2)D(3) formation in the kidney, is also expressed in extrarenal tissues, we hypothesize that dietary vitamin D(3) will be converted to 25(OH)D(3) in the body and then to 1,25(OH)(2)D(3) locally in the cancer microenvironment in

which it will exert autocrine/paracrine anticancer actions. Immunocompromised mice bearing MCF-7 breast cancer xenografts showed significant tumor shrinkage (>50%) after ingestion of a vitamin D(3)-supplemented diet (5000 IU/kg) compared with a control diet (1000 IU/kg). Dietary vitamin D(3) inhibition of tumor growth was equivalent to administered calcitriol (0.025, 0.05, or 0.1 mug/mouse, three times a week). Both treatments equivalently inhibited PC-3 prostate cancer xenograft growth but to a lesser extent than the MCF-7 tumors. Calcitriol at 0.05 mug and 0.1 mug caused modest but statistically significant increases in serum calcium levels indicating that the dietary vitamin D(3) comparison was to a maximally safe calcitriol dose. Dietary vitamin D(3) did not increase serum calcium, demonstrating its safety at the concentration tested. The vitamin D(3) diet raised circulating 1,25 dihydroxyvitamin D levels and did not alter CYP27B1 mRNA in the kidney but increased it in the tumors, suggesting that extrarenal sources including the tumors contributed to the elevated circulating 1,25 dihydroxyvitamin D(3). Both calcitriol and dietary vitamin D(3) were equipotent in suppressing estrogen synthesis and signaling and other proinflammatory and growth signaling pathways. These preclinical data demonstrate the potential utility of dietary vitamin D(3) supplementation in cancer prevention and therapy

Oades GM, Dredge K, Kirby RS, Colston KW. Vitamin D receptor-dependent antitumour effects of 1,25-dihydroxyvitamin D3 and two synthetic analogues in three in vivo models of prostate cancer. *BJU Int* 2002 October;90(6):607-16.

Abstract: OBJECTIVE: To determine the in vitro and in vivo effects of 1,25-dihydroxyvitamin D3 (calcitriol) and two newer less hypercalcaemic analogues, EB1089 and CB1093 (as the use of calcitriol as a therapeutic agent in humans has been limited by hypercalcaemia) in three rodent models of prostate cancer. MATERIALS AND METHODS: The highly metastatic MAT LyLu Dunning prostate model, P4III tumours in Lobund-Wistar rats and LNCaP xenografts in nude mice were used. Vitamin D receptor (VDR) expression and binding were assessed in all cell lines. The effects of calcitriol, EB1089 and CB1093 on tumour growth, cell cycle and angiogenesis in vitro, and growth and serum calcium levels in vivo, were assessed. RESULTS: The growth of prostate adenocarcinoma was inhibited by calcitriol, EB1089 and CB1093 in the Dunning prostate model. Although both analogues increased serum calcium levels, the levels were significantly less than in rats treated with calcitriol. Tumour growth was also inhibited in male athymic nu/nu mice with LNCaP tumour xenografts. P4III cells failed to express functional VDR and were insensitive to calcitriol and its analogues, either in vitro or in vivo. The analogues of calcitriol did not inhibit angiogenesis in a rat aorta assay. CONCLUSION: This is the first report comparing the actions of calcitriol and its analogues in different in vivo models. The results suggest that the newer less hypercalcaemic analogues of calcitriol may offer a novel therapeutic option for treating prostate cancer. VDR-dependent growth inhibition and not the inhibition of angiogenesis is the main mechanism of action of these compounds in vivo

### **Spirulina (inhibitor of NADPH oxidase)**

Floriano-Sanchez E, Castro-Marin M, Cardenas-Rodriguez N, Lara-Padilla E. [Evaluation of the expression of p22 phox subunit of NADPH oxidase (NOX) in prostate cancer and benign prostatic hyperplasia: a comparative study]. *Actas Urol Esp* 2010 April;34(4):340-5.

Abstract: INTRODUCTION AND OBJECTIVE: Recent reports found that prostate cancer is the second most common cancer and second leading cause of cancer death in men. METHODS AND RESULTS: 62 samples were obtained (30 of patients with cancer and 32 of patients with hyperplasia) collected from January 2004 to december 2007. Was conducted a clinical, experimental, transversal, comparative and descriptive trial. Were followed the inclusion (cancer or hyperplasia diagnosis), exclusion (patients not authorized to participate in the study or not candidates for resection of

prostate) and elimination (damage tissue) criteria. Was detected by immunohistochemistry the presence of p22 phox NADPH oxidase subunit in patients with prostate cancer and prostatic hyperplasia from the formation of avidin-biotin complex using diaminobenzidine as a dye contrast. The statistical analysis was determined with t test (Graph Prism 3.0 software) considering  $p < 0.05$  for statistical differences. The results of the immunoreactivity of p22 phox in the stroma and gland of the prostate showed an increase in prostate cancer ( $8.45 \pm 3.6$  and  $25.08 \pm 7.5\%$   $p < 0.0001$ , respectively) in comparison with the results for prostatic hyperplasia ( $4.8 \pm 2.8$  and  $6.7 \pm 3.1\%$   $p < 0.0001$ , respectively). CONCLUSIONS: The over-expression of the NADPH oxidase is involved in the prostate cancer. Moreover, we suggested that the NADPH oxidase, in combination with other classical markers, could be an indicator for the post-treatment monitoring of the patients diagnosed with hyperplasia and others minors pathologies of the prostate

Sun Y, St Clair DK, Xu Y, Crooks PA, St Clair WH. A NADPH oxidase-dependent redox signaling pathway mediates the selective radiosensitization effect of parthenolide in prostate cancer cells. *Cancer Res* 2010 April 1;70(7):2880-90.

Abstract: Cancer cells are usually under higher oxidative stress compared with normal cells. We hypothesize that introducing additional reactive oxygen species (ROS) insults or suppressing antioxidant capacity may selectively enhance cancer cell killing by oxidative stress-generating agents through stress overload or stress sensitization, whereas normal cells may be able to maintain redox homeostasis under exogenous ROS by adaptive response. Here, we show that parthenolide, a sesquiterpene lactone, selectively exhibits a radiosensitization effect on prostate cancer PC3 cells but not on normal prostate epithelial PrEC cells. Parthenolide causes oxidative stress in PC3 cells but not in PrEC cells, as determined by the oxidation of the ROS-sensitive probe H(2)DCFDA and intracellular reduced thiol and disulfide levels. In PC3 but not PrEC cells, parthenolide activates NADPH oxidase, leading to a decrease in the level of reduced thioredoxin, activation of phosphoinositide 3-kinase/Akt, and consequent FOXO3a phosphorylation, which results in the downregulation of FOXO3a targets antioxidant enzyme manganese superoxide dismutase and catalase. Importantly, when combined with radiation, parthenolide further increases ROS levels in PC3 cells whereas it decreases radiation-induced oxidative stress in PrEC cells, possibly by increasing reduced glutathione levels. Together, the results show that parthenolide selectively activates NADPH oxidase and mediates intense oxidative stress in prostate cancer cells by both increasing ROS generation and decreasing antioxidant defense capacity. The results support the concept of exploiting the intrinsic differences in the redox status of cancer cells and normal cells as targets for selective cancer killing

Jajoo S, Mukherjea D, Watabe K, Ramkumar V. Adenosine A(3) receptor suppresses prostate cancer metastasis by inhibiting NADPH oxidase activity. *Neoplasia* 2009 November;11(11):1132-45.

Abstract: Prostate cancer is the most commonly diagnosed and second most lethal malignancy in men, due mainly to a lack of effective treatment for the metastatic disease. A number of recent studies have shown that activation of the purine nucleoside receptor, adenosine A(3) receptor (A(3)AR), attenuates proliferation of melanoma, colon, and prostate cancer cells. In the present study, we determined whether activation of the A(3)AR reduces the ability of prostate cancer cells to migrate in vitro and metastasize in vivo. Using severe combined immunodeficient mice, we show that proliferation and metastasis of AT6.1 rat prostate cancer cells were decreased by the administration of A(3)AR agonist N(6)-(3-iodobenzyl) adenosine-5'-N-methyluronamide. In vitro studies show that activation of A(3)AR decreased high basal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity present in these cells, along with the expression of Rac1 and p47(phox) subunits of this enzyme. Inhibition of NADPH oxidase activity by the dominant-negative RacN17 or short interfering (si)RNA against p47(phox) reduced both the generation of reactive oxygen species and the

invasion of these cells on Matrigel. In addition, we show that membrane association of p47(phox) and activation of NADPH oxidase is dependent on the activity of the extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein kinase pathway. We also provide evidence that A(3)AR inhibits ERK1/2 activity in prostate cancer cells through inhibition of adenylyl cyclase and protein kinase A. We conclude that activation of the A(3)AR in prostate cancer cells reduces protein kinase A-mediated stimulation of ERK1/2, leading to reduced NADPH oxidase activity and cancer cell invasiveness

Lu JP, Monardo L, Bryskin I et al. Androgens induce oxidative stress and radiation resistance in prostate cancer cells through NADPH oxidase. *Prostate Cancer Prostatic Dis* 2010 March;13(1):39-46.

Abstract: Androgen deprivation therapy (ADT) facilitates the response of prostate cancer (PC) to radiation. Androgens have been shown to induce elevated basal levels of reactive oxygen species (ROS) in PC, leading to adaptation to radiation-induced cytotoxic oxidative stress. Here, we show that androgens increase the expression of p22(phox) and gp91(phox) subunits of NADPH oxidase (NOX) and ROS production by NOX2 and NOX4 in PC. Pre-radiation treatment of 22Rv1 human PC cells with NOX inhibitors sensitize the cells to radiation similarly to ADT, suggesting that their future usage may spare the need for adjuvant ADT in PC patients undergoing radiation

Kumar B, Koul S, Khandrika L, Meacham RB, Koul HK. Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. *Cancer Res* 2008 March 15;68(6):1777-85.

Abstract: Reactive oxygen species (ROS) and the coupled oxidative stress have been associated with tumor formation. Several studies suggested that ROS can act as secondary messengers and control various signaling cascades. In the present studies, we characterized the oxidative stress status in three different prostate cancer cells (PC3, DU145, and LNCaP) exhibiting various degree of aggressiveness and normal prostate cells in culture (WPMY1, RWPE1, and primary cultures of normal epithelial cells). We observed increased ROS generation in cancer cells compared with normal cells, and that extramitochondrial source of ROS generator, NAD(P)H oxidase (Nox) systems, are associated with the ROS generation and are critical for the malignant phenotype of prostate cancer cells. Moreover, diphenyliodonium, a specific Nox inhibitor, blocked proliferation, modulated the activity of growth signaling cascades extracellular signal-regulated kinase (ERK)1/ERK2 and p38 mitogen-activated protein kinase as well as AKT protein kinase B, and caused cyclin B-dependent G(2)-M cell cycle arrest. We also observed higher degrees of ROS generation in the PC3 cells than DU145 and LNCaP, and that ROS generation is critical for migratory/invasiveness phenotypes. Furthermore, blocking of the ROS production rather than ROS neutralization resulted in decreased matrix metalloproteinase 9 activity as well as loss of mitochondrial potential, plausible reasons for decreased cell invasion and increased cell death. Taken together, these studies show, for the first time, the essential role of ROS production by extramitochondrial source in prostate cancer and suggest that therapies aimed at reducing ROS production might offer effective means of combating prostate cancer in particular, and perhaps other malignancies in general

Wartenberg M, Hoffmann E, Schwindt H et al. Reactive oxygen species-linked regulation of the multidrug resistance transporter P-glycoprotein in Nox-1 overexpressing prostate tumor spheroids. *FEBS Lett* 2005 August 15;579(20):4541-9.

Abstract: Expression of the multidrug resistance (MDR) transporter P-glycoprotein (P-gp) has been demonstrated to be regulated by hypoxia-inducible factor-1alpha (HIF-1alpha) and inhibited by intracellular reactive oxygen species (ROS). Herein, P-gp and HIF-1alpha expression were investigated in multicellular prostate tumor spheroids overexpressing the ROS-generating enzyme Nox-1 in comparison to the mother cell line DU-145. In Nox-1-overexpressing tumor spheroids (DU-

145Nox1) generation of ROS as well as expression of Nox-1 was significantly increased as compared to DU-145 tumor spheroids. ROS generation was significantly inhibited in the presence of the NADPH-oxidase antagonists diphenylen-iodonium chloride (DPI) and 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF). Albeit growth kinetic of DU-145Nox1 tumor spheroids was decreased as compared to DU-145 spheroids, elevated expression of Ki-67 was observed indicating increased cell cycle activity. In DU-145Nox1 tumor spheroids, expression of HIF-1alpha as well as P-gp was significantly decreased as compared to DU-145 spheroids, which resulted in an increased retention of the anticancer agent doxorubicin. Pretreatment with the free radical scavengers vitamin E and vitamin C increased the expression of P-gp as well as HIF-1alpha in Nox-1-overexpressing cells, whereas no effect of free radical scavengers was observed on *mdr-1* mRNA expression. In summary, the data of the present study demonstrate that the development of P-gp-mediated MDR is abolished under conditions of elevated ROS levels, suggesting that the MDR phenotype can be circumvented by modest increase of intracellular ROS generation

Brar SS, Corbin Z, Kennedy TP et al. NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells. *Am J Physiol Cell Physiol* 2003 August;285(2):C353-C369.

Abstract: Reactive oxygen species (ROS) appear to play an important role in regulating growth and survival of prostate cancer. However, the sources for ROS production in prostate cancer cells have not been determined. We report that ROS are generated by intact American Type Culture Collection DU 145 cells and by their membranes through a mechanism blocked by NAD(P)H oxidase inhibitors. ROS are critical for growth in these cells, because NAD(P)H oxidase inhibitors and antioxidants blocked proliferation. Components of the human phagocyte NAD(P)H oxidase, p22phox and gp91phox, as well as the Ca<sup>2+</sup> concentration-responsive gp91phox homolog NOX5 were demonstrated in DU 145 cells by RT-PCR and sequencing. Although the protein product for p22phox was not detectable, both gp91phox and NOX5 were identified throughout the cell by immunostaining and confocal microscopy and NOX5 immunostaining was enhanced in a perinuclear location, corresponding to enhanced ROS production adjacent to the nuclear membrane imaged by 2',7'-dichlorofluorescein diacetate oxidation. The calcium ionophore ionomycin dramatically stimulated ferricytochrome c reduction in cell media, further supporting the importance of NOX5 for ROS production. Antisense oligonucleotides for NOX5 inhibited ROS production and cell proliferation in DU 145 cells. In contrast, antisense oligonucleotides to p22phox or gp91phox did not impair cell growth. Inhibition of ROS generation with antioxidants or NAD(P)H oxidase inhibitors increased apoptosis in cells. These results indicate that ROS generated by the newly described NOX5 oxidase are essential for prostate cancer growth, possibly by providing trophic intracellular oxidant tone that retards programmed cell death

Floriano-Sanchez E, Castro-Marin M, Cardenas-Rodriguez N, Lara-Padilla E. [Evaluation of the expression of p22 phox subunit of NADPH oxidase (NOX) in prostate cancer and benign prostatic hyperplasia: a comparative study]. *Actas Urol Esp* 2010 April;34(4):340-5.

Abstract: INTRODUCTION AND OBJECTIVE: Recent reports found that prostate cancer is the second most common cancer and second leading cause of cancer death in men. METHODS AND RESULTS: 62 samples were obtained (30 of patients with cancer and 32 of patients with hyperplasia) collected from January 2004 to december 2007. Was conducted a clinical, experimental, transversal, comparative and descriptive trial. Were followed the inclusion (cancer or hyperplasia diagnosis), exclusion (patients not authorized to participate in the study or not candidates for resection of prostate) and elimination (damage tissue) criteria. Was detected by immunohistochemistry the presence of p22 phox NADPH oxidase subunit in patients with prostate cancer and prostatic hyperplasia from the formation of avidin-biotin complex using diaminobenzidine as a dye contrast. The statistical analysis was determined with t test (Graph Prism 3.0 software) considering  $p < 0.05$  for

statistical differences. The results of the immunoreactivity of p22 phox in the stroma and gland of the prostate showed an increase in prostate cancer (8.45+/-3.6 and 25.08+/-7.5% p<0.0001, respectively) in comparison with the results for prostatic hyperplasia (4.8+/-2.8 and 6.7+/-3.1% p<0.0001, respectively). CONCLUSIONS: The over-expression of the NADPH oxidase is involved in the prostate cancer. Moreover, we suggested that the NADPH oxidase, in combination with other classical markers, could be an indicator for the post-treatment monitoring of the patients diagnosed with hyperplasia and others minors pathologies of the prostate

Sun Y, St Clair DK, Xu Y, Crooks PA, St Clair WH. A NADPH oxidase-dependent redox signaling pathway mediates the selective radiosensitization effect of parthenolide in prostate cancer cells. *Cancer Res* 2010 April 1;70(7):2880-90.

Abstract: Cancer cells are usually under higher oxidative stress compared with normal cells. We hypothesize that introducing additional reactive oxygen species (ROS) insults or suppressing antioxidant capacity may selectively enhance cancer cell killing by oxidative stress-generating agents through stress overload or stress sensitization, whereas normal cells may be able to maintain redox homeostasis under exogenous ROS by adaptive response. Here, we show that parthenolide, a sesquiterpene lactone, selectively exhibits a radiosensitization effect on prostate cancer PC3 cells but not on normal prostate epithelial PrEC cells. Parthenolide causes oxidative stress in PC3 cells but not in PrEC cells, as determined by the oxidation of the ROS-sensitive probe H(2)DCFDA and intracellular reduced thiol and disulfide levels. In PC3 but not PrEC cells, parthenolide activates NADPH oxidase, leading to a decrease in the level of reduced thioredoxin, activation of phosphoinositide 3-kinase/Akt, and consequent FOXO3a phosphorylation, which results in the downregulation of FOXO3a targets antioxidant enzyme manganese superoxide dismutase and catalase. Importantly, when combined with radiation, parthenolide further increases ROS levels in PC3 cells whereas it decreases radiation-induced oxidative stress in PrEC cells, possibly by increasing reduced glutathione levels. Together, the results show that parthenolide selectively activates NADPH oxidase and mediates intense oxidative stress in prostate cancer cells by both increasing ROS generation and decreasing antioxidant defense capacity. The results support the concept of exploiting the intrinsic differences in the redox status of cancer cells and normal cells as targets for selective cancer killing

Jajoo S, Mukherjea D, Watabe K, Ramkumar V. Adenosine A(3) receptor suppresses prostate cancer metastasis by inhibiting NADPH oxidase activity. *Neoplasia* 2009 November;11(11):1132-45.

Abstract: Prostate cancer is the most commonly diagnosed and second most lethal malignancy in men, due mainly to a lack of effective treatment for the metastatic disease. A number of recent studies have shown that activation of the purine nucleoside receptor, adenosine A(3) receptor (A(3)AR), attenuates proliferation of melanoma, colon, and prostate cancer cells. In the present study, we determined whether activation of the A(3)AR reduces the ability of prostate cancer cells to migrate in vitro and metastasize in vivo. Using severe combined immunodeficient mice, we show that proliferation and metastasis of AT6.1 rat prostate cancer cells were decreased by the administration of A(3)AR agonist N(6)-(3-iodobenzyl) adenosine-5'-N-methyluronamide. In vitro studies show that activation of A(3)AR decreased high basal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity present in these cells, along with the expression of Rac1 and p47(phox) subunits of this enzyme. Inhibition of NADPH oxidase activity by the dominant-negative RacN17 or short interfering (si)RNA against p47(phox) reduced both the generation of reactive oxygen species and the invasion of these cells on Matrigel. In addition, we show that membrane association of p47(phox) and activation of NADPH oxidase is dependent on the activity of the extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein kinase pathway. We also provide evidence that A(3)AR inhibits ERK1/2 activity in prostate cancer cells through inhibition of adenylyl cyclase and protein kinase A.

We conclude that activation of the A(3)AR in prostate cancer cells reduces protein kinase A-mediated stimulation of ERK1/2, leading to reduced NADPH oxidase activity and cancer cell invasiveness

Lu JP, Monardo L, Bryskin I et al. Androgens induce oxidative stress and radiation resistance in prostate cancer cells through NADPH oxidase. *Prostate Cancer Prostatic Dis* 2010 March;13(1):39-46.

Abstract: Androgen deprivation therapy (ADT) facilitates the response of prostate cancer (PC) to radiation. Androgens have been shown to induce elevated basal levels of reactive oxygen species (ROS) in PC, leading to adaptation to radiation-induced cytotoxic oxidative stress. Here, we show that androgens increase the expression of p22(phox) and gp91(phox) subunits of NADPH oxidase (NOX) and ROS production by NOX2 and NOX4 in PC. Pre-radiation treatment of 22Rv1 human PC cells with NOX inhibitors sensitize the cells to radiation similarly to ADT, suggesting that their future usage may spare the need for adjuvant ADT in PC patients undergoing radiation

Kumar B, Koul S, Khandrika L, Meacham RB, Koul HK. Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. *Cancer Res* 2008 March 15;68(6):1777-85.

Abstract: Reactive oxygen species (ROS) and the coupled oxidative stress have been associated with tumor formation. Several studies suggested that ROS can act as secondary messengers and control various signaling cascades. In the present studies, we characterized the oxidative stress status in three different prostate cancer cells (PC3, DU145, and LNCaP) exhibiting various degrees of aggressiveness and normal prostate cells in culture (WPMY1, RWPE1, and primary cultures of normal epithelial cells). We observed increased ROS generation in cancer cells compared with normal cells, and that extramitochondrial source of ROS generator, NAD(P)H oxidase (Nox) systems, are associated with the ROS generation and are critical for the malignant phenotype of prostate cancer cells. Moreover, diphenyliodonium, a specific Nox inhibitor, blocked proliferation, modulated the activity of growth signaling cascades extracellular signal-regulated kinase (ERK)1/ERK2 and p38 mitogen-activated protein kinase as well as AKT protein kinase B, and caused cyclin B-dependent G(2)-M cell cycle arrest. We also observed higher degrees of ROS generation in the PC3 cells than DU145 and LNCaP, and that ROS generation is critical for migratory/invasiveness phenotypes. Furthermore, blocking of the ROS production rather than ROS neutralization resulted in decreased matrix metalloproteinase 9 activity as well as loss of mitochondrial potential, plausible reasons for decreased cell invasion and increased cell death. Taken together, these studies show, for the first time, the essential role of ROS production by extramitochondrial source in prostate cancer and suggest that therapies aimed at reducing ROS production might offer effective means of combating prostate cancer in particular, and perhaps other malignancies in general

Wartenberg M, Hoffmann E, Schwindt H et al. Reactive oxygen species-linked regulation of the multidrug resistance transporter P-glycoprotein in Nox-1 overexpressing prostate tumor spheroids. *FEBS Lett* 2005 August 15;579(20):4541-9.

Abstract: Expression of the multidrug resistance (MDR) transporter P-glycoprotein (P-gp) has been demonstrated to be regulated by hypoxia-inducible factor-1alpha (HIF-1alpha) and inhibited by intracellular reactive oxygen species (ROS). Herein, P-gp and HIF-1alpha expression were investigated in multicellular prostate tumor spheroids overexpressing the ROS-generating enzyme Nox-1 in comparison to the mother cell line DU-145. In Nox-1-overexpressing tumor spheroids (DU-145Nox1) generation of ROS as well as expression of Nox-1 was significantly increased as compared to DU-145 tumor spheroids. ROS generation was significantly inhibited in the presence of the NADPH-oxidase antagonists diphenylen-iodonium chloride (DPI) and 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF). Albeit growth kinetic of DU-145Nox1 tumor

spheroids was decreased as compared to DU-145 spheroids, elevated expression of Ki-67 was observed indicating increased cell cycle activity. In DU-145Nox1 tumor spheroids, expression of HIF-1 $\alpha$  as well as P-gp was significantly decreased as compared to DU-145 spheroids, which resulted in an increased retention of the anticancer agent doxorubicin. Pretreatment with the free radical scavengers vitamin E and vitamin C increased the expression of P-gp as well as HIF-1 $\alpha$  in Nox-1-overexpressing cells, whereas no effect of free radical scavengers was observed on mdr-1 mRNA expression. In summary, the data of the present study demonstrate that the development of P-gp-mediated MDR is abolished under conditions of elevated ROS levels, suggesting that the MDR phenotype can be circumvented by modest increase of intracellular ROS generation

Brar SS, Corbin Z, Kennedy TP et al. NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells. *Am J Physiol Cell Physiol* 2003 August;285(2):C353-C369.

Abstract: Reactive oxygen species (ROS) appear to play an important role in regulating growth and survival of prostate cancer. However, the sources for ROS production in prostate cancer cells have not been determined. We report that ROS are generated by intact American Type Culture Collection DU 145 cells and by their membranes through a mechanism blocked by NAD(P)H oxidase inhibitors. ROS are critical for growth in these cells, because NAD(P)H oxidase inhibitors and antioxidants blocked proliferation. Components of the human phagocyte NAD(P)H oxidase, p22phox and gp91phox, as well as the Ca<sup>2+</sup> concentration-responsive gp91phox homolog NOX5 were demonstrated in DU 145 cells by RT-PCR and sequencing. Although the protein product for p22phox was not detectable, both gp91phox and NOX5 were identified throughout the cell by immunostaining and confocal microscopy and NOX5 immunostaining was enhanced in a perinuclear location, corresponding to enhanced ROS production adjacent to the nuclear membrane imaged by 2',7'-dichlorofluorescein diacetate oxidation. The calcium ionophore ionomycin dramatically stimulated ferricytochrome c reduction in cell media, further supporting the importance of NOX5 for ROS production. Antisense oligonucleotides for NOX5 inhibited ROS production and cell proliferation in DU 145 cells. In contrast, antisense oligonucleotides to p22phox or gp91phox did not impair cell growth. Inhibition of ROS generation with antioxidants or NAD(P)H oxidase inhibitors increased apoptosis in cells. These results indicate that ROS generated by the newly described NOX5 oxidase are essential for prostate cancer growth, possibly by providing trophic intracellular oxidant tone that retards programmed cell death

McCarty MF. Clinical potential of Spirulina as a source of phycocyanobilin. *J Med Food* 2007 December;10(4):566-70.

Abstract: Recent research reveals that free bilirubin functions physiologically as a potent inhibitor of NADPH oxidase activity. The chromophore phycocyanobilin (PCB), found in blue-green algae and cyanobacteria such as Spirulina, also has been found to be a potent inhibitor of this enzyme complex, likely because in mammalian cells it is rapidly reduced to phycocyanorubin, a close homolog of bilirubin. In light of the protean roles of NADPH oxidase activation in pathology, it thus appears likely that PCB supplementation may have versatile potential in prevention and therapy -- particularly in light of rodent studies demonstrating that orally administered Spirulina or phycocyanin (the Spirulina holoprotein that contains PCB) can exert a wide range of anti-inflammatory effects. Until PCB-enriched Spirulina extracts or synthetically produced PCB are commercially available, the most feasible and least expensive way to administer PCB is by ingestion of whole Spirulina. A heaping tablespoon (about 15 g) of Spirulina can be expected to provide about 100 mg of PCB. By extrapolating from rodent studies, it can be concluded that an intake of 2 heaping tablespoons daily would be likely to have important antioxidant activity in humans -- assuming that humans and rodents digest and absorb Spirulina-bound PCB in a comparable manner. An intake of this magnitude can be clinically feasible if Spirulina is incorporated into "smoothies" featuring such ingredients as soy milk, fruit juices, and whole fruits. Such

a regimen should be evaluated in clinical syndromes characterized and in part mediated by NADPH oxidase overactivity in affected tissues

## **Grape Seed Extract**

Brasky TM, Kristal AR, Navarro SL et al. Specialty supplements and prostate cancer risk in the VITamins and Lifestyle (VITAL) cohort. *Nutr Cancer* 2011;63(4):573-82.

Abstract: Although there is evidence from studies of prostate cancer cell lines and rodent models that several supplements may have antiinflammatory, antioxidant, or other anticancer properties, few epidemiologic studies have examined the association between nonvitamin, nonmineral, "specialty" supplement use and prostate cancer risk. Participants, 50-76 yr, were 35,239 male members of the VITamins and Lifestyle (VITAL) cohort who were residents of western Washington state, and who completed an extensive baseline questionnaire in 2000-2002. Participants responded about their frequency (days/wk) and duration (yr) of specialty supplement uses. 1,602 incident invasive prostate cancers were obtained from the Surveillance, Epidemiology, and End Results registry. Multivariate-adjusted hazards ratios (HR) and 95% confidence intervals (95% CI) were estimated by Cox proportional hazards models. Any use of grapeseed supplements was associated with a 41% (HR 0.59, 95% CI: 0.40-0.86) reduced risk of total prostate cancer. There were no associations for use of chondroitin, coenzyme Q10, fish oil, garlic, ginkgo biloba, ginseng, glucosamine, or saw palmetto. Grapeseed may be a potential chemopreventive agent; however, as current evidence is limited, it should not yet be promoted for prevention of prostate cancer

Kaur M, Velmurugan B, Rajamanickam S, Agarwal R, Agarwal C. Gallic acid, an active constituent of grape seed extract, exhibits anti-proliferative, pro-apoptotic and anti-tumorigenic effects against prostate carcinoma xenograft growth in nude mice. *Pharm Res* 2009 September;26(9):2133-40.

Abstract: PURPOSE: Gallic acid, a natural agent present in a wide-range of fruits and vegetables, has been of potential interest as an anti-cancer agent; herein, we evaluated its efficacy in androgen-independent DU145 and androgen-dependent-22Rv1 human prostate cancer (PCa) cells. MATERIALS AND METHODS: Cell viability was determined by MTT and apoptosis by Annexin V-PI assays. In vivo anti-cancer efficacy was assessed by DU145 and 22Rv1 xenograft growth in nude mice given normal drinking water or one supplemented with 0.3% or 1% (w/v) gallic acid. PCNA, TUNEL and CD31 immunostaining was performed in tumor tissues for in vivo anti-proliferative, apoptotic and anti-angiogenic effects of gallic acid. RESULTS: Gallic acid decreased cell viability in a dose-dependent manner in both DU145 and 22Rv1 cells largely via apoptosis induction. In tumor studies, gallic acid feeding inhibited the growth of DU145 and 22Rv1 PCa xenografts in nude mice. Immunohistochemical analysis revealed significant inhibition of tumor cell proliferation, induction of apoptosis, and reduction of microvessel density in tumor xenografts from gallic acid-fed mice as compared to controls in both DU145 and 22Rv1 models. CONCLUSION: Taken together, our findings show the anti-PCa efficacy of gallic acid and provide a rationale for additional studies with this naturally-occurring agent for its efficacy against PCa

Raina K, Rajamanickam S, Deep G, Singh M, Agarwal R, Agarwal C. Chemopreventive effects of oral gallic acid feeding on tumor growth and progression in TRAMP mice. *Mol Cancer Ther* 2008 May;7(5):1258-67.

Abstract: Our recent studies have identified gallic acid as one of the major constituents of grape seed extract showing strong in vitro anticancer efficacy against human prostate cancer cells. Herein, for the first time, we established the in vivo chemopreventive efficacy of gallic acid against prostate cancer by evaluating its activity against prostate tumor growth and progression in transgenic adenocarcinoma of

the mouse prostate (TRAMP) model. At 4 weeks of age, male TRAMP mice were fed with drinking water supplemented with 0.3% and 1% (w/v) gallic acid until 24 weeks of age. Positive control group was fed with regular drinking water for the same period. Our results showed that gallic acid-fed groups had a higher incidence of differentiated lower-grade prostatic tumors at the expense of strong decrease (approximately 60%;  $P < 0.01$ ) in poorly differentiated tumors. Immunohistochemical analysis of prostate tissue showed a decrease in proliferative index by 36% to 41% ( $P < 0.05$ ) in 0.3% to 1% gallic acid-fed groups, with an increase in the apoptotic cells by 3-fold ( $P < 0.05$ ). Further, both doses of gallic acid completely diminished the expression of Cdc2 in the prostatic tissue together with strong decrease in the expression of Cdk2, Cdk4, and Cdk6. The protein levels of cyclin B1 and E were also decreased by gallic acid feeding. Together, for the first time, we identified that oral gallic acid feeding inhibits prostate cancer growth and progression to advanced-stage adenocarcinoma in TRAMP mice via a strong suppression of cell cycle progression and cell proliferation and an increase in apoptosis

Raina K, Singh RP, Agarwal R, Agarwal C. Oral grape seed extract inhibits prostate tumor growth and progression in TRAMP mice. *Cancer Res* 2007 June 15;67(12):5976-82.

Abstract: Prostate cancer chemoprevention is an alternative and potential strategy to control this malignancy. Herein, we evaluated the chemopreventive efficacy of grape seed extract (GSE) against prostate cancer in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice where animals were fed with GSE by oral gavage at 200 mg/kg body weight dose during 4 to 28 weeks of age. Our results showed a significant reduction (46%,  $P < 0.01$ ) in the weight of genitourinary tract organs in the GSE-fed mice. The GSE-fed group of mice had a higher incidence of prostatic intraepithelial neoplasia but showed strong reduction in the incidence of adenocarcinoma compared with mice in control group. Prostate tissue from the GSE group showed approximately 50% ( $P < 0.001$ ) decrease in proliferating cell nuclear antigen (PCNA)-positive cells and 64% ( $P < 0.01$ ) reduction in total PCNA protein level compared with the control group; however, GSE increased apoptotic cells by 8-fold. Furthermore, GSE strongly decreased the protein levels of cyclin B1, cyclin A, and cyclin E by 84% ( $P < 0.05$ ), 96% ( $P < 0.05$ ), and 89% ( $P < 0.001$ ), respectively. The protein expression of cyclin-dependent kinases 2 and 6 and Cdc2 was also decreased by more than 90% ( $P < 0.05$ ) in the prostate from the GSE-fed group. Together, for the first time, we identified that oral GSE inhibits prostate cancer growth and progression in TRAMP mice, which could be mediated via a strong suppression of cell cycle progression and cell proliferation and an increase in apoptosis

Veluri R, Singh RP, Liu Z, Thompson JA, Agarwal R, Agarwal C. Fractionation of grape seed extract and identification of gallic acid as one of the major active constituents causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. *Carcinogenesis* 2006 July;27(7):1445-53.

Abstract: The anti-cancer efficacy of grape seed extract (GSE) against prostate cancer (PCA) via its anti-proliferative, pro-apoptotic and anti-angiogenic activities in both cell culture and animal models have recently been described by us. GSE is a complex mixture containing gallic acid (GA), catechin (C), epicatechin (EC) and several oligomers (procyanidins) of C and/or EC, some of which are esterified to GA. To determine which components are most active against PCA, an ethyl acetate extract of GSE was separated by reverse-phase high-performance liquid chromatography (HPLC) into three fractions. Fraction 1 was far more effective than others in causing growth inhibition and apoptotic death of human PCA DU145 cells. Of the components in this fraction, GA showed a very strong dose- and time-dependent growth inhibition and apoptotic death of DU145 cells, but C and procyanidins B1 (EC-C dimer), B2 (EC-EC dimer) and B3 (C-C dimer) were nearly ineffective. Mechanistic studies demonstrated a strong caspase-9, caspase-3 and poly (ADP-ribose) polymerase (PARP) cleavages by GA in DU145 cells. Procyanidin oligomers eluting in HPLC Fractions 2 and 3 were obtained in larger quantities by separating GSE into eight fractions (I-VIII) on a gel filtration column. All fractions were analyzed by HPLC-UV and negative-ion electrospray mass spectrometry. Fractions I-III contained the

active compound GA and inactive components C, EC, B1 and B2. Fraction IV contained other dimers and a dimer-GA ester and was also less active than GSE in DU145 cells. Fractions V-VIII, however, caused significant growth inhibition and apoptosis with the highest activity present in the later fractions that contained procyanidin trimers and GA esters of dimers and trimers. Together, these observations identify GA as one of the major active constituents in GSE. Several procyanidins, however, and especially the gallate esters of dimers and trimers also may be efficacious against PCA and merit further investigation

Dhanalakshmi S, Agarwal R, Agarwal C. Inhibition of NF-kappaB pathway in grape seed extract-induced apoptotic death of human prostate carcinoma DU145 cells. *Int J Oncol* 2003 September;23(3):721-7.

Abstract: The alarmingly high rate of prostate cancer (PCA) mortality as well as the limited success in the treatment of advanced PCA suggest that additional approaches are needed to control PCA growth and its metastatic potential. A constitutive activation of NF-kappaB family of transcription factors is known to play a major role in chemotherapy resistance in advanced PCA. In recent studies we showed that grape seed extract (GSE) inhibits advanced human PCA growth and induces apoptosis in cell culture and in nude mice. Accordingly, here we assessed the effect of GSE on constitutive and TNFalpha-induced NF-kappaB DNA binding activity and apoptotic death in advanced human prostate carcinoma DU145 cells. Constitutive and TNFalpha-induced NF-kappaB DNA binding activity was inhibited by GSE at doses  $\geq$  50 microg/ml and treatments for  $\geq$  12 h. This was accompanied by inhibition of IkappaBalpha phosphorylation and IKKalpha kinase activity. A strong induction of apoptosis ( $P < 0.01$ ) was also observed following GSE treatment, while a combination with TNFalpha strongly potentiated apoptosis induction. Our results indicate the potential of developing GSE as an effective cancer therapeutic agent, both alone and in combination with TNFalpha-based chemotherapy of advanced human prostate carcinoma that might prove to be a more effective and less toxic alternative in clinical therapy of PCA

### **NF-kappaB Inhibitors - Salsalate and Anti-inflammatory Phytochemicals**

McCarty MF, Block KI. Preadministration of high-dose salicylates, suppressors of NF-kappaB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. *Integr Cancer Ther* 2006 September;5(3):252-68.

Abstract: NF-kappaB activity is elevated in a high proportion of cancers, particularly advanced cancers that have been treated previously. Cytotoxic treatment selects for such up-regulation inasmuch as NF-kappaB promotes transcription of a large number of proteins that inhibit both the intrinsic and extrinsic pathways of apoptosis; NF-kappaB also boosts expression of *mdr1*, which expels many drugs from cells. Indeed, high NF-kappaB activity appears to be largely responsible for the chemo- and radioresistance of many cancers. Thus, agents that suppress NF-kappaB activity should be useful as adjuvants to cytotoxic cancer therapy. Of the compounds that are known to be NF-kappaB antagonists, the most practical for current use may be the nonsteroidal anti-inflammatory drugs aspirin, salicylic acid, and sulindac, each of which binds to and inhibits Ikappa kinase- beta, a central mediator of NF-kappa activation; the low millimolar plasma concentrations of salicylate required for effective inhibition of this kinase in vivo can be achieved with high-dose regimens traditionally used to manage rheumatic disorders. The gastrointestinal toxicity of such regimens could be minimized by using salsalate or enteric-coated sodium salicylate or by administering misoprostol in conjunction with aspirin therapy. Presumably, best results would be seen if these agents were administered for several days prior to a course of chemo- or radiotherapy, continuing throughout the course. This concept should first be tested in nude mice bearing xenografts of chemoresistant human tumors known to have elevated NF-kappa activity. Ultimately, more complex adjuvant regimens can be envisioned in which salicylates are used

in conjunction with other NF-kappa antagonists and/or agents that target other mediators of down-regulated apoptosis in cancer, such as Stat3; coadministration of salicylate and organic selenium may have intriguing potential in this regard. These strategies may also have potential as adjuvants to metronomic chemotherapy, which seeks to suppress angiogenesis by targeting cycling endothelial cells in tumors

Kozakai N, Kikuchi E, Hasegawa M et al. Enhancement of radiosensitivity by a unique novel NF-kappaB inhibitor, DHMEQ, in prostate cancer. *Br J Cancer* 2012 August 7;107(4):652-7.

Abstract: Background: Inducible activation of nuclear factor (NF)-kappaB is one of the principal mechanisms through which resistant prostate cancer cells are protected from radiotherapy. We hypothesised that inactivation of inducible NF-kappaB with a novel NF-kappaB inhibitor, DHMEQ, would increase the therapeutic effects of radiotherapy. Methods: PC-3 and LNCaP cells were exposed to irradiation and/or DHMEQ. Cell viability, cell cycle analysis, western blotting assay, and NF-kappaB activity were measured. The antitumour effect of irradiation combined with DHMEQ in vivo was also assessed. Results: The combination of DHMEQ with irradiation resulted in cell growth inhibition and G2/M arrest relative to treatment with irradiation alone. Inducible NF-kappaB activity by irradiation was inhibited by DHMEQ treatment. The expression of p53 and p21 in LNCaP, and of 14-3-3sigma in PC-3 cells, was increased in the combination treatment. In the in vivo study, 64 days after the start of treatment, tumour size was 85.1%, 77.1%, and 64.7% smaller in the combination treatment group than that of the untreated control, DHMEQ-treated alone, and irradiation alone groups, respectively. Conclusion: Blockade of NF-kappaB activity induced by radiation with DHMEQ could overcome radio-resistant responses and may become a new therapeutic modality for treating prostate cancer

Jain G, Voogdt C, Tobias A et al. IkappaB kinases modulate the activity of the androgen receptor in prostate carcinoma cell lines. *Neoplasia* 2012 March;14(3):178-89.

Abstract: Enhanced nuclear localization of nuclear factor kappaB (NF-kappaB) in prostate cancer (PCa) samples and constitutive NF-kappaB signaling in a class of PCa cell lines with low androgen receptor (AR) expression (PC3 and DU-145) imply an important role of the IkappaB kinase (IKK)/NF-kappaB system in PCa. However, most PCa and PCa cell lines depend on the activity of the AR, and the role of NF-kappaB in these AR-expressing PCa remains unclear. Here, we demonstrate that inhibition of NF-kappaB signaling by the IKK inhibitor BMS345541 reduced proliferation and increased apoptosis in AR-expressing PCa cell lines. Furthermore, AR activity and target gene expression were distinctively reduced, whereas AR protein levels remained unaltered on BMS345541 treatment. Similar effects were observed particularly after small interfering RNA (siRNA)-mediated knockdown of IKK1, but not by siRNA-mediated suppression of IKK2. Moreover, IKK1 overexpression augmented 5alpha-dihydrotestosterone-induced nuclear AR translocation, whereas nuclear AR was reduced by IKK1 knockdown or BMS345541. However, because IKK1 also enhances the activity of a chronically nuclear AR mutant, modulation of the subcellular distribution seems not to be the only mechanism by which IKK1 enhances AR activity. Finally, reduced in vivo AR phosphorylation after BMS345541 treatment and in vitro AR phosphorylation by IKK1 or IKK2 imply that AR constitutes a novel IKK target. Taken together, our data identify IKK1 as a potentially target structure for future therapeutic intervention in PCa

Jain G, Cronauer MV, Schrader M, Moller P, Marienfeld RB. NF-kappaB signaling in prostate cancer: a promising therapeutic target? *World J Urol* 2012 June;30(3):303-10.

Abstract: Prostate carcinoma (PCa) displays a wide variety of genetic alterations, versatile expression

profiles as well as cell surface markers. Despite this heterogeneity, a common treatment for advanced PCa is androgen deprivation therapy (ADT). ADT targets the androgen receptor-a member of the nuclear receptor superfamily-which is required for development and function of the prostate and critical for PCa growth and survival. After an initial regression of the tumor during ADT, a large fraction of tumors progress to so-called castration-resistant prostate carcinoma (CRPCa) which is highly resistant toward chemotherapy. The ensuing high mortality rates illustrate the importance of novel therapeutic targets for CRPCa. The transcription factor NF-kappaB was recently proposed as such a potential target for therapeutic intervention in CRPCa. Although NF-kappaB is essential for the regulation of innate and adaptive immunity recent data suggest a role of NF-kappaB in cancer initiation and progression. However, the exact function of NF-kappaB signaling in PCa is still a matter of debate. Here, we review known roles of NF-kappaB signaling in PCa and emphasize the crosstalk of NF-kappaB and androgen receptor signaling. Finally, we discuss potential therapeutic relevance of blocking NF-kappaB in PCa

Liu S, Wang Z, Hu Z et al. Anti-tumor activity of curcumin against androgen-independent prostate cancer cells via inhibition of NF-kappaB and AP-1 pathway in vitro. *J Huazhong Univ Sci Technolog Med Sci* 2011 August;31(4):530-4.

Abstract: The anti-tumor activity of curcumin against androgen-independent prostate cancer cells in vitro and the possible mechanism were investigated. After curcumin treatment, the effect of curcumin on the proliferation of prostate cancer PC-3 cells was assessed by CFSE staining. Flow cytometry (FCM) was performed to analyze the cell cycle and the induction of apoptosis of tumor cells. A luciferase reporter gene assay was used to determine the effects of curcumin on the activities of intracellular NF-kappaB and AP-1 signaling pathways. The results showed curcumin could effectively inhibit the proliferation of PC-3 cells in vitro ( $P < 0.05$ ). Cells were arrested at G(2)/M phase. After curcumin treatment, the percentage of apoptotic cells was significantly higher than in control group ( $P < 0.05$ ). The results of the luciferase assay revealed that curcumin selectively inhibited the activities of the NF-kappaB and AP-1 signaling pathways in PC-3 cells significantly. It was suggested that curcumin could exert anti-tumor activity against androgen-independent prostate cancer cells in vitro by inhibiting cellular proliferation and inducing apoptosis, which was probably contributed to the inhibition of transcription factors NF-kappaB and AP-1

Hayashi S, Koshiba K, Hatashita M et al. Thermosensitization and induction of apoptosis or cell-cycle arrest via the MAPK cascade by parthenolide, an NF-kappaB inhibitor, in human prostate cancer androgen-independent cell lines. *Int J Mol Med* 2011 December;28(6):1033-42.

Abstract: Parthenolide (PTL), a nuclear factor-kappaB (NF-kappaB) inhibitor, has a significant thermo-enhancement effect. Modification of thermosensitivity by treatment with PTL prior to hyperthermia was investigated in the human prostate cancer androgen-independent cell lines PC3 and DU145. In addition, we analyzed the mechanisms related to induction of apoptosis or G(2)/M cell-cycle arrest via the effects of ERK1/2, p38 and SAPK/JNK signaling on mitogen-activated protein kinase (MAPK). Lethal damage caused by mild hyperthermia at 41.0 C or 42.0 C in both cell lines resulted in a low level of thermosensitivity, while sequential combination with PTL showed significant thermosensitization. Step-up hyperthermia (SUH) (42 C for 30 min, 43.0 C or 43.5 C for various periods) reduced the thermosensitivity of the cells to second heating. However, PTL given as pre-treatment prior to SUH prevented SUH-induced thermal tolerance and resulted in significant thermosensitization. Induction of apoptosis by the combination of PTL and hyperthermia at 44.0 C was determined by the ratio of sub-G1 division cells using flow cytometry, which was increased significantly in comparison with single treatment, and was more effective in PC3 than DU145 cells. The behavior of ERK1/2, p38, and SAPK/JNK signaling in the MAPK cascade by treatment with PTL and hyperthermia were examined by Western blotting. As for PC3 cells, ras-downstream p-

ERK1/2 was activated and p-p38 slightly activated by combined treatment with PTL and hyperthermia in comparison with each alone. As for DU145 cells, ERK1/2 was not changed, while p38 and SAPK/JNK were slightly activated by combination treatment. These results were related to increases in the induction of apoptosis, G(2)/M cell cycle arrest, and lethal damage of cells via the MAPK cascade. Together, our findings demonstrate that PTL is an effective thermosensitizing agent for multidisciplinary therapy for human prostate cancer

O'Neill AJ, Prencipe M, Dowling C et al. Characterisation and manipulation of docetaxel resistant prostate cancer cell lines. *Mol Cancer* 2011;10:126.

Abstract: BACKGROUND: There is no effective treatment strategy for advanced castration-resistant prostate cancer. Although Docetaxel (Taxotere(R)) represents the most active chemotherapeutic agent it only gives a modest survival advantage with most patients eventually progressing because of inherent or acquired drug resistance. The aims of this study were to further investigate the mechanisms of resistance to Docetaxel. Three Docetaxel resistant sub-lines were generated and confirmed to be resistant to the apoptotic and anti-proliferative effects of increasing concentrations of Docetaxel. RESULTS: The resistant DU-145 R and 22RV1 R had expression of P-glycoprotein and its inhibition with Elacridar partially and totally reversed the resistant phenotype in the two cell lines respectively, which was not seen in the PC-3 resistant sublines. Resistance was also not mediated in the PC-3 cells by cellular senescence or autophagy but multiple changes in pro- and anti-apoptotic genes and proteins were demonstrated. Even though there were lower basal levels of NF-kappaB activity in the PC-3 D12 cells compared to the Parental PC-3, docetaxel induced higher NF-kappaB activity and IkappaB phosphorylation at 3 and 6 hours with only minor changes in the DU-145 cells. Inhibition of NF-kappaB with the BAY 11-7082 inhibitor reversed the resistance to Docetaxel. CONCLUSION: This study confirms that multiple mechanisms contribute to Docetaxel resistance and the central transcription factor NF-kappaB plays an immensely important role in determining docetaxel-resistance which may represent an appropriate therapeutic target

Heymach JV, Shackelford TJ, Tran HT et al. Effect of low-fat diets on plasma levels of NF-kappaB-regulated inflammatory cytokines and angiogenic factors in men with prostate cancer. *Cancer Prev Res (Phila)* 2011 October;4(10):1590-8.

Abstract: Diet, nutritional status, and certain dietary supplements are postulated to influence the development and progression of prostate cancer. Angiogenesis and inflammation are central to tumor growth and progression, but the effect of diet on these processes remains uncertain. We explored changes in 50 plasma cytokines and angiogenic factors (CAF) in 145 men with prostate cancer enrolled in a preoperative, randomized controlled phase II trial with four arms: control (usual diet), low-fat (LF) diet, flaxseed-supplemented (FS) diet, and FS+LS diet. The mean duration of dietary intervention was 30 to 31 days. Among the individual arms, the largest number of significant changes (baseline vs. preoperative follow-up) was observed in the LF arm, with 19 CAFs decreasing and one increasing ( $P < 0.05$ ). Compared with the control arm, 6 CAFs-including proangiogenic factors (stromal-cell derived-1alpha) and myeloid factors (granulocyte-colony-stimulating factor, macrophage colony-stimulating factor)-all decreased in the LF arm compared with controls; three and four CAFs changed in the FS and FS+LF arms, respectively. Weight loss occurred in the LF arms and significantly correlated with VEGF decreases ( $P < 0.001$ ). The CAFs that changed in the LF arm are all known to be regulated by NF-kappaB, and a pathway analysis identified NF-kappaB as the most likely regulatory network associated with these changes in the LF arm but not in the FS-containing arms. These results suggest that a LF diet without flaxseed may reduce levels of specific inflammatory CAFs and suggests that the NF-kappaB pathway may be a mediator of these changes

Cai C, Jiang FN, Liang YX et al. Classical and alternative nuclear factor-kappaB pathways: a comparison among normal prostate, benign prostate hyperplasia and prostate cancer. *Pathol Oncol Res* 2011 December;17(4):873-8.

Abstract: Nuclear factor-kappaB (NF-kappaB) is controlled by the classical and alternative NF-kappaB pathways, the role of which in prostate cancer (PCa) is not clearly defined. To provide this missing translational link, we compared the classical and alternative NF-kappaB pathways in normal prostate, benign prostate hyperplasia (BPH) and PCa. Prostate specimens were divided into three groups: group A, PCa (n = 68); group B, BPH (n = 60); and group C, normal prostates (n = 15). The gene expression levels of NF-kappaB1 and NF-kappaB2 were determined by real-time quantitative RT-PCR. Additionally, we analyzed the expression and sub-cellular localization of phosphorylated P50 (p-P50) and phosphorylated P52 (p-P52) proteins by immunohistochemical staining. Furthermore, associations were made between NF-kappaB pathway proteins and patients' prognosis. Compared with BPH and normal prostate tissues, the expression of NF-kappaB1 gene was differentially down-regulated by >1.5-fold, whereas NF-kappaB2 gene was differentially up-regulated by >2-fold in PCa tissues. The proportion of p-P50 positive patients in group A (26.5%) was significantly lower than in group B (88.3%, p = 0.005) and C (100%, p = 0.002). The proportion of p-P52 positive patients in group A (42.6%) was significantly higher than in group B (11.7%, p = 0.009) and C (6.7%, p = 0.008). Comparison of the survival curves in group A according to p-P52 expression showed a significant difference between positive and negative patients. The p-P52 positive patients showed worse prognosis (p = 0.019). Our findings suggest for the first time that the classical and alternative NF-kappaB pathways have an important role in PCa. p-P52 might be a predictor of poor prognosis for PCa

Shanmugam MK, Rajendran P, Li F et al. Ursolic acid inhibits multiple cell survival pathways leading to suppression of growth of prostate cancer xenograft in nude mice. *J Mol Med (Berl)* 2011 July;89(7):713-27.

Abstract: Activation of transcription factors nuclear factor-kappaB (NF-kappaB) and signal transducer and activator of transcription 3 (STAT3) is frequently observed in prostate cancer and has been linked with tumor cell proliferation, invasion, metastasis, and angiogenesis. In this study, we investigated the effect of ursolic acid (UA) on NF-kappaB and STAT3 signaling pathways in both androgen-independent (DU145) and androgen-dependent (LNCaP) prostate cancer cell lines and also prospectively tested the hypothesis of NF-kappaB and STAT3 inhibition using a virtual predictive functional proteomics tumor pathway technology platform. We found that UA inhibited constitutive and TNF-alpha-induced activation of NF-kappaB in DU145 and LNCaP cells in a dose-dependent manner. The suppression was mediated through the inhibition of constitutive and TNF-alpha-induced IkappaB kinase (IKK) activation, phosphorylation of IkappaBalpha and p65 and NF-kappaB-dependent reporter activity. Furthermore, UA suppressed both constitutive and inducible STAT3 activation in prostate cancer cells concomitant with suppression of activation of upstream kinases (Src and JAK2) and STAT3-dependent reporter gene activity. UA also downregulated the expression of various NF-kappaB and STAT3 regulated gene products involved in proliferation, survival, and angiogenesis and induced apoptosis in both cells lines as evidenced by DNA fragmentation and annexin V staining. In vivo, UA (200 mg/kg b.w.) treated for 6 weeks inhibited the growth of DU145 cells in nude mice without any significant effect on body weight. Overall, our results from experimental and predictive studies suggest that UA mediates its anti-tumor effects through suppression of NF-kappaB and STAT3 pathways in prostate cancer

Cansino JR, Vera R, Rodriguez de BF et al. [Prostate specific antigen and NF-kB in prostatic disease: relation with malignancy]. *Actas Urol Esp* 2011 January;35(1):16-21.

Abstract: INTRODUCTION: NF- $\kappa$ B (p50/p65) is a transcription factor involved in TNF- $\alpha$ -induced cell death resistance by promoting several antiapoptotic genes. We intend to relate the expression of NF- $\kappa$ B (p50 and p65) with serum levels of prostate-specific antigen (PSA), both in normal males and in those with pathologic conditions of the prostate. MATERIALS AND METHODS: this study was carried out in 5 normal, 24 benign prostatic hyperplastic (BPH) and 19 patients with prostate cancer (PC). Immunohistochemical and Western blot analyses were performed on tissue and serum PSA was assayed by PSA DPC Immulite assays (Diagnostics Products Corporation, Los Angeles, CA). RESULTS: in controls, p65 NF- $\kappa$ B was not found and p50 was scantily detected in 60% normal samples in the cytoplasm of epithelial cells. Both p50 and p65 were expressed in 62.5% of the samples with BPH and in 63.2% of those with PC. Both increased its frequency of expression with higher PSA serum levels. CONCLUSIONS: Activation of NF- $\kappa$ B revealed by its nuclear translocation in prostate cancer could be related to cancer progression and elevated seric PSA levels. A better understanding of the biologic mechanism by which circulating PSA levels increase and its relation with NF- $\kappa$ B expression is needed. Possibly, NF- $\kappa$ B blockage could be used as a therapeutic target to counteract proliferation in prostate cancer

Rajasekhar VK, Studer L, Gerald W, Socci ND, Scher HI. Tumour-initiating stem-like cells in human prostate cancer exhibit increased NF- $\kappa$ B signalling. *Nat Commun* 2011 January 18;2:162.

Abstract: Androgen depletion is a key strategy for treating human prostate cancer, but the presence of hormone-independent cells escaping treatment remains a major therapeutic challenge. Here, we identify a minor subset of stem-like human prostate tumour-initiating cells (TICs) that do not express prostate cancer markers, such as androgen receptor or prostate specific antigen. These TICs possess stem cell characteristics and multipotency as demonstrated by in vitro sphere-formation and in vivo tumour-initiation, respectively. The cells represent an undifferentiated subtype of basal cells and can be purified from prostate tumours based on coexpression of the human pluripotent stem cell marker TRA-1-60 with CD151 and CD166. Such triple-marker-positive TICs recapitulate the original parent tumour heterogeneity in serial xeno-transplantations indicating a tumour cell hierarchy in human prostate cancer development. These TICs exhibit increased nuclear factor- $\kappa$ B activity. These findings are important in understanding the molecular basis of human prostate cancer

Parrondo R, de las PA, Reiner T, Rai P, Perez-Stable C. NF- $\kappa$ B activation enhances cell death by antimetabolic drugs in human prostate cancer cells. *Mol Cancer* 2010;9:182.

Abstract: BACKGROUND: NF- $\kappa$ B is a transcription factor that promotes inhibition of apoptosis and resistance to chemotherapy. It is commonly believed that inhibition of NF- $\kappa$ B activity can increase sensitivity of cancer cells to chemotherapy. However, there is evidence that NF- $\kappa$ B activation can sensitize cells to apoptosis and that inhibition of NF- $\kappa$ B results in resistance to chemotherapy. In prostate cancer, it is not clear in the different cell types (androgen-dependent and castration-resistant) if activation or inhibition of NF- $\kappa$ B is required for stimulation of apoptosis by chemotherapy. RESULTS: Our data indicate that the response of prostate cancer (PC) cells to the antimetabolic drugs docetaxel (Doc) and 2-methoxyestradiol (2ME2) is dependent on the levels of NF- $\kappa$ B activity. In androgen-dependent LNCaP cells, Doc and 2ME2 treatment increased the low basal NF- $\kappa$ B activity, as determined by Western blot analysis of phospho-I $\kappa$ B $\alpha$ /p65, NF- $\kappa$ B promoter reporter assays, and p65 localization. Treatment of LNCaP cells with parthenolide, a pharmacologic inhibitor of NF- $\kappa$ B, or introduction of dominant-negative I $\kappa$ B $\alpha$ , or an shRNA specific for p65, a component of the NF- $\kappa$ B heterodimer, blocked apoptosis induced by Doc and 2ME2. In castration-resistant DU145 and PC3 cells, Doc and 2ME2 had little effect on the high basal NF- $\kappa$ B activity and addition of parthenolide did not enhance cell death. However, the combination of Doc or 2ME2 with betulonic acid (BA), a triterpenoid that activates NF- $\kappa$ B, stimulated apoptosis in LNCaP and non-apoptotic cell death in DU145 and PC3 cells. Increased

sensitivity to cell death mediated by the Doc or 2ME2 + BA combination is likely due to increased NF-kappaB activity. CONCLUSIONS: Our data suggest that the combination of antimitotic drugs with NF-kappaB inhibitors will have antagonistic effects in a common type of PC cell typical of LNCaP. However, combination strategies utilizing antimitotic drugs with BA, an activator of NF-kappaB, will universally enhance cell death in PC cells, notably in the aggressive, castration-resistant variety that does not respond to conventional therapies

Wu K, Zeng J, Li L et al. Silibinin reverses epithelial-to-mesenchymal transition in metastatic prostate cancer cells by targeting transcription factors. *Oncol Rep* 2010 June;23(6):1545-52.

Abstract: Silibinin, a naturally occurring flavanone isolated from milk thistle extract, has been shown to possess strong anticancer efficacy against both androgen-dependent and androgen-independent prostate cancer, wherein it inhibits not only cell growth, but also cell invasion and metastasis. Inhibitory effects of silibinin on prostate cancer invasion, motility and migration were previously observed in the highly bone metastatic ARCaP M cell line; however, mechanisms of such efficacy are not completely elucidated. The epithelial-to-mesenchymal transition (EMT) is a crucial step in the progression of prostate cancer, reversal or inhibition of EMT by drugs thus provides a new approach to prostate cancer therapy. In the present study, we found that silibinin treatment resulted in the up-regulation of cytokeratin-18 and down-regulation of vimentin and MMP2, which was consistent with morphologic reversal of EMT phenotype leading to be epithelial. Moreover, we found that silibinin could inhibit the nuclear factor kappaB (NF-kappaB) p50 translocation via the up-regulation of I kappaB alpha protein, and possibly subsequently down-regulated the expression of two major EMT regulators, ZEB1 and SLUG transcription factors. Overall these findings demonstrate silibinin was able to reverse EMT to suppress the invasive property of metastatic prostate cancer cells at the transcriptional level

Shanmugam R, Kusumanchi P, Cheng L et al. A water-soluble parthenolide analogue suppresses in vivo prostate cancer growth by targeting NFkappaB and generating reactive oxygen species. *Prostate* 2010 July 1;70(10):1074-86.

Abstract: BACKGROUND: To characterize the molecular changes associated with DMAPT-induced prostate cancer cell death and its in vivo activity. METHODS: CWR22Rv1 and PC-3 were subjected to flow cytometry, electrophoretic mobility shift assays, and Western blot studies to measure DMAPT's ability to generate reactive oxygen species (ROS), inhibit NFkappaB DNA binding, and cause changes in anti-apoptotic proteins. N-acetyl cysteine (NAC) and short hairpin RNA (shRNA) were used to determine the contribution of ROS and JNK2 activation, respectively. The BrdU incorporation assay was used to measure proliferation and trypan blue studies assessed cell viability after DMAPT treatment. The in vivo activity of DMAPT as a single agent and in combination with bicalutamide or docetaxel was assessed in a subcutaneous xenograft model with athymic nude female mice. RESULTS: DMAPT generated ROS with subsequent JNK activation and inhibited NFkappaB DNA binding and expression of NFkappaB-regulated anti-apoptotic proteins. DMAPT increased necrotic and apoptotic cell death in a cell-type-dependent manner and both types of cell death were blocked by NAC. Additionally, shRNA JNK2 partially blocked the anti-proliferative activity of DMAPT. DMAPT inhibited CWR22Rv1 and PC-3 cellular proliferation by 100% with 10 and 20 microM respectively and in vivo, DMAPT was more effective at inhibiting growth than bicalutamide (CWR22v1) and docetaxel (PC-3). CONCLUSIONS: DMAPT promotes cell death by both generating ROS and inhibition of NFkappaB. Its in vivo activity supports the conduct of clinical trials in patients with castrate-resistant disease

Bao BY, Ting HJ, Hsu JW, Yasmin-Karim S, Messing E, Lee YF. Down-regulation of NF-kappaB signals is involved in loss of 1alpha,25-dihydroxyvitamin D3 responsiveness. *J Steroid Biochem Mol*

*Biol* 2010 May;120(1):11-21.

Abstract: Vitamin D anti-tumor effect is often found reduced in the late stages of cancer. To uncover vitamin D resistance mechanism, we established a vitamin D-resistant human prostate cancer LNCaP cell line, LNCaP-R, by chronic exposure of cells to 1 $\alpha$ ,25-dihydroxyvitamin D(3) (1,25-VD). The vitamin D receptor (VDR)-mediated transcriptional activity was reduced in LNCaP-R, whereas VDR expression level and DNA-binding capacity were similar compared to parental cells (LNCaP-P). The expressions of the key factors involved in VDR transactivity, including CYP24A1 and VDR-associated proteins are all increased in LNCaP-R cells, and yet treatment with ketoconazole, P450 enzymes inhibitor, as well as trichostatin A (TSA), a histone deacetylase inhibitor, did not sensitize LNCaP-R cells response to vitamin D, suggesting that neither a local 1,25-VD availability, nor VDR-associated proteins are responsible for the vitamin D resistance. Interestingly, nuclear factor-kappaB (NF-kappaB) signaling, which is critical for 1,25-VD/VDR activity was found reduced in LNCaP-R cells, thereby treatment with NF-kappaB activator, 12-O-tetradecanoylphorbol-13-acetate (TPA), can sensitize LNCaP-R vitamin D response. Together, we conclude that NF-kappaB signaling is critical for vitamin D sensitivity, and dysregulation of this pathway would result in vitamin D resistance and disease progression

Miller SC, Huang R, Sakamuru S et al. Identification of known drugs that act as inhibitors of NF-kappaB signaling and their mechanism of action. *Biochem Pharmacol* 2010 May 1;79(9):1272-80.

Abstract: Nuclear factor-kappa B (NF-kappaB) is a transcription factor that plays a critical role across many cellular processes including embryonic and neuronal development, cell proliferation, apoptosis, and immune responses to infection and inflammation. Dysregulation of NF-kappaB signaling is associated with inflammatory diseases and certain cancers. Constitutive activation of NF-kappaB signaling has been found in some types of tumors including breast, colon, prostate, skin and lymphoid, hence therapeutic blockade of NF-kappaB signaling in cancer cells provides an attractive strategy for the development of anticancer drugs. To identify small molecule inhibitors of NF-kappaB signaling, we screened approximately 2800 clinically approved drugs and bioactive compounds from the NIH Chemical Genomics Center Pharmaceutical Collection (NPC) in a NF-kappaB mediated beta-lactamase reporter gene assay. Each compound was tested at fifteen different concentrations in a quantitative high throughput screening format. We identified nineteen drugs that inhibited NF-kappaB signaling, with potencies as low as 20 nM. Many of these drugs, including emetine, fluorosalan, sunitinib malate, bithionol, narasin, tribromsalan, and lestaurtinib, inhibited NF-kappaB signaling via inhibition of IkappaBalpha phosphorylation. Others, such as ectinascidin 743, chromomycin A3 and bortezomib utilized other mechanisms. Furthermore, many of these drugs induced caspase 3/7 activity and had an inhibitory effect on cervical cancer cell growth. Our results indicate that many currently approved pharmaceuticals have previously unappreciated effects on NF-kappaB signaling, which may contribute to anticancer therapeutic effects. Comprehensive profiling of approved drugs provides insight into their molecular mechanisms, thus providing a basis for drug repurposing

Karin M. NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol* 2009 November;1(5):a000141.

Abstract: NF-kappaB transcription factors have been suspected to be involved in cancer development since their discovery because of their kinship with the v-Rel oncogene product. Subsequent work led to identification of oncogenic mutations that result in NF-kappaB activation in lymphoid malignancies, but most of these mutations affect upstream components of NF-kappaB signaling pathways, rather than NF-kappaB family members themselves. NF-kappaB activation has also been observed in many solid tumors, but so far no oncogenic mutations responsible for NF-kappaB

activation in carcinomas have been identified. In such cancers, NF-kappaB activation is a result of underlying inflammation or the consequence of formation of an inflammatory microenvironment during malignant progression. Most importantly, through its ability to up-regulate the expression of tumor promoting cytokines, such as IL-6 or TNF-alpha, and survival genes, such as Bcl-X(L), NF-kappaB provides a critical link between inflammation and cancer

Srinivasan S, Kumar R, Koduru S, Chandramouli A, Damodaran C. Inhibiting TNF-mediated signaling: a novel therapeutic paradigm for androgen independent prostate cancer. *Apoptosis* 2010 February;15(2):153-61.

Abstract: The tumor necrosis factor (TNF) receptor super family comprises of members that induce two distinct signaling cascades, leading to either cell survival or apoptosis. However, in prostate cancer (PCa), TNF-mediated prosurvival signaling is the predominant pathway that leads to cell survival and resistance to therapy. Although inhibition of TNF signaling by pharmacological agents or monoclonal antibodies has gained importance in the field of cancer therapy, toxicity to normal cells has impaired their extensive use for cancer treatment. We previously identified a natural, nontoxic compound psoralidin that inhibited viability and induced apoptosis in androgen independent prostate cancer (AIPC) cells. Thus, the goal of our study is to investigate whether psoralidin inhibits TNF-mediated prosurvival signaling in AIPC cells. Our results suggest that psoralidin inhibits constitutive and TNF-induced expression of TNF-alpha and its downstream prosurvival signaling molecules such as NF-kappaB and Bcl-2 in AIPC cells. On the other hand, psoralidin simultaneously induces the death receptor (DR)-mediated apoptotic signaling eventually causing the activation of caspase cascade and resultant induction of apoptosis. Oral administration of psoralidin inhibits expression of TNF-alpha and NF-kappaB/p65 in tumor sections, resulting in tumor regression in PC-3 xenografts. Our results suggest that psoralidin inhibits TNF-mediated survival signaling in AIPC and thus is a potent therapeutic agent for prostate cancer

Lee SY, Cho JS, Yuk DY et al. Obovatol enhances docetaxel-induced prostate and colon cancer cell death through inactivation of nuclear transcription factor-kappaB. *J Pharmacol Sci* 2009 October;111(2):124-36.

Abstract: Nuclear transcription factor-kappaB (NF-kappaB) is constitutively activated in prostate and colon cancers and is related with the resistance of cancer cells against chemotherapeutics. Previously, we found that obovatol, an active compound isolated from *Magnolia obovata*, inhibited cancer cell growth through inhibition of NF-kappaB activity. We investigated here whether obovatol could sensitize cancer cells against docetaxel through inhibition of NF-kappaB activity in prostate cancer (LNCaP and PC-3) and colon cancer (SW620 and HCT116) cells. The combination treatment with each drug at one half the respective IC(50) dose (5 microM obovatol + 5 nM docetaxel) was more effective and significant (60%-70%) in the inhibition of cancer cell growth than single treatment by each drug (20%-40%); inhibition was exerted through a significant increase of apoptosis induction (60%-80%) by the combination treatment compared to the single treatment (10%-30%). Correlating well with the synergistic inhibition (combination indices are less than 1 in all cell types), the combination significantly inhibited NF-kappaB activities as well as expression of NF-kappaB target apoptotic cell death proteins, but decreased anti-apoptotic cell death proteins. Similar combination effects of obovatol with other chemotherapeutic agents (paclitaxel, cisplatin, and doxorubicin) on the inhibition of cell growth and NF-kappaB activity were also found. These results indicate that obovatol augments cell growth inhibition by chemotherapeutics through inactivation of NF-kappaB and suggest that obovatol may have therapeutic advantages in the combination treatment with other chemotherapeutics. [Supplementary Figure: available only at <http://dx.doi.org/10.1254/jphs.09048FP>]

Zhang L, Altuwaijri S, Deng F et al. NF-kappaB regulates androgen receptor expression and prostate cancer growth. *Am J Pathol* 2009 August;175(2):489-99.

Abstract: Prostate cancers that progress during androgen-deprivation therapy often overexpress the androgen receptor (AR) and depend on AR signaling for growth. In most cases, increased AR expression occurs without gene amplification and may be due to altered transcriptional regulation. The transcription factor nuclear factor (NF)-kappaB, which is implicated in tumorigenesis, functions as an important downstream substrate of mitogen-activated protein kinase, phosphatidylinositol 3-kinase, AKT, and protein kinase C and plays a role in other cancer-associated signaling pathways. NF-kappaB is an important determinant of prostate cancer clinical biology, and therefore we investigated its role in the regulation of AR expression. We found that NF-kappaB expression in prostate cancer cells significantly increased AR mRNA and protein levels, AR transactivation activity, serum prostate-specific antigen levels, and cell proliferation. NF-kappaB inhibitors decrease AR expression levels, prostate-specific antigen secretion, and proliferation of prostate cancer cells in vitro. Furthermore, inhibitors of NF-kappaB demonstrated anti-tumor activity in androgen deprivation-resistant prostate cancer xenografts. In addition, levels of both NF-kappaB and AR were strongly correlated in human prostate cancer. Our data suggest that NF-kappaB can regulate AR expression in prostate cancer and that NF-kappaB inhibitors may have therapeutic potential

Benitez DA, Hermoso MA, Pozo-Guisado E, Fernandez-Salguero PM, Castellon EA. Regulation of cell survival by resveratrol involves inhibition of NF kappa B-regulated gene expression in prostate cancer cells. *Prostate* 2009 July 1;69(10):1045-54.

Abstract: BACKGROUND: Polyphenols have been proposed as antitumoral agents. We have shown that resveratrol (RES) induced cell cycle arrest and promoted apoptosis in prostate cancer cells by inhibition of the PI3K pathway. The RES effects on NF kappaB activity in LNCaP cells (inducible NF kappaB), and PC-3 cells (constitutive NF kappaB) are reported. METHODS: Cells were treated with 1-150 microM of RES during 36 hr. NF kappaB subcellular localization was analyzed by western blot and immunofluorescence. I kappaB alpha was evaluated by immunoprecipitation followed by Western blot. Specific DNA binding of NF kappaB was determined by EMSA assays and NF kappaB-mediated transcriptional activity by transient transfection with a luciferase gene reporter system. RESULTS: RES induced a dose-dependent cytoplasmic retention of NF kappaB mediated by I kappaB alpha in PC-3 cells but not in LNCaP. RES-induced inhibition of NF kappaB specific binding to DNA was more significant in PC-3 cells. NF kappaB-mediated transcriptional activity induced by EGF and TNFalpha were inhibited by RES in both cell lines. LY294002 mimicked RES effects on NF kappaB activity. CONCLUSION: Antiproliferative and apoptotic effects of RES on human prostate cancer cells may be mediated by the inhibition of NF kappaB activity. This mechanism seems to be associated to RES-induced PI3K inhibition. RES could have therapeutic potential for prostate cancer treatment

Zhang X, Huang X, Olumi AF. Repression of NF-kappaB and activation of AP-1 enhance apoptosis in prostate cancer cells. *Int J Cancer* 2009 April 15;124(8):1980-9.

Abstract: TNFalpha and TRAIL, 2 members of the tumor necrosis factor family, share many common signaling pathways to induce apoptosis. Although many cancer cells are sensitive to these proapoptotic agents, some develop resistance. Recently, we have demonstrated that upregulation of c-Fos/AP-1 is necessary, but insufficient for cancer cells to undergo TRAIL-induced apoptosis. Here we present a prostate cancer model with differential sensitivity to TNFalpha and TRAIL. We show that inhibition of NF-kappaB or activation of AP-1 can only partially sensitize resistant prostate cancer cells to proapoptotic effects of TNFalpha or TRAIL. Inhibition of NF-kappaB by silencing TRAF2, by silencing RIP or by ectopic expression of IkappaB partially sensitized resistant prostate

cancer. Similarly, activation of c-Fos/AP-1 only partially sensitized resistant cancer cells to proapoptotic effects of TNFalpha or TRAIL. However, concomitant repression of NF-kappaB and activation of c-Fos/AP-1 significantly enhanced the proapoptotic effects of TNFalpha and TRAIL in resistant prostate cancer cells. Therefore, multiple molecular pathways may need to be modified, to overcome cancers that are resistant to proapoptotic therapies

Shankar S, Ganapathy S, Chen Q, Srivastava RK. Curcumin sensitizes TRAIL-resistant xenografts: molecular mechanisms of apoptosis, metastasis and angiogenesis. *Mol Cancer* 2008;7:16.

Abstract: **BACKGROUND:** We have recently shown that curcumin (a diferuloylmethane, the yellow pigment in turmeric) enhances apoptosis-inducing potential of TRAIL in prostate cancer PC-3 cells, and sensitizes TRAIL-resistant LNCaP cells in vitro through multiple mechanisms. The objectives of this study were to investigate the molecular mechanisms by which curcumin sensitized TRAIL-resistant LNCaP xenografts in vivo. **METHODS:** Prostate cancer TRAIL-resistant LNCaP cells were implanted in Balb c nude mice to examine the effects of curcumin and/or TRAIL on tumor growth and genes related to apoptosis, metastasis and angiogenesis. **RESULTS:** Curcumin inhibited growth of LNCaP xenografts in nude mice by inducing apoptosis (TUNEL staining) and inhibiting proliferation (PCNA and Ki67 staining), and sensitized these tumors to undergo apoptosis by TRAIL. In xenografted tumors, curcumin upregulated the expression of TRAIL-R1/DR4, TRAIL-R2/DR5, Bax, Bak, p21/WAF1, and p27/KIP1, and inhibited the activation of NFkappaB and its gene products such as cyclin D1, VEGF, uPA, MMP-2, MMP-9, Bcl-2 and Bcl-XL. The regulation of death receptors and members of Bcl-2 family, and inactivation of NFkappaB may sensitize TRAIL-resistant LNCaP xenografts. Curcumin also inhibited number of blood vessels in tumors, and circulating endothelial growth factor receptor 2-positive endothelial cells in mice. **CONCLUSION:** The ability of curcumin to inhibit tumor growth, metastasis and angiogenesis, and enhance the therapeutic potential of TRAIL suggests that curcumin alone or in combination with TRAIL can be used for prostate cancer prevention and/or therapy

Araki S, Omori Y, Lyn D et al. Interleukin-8 is a molecular determinant of androgen independence and progression in prostate cancer. *Cancer Res* 2007 July 15;67(14):6854-62.

Abstract: The proinflammatory chemokine interleukin-8 (IL-8) is undetectable in androgen-responsive prostate cancer cells (e.g., LNCaP and LAPC-4), but it is highly expressed in androgen-independent metastatic cells, such as PC-3. In this report, we show IL-8 functions in androgen independence, chemoresistance, tumor growth, and angiogenesis. We stably transfected LNCaP and LAPC-4 cells with IL-8 cDNA and selected IL-8-secreting (IL8-S) transfectants. The IL8-S transfectants that secreted IL-8 at levels similar to that secreted by PC-3 cells (100-170 ng/10(6) cells) were characterized. Continuous or transient exposure of LNCaP and LAPC-4 cells to IL-8 reduced their dependence on androgen for growth and decreased sensitivity (>3.5x) to an antiandrogen. IL-8-induced cell proliferation was mediated through CXCR1 and was independent of androgen receptor (AR). Quantitative PCR, immunoblotting, and transfection studies showed that IL8-S cells or IL-8-treated LAPC-4 cells exhibit a 2- to 3-fold reduction in PSA and AR levels, when compared with vector transfectants. IL8-S cells expressed 2- to 3-fold higher levels of phospho-EGFR, src, Akt, and nuclear factor kappaB (NF-kappaB) and showed increased survival when treated with docetaxel. This increase was blocked by NF-kappaB and src inhibitors, but not by an Akt inhibitor. IL8-S transfectants displayed a 3- to 5-fold increased motility, invasion, matrix metalloproteinase-9 and vascular endothelial growth factor production. LNCaP IL8-S cells grew rapidly as tumors, with increased microvessel density and abnormal tumor vasculature when compared with the tumors derived from their vector-transfected counterparts. Therefore, IL-8 is a molecular determinant of androgen-independent prostate cancer growth and progression

Paule B, Terry S, Kheuang L, Soyeux P, Vacherot F, de la Taille A. The NF-kappaB/IL-6 pathway in metastatic androgen-independent prostate cancer: new therapeutic approaches? *World J Urol* 2007 October;25(5):477-89.

Abstract: The nuclear factor of kappa beta (NF-kappaB) transcription factor regulates the transcription of numerous genes including that of interleukin 6 (IL-6). The IL-6 acts as an autocrine and paracrine growth factor of androgen-independent prostate cancer. An aberrant expression of the IL-6 gene and an increase in IL-6 expression are detected in bone metastatic and hormone-refractory prostate cancer. IL-6 has been suggested to have a crucial role in the resistance to chemotherapy or hormonal therapy involving apoptotic cell death. The NF-kappaB/IL-6 dependent pathways promote tumour-cell survival and in most situations protect cells against apoptotic stimuli. These data provide a rational framework for targeting NF-kappaB and IL-6 activity in novel biologically based therapies for aggressive and androgen independent prostate cancers

Shanmugam R, Jayaprakasan V, Gokmen-Polar Y et al. Restoring chemotherapy and hormone therapy sensitivity by parthenolide in a xenograft hormone refractory prostate cancer model. *Prostate* 2006 October 1;66(14):1498-511.

Abstract: BACKGROUND: Nuclear Factor kappa B (NFkappaB) is a eukaryotic transcription factor that is constitutively active in human cancers and can be inhibited by the naturally occurring sesquiterpene lactone, parthenolide (P). METHODS: The in vitro effects of P were assessed using the androgen independent cell line, CWR22Rv1, and human umbilical endothelial cells (HUVECs). The in vivo activity of P as a single agent and its ability to augment the efficacy of docetaxel and the anti-androgen, bicalutamide, were determined using the CWR22Rv1 xenograft model. RESULTS: Parthenolide at low micromolar concentration inhibited proliferation of CWR22Rv1 and HUVEC cells, promoted apoptosis and abrogated NFkappaB-DNA binding. Parthenolide downregulated anti-apoptotic genes under NFkappaB control, TRAF 1 and 2, and promoted sustained activation of c-jun-NH2 kinase (JNK). Parthenolide also augmented the in vivo efficacy of docetaxel and restored sensitivity to anti-androgen therapy. CONCLUSION: These studies demonstrate parthenolide's anti-tumor and anti-angiogenic activity, and its potential to augment the efficacy of chemotherapy and hormonal therapy

Deeb DD, Jiang H, Gao X, Divine G, Dulchavsky SA, Gautam SC. Chemosensitization of hormone-refractory prostate cancer cells by curcumin to TRAIL-induced apoptosis. *J Exp Ther Oncol* 2005;5(2):81-91.

Abstract: Failure to undergo apoptosis has been implicated in the resistance of tumor cells to anticancer therapies. Promotion of apoptosis in tumor cells could potentially increase the efficacy of conventional treatment regimens and improve prognosis. Prostate cancer cells are generally resistant to induction of apoptosis by anticancer agents and death ligands. We investigated the sensitization of prostate cancer cell lines by curcumin (diferuloyl-methane) to TNF-related apoptosis inducing ligand (TRAIL)-induced apoptosis. Prostate cancer cells treated with curcumin or TRAIL or curcumin and TRAIL together were assessed for induction of apoptosis and pathway of apoptosis was determined from the activation of procaspases and release of cytochrome c from mitochondria. Curcumin sensitized LNCaP, DU145 and PC3 tumor cell lines to TRAIL. Combined curcumin and TRAIL treatment produced the most loss of viable cells by inducing apoptosis as revealed by accumulation of hypodiploid cells in sub-G1 phase, enhanced annexin V binding, DNA fragmentation, cleavage of procaspases-3, -8, and 9, truncation of proapoptotic Bid, and release of cytochrome c from mitochondria. Tumor cells expressed constitutively active NF-kappaB and sensitization to TRAIL involved inhibition of NF-kappaB by curcumin. These findings suggest that combined

curcumin/TRAIL chemo-immunotherapy may be a beneficial adjunct to the standard therapeutic regimens for prostate cancer

Kuroda K, Horiguchi Y, Nakashima J et al. Prevention of cancer cachexia by a novel nuclear factor {kappa}B inhibitor in prostate cancer. *Clin Cancer Res* 2005 August 1;11(15):5590-4.

Abstract: PURPOSE: To investigate the association between serum interleukin-6 (IL-6) and cachexia in patients with prostate cancer and the inhibitory effect of a new nuclear factor kappaB (NF-kappaB) inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), on IL-6 production and cachexia in an animal model of hormone-refractory prostate cancer. EXPERIMENTAL DESIGN: The association between serum IL-6 levels and variables of cachexia was evaluated in 98 patients with prostate cancer. The inhibitory effects of DHMEQ on IL-6 secretion and cachexia were investigated in in vitro and in vivo studies using JCA-1 cells derived from human prostate cancer. RESULTS: Serum IL-6 levels were significantly elevated and cachexia developed in JCA-1 tumor-bearing mice as well as in prostate cancer patients with progressive disease. IL-6 secretion was significantly inhibited in JCA-1 cells exposed to DHMEQ. Intraperitoneal administration of DHMEQ (8 mg/kg) to tumor-bearing mice produced a significant amelioration of the reduction in body weight, epididymal fat weight, gastrocnemius muscle weight, hematocrit, and serum levels of triglyceride and albumin when compared with administration of DMSO or no treatment. DHMEQ caused a significant decrease of serum IL-6 level in JCA-1 tumor-bearing mice (all  $P < 0.05$ ). CONCLUSIONS: These results suggested an association between serum IL-6 and cachexia in patients with prostate cancer and in JCA-1 tumor-bearing mice and that a new NF-kappaB inhibitor, DHMEQ, could prevent the development of cachexia in JCA-1 tumor-bearing mice presumably through the inhibition of IL-6 secretion. DHMEQ seems to show promise as a novel and unique anticachectic agent in hormone-refractory prostate cancer

Xu C, Shen G, Chen C, Gelinas C, Kong AN. Suppression of NF-kappaB and NF-kappaB-regulated gene expression by sulforaphane and PEITC through IkappaBalpha, IKK pathway in human prostate cancer PC-3 cells. *Oncogene* 2005 June 30;24(28):4486-95.

Abstract: Recent studies indicate that natural isothiocyanates, such as sulforaphane (SFN) and phenethyl isothiocyanate (PEITC) possess strong antitumor activities in vitro and in vivo. The nuclear factor kappa B (NF-kappaB) is believed to play an important role in cancer chemoprevention due to its involvement in tumor cell growth, proliferation, angiogenesis, invasion, apoptosis, and survival. In this study, we investigated the effects and the molecular mechanisms of SFN and PEITC on NF-kappaB transcriptional activation and NF-kappaB-regulated gene expression in human prostate cancer PC-3 C4 cells. Treatment with SFN (20 and 30 microM) and PEITC (5 and 7.5 microM) significantly inhibited NF-kappaB transcriptional activity, nuclear translocation of p65, and gene expression of NF-kappaB-regulated VEGF, cyclin D1, and Bcl-X(L) in PC-3 C4 cells. To further elucidate the mechanism, we utilized the dominant-negative mutant of inhibitor of NF-kappaB alpha (IkappaBalpha) (SR-IkappaBalpha). Analogous to treatments with SFN and PEITC, SR-IkappaBalpha also strongly inhibited NF-kappaB transcriptional activity as well as VEGF, cyclin D1, and Bcl-X(L) expression. Furthermore, SFN and PEITC also inhibited the basal and UVC-induced phosphorylation of IkappaBalpha and blocked UVC-induced IkappaBalpha degradation in PC-3 C4 cells. In examining the upstream signaling, we found that the dominant-negative mutant of IKKbeta (dnIKKbeta) possessed inhibitory effects similar to SFN and PEITC on NF-kappaB, VEGF, cyclin D1, Bcl-X(L) as well as IkappaBalpha phosphorylation. In addition, treatment with SFN and PEITC potently inhibited phosphorylation of both IKKbeta and IKKalpha and significantly inhibited the in vitro phosphorylation of IkappaBalpha mediated by IKKbeta. Taken together, these results suggest that the inhibition of SFN and PEITC on NF-kappaB transcriptional activation as well as NF-kappaB-regulated VEGF, cyclin D1, and Bcl-X(L) gene expression is mainly mediated through the inhibition

of IKK phosphorylation, particularly IKKbeta, and the inhibition of IkappaBalpha phosphorylation and degradation, as well as the decrease of nuclear translocation of p65 in PC-3 cells

Sweeney C, Li L, Shanmugam R et al. Nuclear factor-kappaB is constitutively activated in prostate cancer in vitro and is overexpressed in prostatic intraepithelial neoplasia and adenocarcinoma of the prostate. *Clin Cancer Res* 2004 August 15;10(16):5501-7.

Abstract: PURPOSE: The transcription factor nuclear factor-kappaB (NF-kappaB) promotes the production of angiogenic, antiapoptotic, and prometastatic factors that are involved in carcinogenesis. EXPERIMENTAL DESIGN: Electromobility gel shift assays were used to evaluate NF-kappaB DNA binding in vitro. The functional relevance of NF-kappaB DNA binding was assessed by both cDNA array analyses and proliferation assays of prostate cancer cells with and without exposure to an NF-kappaB inhibitor, parthenolide. Immunohistochemistry staining for the p65 NF-kappaB subunit was used to determine the frequency and location of NF-kappaB in 97 prostatectomy specimens. The amount of staining was quantified on a 0-3+ scale. RESULTS: An electromobility gel shift assay confirmed the presence of NFkappaB DNA binding in all four prostate cancer cell lines tested. The binding was inhibited by parthenolide, and this agent also decreased multiple gene transcripts under the control of NF-kappaB and inhibited proliferation of prostate cancer cells. The staining results revealed overexpression of p65 in the prostatic intraepithelial neoplasia and cancer compared with the benign epithelium. Specifically, there was a predominance of 1+ and 2+ with no 3+ staining in benign epithelium, whereas there was only 2+ and 3+ staining (30 and 70%, respectively) in the cancerous areas. These differences were statistically different. There was no correlation with tumor grade or stage. CONCLUSIONS: NF-kappaB is constitutively activated in prostate cancer and functionally relevant in vitro. Immunohistochemistry of human prostatectomy specimens demonstrated overexpression of the active subunit of NF-kappaB, p65, and that this occurs at an early stage in the genesis of prostate cancer. This work supports the rationale for targeting NF-kappaB for the prevention and/or treatment of prostate cancer

Raj GV, Sekula JA, Guo R, Madden JF, Daaka Y. Lysophosphatidic acid promotes survival of androgen-insensitive prostate cancer PC3 cells via activation of NF-kappaB. *Prostate* 2004 October 1;61(2):105-13.

Abstract: BACKGROUND: Dysregulated cell survival contributes to the poor efficacy of many chemotherapeutic regimens for patients with advanced prostate cancer. In this study we examined ability of the lipid growth factor lysophosphatidic acid (LPA), a G protein-coupled receptor (GPCR) ligand, to promote prostate cell survival. METHODS: PC3 cells were used as a model to study mechanisms involved in survival of androgen-insensitive prostate cancer cells. Cell survival was measured by FACS analysis of cell cycle parameters after propidium iodide or annexin V and 7-AAD immunostaining. Activation state of nuclear factor-kappaB (NF-kappaB) was determined biochemically by nuclear translocation and transcriptional activation. Human tissue was analyzed for nuclear expression of NF-kappaB by immunohistochemistry. RESULTS: Molecular dissection of the LPA-regulated PC3 cell survival revealed the sequential phosphorylation of Akt, IkappaB, and transcriptional activation of NF-kappaB. Both Akt and NF-kappaB were required to escape serum deprivation-induced cell death since their inhibition abrogated the LPA-mediated PC3 cell survival. Data from archival human tissue show that NF-kappaB is constitutively activated in prostate cancers, but not in benign prostate tissues. CONCLUSIONS: Targeted disruption of the LPA receptor-Akt-NF-kappaB signaling axis may be effective for the treatment of androgen-insensitive prostate cancer

Deeb D, Jiang H, Gao X et al. Curcumin sensitizes prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand/Apo2L by inhibiting nuclear factor-kappaB through suppression of IkappaBalpha phosphorylation. *Mol Cancer Ther* 2004 July;3(7):803-12.

Abstract: Epidemiologic studies suggest that diet rich in plant-derived foods plays an important role in the prevention of prostate cancer. Curcumin, the yellow pigment in the spice turmeric, has been shown to exhibit chemopreventive and growth inhibitory activities against multiple tumor cell lines. We have shown previously that curcumin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L interact to induce cytotoxicity in the LNCaP prostate cancer cell line. In this study, we investigated the mechanism by which curcumin augments TRAIL-induced cytotoxicity in LNCaP cells. Subtoxic concentrations of the curcumin-TRAIL combination induced strong apoptotic response in LNCaP cells as demonstrated by the binding of Annexin V-FITC and cleavage of procaspase-3. Furthermore, LNCaP cells express constitutively active nuclear factor-kappaB (NF-kappaB), which is inhibited by curcumin. Because NF-kappaB has been shown to mediate resistance to TRAIL-induced apoptosis in tumor cells, we investigated whether there is a relationship between NF-kappaB activation and resistance to TRAIL in LNCaP prostate cancer cells. Pretreatment with curcumin inhibited the activation of NF-kappaB and sensitized LNCaP cells to TRAIL. A similar increase in the sensitivity of LNCaP cells to TRAIL-induced apoptosis was observed following inhibition of NF-kappaB by dominant negative mutant IkappaBalpha, an inhibitor of NF-kappaB. Finally, curcumin was found to inhibit NF-kappaB by blocking phosphorylation of IkappaBalpha. We conclude that NF-kappaB mediates resistance of LNCaP cells to TRAIL and that curcumin enhances the sensitivity of these tumor cells to TRAIL by inhibiting NF-kappaB activation by blocking phosphorylation of IkappaBalpha and its degradation

Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MM. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. *Oncogene* 2004 February 26;23(8):1599-607.

Abstract: Curcumin (Diferuloylmethane) is a major chemical component of turmeric (*curcuma longa*) and is used as a spice to give a specific flavor and yellow color in Asian food. Curcumin exhibits growth inhibitory effects in a broad range of tumors as well as in TPA-induced skin tumors in mice. This study was undertaken to investigate the radiosensitizing effects of curcumin in p53 mutant prostate cancer cell line PC-3. Compared to cells that were irradiated alone (SF(2)=0.635; D(0)=231 cGy), curcumin at 2 and 4 microM concentrations in combination with radiation showed significant enhancement to radiation-induced clonogenic inhibition (SF(2)=0.224; D(0)=97 cGy and SF(2)=0.080; D(0)=38 cGy) and apoptosis. It has been reported that curcumin inhibits TNF-alpha-induced NFkappaB activity that is essential for Bcl-2 protein induction. In PC-3 cells, radiation upregulated TNF-alpha protein leading to an increase in NFkappaB activity resulting in the induction of Bcl-2 protein. However, curcumin in combination with radiation treated showed inhibition of TNF-alpha-mediated NFkappaB activity resulting in bcl-2 protein downregulation. Bax protein levels remained constant in these cells after radiation or curcumin plus radiation treatments. However, the downregulation of Bcl-2 and no changes in Bax protein levels in curcumin plus radiation-treated PC-3 cells, together, altered the Bcl2 : Bax ratio and this caused the enhanced radiosensitization effect. In addition, significant activation of cytochrome c and caspase-9 and -3 were observed in curcumin plus radiation treatments. Together, these mechanisms strongly suggest that the natural compound curcumin is a potent radiosensitizer, and it acts by overcoming the effects of radiation-induced prosurvival gene expression in prostate cancer

Suh J, Rabson AB. NF-kappaB activation in human prostate cancer: important mediator or epiphenomenon? *J Cell Biochem* 2004 January 1;91(1):100-17.

Abstract: The NF-kappaB family of transcription factors has been shown to be constitutively activated in various human malignancies, including leukemias, lymphomas, and a number of solid tumors. NF-kappaB is hypothesized to contribute to development and/or progression of malignancy by regulating the expression of genes involved in cell growth and proliferation, anti-apoptosis,

angiogenesis, and metastasis. Prostate cancer cells have been reported to have constitutive NF-kappaB activity due to increased activity of the IkappaB kinase complex. Furthermore, an inverse correlation between androgen receptor (AR) status and NF-kappaB activity was observed in prostate cancer cell lines. NF-kappaB may promote cell growth and proliferation in prostate cancer cells by regulating expression of genes such as c-myc, cyclin D1, and IL-6. NF-kappaB may also inhibit apoptosis in prostate cancer cells through activation of expression of anti-apoptotic genes, such as Bcl-2, although pro-apoptotic activity of NF-kappaB has also been reported. NF-kappaB-mediated expression of genes involved in angiogenesis (IL-8, VEGF), and invasion and metastasis (MMP9, uPA, uPA receptor) may further contribute to the progression of prostate cancer. Constitutive NF-kappaB activity has also been demonstrated in primary prostate cancer tissue samples and suggested to have prognostic importance for a subset of primary tumors. The limited number of samples analyzed in those studies and the relative lack of NF-kappaB target genes identified in RNA expression microarray analyses of prostate cancer cells suggest that further studies will be required in order to determine if NF-kappaB actually plays a role in human prostate cancer development, and/or progression, and to characterize its potential as a therapeutic target

Andela VB, Gordon AH, Zotalis G et al. NFkappaB: a pivotal transcription factor in prostate cancer metastasis to bone. *Clin Orthop Relat Res* 2003 October;(415 Suppl):S75-S85.

Abstract: Prostate cancers frequently metastasize to bone and this accounts for substantial morbidity. We investigated the potential role of the transcription factor NFkappaB as a central regulator of prostate cancer metastasis using the prostate adenocarcinoma cell line, PC-3, in a series of in vitro studies. Wild type PC-3 cells (PC-3.WT) have high basal levels of NFkappaB signaling, otherwise absent in PC-3 cells stably expressing a mutant form of the inhibitory kappa B (IkappaB) protein alpha (PC-3.mIkappaB). Although PC-3.WT cells in co-culture with rat bone marrow cells enhance bone resorption, no increase was observed in co-cultures with PC-3.mIkappaB cells. Similarly, although PC-3.WT cells were invasive in a chicken chorioallantoic membrane extravasation model, PC-3.mIkappaB cells lose this capacity to invade. NFkappaB reciprocally regulated genes involved in cellular invasion, with upregulation of MMP-9 and downregulation of its inhibitor, TIMP-1 in PC-3.WT cells, whereas MMP-9 was downregulated and TIMP-1 was upregulated in PC-3.mIkappaB cells. Finally, high basal gene and protein expression of the osteoclast-activating cytokine IL-6, observed in PC-3.WT cells, was absent in PC-3.mIkappaB cells. These in vitro experiments suggest NFkappaB as an important target to prevent prostate cancer bone metastasis and provide a rationale for further study of this transcription factor in metastatic disease

Lloyd FP, Jr., Slivova V, Valachovicova T, Sliva D. Aspirin inhibits highly invasive prostate cancer cells. *Int J Oncol* 2003 November;23(5):1277-83.

Abstract: Cell adhesion, proteolytic degradation and cell migration are interrelated processes responsible for the invasion and metastasis of cancer. One of the crucial molecules involved in cancer metastasis is urokinase-type plasminogen activator (uPA). An elevated concentration of uPA is a strong indicator of poor prognosis. In addition to the proteolytic activity of uPA, which degrades the extracellular matrix, uPA also binds to its receptor (uPAR) and controls cell adhesion and migration through the reorganization of actin cytoskeleton. We have recently demonstrated that constitutively active nuclear factor-kappa B (NF-kappaB) is responsible for the increased secretion of uPA and that inhibition of NF-kappaB suppresses secretion of uPA and cell migration of highly invasive cancer cells. Aspirin and other nonsteroidal anti-inflammatory drugs have been recently shown to have a chemopreventive effect in colon and pancreatic cancers. Here we show that aspirin inhibits NF-kappaB, resulting in the suppression of uPA secretion from the highly invasive human prostate cancer cells PC-3. Furthermore, aspirin inhibited migration of PC-3 cells, suggesting an effect on the uPA-uPAR signaling complex. Finally, aspirin suppressed adhesion of PC-3 cells to fibronectin (FN),

which binds to an alpha3beta1 integrin receptor, and to vitronectin (VN), which binds to alphavbeta3 integrin receptor. Altogether, our data suggests that aspirin inhibits the formation of uPA-uPAR-FN-alpha3beta1 and uPA-uPAR-VN-alphavbeta3 complexes, resulting in the suppression of cell adhesion and cell motility of the highly invasive prostate cancer cells PC-3. These results indicate that aspirin may contribute directly to reducing invasion and metastasis of prostate cancers by inhibiting cell migration and invasion

Lessard L, Mes-Masson AM, Lamarre L, Wall L, Lattouf JB, Saad F. NF-kappa B nuclear localization and its prognostic significance in prostate cancer. *BJU Int* 2003 March;91(4):417-20.

Abstract: OBJECTIVE: To detect the subcellular localization of NF-kappa B (p65) in human prostate cancer tissues of different histological grades, and to test whether NF-kappa B localization alone, or combined with the histological grade, can be used to predict patient outcome. PATIENTS AND METHODS: Prostate cancer tissues were obtained from radical prostatectomy specimens; the histological grade was determined using the Gleason grading system. Clinical outcomes were defined as good (5-year disease-free survival with undetectable levels of prostate specific antigen) or poor (progression to bone metastases). The subcellular localization of NF-kappa B was visualized by immunohistochemistry using an anti-p65 antibody. RESULTS: The NF-kappa B subcellular localization was initially assessed in 45 specimens; in these samples a nuclear localization of NF-kappa B was specific to cancer tissues, but did not correlate with the Gleason score (P = 0.089). NF-kappa B was then assessed as a prognostic marker to complement Gleason score in predicting cancer progression. Tumour tissues from 30 men with a known clinical outcome were included; 10 of 17 patients who had a poor outcome were positive for NF-kappa B nuclear staining, whereas only two of 13 with a good outcome were positive (P = 0.026). When NF-kappa B subcellular localization and Gleason score were combined, two risk categories of progression were defined. Eleven of 13 specimens from those with a good outcome were in the low-risk category (Gleason 2-4 or Gleason 5-7 with negative nuclear NF-kappa B) and 12 of 17 in the poor outcome group were in the high-risk category (Gleason 8-10 or Gleason 5-7 with positive nuclear NF-kappa B; P = 0.004). CONCLUSION: NF-kappa B is detectable in the nucleus in prostate cancer tissues and positivity can be used to help predict patient outcome. Multivariate analyses using other clinical and molecular variables are underway, and will validate the usefulness of NF-kappa B as a prognostic factor

Kikuchi E, Horiguchi Y, Nakashima J et al. Suppression of hormone-refractory prostate cancer by a novel nuclear factor kappaB inhibitor in nude mice. *Cancer Res* 2003 January 1;63(1):107-10.

Abstract: We have synthesized and explored the feasibility of using a novel nuclear factor (NF) kappaB inhibitor, a dehydroxymethylepoxyquinomicin designated as DHMEQ, against prostate cancer. The activity of NFkappaB, evaluated by transient transfection of a luciferase reporter DNA containing a specific binding sequence for NFkappaB, was inhibited by DHMEQ in three human hormone-refractory prostate cancer cell lines, DU145, JCA-1, and PC-3. Statistically significant growth inhibition was achieved by 20 micro g/ml of DHMEQ, and marked levels of apoptosis were induced 48 h after DHMEQ administration in vitro. Electrophoretic mobility shift assay showed that DHMEQ completely inhibited NFkappaB DNA binding activity in JCA-1 cells. Furthermore, i.p. administrations of DHMEQ significantly inhibited pre-established JCA-1 s.c. tumor growth in nude mice without any side effects. Our result indicates the possibility of using a novel NFkappaB activation inhibitor, DHMEQ, as a new treatment strategy against hormone-refractory prostate cancer

Hour TC, Chen J, Huang CY, Guan JY, Lu SH, Pu YS. Curcumin enhances cytotoxicity of chemotherapeutic agents in prostate cancer cells by inducing p21(WAF1/CIP1) and C/EBPbeta expressions and suppressing NF-kappaB activation. *Prostate* 2002 May 15;51(3):211-8.

Abstract: **BACKGROUND:** The modulatory effects and molecular mechanisms of curcumin (CCM) on the cytotoxicity of chemotherapeutic agents to prostate cancer cells were explored. **METHODS:** The combined effects of CCM and chemotherapeutic agents were examined by three different administration schedules (one concurrent and two sequential treatments) in two androgen-independent prostate cancer (AIPC) cells (PC-3 and DU145). Alteration of cell cycle progression, protein levels, and transcriptional activation in PC-3 cells were assayed by flow cytometry, Western blotting, and gel shift assay, respectively. **RESULTS:** The combined effects of CCM --> chemotherapeutic agent schedule showed the greatest synergistic cytotoxicity when compared to the other two schedules in both cells. CCM induced a significant G1 arrest in PC-3, which may be mediated by the induction of p21(WAF1/CIP1) and C/EBPbeta. Moreover, CCM was able to inhibit both the constitutional and TNF-alpha-induced NF-kappaB activation in a time-dependent manner. **CONCLUSIONS:** The incorporation of CCM into cytotoxic therapies may be a promising strategy for the treatment of AIPC

Chen CD, Sawyers CL. NF-kappa B activates prostate-specific antigen expression and is upregulated in androgen-independent prostate cancer. *Mol Cell Biol* 2002 April;22(8):2862-70.

Abstract: The transcription factor NF-kappa B regulates gene expression involved in cell growth and survival and has been implicated in progression of hormone-independent breast cancer. By expressing a dominant-active form of mitogen-activated protein kinase kinase kinase 1, by exposure to tumor necrosis factor alpha, or by overexpression of p50/p65, we show that NF-kappa B activates a transcription regulatory element of the prostate-specific antigen (PSA)-encoding gene, a marker for prostate cancer development, treatment, and progression. By DNase I footprinting, we identified four NF-kappa B binding sites in the PSA core enhancer. We also demonstrate that androgen-independent prostate cancer xenografts have higher constitutive NF-kappa B binding activity than their androgen-dependent counterparts. These results suggest a role of NF-kappa B in prostate cancer progression

Dhanalakshmi S, Singh RP, Agarwal C, Agarwal R. Silibinin inhibits constitutive and TNFalpha-induced activation of NF-kappaB and sensitizes human prostate carcinoma DU145 cells to TNFalpha-induced apoptosis. *Oncogene* 2002 March 7;21(11):1759-67.

Abstract: Prostate cancer (PCA) is one of the most common invasive malignancies of men in the US, however, there have been limited successes so far in its therapy. Even most potent agents (e.g. TNFalpha) are ineffective in killing human PCA cells possibly due to constitutive activation of NF-kappaB that subsequently activates a large number of anti-apoptotic genes. In such a scenario, strong apoptotic agent TNFalpha, further induces NF-kappaB activation rather than inducing apoptosis. In several recent studies, we have demonstrated both cancer preventive and anti-cancer efficacy of silymarin and its constituent silibinin in a variety of experimental tumor models and cell culture systems. Here we examined whether silibinin is effective in inhibiting constitutive NF-kappaB activation in human PCA cells, which would help in overcoming TNFalpha-insensitivity. Our studies reveal that silibinin effectively inhibits constitutive activation of NF-kappaB in advanced human prostate carcinoma DU145 cells. Consistent with this, nuclear levels of p65 and p50 sub-units of NF-kappaB were also reduced. In the studies assessing molecular mechanism of this effect, silibinin treatment resulted in a significant increase in the level of IkappaBalpha with a concomitant decrease in phospho-IkappaBalpha. Kinase assays revealed that silibinin dose-dependently decreases IKKalpha kinase activity. The effect of silibinin on IKKalpha seemed to be direct as evidenced by the in vitro kinase assay, where immunoprecipitated IKKalpha was incubated with silibinin. This shows that silibinin does not necessarily need an upstream event to bring about its inhibitory effect on IKKalpha and downstream effectors. Additional studies showed that silibinin also inhibits TNFalpha-induced activation of NF-kappaB via IkappaBalpha pathway and subsequently sensitizes DU145 cells to

TNF $\alpha$ -induced apoptosis. These results indicate that silibinin could be used to enhance the effectiveness of TNF $\alpha$ -based chemotherapy in advanced PCA

Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. *Oncogene* 2001 November 15;20(52):7597-609.

Abstract: While the role of nuclear transcription factor activator protein-1 (AP-1) in cell proliferation, and of nuclear factor-kappaB (NF-kappaB) in the suppression of apoptosis are known, their role in survival of prostate cancer cells is not well understood. We investigated the role of NF-kappaB and AP-1 in the survival of human androgen-independent (DU145) and -dependent (LNCaP) prostate cancer cell lines. Our results show that the faster rate of proliferation of DU145 cells when compared to LNCaP cells correlated with the constitutive expression of activated NF-kappaB and AP-1 in DU145 cells. The lack of constitutive expression of NF-kappaB and AP-1 in LNCaP cells also correlated with their sensitivity to the antiproliferative effects of tumor necrosis factor (TNF). TNF induced NF-kappaB activation but not AP-1 activation in LNCaP cells. In DU145 cells both c-Fos and c-Jun were expressed and treatment with TNF activated c-Jun NH2-terminal kinase (JNK), needed for AP-1 activation. In LNCaP cells, however, only low levels of c-Jun was expressed and treatment with TNF minimally activated JNK. Treatment of cells with curcumin, a chemopreventive agent, suppressed both constitutive (DU145) and inducible (LNCaP) NF-kappaB activation, and potentiated TNF-induced apoptosis. Curcumin alone induced apoptosis in both cell types, which correlated with the downregulation of the expression of Bcl-2 and Bcl-xL and the activation of procaspase-3 and procaspase-8. Overall, our results suggest that NF-kappaB and AP-1 may play a role in the survival of prostate cancer cells, and curcumin abrogates their survival mechanisms

Lindholm PF, Bub J, Kaul S, Shidham VB, Kajdacsy-Balla A. The role of constitutive NF-kappaB activity in PC-3 human prostate cancer cell invasive behavior. *Clin Exp Metastasis* 2000;18(6):471-9.

Abstract: The purpose of this study was to determine if increased NF-kappaB activity of highly invasive PC-3 cells contributed to their invasive behavior. Increased NF-kappaB activity has been observed in several malignant tumors and it may have an important role in tumorigenesis, progression and chemotherapy resistance. By serial selection, we obtained invasion variant PC-3 cell sublines. The PC-3 High Invasive cells invade readily through a Matrigel reconstituted basement membrane while PC-3 Low Invasive cells have low baseline invasion activity. In these studies, we discovered that NF-kappaB DNA binding activity was increased in PC-3 High Invasive cells when compared to PC-3 Low Invasive cells by electrophoretic mobility shift assay (EMSA). Gel supershift assays showed a 4-fold increase in p65 containing complexes and a 2.2-fold increase in the p50 containing complexes in the PC-3 High Invasive cells. Luciferase reporter assays showed that NF-kappaB dependent transcription activity was increased 10.2 +/- 2.5-fold in the highly invasive cells ( $P < 0.002$ ). The PC-3 High Invasive cells showed a constitutive increase in phospho-IkappaB alpha and introduction of the super-repressor IkappaB alpha S32/36A inhibited NF-kappaB activity to 19.2 +/- 2.5 percent of control transfected cells ( $P < 0.001$ ). The IkappaBa super-repressor reduced the basement membrane invasion of PC-3 High Invasive cells from 6.2 +/- 1.1 to 3.8 +/- 0.4 percent ( $P < 0.002$ ) with no decrease in cell viability or proliferation. These results demonstrate that increased NF-kappaB activity contributed directly to the invasive behavior of PC-3 High Invasive prostate cancer cells

Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. *Oncogene* 2001 July 12;20(31):4188-97.

Abstract: Since the NF-kappaB/relA transcription factor is constitutively activated in human prostate cancer cells, we determined whether blocking NF-kappaB/relA activity in human prostate cancer cells affected their angiogenesis, growth, and metastasis in an orthotopic nude mouse model. Highly metastatic PC-3M human prostate cancer cells were transfected with a mutated IkappaBalpha (IkappaBalphaM), which blocks NF-kappaB activity. Parental (PC-3M), control vector-transfected (PC-3M-Neo), and IkappaBalphaM-transfected (PC-3M-IkappaBalphaM) cells were injected into the prostate gland of nude mice. PC-3M and PC-3M-Neo cells produced rapidly growing tumors and regional lymph node metastasis, whereas PC-3M-IkappaBalphaM cells produced slow growing tumors with low metastatic potential. NF-kappaB signaling blockade significantly inhibited in vitro and in vivo expression of three major proangiogenic molecules, VEGF, IL-8, and MMP-9, and hence decreased neoplastic angiogenesis. Inhibition of NF-kappaB activity in PC-3M cells also resulted in the downregulation of MMP-9 mRNA and collagenase activity, resulting in decreased invasion through Matrigel. Collectively, these data suggest that blockade of NF-kappaB activity in PC-3M cells inhibits angiogenesis, inv

## Soy Isoflavones

Pendleton JM, Tan WW, Anai S et al. Phase II trial of isoflavone in prostate-specific antigen recurrent prostate cancer after previous local therapy. *BMC Cancer* 2008;8:132.

Abstract: BACKGROUND: Data exist that demonstrate isoflavones' potent antiproliferative effects on prostate cancer cells. We evaluated the efficacy of isoflavone in patients with PSA recurrent prostate cancer after prior therapy. We postulated that isoflavone therapy would slow the rate of rise of serum PSA. METHODS: Twenty patients with rising PSA after prior local therapy were enrolled in this open-labeled, Phase II, nonrandomized trial (Trial registration # NCT00596895). Patients were treated with soy milk containing 47 mg of isoflavonoid per 8 oz serving three times per day for 12 months. Serum PSA, testosterone, lipids, isoflavone levels (genistein, daidzein, and equol), and quality of life (QOL) were measured at various time points from 0 to 12 months. PSA outcome was evaluated. RESULTS: Within the mixed regression model, it was estimated that PSA had increased 56% per year before study entry and only increased 20% per year for the 12-month study period ( $p = 0.05$ ). Specifically, the slope of PSA after study entry was significantly lower than that before study entry in 6 patients and the slope of PSA after study entry was significantly higher than before study entry in 2 patients. For the remaining 12 patients, the change in slope was statistically insignificant. Nearly two thirds of the patients were noted to have significant levels of free equol in their serum while on therapy. CONCLUSION: Dietary intervention with isoflavone supplementation may have biologic activity in men with biochemical recurrent prostate cancer as shown by a decline in the slope of PSA. This study may lend support to the literature that nutritional supplements have biologic activity in prostate cancer and therefore, further studies with these agents in randomized clinical trials should be encouraged

Napora JK, Short RG, Muller DC et al. High-dose isoflavones do not improve metabolic and inflammatory parameters in androgen-deprived men with prostate cancer. *J Androl* 2011 January;32(1):40-8.

Abstract: The profound hypogonadism that occurs with androgen deprivation therapy (ADT) for prostate cancer (PCa) results in complications such as diabetes and metabolic syndrome that predispose to cardiovascular disease. Because phytoestrogens have been associated with an improvement in metabolic parameters, we evaluated their role in men undergoing ADT. Our objective was to evaluate the effects of high-dose isoflavones on metabolic and inflammatory parameters in men undergoing ADT. This was a randomized, double-blind, placebo-controlled, 12-week pilot study. Participants were randomly assigned to receive 20 g of soy protein containing 160 mg of total

isoflavones vs taste-matched placebo (20 g whole milk protein). The study was conducted at a tertiary care center in the United States. Thirty-three men (isoflavones = 17, placebo = 16) undergoing ADT for PCa completed this pilot study. Mean age in the 2 groups was 69 years and the majority of men were Caucasians. Mean duration of ADT in both groups was approximately 2 years ( $P = .70$ ). The 2 groups were well matched at baseline. After 12 weeks of intervention, there was no significant difference in either metabolic or inflammatory parameters between the 2 groups. We found that high-dose isoflavones over a course of 12 weeks do not improve metabolic or inflammatory parameters in androgen-deprived men

Kwan W, Duncan G, Van PC, Liu M, Lim J. A phase II trial of a soy beverage for subjects without clinical disease with rising prostate-specific antigen after radical radiation for prostate cancer. *Nutr Cancer* 2010;62(2):198-207.

Abstract: Our objective was to evaluate the tolerability and effect of a daily soy beverage in prostate cancer patients with biochemical failure after radiotherapy. Patients with rising prostate-specific antigen (PSA) after radical radiation for prostate cancer were instructed to consume 500 ml of soy beverage daily for 6 mo. Tolerability of the soy beverage and compliance were assessed. PSA doubling times before and after the consumption of soy were compared. Thirty-four subjects were enrolled; 5 withdrew before 1 mo of soy for reasons unrelated to soy consumption. All remaining 29 subjects were included in the analysis. Mean consumption of the assigned soy beverage was 93%. Mild gastrointestinal upset (38%) not affecting soy consumption was the commonest side effect. PSA showed a declining trend in 4 patients (13.8%), and there was a  $> 100\%$  prolongation of PSA doubling time in 8 patients (27.6%). However, PSA doubling time also showed a 50% or more shortening in 5 patients (17.2%). In our cohort of North American subjects, 6 mo of a daily soy beverage was well tolerated and was associated with a declining trend or more than 2 times prolongation of PSA doubling time in 41% of subjects. Confirmatory studies are warranted

Hussain M, Banerjee M, Sarkar FH et al. Soy isoflavones in the treatment of prostate cancer. *Nutr Cancer* 2003;47(2):111-7.

Abstract: Epidemiological studies suggest an inverse association between soy intake and prostate cancer (Pca) risk. We have previously observed that soy isoflavone genistein induces apoptosis and inhibits growth of both androgen-sensitive and androgen-independent Pca cells in vitro. To determine the clinical effects of soy isoflavones on Pca we conducted a pilot study in patients with Pca who had rising serum prostate-specific antigen (PSA) levels. Patients with Pca were enrolled in the study if they had either newly diagnosed and untreated disease under watchful waiting with rising PSA (group I) or had increasing serum PSA following local therapy (group II) or while receiving hormone therapy (group III). The study intervention consisted of 100 mg of soy isoflavone (Novasoy) taken by mouth twice daily for a minimum of 3 or maximum of 6 mo. Forty-one patients were enrolled (4 in group I, 18 in group II, and 19 in group III) and had a median PSA level of 13.3 ng/ml. Thirty-nine patients could be assessed for response. Soy isoflavone supplementation was given for a median of 5.5 (range 0.8-6) mo per patient. Although there were no sustained decreases in PSA qualifying for a complete or partial response, stabilization of the PSA occurred in 83% of patients in hormone-sensitive (group II) and 35% of hormone-refractory (group III) patients. There was a decrease in the rate of the rise of serum PSA in the whole group ( $P = 0.01$ ) with rates of rise decreasing from 14 to 6% in group II ( $P = 0.21$ ) and from 31 to 9% in group III ( $P = 0.05$ ) following the soy isoflavone intervention. Serum genistein and daidzein levels increased during supplementation from 0.11 to 0.65 microM ( $P = 0.00002$ ) and from 0.11 to 0.51 microM ( $P = 0.00001$ ), respectively. No significant changes were observed in serum levels of testosterone, IGF-1, IGFBP-3, or 5-OHmdU. These data suggest that soy isoflavones may benefit some patients with Pca

## Diindolylmethane

Beaver LM, Yu TW, Sokolowski EI, Williams DE, Dashwood RH, Ho E. 3,3'-Diindolylmethane, but not indole-3-carbinol, inhibits histone deacetylase activity in prostate cancer cells. *Toxicol Appl Pharmacol* 2012 September 15;263(3):345-51.

Abstract: Increased consumption of cruciferous vegetables is associated with a reduced risk of developing prostate cancer. Indole-3-carbinol (I3C) and 3,3'-diindolylmethane (DIM) are phytochemicals derived from cruciferous vegetables that have shown promise in inhibiting prostate cancer in experimental models. Histone deacetylase (HDAC) inhibition is an emerging target for cancer prevention and therapy. We sought to examine the effects of I3C and DIM on HDACs in human prostate cancer cell lines: androgen insensitive PC-3 cells and androgen sensitive LNCaP cells. I3C modestly inhibited HDAC activity in LNCaP cells by 25% but no inhibition of HDAC activity was detected in PC-3 cells. In contrast, DIM significantly inhibited HDAC activity in both cell lines by as much as 66%. Decreases in HDAC activity correlated with increased expression of p21, a known target of HDAC inhibitors. DIM treatment caused a significant decrease in the expression of HDAC2 protein in both cancer cell lines but no significant change in the protein levels of HDAC1, HDAC3, HDAC4, HDAC6 or HDAC8 was detected. Taken together, these results show that inhibition of HDAC activity by DIM may contribute to the phytochemicals' anti-proliferative effects in the prostate. The ability of DIM to target aberrant epigenetic patterns, in addition to its effects on detoxification of carcinogens, may make it an effective chemopreventive agent by targeting multiple stages of prostate carcinogenesis

Kong D, Heath E, Chen W et al. Loss of let-7 up-regulates EZH2 in prostate cancer consistent with the acquisition of cancer stem cell signatures that are attenuated by BR-DIM. *PLoS ONE* 2012;7(3):e33729.

Abstract: The emergence of castrate-resistant prostate cancer (CRPC) contributes to the high mortality of patients diagnosed with prostate cancer (PCa), which in part could be attributed to the existence and the emergence of cancer stem cells (CSCs). Recent studies have shown that deregulated expression of microRNAs (miRNAs) contributes to the initiation and progression of PCa. Among several known miRNAs, let-7 family appears to play a key role in the recurrence and progression of PCa by regulating CSCs; however, the mechanism by which let-7 family contributes to PCa aggressiveness is unclear. Enhancer of Zeste homolog 2 (EZH2), a putative target of let-7 family, was demonstrated to control stem cell function. In this study, we found loss of let-7 family with corresponding over-expression of EZH2 in human PCa tissue specimens, especially in higher Gleason grade tumors. Overexpression of let-7 by transfection of let-7 precursors decreased EZH2 expression and repressed clonogenic ability and sphere-forming capacity of PCa cells, which was consistent with inhibition of EZH2 3'UTR luciferase activity. We also found that the treatment of PCa cells with BR-DIM (formulated DIM: 3,3'-diindolylmethane by Bio Response, Boulder, CO, abbreviated as BR-DIM) up-regulated let-7 and down-regulated EZH2 expression, consistent with inhibition of self-renewal and clonogenic capacity. Moreover, BR-DIM intervention in our on-going phase II clinical trial in patients prior to radical prostatectomy showed upregulation of let-7 consistent with down-regulation of EZH2 expression in PCa tissue specimens after BR-DIM intervention. These results suggest that the loss of let-7 mediated increased expression of EZH2 contributes to PCa aggressiveness, which could be attenuated by BR-DIM treatment, and thus BR-DIM is likely to have clinical impact

Singh-Gupta V, Banerjee S, Yunker CK et al. B-DIM impairs radiation-induced survival pathways independently of androgen receptor expression and augments radiation efficacy in prostate cancer. *Cancer Lett* 2012 May 1;318(1):86-92.

Abstract: Increased consumption of cruciferous vegetables is associated with decreased risk in prostate cancer (PCa). The active compound in cruciferous vegetables appears to be the self dimerized product [3,3'-diindolylmethane (DIM)] of indole-3-carbinol (I3C). Nutritional grade B-DIM (absorption-enhanced) has proven safe in a Phase I trial in PCa. We investigated the anti-cancer activity of B-DIM as a new biological approach to improve the effects of radiotherapy for hormone refractory prostate cancer cells, which were either positive or negative for androgen receptor (AR) expression. B-DIM inhibited cell growth in a dose-dependent manner in both PC-3 (AR-) and C4-2B (AR+) cell lines. B-DIM was effective at increasing radiation-induced cell killing in both cell lines, independently of AR expression. B-DIM inhibited NF-kappaB and HIF-1alpha DNA activities and blocked radiation-induced activation of these transcription factors in both PC-3 and C4-2B cells. In C4-2B (AR+) cells, AR expression and nuclear localization were significantly increased by radiation. However, B-DIM abrogated the radiation-induced AR increased expression and trafficking to the nucleus, which was consistent with decreased PSA secretion. In vivo, treatment of PC-3 prostate tumors in nude mice with B-DIM and radiation resulted in significant primary tumor growth inhibition and control of metastasis to para-aortic lymph nodes. These studies demonstrate that B-DIM augments radiation-induced cell killing and tumor growth inhibition. B-DIM impairs critical survival signaling pathways activated by radiation, leading to enhanced cell killing. These novel observations suggest that B-DIM could be used as a safe compound to enhance the efficacy of radiotherapy for castrate-resistant PCa

Wang TT, Schoene NW, Milner JA, Kim YS. Broccoli-derived phytochemicals indole-3-carbinol and 3,3'-diindolylmethane exerts concentration-dependent pleiotropic effects on prostate cancer cells: comparison with other cancer preventive phytochemicals. *Mol Carcinog* 2012 March;51(3):244-56.

Abstract: In the present studies, we utilized prostate cancer cell culture models to elucidate the mechanisms of action of broccoli-derived phytochemicals 3,3'-diindolylmethane (DIM) and indole-3-carbinol (I3C). We found DIM and I3C at 1-5 microM inhibited androgen and estrogen-mediated pathways and induced xenobiotic metabolism pathway. By contrast, DIM and I3C induced cyclin inhibitors, indicators of stress/DNA damage, only at  $\geq 25$  microM. We also demonstrated that an inhibitory effect of DIM and I3C on cell growth involves inhibition of insulin-like growth factor-1 receptor expression. More importantly, we showed that differences in efficacies and mechanisms existed between DIM and I3C. These included differences in effective concentrations, a differential effect on androgen receptor binding, and a differential effect on xenobiotic metabolic pathway through aryl hydrocarbon receptor-dependent and -independent mechanism. Furthermore we determined that several other diet-derived cancer protective compounds, similar to DIM and I3C, exhibited pleiotropic effects on signaling pathways that included proliferation, cell cycle, and nuclear receptors-mediated pathways. However, the efficacies and mechanisms of these compounds vary. We also showed that some cellular pathways are not likely to be affected by DIM or I3C when circulating concentration of orally ingested DIM or I3C is considered. Based on our results, a model for cancer protective effects of DIM and I3C was proposed

Heath EI, Heilbrun LK, Li J et al. A phase I dose-escalation study of oral BR-DIM (BioResponse 3,3'-Diindolylmethane) in castrate-resistant, non-metastatic prostate cancer. *Am J Transl Res* 2010;2(4):402-11.

Abstract: 3, 3'-diindolylmethane (DIM) modulates estrogen metabolism and acts as an anti-androgen which down-regulates the androgen receptor and prostate specific antigen (PSA). We conducted a

dose-escalation, phase I study of BioResponse (BR)-DIM with objectives to determine the maximum tolerated dose (MTD), toxicity profile, and pharmacokinetics (PK) of BR-DIM, and to assess its effects on serum PSA and quality of life (QoL). PATIENTS AND METHODS: Cohorts of 3-6 patients received escalating doses of twice daily oral BR-DIM providing DIM at 75 mg, then 150 mg, 225 mg, and 300 mg. Toxicity was evaluated monthly. Serum PSA and QoL were measured at baseline, monthly during treatment, and at end of study. RESULTS: 12 patients with castrate-resistant, non-metastatic, PSA relapse prostate cancer were treated over 4 dose cohorts; 2 patients (at 150 mg and 225 mg, respectively) underwent intra-patient dose escalation, by one dose level. After oral administration of the first dose of BR-DIM, the plasma exposure to DIM appeared dose proportional at doses ranging from 75 to 300 mg, with the mean C(max) and mean AUC(last) increasing from 41.6 to 236.4 ng/ml and from 192.0 to 899.0 ng/ml\*h, respectively. Continued relatively stable systemic exposure to DIM was achieved following twice daily oral administration of BR-DIM. Minimal toxicity was observed. Two of the four patients treated at 300 mg had grade 3 asymptomatic hyponatremia (AH) discovered on routine blood work. The other 2 patients at this dose had no AH. Therefore, the maximum tolerated dose (MTD) was deemed to be 300 mg and the recommended phase II dose (RP2D) of BR-DIM was 225 mg twice daily. One patient without AH at 225 mg experienced a 50% PSA decline. One patient with BR-DIM dose of 225 mg had PSA stabilization. The other 10 patients had an initial deceleration of their PSA rise (decrease in slope), but eventually progressed based on continual PSA rise or evidence of metastatic disease. Ten patients completed monthly QoL reports for a mean of 6 months (range: 1-13). QoL measures emotional functioning may have held up somewhat better over time than their physical functioning. CONCLUSION: BR-DIM was well tolerated. Increasing systemic exposure to DIM was achieved with the increase of BR-DIM dose. Modest efficacy was demonstrated. Patients' QoL varied over time with length of treatment. Phase II studies are recommended at the dose of 225 mg orally twice daily

Rahman KM, Banerjee S, Ali S et al. 3,3'-Diindolylmethane enhances taxotere-induced apoptosis in hormone-refractory prostate cancer cells through survivin down-regulation. *Cancer Res* 2009 May 15;69(10):4468-75.

Abstract: Survivin, a member of inhibitor of apoptosis family, is associated with both prostate cancer progression and drug resistance. Therefore, we hypothesized that survivin may play a potentially important role in hormone-refractory prostate cancer (HRPC) and bone metastatic disease; thus, targeting of survivin signaling could enhance therapeutic efficacy in prostate cancer. 3,3'-Diindolylmethane (DIM) has been known to have cancer chemoprevention activity. However, no information is available regarding the down-regulation of survivin by DIM, which could result in the chemosensitization of HRPC cells to Taxotere-induced killing. We investigated the effect of DIM alone or in combination with Taxotere using LNCaP and C4-2B prostate cancer cells. We observed that DIM enhanced Taxotere-induced apoptotic death in both cell lines. These enhancing effects were related to a decrease in survivin expression as well as androgen receptor and nuclear factor-kappaB (NF-kappaB) DNA-binding activity. We also found that knockdown of survivin expression by small interfering RNA transfection increased DIM-induced cell growth inhibition and apoptosis, whereas overexpression of survivin by cDNA transfection abrogated DIM-induced cell growth inhibition and apoptosis in both prostate cancer cells. Importantly, luciferase assays showed a significant reduction of survivin-Luc and NF-kappaB-Luc activity in prostate cancer cells exposed to DIM and Taxotere. Furthermore, combination treatment significantly inhibited C4-2B bone tumor growth, and the results were correlated with the down-regulation of survivin. From these results, we conclude that inactivation of survivin by DIM enhanced the therapeutic efficacy of Taxotere in prostate cancer in general, which could be useful for the treatment of HRPC and metastatic prostate cancer

Vivar OI, Lin CL, Firestone GL, Bjeldanes LF. 3,3'-Diindolylmethane induces a G(1) arrest in human prostate cancer cells irrespective of androgen receptor and p53 status. *Biochem Pharmacol* 2009 September 1;78(5):469-76.

Abstract: 3,3'-Diindolylmethane (DIM) is a potential chemopreventive phytochemical derived from Brassica vegetables. In this study we characterized the effect of DIM on cell cycle regulation in both androgen-dependent LNCaP and androgen receptor negative p53 mutant DU145 human prostate cancer cells. DIM had an anti-proliferative effect on both LNCaP and DU145 cells, as it significantly inhibited [3H]-thymidine incorporation. FACS analysis revealed a DIM-mediated G(1) cell cycle arrest. DIM strongly inhibited the expression of cdk2 and cdk4 protein and increased the expression of the cell cycle inhibitor p27(Kip1) protein in LNCaP and DU145 cells. Promoter deletion studies with p27(Kip1) reporter gene constructs showed that this DIM-mediated increase in p27(Kip1) was dependent on the Sp1 transcription factor. Moreover, using a dominant negative inhibitor of p38 MAPK, we showed that the induction of p27(Kip1) and subsequent G(1) arrest by DIM involve activation of the p38 MAPK pathway in the DU145 cells. Taken together, our results indicate that DIM is able to stop the cell cycle progression of human prostate cancer cells regardless of their androgen-dependence and p53 status, by differentially modulating cell cycle regulatory pathways. The Sp1 and p38 MAPK pathways mediate the DIM cell cycle regulatory effect in DU145 cells

Chinnakannu K, Chen D, Li Y et al. Cell cycle-dependent effects of 3,3'-diindolylmethane on proliferation and apoptosis of prostate cancer cells. *J Cell Physiol* 2009 April;219(1):94-9.

Abstract: Epidemiological studies have shown that a diet rich in fruits and cruciferous vegetables is associated with a lower risk of prostate cancer. Indole-3-carbinol (I3C) and its dimeric product 3,3'-diindolylmethane (DIM) have been shown to exhibit anti-tumor activity both in vitro and in vivo. Recently, we have reported that a formulated DIM (B-DIM) induced apoptosis and inhibited growth, angiogenesis, and invasion of prostate cancer cells by regulating Akt, NF-kappaB, VEGF and the androgen receptor (AR) signaling pathway. However, the precise molecular mechanism(s) by which B-DIM inhibits prostate cancer cell growth and induces apoptosis have not been fully elucidated. Most importantly, it is not known how B-DIM affects cell cycle regulators and proteasome activity, which are critically involved in cell growth and apoptosis. In this study, we investigated the effects of B-DIM on proteasome activity and AR transactivation with respect to B-DIM-mediated cell cycle regulation and induction of apoptosis in both androgen-sensitive LNCaP and androgen-insensitive C4-2B prostate cancer cells. We believe that our results show for the first time the cell cycle-dependent effects of B-DIM on proliferation and apoptosis of synchronized prostate cancer cells progressing from G(1) to S phase. B-DIM inhibited this progression by induction of p27(Kip1) and down-regulation of AR. We also show for the first time that B-DIM inhibits proteasome activity in S phase, leading to the inactivation of NF-kappaB signaling and induction of apoptosis in LNCaP and C4-2B cells. These results suggest that B-DIM could be a potent agent for the prevention and/or treatment of both hormone sensitive as well as hormone-refractory prostate cancer

Ahmad A, Kong D, Sarkar SH, Wang Z, Banerjee S, Sarkar FH. Inactivation of uPA and its receptor uPAR by 3,3'-diindolylmethane (DIM) leads to the inhibition of prostate cancer cell growth and migration. *J Cell Biochem* 2009 June 1;107(3):516-27.

Abstract: 3,3'-Diindolylmethane (DIM) has been studied for its putative anti-cancer properties, especially against prostate cancer; however, its exact mechanism of action remains unclear. We recently provided preliminary data suggesting down-regulation of uPA during B-DIM (a clinically active DIM)-induced inhibition of invasion and angiogenesis in prostate cancer cells. Since the expression and activation of uPA plays important role in tumorigenicity, and high endogenous levels of uPA and uPAR are found in advanced metastatic cancers, we investigated their role in B-DIM-

mediated inhibition of prostate cancer cell growth and motility. Using PC3 cells, we found that B-DIM treatment as well as the silencing of uPA and uPAR by siRNAs led to the inhibition of cell growth and motility. Conversely, over-expression of uPA/uPAR in LNCaP and C4-2B cells resulted in increased cell growth and motility, which was effectively inhibited by B-DIM. Moreover, we found that uPA as well as uPAR induced the production of VEGF and MMP-9, and that the down-regulation of uPA/uPAR by siRNAs or B-DIM treatment resulted in the inhibition of VEGF and MMP-9 secretion which could be responsible for the observed inhibition of cell migration. Interestingly, silencing of uPA/uPAR led to decreased sensitivity to B-DIM indicating important role of uPA/uPAR in B-DIM-mediated regulation of prostate cancer cell growth and migration. Our data suggest that chemopreventive and/or therapeutic activity of B-DIM is in part due to down-regulation of uPA-uPAR leading to reduced production of VEGF/MMP-9 which ultimately leads to the inhibition of cell growth and migration of aggressive prostate cancer cells

Zhao YY, Zhou L, Pan YZ et al. [3,3-diindolylmethane enhances the inhibitory effect of idarubicin on the growth of human prostate cancer cells]. *Zhonghua Yi Xue Za Zhi* 2008 March 11;88(10):661-4.

Abstract: OBJECTIVE: To study the effects of idarubicin (IDA) combined with 3, 3'-diindolylmethane (DIM) on the growth inhibition of human prostate cancer cells. METHODS: Human prostate cancer cells of the line PC-3M were cultured and then divided into the following groups: control group with solvent added into the culture fluid; IDA groups, with IDA of the terminal concentrations of 0.5, 1 or 5 mg/L added into the culture fluid; DIM groups, with DIM of the terminal concentrations of 30, 60 or 100 micromol/L added into the culture fluid; and DIM + IDA groups, with 0.5 mg/L IDA and DIM 30, 60 or 100 micromol/L added into the culture fluid. 48 h later the cell growth inhibition rate was detected by MTT assay. Flow cytometry and acridine orange staining were used to detect the cell cycle and apoptosis. RT-PCR and Western blotting were used to detect the mRNA and protein expression of caspase 9, an apoptosis gene. RESULTS: Both IDA and DIM dose-dependently inhibited the growth of the PC-3M cells. The growth inhibition rate of the 60 micromol/L DIM + 0.5 mg/L IDA group was 69.9%, almost 10 times as that of the 0.5 mg/L IDA group. The apoptosis rate of the 60 micromol/L DIM + 0.5 mg/L IDA group was 47.0%, significantly higher than that of the 0.5 mg/L IDA group (3.2%,  $P < 0.05$ ). RT-PCR and Western blotting showed that the combination of DIM and IDA significantly enhanced the mRNA and protein expression of caspase 9. CONCLUSION: DIM enhances the growth inhibition effect of IDA on human prostate cancer cells by the mechanism of induction of apoptosis

Kong D, Banerjee S, Huang W et al. Mammalian target of rapamycin repression by 3,3'-diindolylmethane inhibits invasion and angiogenesis in platelet-derived growth factor-D-overexpressing PC3 cells. *Cancer Res* 2008 March 15;68(6):1927-34.

Abstract: Platelet-derived growth factor-D (PDGF-D) is a newly recognized growth factor known to regulate many cellular processes, including cell proliferation, transformation, invasion, and angiogenesis. Recent studies have shown that PDGF-D and its cognate receptor PDGFR-beta are expressed in prostate tumor tissues, suggesting that PDGF-D might play an important role in the development and progression of prostate cancer. However, the biological role of PDGF-D in tumorigenesis remains elusive. In this study, we found that PDGF-D-overexpressing PC3 cells (PC3 cells stably transfected with PDGF-D cDNA and referred to as PC3 PDGF-D) exhibited a rapid growth rate and enhanced cell invasion that was associated with the activation of mammalian target of rapamycin (mTOR) and reduced Akt activity. Rapamycin repressed mTOR activity and concomitantly resulted in the activation of Akt, which could attenuate the therapeutic effects of mTOR inhibitors. In contrast, B-DIM (BR-DIM from Bioresponse, Inc.; a chemopreventive agent) significantly inhibited both mTOR and Akt in PC3 PDGF-D cells, which were correlated with decreased cell proliferation and invasion. Moreover, conditioned medium from PC3 PDGF-D cells

significantly increased the tube formation of human umbilical vein endothelial cells, which was inhibited by B-DIM treatment concomitant with reduced full-length and active form of PDGF-D. Our results suggest that B-DIM could serve as a novel and efficient chemopreventive and/or therapeutic agent by inactivation of both mTOR and Akt activity in PDGF-D-overexpressing prostate cancer

Li Y, Wang Z, Kong D et al. Regulation of FOXO3a/beta-catenin/GSK-3beta signaling by 3,3'-diindolylmethane contributes to inhibition of cell proliferation and induction of apoptosis in prostate cancer cells. *J Biol Chem* 2007 July 20;282(29):21542-50.

Abstract: Previous studies from our laboratory have shown anti-proliferative and pro-apoptotic effects of 3,3'-diindolylmethane (DIM) through regulation of Akt and androgen receptor (AR) in prostate cancer cells. However, the mechanism by which DIM regulates Akt and AR signaling pathways has not been fully investigated. It has been known that FOXO3a and glycogen synthase kinase-3beta (GSK-3beta), two targets of activated Akt, interact with beta-catenin, regulating cell proliferation and apoptotic cell death. More importantly, FOXO3a, GSK-3beta, and beta-catenin are all AR coregulators and regulate the activity of AR, mediating the development and progression of prostate cancers. Here, we investigated the molecular effects of B-DIM, a formulated DIM with higher bioavailability, on Akt/FOXO3a/GSK-3beta/beta-catenin/AR signaling in hormone-sensitive LNCaP and hormone-insensitive C4-2B prostate cancer cells. We found that B-DIM significantly inhibited the phosphorylation of Akt and FOXO3a and increased the phosphorylation of beta-catenin, leading to the inhibition of cell growth and induction of apoptosis. We also found that B-DIM significantly inhibited beta-catenin nuclear translocation. By electrophoretic mobility shift and chromatin immunoprecipitation assays, we found that B-DIM inhibited FOXO3a binding to the promoter of AR and promoted FOXO3a binding to the p27(KIP1) promoter, resulting in the alteration of AR and p27(KIP1) expression, the inhibition of cell proliferation, and the induction of apoptosis in both androgen-sensitive and -insensitive prostate cancer cells. These results suggest that B-DIM-induced cell growth inhibition and apoptosis induction are partly mediated through the regulation of Akt/FOXO3a/GSK-3beta/beta-catenin/AR signaling. Therefore, B-DIM could be a promising non-toxic agent for possible treatment of hormone-sensitive but most importantly hormone-refractory prostate cancers

Kong D, Li Y, Wang Z, Banerjee S, Sarkar FH. Inhibition of angiogenesis and invasion by 3,3'-diindolylmethane is mediated by the nuclear factor-kappaB downstream target genes MMP-9 and uPA that regulated bioavailability of vascular endothelial growth factor in prostate cancer. *Cancer Res* 2007 April 1;67(7):3310-9.

Abstract: Progression of prostate cancer is believed to be dependent on angiogenesis induced by tumor cells. 3,3'-Diindolylmethane (DIM) has been shown to repress neovascularization in a Matrigel plug assay and inhibit cell proliferation, migration, invasion, and capillary tube formation of cultured human umbilical vein endothelial cells. However, the molecular mechanism, by which DIM inhibits angiogenesis and invasion, has not been fully elucidated. Therefore, we sought to explore the molecular mechanism by which DIM inhibits angiogenesis and invasion, specifically by investigating the role of angiogenic factors secreted by prostate cancer cells which control all steps of angiogenesis. We found that BioResponse DIM (B-DIM), a formulated DIM with higher bioavailability, inhibited angiogenesis and invasion by reducing the bioavailability of vascular endothelial growth factor (VEGF) via repressing extracellular matrix-degrading proteases, such as matrix metalloproteinase (MMP)-9 and urokinase-type plasminogen activator (uPA), in human prostate cancer cells and reduced vascularity (angiogenesis) in vivo using Matrigel plug assay. We also found that B-DIM treatment inhibited DNA binding activity of nuclear factor-kappaB (NF-kappaB), which is known to mediate the expression of many NF-kappaB downstream target genes, including VEGF, IL-8, uPA, and MMP-9, all of which are involved in angiogenesis, invasion, and metastasis. Our data suggest

that inhibition of NF-kappaB DNA binding activity by B-DIM contributes to the regulated bioavailability of VEGF by MMP-9 and uPA and, in turn, inhibits invasion and angiogenesis, which could be mechanistically linked with the antitumor activity of B-DIM as observed previously by our laboratory in a prostate cancer animal model

Bhuiyan MM, Li Y, Banerjee S et al. Down-regulation of androgen receptor by 3,3'-diindolylmethane contributes to inhibition of cell proliferation and induction of apoptosis in both hormone-sensitive LNCaP and insensitive C4-2B prostate cancer cells. *Cancer Res* 2006 October 15;66(20):10064-72.

Abstract: Despite the initial efficacy of androgen deprivation therapy, most patients with advanced prostate cancer eventually progress to hormone-refractory prostate cancer, for which there is no curative therapy. Previous studies from our laboratory and others have shown the antiproliferative and proapoptotic effects of 3,3'-diindolylmethane (DIM) in prostate cancer cells. However, the molecular mechanism of action of DIM has not been investigated in androgen receptor (AR)-positive hormone-responsive and -nonresponsive prostate cancer cells. Therefore, we investigated the effects of B-DIM, a formulated DIM with greater bioavailability, on AR, Akt, and nuclear factor kappaB (NF-kappaB) signaling in hormone-sensitive LNCaP (AR+) and hormone-insensitive C4-2B (AR-) prostate cancer cells. We found that B-DIM significantly inhibited cell proliferation and induced apoptosis in both cell lines. By Akt gene transfection, reverse transcription-PCR, Western blot analysis, and electrophoretic mobility shift assay, we found a potential crosstalk between Akt, NF-kappaB, and AR. Importantly, B-DIM significantly inhibited Akt activation, NF-kappaB DNA binding activity, AR phosphorylation, and the expressions of AR and prostate-specific antigen, suggesting that B-DIM could interrupt the crosstalk. Confocal studies revealed that B-DIM inhibited AR nuclear translocation, leading to the down-regulation of AR target genes. Moreover, B-DIM significantly inhibited C4-2B cell growth in a severe combined immunodeficiency-human model of experimental prostate cancer bone metastasis. These results suggest that B-DIM-induced cell proliferation inhibition and apoptosis induction are partly mediated through the down-regulation of AR, Akt, and NF-kappaB signaling. These observations provide a rationale for devising novel therapeutic approaches for the treatment of hormone-sensitive, but more importantly, hormone-refractory prostate cancer by using B-DIM alone or in combination with other therapeutics

Garikapaty VP, Ashok BT, Tadi K, Mittelman A, Tiwari RK. 3,3'-Diindolylmethane downregulates pro-survival pathway in hormone independent prostate cancer. *Biochem Biophys Res Commun* 2006 February 10;340(2):718-25.

Abstract: Epidemiological evidences suggest that the progression and promotion of prostate cancer (CaP) can be modulated by diet. Since all men die with prostate cancer rather than of the disease, it is of particular interest to prevent or delay the progression of the disease by chemopreventive strategies. We have been studying the anticancer properties of compounds present in cruciferous vegetables such as indole-3-carbinol (I3C). Diindolylmethane (DIM) is a dimer of I3C that is formed under acidic conditions and unlike I3C is more stable with higher anti-cancer effects. In the present report, we demonstrate that DIM is a potent anti-proliferative agent compared to I3C in the hormone independent DU 145 CaP cells. The anti-prostate cancer effect is mediated by the inhibition of the Akt signal transduction pathway as DIM, in sharp contrast to I3C, induces the downregulation of Akt, p-Akt, and PI3 kinase. DIM also induced a G1 arrest in DU 145 cells by flow cytometry and downstream concurrent inhibition of cell cycle parameters such as cyclin D1, cdk4, and cdk6. Our data suggest a need for further development of DIM, as a chemopreventive agent for CaP, which justifies epidemiological evidences and molecular targets that are determinants for CaP dissemination/progression. The ingestion of DIM may benefit CaP patients and reduce disease recurrence by eliminating micro-metastases that may be present in patients who undergo radical prostatectomy

Hsu JC, Zhang J, Dev A, Wing A, Bjeldanes LF, Firestone GL. Indole-3-carbinol inhibition of androgen receptor expression and downregulation of androgen responsiveness in human prostate cancer cells. *Carcinogenesis* 2005 November;26(11):1896-904.

Abstract: Indole-3-carbinol (I3C), a naturally occurring compound found in vegetables of the Brassica genus, such as broccoli and cabbage, is a promising anticancer agent previously shown to induce a G(1) cell-cycle arrest in the cells of human lymph node carcinoma of prostate (LNCaP) through regulation of specific G(1)-acting cell-cycle components. Since the androgen receptor (AR) mediates proliferation and differentiation in the prostate and is expressed in nearly all human prostate cancers, the effects of I3C on AR expression and function were examined in LNCaP cells. Immunoblot and quantitative RT-PCR assays revealed that I3C inhibited the expression of AR protein and mRNA levels within 12 h of indole treatment. I3C downregulated the reporter activity of LNCaP cells transiently transfected with an AR promoter-luciferase plasmid, demonstrating that a unique response to I3C is the inhibition of AR promoter activity. In contrast to I3C, the natural I3C dimerization product 3,3'-diindolylmethane, which acts as an androgen antagonist, had no effect on AR expression. To determine the functional significance of the I3C-inhibited expression of AR, the AR-regulated prostate specific antigen (PSA) was utilized as a downstream indicator. I3C downregulated the expression of PSA transcripts and protein levels and inhibited PSA promoter activity, as well as that of a minimal androgen responsive element containing reporter plasmid. Expression of exogenous AR prevented the I3C disruption of androgen-induced PSA expression. Taken together, our results demonstrate that I3C represses AR expression and responsiveness in LNCaP cells as a part of its antiproliferative mechanism

Nachshon-Kedmi M, Fares FA, Yannai S. Therapeutic activity of 3,3'-diindolylmethane on prostate cancer in an in vivo model. *Prostate* 2004 October 1;61(2):153-60.

Abstract: BACKGROUND: Prostate cancer (PC) is the second leading cancer-related death in men in Western countries. Hence, efficient anti-carcinogenic and therapeutic compounds against PC are badly needed. We have previously shown that 3,3'-diindolylmethane (DIM) has a suppressive effect on the growth of human breast and PC cell lines. The objective of this study was examination of the potential therapeutic effects of DIM in an in vivo model. METHODS: TRAMP-C2, a mouse PC cell line, was injected into the flank of male C57BL/6 mice. When tumors appeared, mice were injected intraperitoneally with either corn oil (vehicle) or DIM (2.5, 5, or 10 mg per kg body weight) 3-times a week, for 3 weeks, and tumor volumes were measured bi-weekly with calibermeters. Later, the tumors were removed, their final weights and volumes were measured, and tumor sections were tested for histological studies. RESULTS: DIM had a significant inhibitory effect, caused by diminished tumor growth. Histological examination of tumors from treated groups revealed apoptosis and decreased cell proliferation, compared with the controls. DIM didn't affect body weights or kidney and liver functioning. CONCLUSIONS: The inhibitory action of DIM on tumor growth was demonstrated in vivo. Hence, this compound at the concentrations tested may offer an effective and non toxic therapeutic means against tumor growth in rodents, and may serve as a potential natural anti-carcinogenic compound in humans

Nachshon-Kedmi M, Yannai S, Haj A, Fares FA. Indole-3-carbinol and 3,3'-diindolylmethane induce apoptosis in human prostate cancer cells. *Food Chem Toxicol* 2003 June;41(6):745-52.

Abstract: Cruciferous vegetables contain glucobrassicin which, during metabolism, yields indole-3-carbinol (I3C). In a low pH environment I3C is converted into polymeric products, among which 3,3'-diindolylmethane (DIM) is the main one. The apoptotic effects of I3C and DIM were exhibited in human breast cancer cells. The objectives of this study were: (a) examination of the potential effects of I3C and DIM on the proliferation and induction of apoptosis in human prostate cancer cell lines

with different p53 status; (b) to try to characterise the mechanism(s) involved in these effects. Our results indicate that both indole derivatives suppress the growth of these cells in a dose- and time-dependent manner, by inducing apoptosis. It appears that these indolic compounds may offer effective means against prostate cancer. Induction of apoptosis was p53-independent. Moreover, the indole derivatives employed did not affect the levels of bcl-2, bax and fasL

Le HT, Schaldach CM, Firestone GL, Bjeldanes LF. Plant-derived 3,3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells. *J Biol Chem* 2003 June 6;278(23):21136-45.

Abstract: 3,3'-Diindolylmethane (DIM) is a major digestive product of indole-3-carbinol, a potential anticancer component of cruciferous vegetables. Our results indicate that DIM exhibits potent antiproliferative and antiandrogenic properties in androgen-dependent human prostate cancer cells. DIM suppresses cell proliferation of LNCaP cells and inhibits dihydrotestosterone (DHT) stimulation of DNA synthesis. These activities were not produced in androgen-independent PC-3 cells. Moreover, DIM inhibited endogenous PSA transcription and reduced intracellular and secreted PSA protein levels induced by DHT in LNCaP cells. Also, DIM inhibited, in a concentration-dependent manner, the DHT-induced expression of a prostate-specific antigen promoter-regulated reporter gene construct in transiently transfected LNCaP cells. Similar effects of DIM were observed in PC-3 cells only when these cells were co-transfected with a wild-type androgen receptor expression plasmid. Using fluorescence imaging with green fluorescent protein androgen receptor and Western blot analysis, we demonstrated that DIM inhibited androgen-induced androgen receptor (AR) translocation into the nucleus. Results of receptor binding assays indicated further that DIM is a strong competitive inhibitor of DHT binding to the AR. Results of structural modeling studies showed that DIM is remarkably similar in conformational geometry and surface charge distribution to an established synthetic AR antagonist, although the atomic compositions of the two substances are quite different. Taken together with our published reports of the estrogen agonist activities of DIM, the present results establish DIM as a unique bifunctional hormone disrupter. To our knowledge, DIM is the first example of a pure androgen receptor antagonist from plants

## Tocotrienols

Campbell SE, Rudder B, Phillips RB et al. gamma-Tocotrienol induces growth arrest through a novel pathway with TGFbeta2 in prostate cancer. *Free Radic Biol Med* 2011 May 15;50(10):1344-54.

Abstract: Regions along the Mediterranean and in southern Asia have lower prostate cancer incidence compared to the rest of the world. It has been hypothesized that one of the potential contributing factors for this low incidence includes a higher intake of tocotrienols. Here we examine the potential of gamma-tocotrienol (GT3) to reduce prostate cancer proliferation and focus on elucidating pathways by which GT3 could exert a growth-inhibitory effect on prostate cancer cells. We find that the gamma and delta isoforms of tocotrienol are more effective at inhibiting the growth of prostate cancer cell lines (PC-3 and LNCaP) compared with the gamma and delta forms of tocopherol. Knockout of PPAR-gamma and GT3 treatment show inhibition of prostate cancer cell growth, through a partially PPAR-gamma-dependent mechanism. GT3 treatment increases the levels of the 15-lipoxygenase-2 enzyme, which is responsible for the conversion of arachidonic acid to the PPAR-gamma-activating ligand 15-S-hydroxyeicosatrienoic acid. In addition, the latent precursor and the mature forms of TGFbeta2 are down-regulated after treatment with GT3, with concomitant disruptions in TGFbeta receptor I, SMAD-2, p38, and NF-kappaB signaling

Barve A, Khor TO, Reuhl K, Reddy B, Newmark H, Kong AN. Mixed tocotrienols inhibit prostate carcinogenesis in TRAMP mice. *Nutr Cancer* 2010;62(6):789-94.

Abstract: The biological activities of tocotrienols are receiving increasing attention. Herein, we report the efficacy of a mixed-tocotrienol diet against prostate tumorigenesis in the transgenic adenocarcinoma mouse prostate (TRAMP) mouse model. Male TRAMP mice, 8 wk old, were fed 0.1%, 0.3%, or 1% mixed tocotrienols in AIN-76A diet up to 24 wk old. Likewise, a positive control group consisting of male TRAMP mice and a negative control group consisting of wild-type nontransgenic mice were fed regular AIN-76A diet up to 24 wk old. Our results show that mixed-tocotrienol-fed groups had a lower incidence of tumor formation along with a significant reduction in the average wet weight of genitourinary apparatus. Furthermore, mixed tocotrienols significantly reduced the levels of high-grade neoplastic lesions as compared to the positive controls. This decrease in levels of high-grade neoplastic lesions was found to be associated with increased expression of proapoptotic proteins BAD (Bcl(2) antagonist of cell death) and cleaved caspase-3 and cell cycle regulatory proteins cyclin dependent kinase inhibitors p21 and p27. In contrast, the expression of cyclins A and E were found to be decreased in mixed-tocotrienol groups. Taken together, our results show that by modulating cell cycle regulatory proteins and increasing expression of proapoptotic proteins, mixed tocotrienols suppress prostate tumorigenesis in the TRAMP mice

Luk SU, Yap WN, Chiu YT et al. Gamma-tocotrienol as an effective agent in targeting prostate cancer stem cell-like population. *Int J Cancer* 2011 May 1;128(9):2182-91.

Abstract: Emerging evidence supports that prostate cancer originates from a rare subpopulation of cells, namely prostate cancer stem cells (CSCs). Conventional therapies for prostate cancer are believed to mainly target the majority of differentiated tumor cells but spare CSCs, which may account for the subsequent disease relapse after treatment. Therefore, successful elimination of CSCs may be an effective strategy to achieve complete remission from this disease. Gamma-tocotrienols (gamma-T3) is one of the vitamin-E constituents, which have been shown to have anticancer effects against a wide range of human cancers. Recently, we have reported that gamma-T3 treatment not only inhibits prostate cancer cell invasion but also sensitizes the cells to docetaxel-induced apoptosis, suggesting that gamma-T3 may be an effective therapeutic agent against advanced stage prostate cancer. Here, we demonstrate for the first time that gamma-T3 can downregulate the expression of prostate CSC markers (CD133/CD44) in androgen-independent prostate cancer cell lines (PC-3 and DU145), as evident from Western blotting analysis. Meanwhile, the spheroid formation ability of the prostate cancer cells was significantly hampered by gamma-T3 treatment. In addition, pretreatment of PC-3 cells with gamma-T3 was found to suppress tumor initiation ability of the cells. More importantly, although CD133-enriched PC-3 cells were highly resistant to docetaxel treatment, these cells were as sensitive to gamma-T3 treatment as the CD133-depleted population. Our data suggest that gamma-T3 may be an effective agent in targeting prostate CSCs, which may account for its anticancer and chemosensitizing effects reported in previous studies

Conte C, Floridi A, Aisa C, Piroddi M, Floridi A, Galli F. Gamma-tocotrienol metabolism and antiproliferative effect in prostate cancer cells. *Ann N Y Acad Sci* 2004 December;1031:391-4.

Abstract: In this study, we evaluated the antiproliferative effect of tocotrienols (T3) and the presence of a specific vitamin E metabolism in PC3 and LNCaP prostate cancer cells. These cell lines are able to transform tocopherols (T) and T3 in the corresponding carboxyethyl-hydroxychromans metabolites (CEHCs). The extent of this metabolism and the inhibitory effect on cell growth followed the order of magnitude  $\alpha\text{-T} < \alpha\text{-T3} < \gamma\text{-T} < \gamma\text{-T3}$ . The partial inhibition of gamma-T3 metabolism by ketoconazole did not influence cell proliferation. These early findings may suggest that the transformation of vitamin E to CEHC is mostly a detoxification mechanism useful to maintain the malignant properties of prostate cancer cells

## Modified Citrus Pectin

Yan J, Katz A. PectaSol-C modified citrus pectin induces apoptosis and inhibition of proliferation in human and mouse androgen-dependent and -independent prostate cancer cells. *Integr Cancer Ther* 2010 June;9(2):197-203.

Abstract: AIM: To demonstrate the efficacy of PectaSol-C modified citrus pectin (MCP) on prostate cancer in vitro. METHOD: Cytotoxicity analysis of PectaSol-C was performed by MTT assay, as were parallel studies with the former brand version of MCP called PectaSol. Apoptosis and inhibition of cell growth were investigated by Western blotting. RESULTS: Androgen-dependent and -independent human prostate cancer cell lines (LNCaP and PC3, respectively), androgen-dependent and -independent murine prostate cancer cell lines (CASP2.1 and CASP1.1, respectively), as well as noncancerous human benign prostate hyperplasia BPH-1 cell line, were used in the study. MTT assay revealed that 1.0% PectaSol exerted cytotoxicity on LNCaP, PC3, CASP2.1, CASP1.1, and BPH-1 cells for 4-day treatment by 48.0% +/- 2.1%, 54.4% +/- 0.3%, 15.4% +/- 0.8%, 46.1% +/- 1.7%, and 27.4% +/- 1.6%, respectively; whereas 1.0% PectaSol-C showed cytotoxicity by 52.2% +/- 1.8%, 48.2% +/- 2.9%, 23.0% +/- 2.6%, 49.0% +/- 1.3%, and 26.8% +/- 2.6%, respectively. Western blotting further confirmed that both MCPs inhibit MAP kinase activation, increase the expression level of its downstream target Bim, a pro-apoptotic protein, and induce the cleavage of Caspase-3 in PC3 and CASP1.1 prostate cancer cells. CONCLUSION: PectaSol MCP and PectaSol-C MCP can inhibit cell proliferation and apoptosis in prostate cancer cell lines. Our data suggested that 1.0% PectaSol-C can be used for further chemopreventive and chemotherapeutic analysis in vivo

Jackson CL, Dreaden TM, Theobald LK et al. Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure. *Glycobiology* 2007 August;17(8):805-19.

Abstract: Treatment options for androgen-independent prostate cancer cells are limited. Therefore, it is critical to identify agents that induce death of both androgen-responsive and androgen-insensitive cells. Here we demonstrate that a product of plant cell walls, pectin, is capable of inducing apoptosis in androgen-responsive (LNCaP) and androgen-independent (LNCaP C4-2) human prostate cancer cells. Commercially available fractionated pectin powder (FPP) induced apoptosis (approximately 40-fold above non-treated cells) in both cell lines as determined by the Apoptosense assay and activation of caspase-3 and its substrate, poly(ADP-ribose) polymerase. Conversely, citrus pectin (CP) and the pH-modified CP, PectaSol, had little or no apoptotic activity. Glycosyl residue composition and linkage analyses revealed no significant differences among the pectins. Mild base treatment to remove ester linkages destroyed FPP's apoptotic activity and yielded homogalacturonan (HG) oligosaccharides. The treatment of FPP with pectinmethylesterase to remove galacturonosyl carboxymethylesters and/or with endopolygalacturonase to cleave nonmethylesterified HG caused no major reduction in apoptotic activity, implicating the requirement for a base-sensitive linkage other than the carboxymethylester. Heat treatment of CP (HTCP) led to the induction of significant levels of apoptosis comparable to FPP, suggesting a means for generating apoptotic pectic structures. These results indicate that specific structural elements within pectin are responsible for the apoptotic activity, and that this structure can be generated, or enriched for, by heat treatment of CP. These findings provide the foundation for mechanistic studies of pectin apoptotic activity and a basis for the development of pectin-based pharmaceuticals, nutraceuticals, or recommended diet changes aimed at combating prostate cancer occurrence and progression

Guess BW, Scholz MC, Strum SB, Lam RY, Johnson HJ, Jennrich RI. Modified citrus pectin (MCP) increases the prostate-specific antigen doubling time in men with prostate cancer: a phase II pilot study. *Prostate Cancer Prostatic Dis* 2003;6(4):301-4.

Abstract: This trial investigated the tolerability and effect of modified citrus pectin (Pecta-Sol) in 13 men with prostate cancer and biochemical prostate-specific antigen (PSA) failure after localized treatment, that is, radical prostatectomy, radiation, or cryosurgery. A total of 13 men were evaluated for tolerability and 10 for efficacy. Changes in the prostate-specific antigen doubling time (PSADT) of the 10 men were the primary end point in the study. We found that the PSADT increased (P-value<0.05) in seven (70%) of 10 men after taking MCP for 12 months compared to before taking MCP. This study suggests that MCP may lengthen the PSADT in men with recurrent prostate cancer

Hayashi A, Gillen AC, Lott JR. Effects of daily oral administration of quercetin chalcone and modified citrus pectin on implanted colon-25 tumor growth in Balb-c mice. *Altern Med Rev* 2000 December;5(6):546-52.

Abstract: The health benefits of fruits and vegetables have been the subject of numerous investigations over many years. Two natural substances, quercetin (a flavonoid) and citrus pectin (a polysaccharide found in the cell wall of plants) are of particular interest to cancer researchers. Two modified versions of these substances - quercetin chalcone (QC) and a pH-modified citrus pectin (MCP) - are the focus of this study. Previous research has confirmed that quercetin exhibits antitumor properties, likely due to immune stimulation, free radical scavenging, alteration of the mitotic cycle in tumor cells, gene expression modification, anti-angiogenesis activity, or apoptosis induction, or a combination of these effects. MCP has inhibited metastases in animal studies of prostate cancer and melanoma. To date, no study has demonstrated a reduction in solid tumor growth with MCP, and there is no research into the antitumor effect of QC. This study examines the effects of MCP and QC on the size and weight of colon-25 tumors implanted in balb-c mice. Fifty mice were orally administered either 1 ml distilled water (controls), low-dose QC (0.8 mg/ml), high-dose QC (1.6 mg/ml), low-dose MCP (0.8 mg/ml) or high-dose MCP (1.6 mg/ml) on a daily basis, beginning the first day of tumor palpation (usually eight days post-implantation). A significant reduction in tumor size was noted at day 20 in all groups compared to controls. The groups given low-dose QC and MCP had a 29-percent (NS) and 38-percent (p<0.02) decrease in size, respectively. The high-dose groups had an even more impressive reduction in size; 65 percent in the QC group and 70 percent in the mice given MCP (both p<0.001). This is the first evidence that MCP can reduce the growth of solid primary tumors, and the first research showing QC has antitumor activity. Additional research on these substances and their effect on human cancers is warranted

Pienta KJ, Naik H, Akhtar A et al. Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin. *J Natl Cancer Inst* 1995 March 1;87(5):348-53.

Abstract: BACKGROUND: Prostate cancer is the most common cancer diagnosed in U.S. men and remains incurable once it has metastasized. Many stages of the metastatic cascade involve cellular interactions mediated by cell surface components, such as carbohydrate-binding proteins, including galactoside-binding lectins (galectins). Modified citrus pectin (pH-modified), a soluble component of plant fiber derived from citrus fruit, has been shown to interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. PURPOSE: The aim of this study was to determine whether modified citrus pectin, a complex polysaccharide rich in galactosyl residues, could inhibit spontaneous metastasis of prostate adenocarcinoma cells in the rat. METHODS: The ability of modified citrus pectin to inhibit the adhesion of Dunning rat prostate cancer MAT-LyLu cells to rat endothelial cells was measured by <sup>51</sup>Cr-labeling. Modified citrus pectin inhibition of MAT-LyLu cell anchorage-independent growth was measured by colony formation in agarose. The presence of galectin-

3 in rat MAT-LyLu cells and human prostate carcinoma was demonstrated by immunoblotting and immunohistochemistry. One million MAT-LyLu cells were injected subcutaneously into the hind limb of male Copenhagen rats on day 0. Rats were given 0.0%, 0.01%, 0.1%, or 1.0% (wt/vol) modified citrus pectin continuously in their drinking water (from day 4 until necropsy on day 30). The number of MAT-LyLu tumor colonies in the lungs were counted. RESULTS: Compared with 15 or 16 control rats that had lung metastases on day 30, seven of 14 rats in the 0.1% and nine of 16 rats in the 1.0% modified citrus-pectin group had statistically significant (two-sided;  $P < .03$  and  $P < .001$ , respectively) reductions in lung metastases. The lungs of the 1.0% modified citrus pectin-treated rats had significantly (two-sided;  $P < .05$ ) fewer metastatic colonies than control groups (9 colonies  $\pm$  4 [mean  $\pm$  SE] in the control group compared with 1 colony  $\pm$  1 in the treated group). Modified citrus pectin had no effect on the growth of the primary tumors. In vitro, modified citrus pectin inhibited MAT-LyLu cell adhesion to rat endothelial cells in a time- and dose-dependent manner as well as their colony formation in semisolid medium. CONCLUSIONS: We present a novel therapy in which oral intake of modified citrus pectin acts as a potent inhibitor of spontaneous prostate carcinoma metastasis in the Copenhagen rat. IMPLICATIONS: Further investigations are warranted to determine the following: 1) the role of galectin-3 in normal and cancerous prostate tissues and 2) the ability of modified citrus pectin to inhibit human prostate metastasis in nude mice

### **Macrophage Activating Factor - GcMAF**

Gregory KJ, Zhao B, Bielenberg DR et al. Vitamin D binding protein-macrophage activating factor directly inhibits proliferation, migration, and uPAR expression of prostate cancer cells. *PLoS ONE* 2010;5(10):e13428.

Abstract: BACKGROUND: Vitamin D binding protein-macrophage activating factor (DBP-maf) is a potent inhibitor of tumor growth. Its activity, however, has been attributed to indirect mechanisms such as boosting the immune response by activating macrophages and inhibiting the blood vessel growth necessary for the growth of tumors. METHODS AND FINDINGS: In this study we show for the first time that DBP-maf exhibits a direct and potent effect on prostate tumor cells in the absence of macrophages. DBP-maf demonstrated inhibitory activity in proliferation studies of both LNCaP and PC3 prostate cancer cell lines as well as metastatic clones of these cells. Flow cytometry studies with annexin V and propidium iodide showed that this inhibitory activity is not due to apoptosis or cell death. DBP-maf also had the ability to inhibit migration of prostate cancer cells in vitro. Finally, DBP-maf was shown to cause a reduction in urokinase plasminogen activator receptor (uPAR) expression in prostate tumor cells. There is evidence that activation of this receptor correlates with tumor metastasis. CONCLUSIONS: These studies show strong inhibitory activity of DBP-maf on prostate tumor cells independent of its macrophage activation

Yamamoto N, Suyama H, Yamamoto N. Immunotherapy for Prostate Cancer with Gc Protein-Derived Macrophage-Activating Factor, GcMAF. *Transl Oncol* 2008 July;1(2):65-72.

Abstract: Serum Gc protein (known as vitamin D(3)-binding protein) is the precursor for the principal macrophage-activating factor (MAF). The MAF precursor activity of serum Gc protein of prostate cancer patients was lost or reduced because Gc protein was deglycosylated by serum alpha-N-acetylgalactosaminidase (Nagalase) secreted from cancerous cells. Therefore, macrophages of prostate cancer patients having deglycosylated Gc protein cannot be activated, leading to immunosuppression. Stepwise treatment of purified Gc protein with immobilized beta-galactosidase and sialidase generated the most potent MAF (termed GcMAF) ever discovered, which produces no adverse effect in humans. Macrophages activated by GcMAF develop a considerable variation of receptors that recognize the abnormality in malignant cell surface and are highly tumoricidal. Sixteen nonanemic prostate cancer patients received weekly administration of 100 ng of GcMAF. As the

MAF precursor activity increased, their serum Nagalase activity decreased. Because serum Nagalase activity is proportional to tumor burden, the entire time course analysis for GcMAF therapy was monitored by measuring the serum Nagalase activity. After 14 to 25 weekly administrations of GcMAF (100 ng/week), all 16 patients had very low serum Nagalase levels equivalent to those of healthy control values, indicating that these patients are tumor-free. No recurrence occurred for 7 years

## **Intravenous Ascorbate Therapy**

Chen P, Yu J, Chalmers B et al. Pharmacological ascorbate induces cytotoxicity in prostate cancer cells through ATP depletion and induction of autophagy. *Anticancer Drugs* 2012 April;23(4):437-44.

**Abstract:** Recent studies have revealed the scientific basis for the use of intravenous (i.v.) vitamin C or ascorbic acid (ascorbate) in treating cancers, and raised the possibility of using i.v. ascorbate as a prooxidant anticancer therapy. Through the production of H<sub>2</sub>O<sub>2</sub>, pharmacologic ascorbate can induce some cancer cell death in vitro and inhibit a number of types of tumor growth in animal models. However, the mechanism of cell death triggered by ascorbate is not well understood. In this study, we investigated the cytotoxicity of pharmacological concentrations of ascorbate to human prostate cancer cells and the mechanisms involved. The results showed that ascorbate in the millimolar range induced cytotoxicity in five of the six tested prostate cancer cell lines. The IC<sub>50</sub> values in the sensitive prostate cancer cells ranged from 1.9 to 3.5 mmol/l, concentrations clinically achievable with i.v. ascorbate use. All tested androgen-independent cells were sensitive to ascorbate treatment. The ascorbate-insensitive cell line LaPC4 is hormonally dependent. Whereas the reasons for sensitivity/resistance to ascorbate treatment need to be investigated further, cell death in sensitive cells was dependent on H<sub>2</sub>O<sub>2</sub>. Ascorbate treatment depleted ATP and induced autophagy in sensitive prostate cancer cells, resulting in cell death. Taken together with previous studies, high-dose ascorbate has the potential to be a novel treatment option to hormone-refractory prostate cancer

Fromberg A, Gutsch D, Schulze D et al. Ascorbate exerts anti-proliferative effects through cell cycle inhibition and sensitizes tumor cells towards cytostatic drugs. *Cancer Chemother Pharmacol* 2011 May;67(5):1157-66.

**Abstract:** **PURPOSE:** While the benefits of ascorbic acid (vitamin C, ascorbate) as an essential nutrient are well established, its effects on tumor cells and in tumor treatment are controversial. In particular, conflicting data exist whether ascorbate may increase the cytotoxic effects of antineoplastic drugs or may rather exert adverse effects on drug sensitivity during cancer treatment. Findings are further obscured regarding the distinction between ascorbate and dehydroascorbate (DHA). Thus, the purpose of this study was to evaluate and directly compare the cytotoxic efficacy of ascorbate compared to DHA, and to analyse if ascorbate at pharmacological concentrations affects the efficacy of antineoplastic agents in prostate carcinoma cells. **METHODS:** We directly compare the effects of ascorbate (supplied as 'Pascorbin solution for injection') and DHA on tumor cell viability, and determine IC<sub>50</sub> values for various cell lines. At concentrations well below the IC<sub>50</sub>, ascorbate effects on cell proliferation and cell cycle are analysed. We furthermore determine changes in cellular sensitivity towards various cytostatic drugs upon pre-treatment of cells with ascorbate. **RESULTS:** We demonstrate higher therapeutic efficacy of ascorbate over DHA in various cell lines, independent of cell line-specific differences in ascorbate sensitivity, and identify the extracellular generation of H<sub>2</sub>O<sub>2</sub> as critical mechanism of ascorbate action. We furthermore show that, in addition to pro-apoptotic effects described previously, ascorbate treatment already at concentrations well below the IC<sub>50</sub> exerts anti-proliferative effects on tumor cells. Those are based on interference with the cell cycle, namely by inducing a G<sub>0</sub>/G<sub>1</sub> arrest. Pre-treatment of tumor cells with ascorbate leads to increased cellular sensitivity towards Docetaxel, Epirubicin, Irinotecan and 5-FU, but not towards

Oxaliplatin and Vinorelbine. For Docetaxel and 5-FU, a linear correlation between this sensitizing effect and the ascorbate dosage is observed. CONCLUSIONS: The redox-active form of vitamin C, ascorbate, shows therapeutic efficacy in tumor cells. These antitumor effects of ascorbate are mainly based on its extracellular action and, in addition to the induction of apoptosis, also include an anti-proliferative effect by inducing cell cycle arrest. Furthermore, ascorbate treatment specifically enhances the cytostatic potency of certain chemotherapeutics, which implicates therapeutic benefit during tumor treatment

Pollard HB, Levine MA, Eidelman O, Pollard M. Pharmacological ascorbic acid suppresses syngeneic tumor growth and metastases in hormone-refractory prostate cancer. *In Vivo* 2010 May;24(3):249-55.

Abstract: AIM: The aim of this study was to test for the influence of ascorbic acid on tumorigenicity and metastases of implanted P401 prostate cancer adenocarcinoma cells in syngeneic LW rats. MATERIALS AND METHODS: Hormone-refractory prostate cancer P401 cells were implanted subcutaneously into immunologically intact, Long-Evans (LE) rats. Intraperitoneal pharmacological doses of ascorbic acid were administered each day for the ensuing 30 days. On the 40th day, animals were sacrificed. Local tumor weights were measured, and metastases were counted. RESULTS: At the end of the 40 day experimental period, the primary tumors were found to be significantly reduced in weight ( $p=0.026$ ). In addition, sub-pleural lung metastases were even more profoundly reduced in number and size ( $p=0.009$ ). Grossly enlarged ipsilateral lymph node metastases declined from 7 of 15 rats to 1 of 15 rats. CONCLUSION: Pharmacological doses of ascorbic acid suppress tumor growth and metastases in hormone-refractory prostate cancer

Duconge J, Miranda-Massari JR, Gonzalez MJ et al. Vitamin C pharmacokinetics after continuous infusion in a patient with prostate cancer. *Ann Pharmacother* 2007 June;41(6):1082-3.  
Ref ID: 37760

## Valproate

Zhang L, Wang G, Wang L, Song C, Wang X, Kang J. Valproic acid inhibits prostate cancer cell migration by up-regulating E-cadherin expression. *Pharmazie* 2011 August;66(8):614-8.

Abstract: E-Cadherin plays important roles in cell-cell adhesion, epithelial-to-mesenchymal transition, cancer cell migration and invasion. Valproic acid (VPA), a well-known inhibitor of class I and class II histone deacetylases, has been considered a promising anticancer drug due to its capacity of inducing cancer cell proliferation arrest and death through different mechanisms. However, effects of VPA on E-cadherin mediated cell-cell adhesion and cancer cell migration remain unclear. In the present study, we found that VPA potently induced hyperacetylation of histone H3 and H4, increased the expression of E-cadherin and inhibited cell migration in prostate cancer cells. Furthermore, knock-down of E-cadherin significantly restored the effects of VPA on cell migration, while over-expression of E-cadherin in prostate cancer cells significantly inhibited cell migration to a similar level as VPA treatment. These results thus suggest that up-regulation of E-cadherin and inhibition of cell migration may represent a new anticancer mechanism of VPA

Hudak L, Tezeeh P, Wedel S et al. Low dosed interferon alpha augments the anti-tumor potential of histone deacetylase inhibition on prostate cancer cell growth and invasion. *Prostate* 2012 April 2.

Abstract: We evaluated whether low-dosed interferon alpha (IFN $\alpha$ ) may augment the anti-tumor potential of the histone deacetylase (HDAC)-inhibitor valproic acid (VPA) on prostate cancer cells in vitro and in vivo. PC-3, DU-145, or LNCaP prostate cancer cells were treated with VPA (1 mM),

IFNa (200 U/ml), or with the VPA-IFNa combination. Tumor cell growth, cell cycle progression, and cell cycle regulating proteins were then investigated by the MTT assay, flow cytometry, and western blotting. Tumor cell adhesion to endothelium or to immobilized extracellular matrix proteins, as well as migratory properties of the cells, were evaluated. Integrin alpha and beta adhesion molecules and alterations of cell signaling pathways were analyzed. Finally, effects of the drug treatment on prostate cancer growth in vivo were determined in the NOD/SCID mouse model. VPA reduced tumor cell adhesion, migration, and growth in vitro. A much stronger anti-cancer potential was evoked by the VPA-IFNa combination, although IFNa in itself did not block growth or adhesion. The same effect was seen when tumor growth was evaluated in vivo. Molecular analysis revealed distinct elevation of histone H3 acetylation caused by VPA which was further up-regulated by VPA-IFNa, whereas IFNa alone did not alter H3 acetylation. The combinatorial benefit became obvious in Akt phosphorylation, p21 and p27 and integrin alpha1, alpha3, and beta1 expression. Application of low-dosed IFNa to a VPA based regimen profoundly boosts the anti-tumor properties of VPA. The combined use of VPA and low-dosed IFNa may therefore be an innovative option in treating advanced prostate cancer. Prostate (c) 2012 Wiley Periodicals, Inc

Chou YW, Chaturvedi NK, Ouyang S et al. Histone deacetylase inhibitor valproic acid suppresses the growth and increases the androgen responsiveness of prostate cancer cells. *Cancer Lett* 2011 December 8;311(2):177-86.

Abstract: We identified the molecular target by histone deacetylase (HDAC) inhibitors for exploring their potential prostate cancer (PCa) therapy. Upon HDAC inhibitors-treatment, LNCaP cell growth was suppressed, correlating with increased cellular prostatic acid phosphatase (cPACp) expression, an authentic protein tyrosine phosphatase. In those cells, ErbB-2 was dephosphorylated, histone H3/H4 acetylation and methylation increased and cyclin proteins decreased. In PACp shRNA-transfected C-81 cells, valproic acid (VPA) efficacy of growth suppression was diminished. Further, VPA pre-treatment enhanced androgen responsiveness of C-81, C4-2 and MDA PCa2b-AI cells. Thus, cPACp expression is involved in growth suppression by HDAC inhibitors in PCa cells, and VPA pre-treatments increase androgen responsiveness

Chen X, Wong JY, Wong P, Radany EH. Low-dose valproic acid enhances radiosensitivity of prostate cancer through acetylated p53-dependent modulation of mitochondrial membrane potential and apoptosis. *Mol Cancer Res* 2011 April;9(4):448-61.

Abstract: Histone deacetylase inhibitors (HDI) have shown promise as candidate radiosensitizers for many types of cancers, including prostate cancer. However, the mechanisms of action are not well understood. In this study, we show in prostate cancer cells that valproic acid (VPA) at low concentrations has minimal cytotoxic effects yet can significantly increase radiation-induced apoptosis. VPA seems to stabilize a specific acetyl modification (lysine 120) of the p53 tumor suppressor protein, resulting in an increase in its proapoptotic function at the mitochondrial membrane. These effects of VPA are independent of any action of the p53 protein as a transcription factor in the nucleus, since these effects were also observed in native and engineered prostate cancer cells containing mutant forms of p53 protein having no transcription factor activity. Transcription levels of p53-related or Bcl-2 family member proapoptotic proteins were not affected by VPA exposure. The results of this study suggest that, in addition to nuclear-based pathways previously reported, HDIs may also result in radiosensitization at lower concentrations via a specific p53 acetylation and its mitochondrial-based pathway(s)

Gavrilov V, Leibovich Y, Ariad S, Lavrenkov K, Shany S. A combined pretreatment of 1,25-dihydroxyvitamin D3 and sodium valproate enhances the damaging effect of ionizing radiation on prostate cancer cells. *J Steroid Biochem Mol Biol* 2010 July;121(1-2):391-4.

Abstract: Radiotherapy is one of the curative treatment options for prostate cancer (PCa). However, effective doses of ionizing radiation (IR) have a high risk of side effects. To increase sensitivity of PCa to IR we pretreated human androgen-refractory DU145 PCa cells with a combination of sodium valproate (VPA), a well-tolerated drug with histone deacetylases inhibiting activity, and 1,25-dihydroxyvitamin D3, 1,25(OH)2D3, the active metabolite of vitamin D, a well known anticancer agent. The results show that irradiation (4Gy) of DU145 PCa cells pretreated with a combination of 1 mM VPA and 100 nM 1,25(OH)2D3 efficiently suppressed (87.9%) PCa cell proliferation. IR after combined pretreatment resulted in increased DNA double-strand breaks expressed as levels of phosphorylated histone H2A.X, compared with non-treated cells the increase was 58.1% in pretreated cells and 11.8% in non-pretreated cells ( $p < 0.002$ ). Combined pretreatment enhanced IR-induced activation of DNA damage checkpoint kinase Chk2, 39.0% in pretreated cells compared to 23.8% in non-pretreated cells ( $p < 0.05$ ). These molecular changes led to DNA replication blockade, S-phase cell-cycle arrest and enhanced apoptosis. Cumulatively, the results indicate that combined pretreatment with VPA and 1,25(OH)2D3 followed by IR is a highly effective treatment for human PCa cells. This observation may have important implications for reducing doses of radiation administered to cancer patients thus limiting the severity of side effects

Sharma S, Symanowski J, Wong B, Dino P, Manno P, Vogelzang N. A Phase II Clinical Trial of Oral Valproic Acid in Patients with Castration-Resistant Prostate Cancers Using an Intensive Biomarker Sampling Strategy. *Transl Oncol* 2008 September;1(3):141-7.

Abstract: Oral valproic acid (VPA), which is a histone deacetylase inhibitor, was used in a phase II trial to treat patients with castration-resistant prostate cancer (CRPC). Ten patients with CRPC were treated with oral VPA. Oral VPA was not well tolerated in this patient population at a dose targeted to a serum level less than 50 microg/L. The main toxicities were grades 1 and 2 neurologic events and grades 1 and 2 fatigue that caused interruption in the administration of oral VPA and dose delays. Two (20%) of 10 patients had prostate-specific antigen (PSA) responses, and one response was durable. Intensive biomarker collections (weekly) revealed that PSA levels were inversely correlated with total VPA levels. Histone acetylation could not be consistently observed in peripheral lymphocytes using oral VPA. Oral VPA can be administered to CRPC patients with resultant PSA responses. However, oral VPA cannot be administered reliably to achieve consistent levels or duration to be useful in the treatment of CRPC patients. It is unlikely that PSA responses from oral VPA are related to histone deacetylase inhibition. Development of oral VPA in prostate cancers is not recommended using an oral formulation. An intensive biomarker strategy is useful to develop clinical hypotheses in patients with CRPCs in small numbers of patients

Gao D, Xia Q, Lv J, Zhang H. Chronic administration of valproic acid inhibits PC3 cell growth by suppressing tumor angiogenesis in vivo. *Int J Urol* 2007 September;14(9):838-45.

Abstract: AIM: Chromatin remodeling agents such as histone deacetylase inhibitors have been shown to modulate gene expression in tumor cells and inhibit tumor growth and angiogenesis. We investigated the mechanisms of chronic valproic acid (VPA) inhibiting PC3 cell growth in the study. METHODS: We established tumor xenografts of the PC3 cell line and investigated the effect of VPA chronic administration on tumor growth. Apoptosis in tumor tissue was measured using the TUNEL Detection Kit. We detected the effect of VPA chronic administration on histone acetylation; p21CIP1/WAF1 gene expression; vascular endothelial growth factor (VEGF) expression by reverse-transcription Polymerase Chain Reaction (PCR) analysis; immunohistochemistry; and Western Blotting. RESULT: In mouse models with established subcutaneous prostate (PC3), VPA treatment induced 70% inhibition of tumor growth without overt toxicity. Our result showed that chronic administration of VPA has an effect on tumor growth arrest and the effect was associated with

increased histone acetylation, p21CIP1/WAF1 up-regulation, and VEGF down-regulation.  
CONCLUSION: We conclude that chronic VPA results in profound decreases in the proliferation of PC3 cells, not only by increasing histone H3 acetylation and up-regulating p21CIP1/WAF1 expression, but also by down-regulating VEGF

Angelucci A, Valentini A, Millimaggi D et al. Valproic acid induces apoptosis in prostate carcinoma cell lines by activation of multiple death pathways. *Anticancer Drugs* 2006 November;17(10):1141-50.

Abstract: Valproic acid is a well-known antiepileptic drug that was recently discovered to have a wide-spectrum antitumoral action in several tumors. In our work, we tested the proapoptotic activity of valproic acid in prostate cancer. Valproic acid-induced apoptosis was described by several in-vitro assays in three prostate cancer cell lines: two representing the prototype of advanced, clinically untreatable stages of prostate progression, PC3 and DU145, and one resembling a more differentiated androgen-sensitive tumor, LNCaP. We observed that valproic acid was a potent and early apoptotic inducer, mainly in less-differentiated prostate cancer cell lines. The molecular analysis of the apoptotic machinery involved in valproic acid action revealed a central role in Bcl-2 downmodulation. When prostate cancer cells were treated for a longer time with valproic acid, we detected an enhancement of Fas-dependent apoptosis associated with an overexpression in Fas and Fas ligand. Our data indicate that the use of valproic acid may be a suitable therapeutic agent in the control of prostate cancer progression and its action appears particularly relevant in the control of refractory stages of prostate cancer

Xia Q, Sung J, Chowdhury W et al. Chronic administration of valproic acid inhibits prostate cancer cell growth in vitro and in vivo. *Cancer Res* 2006 July 15;66(14):7237-44.

Abstract: Valproic acid (VPA) is an established drug in the long-term therapy of seizure disorders. Recently, VPA has been associated with anticancer activity, an effect thought to be mediated through the inhibition of cellular histone deacetylase 1. We investigated the effect of various doses of VPA (0, 1.2, and 5.0 mmol/L) administered either acutely or chronically on histone acetylation, p21 gene expression, androgen receptor expression, prostate-specific antigen (PSA) expression, and cell survival and proliferation in prostate cancer cell lines. We also studied the effect of chronic VPA on tumor xenograft growth in vivo. Our results show that acute treatment (3 days) VPA can increase net histone H3 acetylation and up-regulate p21, AR, and cytosolic PSA expression. Interestingly, the effects on AR and PSA are reversed with chronic treatment. In addition, acute VPA reduces cell survival but has no effect on the subsequent proliferation of surviving cells following drug withdrawal. However, when VPA is chronically administered (10-14 days) to prostate cancer cells, even lower doses of VPA result in marked decreases in the net proliferation rate, correlating with increased caspase-2 and caspase-3 activation. These effects are evident in both androgen receptor-positive (LNCaP and C4-2) and androgen receptor-negative (DU145 and PC3) prostate cancer cells. Moreover, chronic VPA treatment results in statistically significant reduction of tumor xenograft growth in vivo. We conclude that acute treatment has nominal effects on prostate cancer cell survival and proliferation, but chronic VPA results in profound decreases in proliferation, independently of androgen regulation

Candelaria M, Gallardo-Rincon D, Arce C et al. A phase II study of epigenetic therapy with hydralazine and magnesium valproate to overcome chemotherapy resistance in refractory solid tumors. *Ann Oncol* 2007 September;18(9):1529-38.

Abstract: BACKGROUND: Epigenetic aberrations lead to chemotherapy resistance; hence, their reversal by inhibitors of DNA methylation and histone deacetylases may overcome it. PATIENTS AND

**METHODS:** Phase II, single-arm study of hydralazine and magnesium valproate added to the same schedule of chemotherapy on which patients were progressing. Schedules comprised cisplatin, carboplatin, paclitaxel, vinorelbine, gemcitabine, pemetrexed, topotecan, doxorubicin, cyclophosphamide, and anastrozole. Patients received hydralazine at 182 mg for rapid, or 83 mg for slow, acetylators, and magnesium valproate at 40 mg/kg, beginning a week before chemotherapy. Response, toxicity, DNA methylation, histone deacetylase activity, plasma valproic acid, and hydralazine levels were evaluated. **RESULTS:** Seventeen patients were evaluable for toxicity and 15 for response. Primary sites included cervix (3), breast (3), lung (1), testis (1), and ovarian (7) carcinomas. A clinical benefit was observed in 12 (80%) patients: four PR, and eight SD. The most significant toxicity was hematologic. Reduction in global DNA methylation, histone deacetylase activity, and promoter demethylation were observed. **CONCLUSIONS:** The clinical benefit noted with the epigenetic agents hydralazine and valproate in this selected patient population progressing to chemotherapy' and re-challenged with the same chemotherapy schedule after initiating hydralazine and valproate' lends support to the epigenetic-driven tumor-cell chemoresistance hypothesis (ClinicalTrials.gov Identifier: NCT00404508)

## **Nelfinavir**

Guan M, Fousek K, Chow WA. Nelfinavir inhibits regulated intramembrane proteolysis of sterol regulatory element binding protein-1 and activating transcription factor 6 in castration-resistant prostate cancer. *FEBS J* 2012 July;279(13):2399-411.

**Abstract:** Nelfinavir induces apoptosis in liposarcoma by inhibiting site-2 protease (S2P) activity, which leads to suppression of regulated intramembrane proteolysis. We postulate similar effects in castration-resistant prostate cancer because it exhibits a lipogenic phenotype. Nelfinavir inhibited androgen receptor activation in androgen-sensitive prostate cancer and the nuclear translocation of the fusion proteins sterol regulatory element binding protein-1 (SREBP-1)-enhanced green fluorescence protein (EGFP) and activating transcription factor 6 (ATF6)-EGFP in castration-resistant prostate cancer cells, viewed under confocal microscopy. Nelfinavir and site-1 protease (S1P) and S2P small interfering RNAs (siRNAs) reduced the proliferation of castration-resistant prostate cancer and induced apoptosis, which was opposed by autophagy. Inhibition of autophagy with hydroxychloroquine was additive to the apoptotic effect of nelfinavir. Western blotting of S1P and S2P siRNA knockdown and/or nelfinavir-treated cells confirmed the accumulation of precursor SREBP-1 and ATF6. 3,4-Dichloroisocoumarin, an S1P inhibitor, did not affect SREBP-1 processing. In contrast, 1,10-phenanthroline, an S2P inhibitor, reproduced the nelfinavir-treated molecular and biological phenotype. Nelfinavir-mediated inhibition of regulated intramembrane proteolysis led to the accumulation of unprocessed SREBP-1 and ATF6. This resulted in sequential endoplasmic reticulum stress, inhibition of the unfolded protein response, reduced fatty acid synthase expression and apoptosis, which was countered by autophagy. Inhibition of autophagy was at least additive to this pro-apoptotic effect. These findings provide new insights into nelfinavir-induced endoplasmic reticulum stress and cancer cell death, and lead us to propose investigating its clinical activity in castration-resistant prostate cancer. This report validates S2P as a therapeutic target in castration-resistant prostate cancer

Yang Y, Ikezoe T, Takeuchi T et al. HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer LNCaP cells in vitro and in vivo in conjunction with blockade of androgen receptor STAT3 and AKT signaling. *Cancer Sci* 2005 July;96(7):425-33.

**Abstract:** This study found that the HIV-1 protease inhibitor nelfinavir (NFV) induced growth arrest and apoptosis of human prostate cancer cells (LNCaP, DU145 and PC-3 cells), as measured by MTT

and terminal deoxyribonucleotide transferase-mediated dUTP nick end labeling (TUNEL) assays, respectively, on the third day of culture. In addition, NFV blocked androgen receptor (AR) signaling in association with downregulation of nuclear levels of AR in LNCaP cells as measured by reporter assay and western blot analysis. As expected, NFV downregulated the level of the AR target molecule prostate specific antigen in these cells. Moreover, NFV disrupted STAT3 signaling; protease inhibitors blocked interleukin-6-induced phosphorylation of STAT3 and inhibited STAT3 DNA binding activity in LNCaP and DU145 cells, as measured by western blot analysis and enzyme-linked immunosorbent assay (ELISA), respectively. Furthermore, NFV blocked AKT signaling in prostate cancer cells as measured by kinase assay with glycogen synthase kinase-3 $\alpha$ / $\beta$  as a substrate. Importantly, NFV inhibited the proliferation of LNCaP cells presented as tumor xenografts in BALB/c nude mice without side-effects. Taken together, NFV inhibited the proliferation of prostate cancer cells in conjunction with blockade of signaling by AR, STAT3, and AKT, suggesting that this family of compounds might be useful for the treatment of individuals with prostate cancer

### **Chloroquine/Hydroxychloroquine**

Kaini RR, Hu CA. Synergistic killing effect of chloroquine and androgen deprivation in LNCaP cells. *Biochem Biophys Res Commun* 2012 August 24;425(2):150-6.

Abstract: Modulation of autophagy is a new paradigm in cancer therapeutics. Recently a novel function of chloroquine (CLQ) in inhibiting degradation of autophagic vesicles has been revealed, which raises the question whether CLQ can be used as an adjuvant in targeting autophagic pro-survival mechanism in prostate cancer (PCa). We previously showed that autophagy played a protective role during hormone ablation therapy, in part, by consuming lipid droplets in PCa cells. In addition, blocking autophagy by genetic and pharmacological means in the presence of androgen deprivation caused cell death in PCa cells. To further investigate the importance of autophagy in PCa survival and dissect the role of CLQ in PCa death, we treated hormone responsive LNCaP cells with CLQ in combination with androgen deprivation. We observed that CLQ synergistically killed LNCaP cells during androgen deprivation in a dose- and time-dependent manner. We further confirmed that CLQ inhibited the maturation of autophagic vesicles and decreased the cytosolic ATP. Moreover, CLQ induced nuclear condensation and DNA fragmentation, a hallmark of apoptosis, in androgen deprived LNCaP cells. Taken together, our finding suggests that CLQ may be an useful adjuvant in hormone ablation therapy to improve the therapeutic efficacy

### **Itraconazole (inhibitor of hedgehog pathway)**

Singh S, Chitkara D, Mehrazin R, Behrman SW, Wake RW, Mahato RI. Chemoresistance in Prostate Cancer Cells Is Regulated by miRNAs and Hedgehog Pathway. *PLoS ONE* 2012;7(6):e40021.

Abstract: Many prostate cancers relapse due to the generation of chemoresistance rendering first-line treatment drugs like paclitaxel (PTX) ineffective. The present study aims to determine the role of miRNAs and Hedgehog (Hh) pathway in chemoresistant prostate cancer and to evaluate the combination therapy using Hh inhibitor cyclopamine (CYA). Studies were conducted on PTX resistant DU145-TXR and PC3-TXR cell lines and clinical prostate tissues. Drug sensitivity and apoptosis assays showed significantly improved cytotoxicity with combination of PTX and CYA. To distinguish the presence of cancer stem cell like side populations (SP), Hoechst 33342 flow cytometry method was used. PTX resistant DU145 and PC3 cells, as well as human prostate cancer tissue possess a distinct SP fraction. Nearly 75% of the SP cells are in the G0/G1 phase compared to 62% for non-SP cells and have higher expression of stem cell markers as well. SP cell fraction was increased following PTX monotherapy and treatment with CYA or CYA plus PTX effectively reduced their numbers suggesting the effectiveness of combination therapy. SP fraction cells were

allowed to differentiate and reanalyzed by Hoechst staining and gene expression analysis. Post differentiation, SP cells constitute 15.8% of total viable cells which decreases to 0.6% on treatment with CYA. The expression levels of P-gp efflux protein were also significantly decreased on treatment with PTX and CYA combination. MicroRNA profiling of DU145-TXR and PC3-TXR cells and prostate cancer tissue from the patients showed decreased expression of tumor suppressor miRNAs such as miR34a and miR200c. Treatment with PTX and CYA combination restored the expression of miR200c and 34a, confirming their role in modulating chemoresistance. We have shown that supplementing mitotic stabilizer drugs such as PTX with Hh-inhibitor CYA can reverse PTX chemoresistance and eliminate SP fraction in androgen independent, metastatic prostate cancer cell lines

Efstathiou E, Karlou M, Wen S et al. Integrated Hedgehog signaling is induced following castration in human and murine prostate cancers. *Prostate* 2012 July 2.

Abstract: BACKGROUND: The interplay between androgen and Hedgehog (Hh) signaling pathways may be associated with prostate cancer progression and resistance to therapy. METHODS: Tissue microarrays from prostatectomy specimens were derived from 53 patients treated preoperatively with androgen ablation (AA) with or without chemotherapy, and from 26 stage- and grade-matched controls. A previously characterized androgen-regulated human prostate cancer xenograft was used to conduct parallel murine studies. Expression of markers of interest was determined on both untreated and castrated tumors. RESULTS: Four-month exposure to AA or AA with chemotherapy led to a uniform increase in Hh signaling as compared to controls, paired with an inverse trend of androgen receptor (AR) and CYP17 expression in clinically derived specimens. Changes in the expression profiles of Hh signaling were observed in the epithelium and stroma, in response to genotoxic stress of androgen ablation and chemotherapy. A reduced expression of KI67 and increased bcl2 expression was observed in the malignant epithelial compartment. CONCLUSION: To our knowledge, this is the first clinical evidence that Hh signaling is induced by AA or the combination of AA and chemotherapy and, by inference, contributes to castrate-resistant progression of prostate cancer as supported by parallel human and murine studies. These data are in agreement with previous reports that implicate Hh signaling in castrate-resistant progression of prostate cancer. Based on these findings, we are pursuing parallel clinical and murine investigations to determine if Hh signaling inhibition combined with AA will be more effective than AA alone. *Prostate* (c) 2012 Wiley Periodicals, Inc

Chen M, Carkner R, Buttyan R. The hedgehog/Gli signaling paradigm in prostate cancer. *Expert Rev Endocrinol Metab* 2011;6(3):453-67.

Abstract: Hedgehog is a ligand-activated signaling pathway that regulates Gli-mediated transcription. Although most noted for its role as an embryonic morphogen, hyperactive hedgehog also causes human skin and brain malignancies. The hedgehog-related gene anomalies found in these tumors are rarely found in prostate cancer. Yet surveys of human prostate tumors show concordance of high expression of hedgehog ligands and Gli2 that correlate with the potential for metastasis and therapy-resistant behavior. Likewise, prostate cancer cell lines express hedgehog target genes, and their growth and survival is affected by hedgehog/Gli inhibitors. To date, the preponderance of data supports the idea that prostate tumors benefit from a paracrine hedgehog microenvironment similar to the developing prostate. Uncertainty remains as to whether hedgehog's influence in prostate cancer also includes aspects of tumor cell autocrine-like signaling. The recent findings that Gli proteins interact with the androgen receptor and affect its transcriptional output have helped to identify a novel pathway through which hedgehog/Gli might affect prostate tumor behavior and raises questions as to whether hedgehog signaling in prostate cancer cells is suitably measured by the expression of Gli target genes alone

Chen G, Goto Y, Sakamoto R et al. GLI1, a crucial mediator of sonic hedgehog signaling in prostate cancer, functions as a negative modulator for androgen receptor. *Biochem Biophys Res Commun* 2011 January 21;404(3):809-15.

Abstract: Sonic hedgehog (SHH) signaling, acting in a combinatorial manner with androgen signaling, is essential for prostate patterning and development. Recently, elevated activation of SHH signaling has been shown to play important roles in proliferation, progression and metastasis of prostate cancer. In this report, we demonstrate for the first time, that GLI1, which has been shown to play a central role in SHH signaling in prostate cancer, can act as a co-repressor to substantially block androgen receptor (AR)-mediated transactivation, at least in part, by directly interacting with AR. Our observations suggest that the SHH-GLI pathway might be one of determinants governing the transition of prostate cancer from an androgen-dependent to an androgen-independent state by compensating, or even superseding androgen signaling

Kim J, Tang JY, Gong R et al. Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth. *Cancer Cell* 2010 April 13;17(4):388-99.

Abstract: In a screen of drugs previously tested in humans we identified itraconazole, a systemic antifungal, as a potent antagonist of the Hedgehog (Hh) signaling pathway that acts by a mechanism distinct from its inhibitory effect on fungal sterol biosynthesis. Systemically administered itraconazole, like other Hh pathway antagonists, can suppress Hh pathway activity and the growth of medulloblastoma in a mouse allograft model and does so at serum levels comparable to those in patients undergoing antifungal therapy. Mechanistically, itraconazole appears to act on the essential Hh pathway component Smoothened (SMO) by a mechanism distinct from that of cyclopamine and other known SMO antagonists, and prevents the ciliary accumulation of SMO normally caused by Hh stimulation

## **Metronomic Cyclophosphamide**

Bojko P, Schimmel G, Bosse D, Abenhardt W. Metronomic oral cyclophosphamide in patients with advanced solid tumors. *Onkologie* 2012;35(1-2):35-8.

Abstract: BACKGROUND: Cure is rarely achieved in patients with advanced metastatic solid tumors, and quality of life including times without burdening therapies is an important endpoint. Metronomic oral cyclophosphamide (Cy) has been studied before and is a reasonable option. PATIENTS AND METHODS: 24 patients with a mean age of 64.4 years (range 36-82 years) were studied. 18 patients had breast cancer, 4 prostate cancer, 1 uterine carcinoma, and 1 carcinoma of unknown primary. RESULTS: All patients had advanced disease with a mean of 2 metastatic sites. Cy was given at a mean dosage of 52 mg daily. Time from diagnosis to start of Cy was 108.6 +/- 7.6 months, and from occurrence of metastatic disease to Cy 45.8 +/- 45.6 months. Patients had received a mean of 4.2 +/- 2.1 prior regimens for metastatic disease. The mean time to treatment failure was 6.4 +/- 5.4 months, and mean overall survival was 12.7 +/- 7.3 months. Patients received 2.1 +/- 1.4 further treatments upon progression. Main toxicities were grade 1 and 2 (n = 25); 3 patients had grade 3 nausea, leucopenia, and elevated gamma glutamyl transferase, respectively. CONCLUSION: Low-dose oral Cy is a reasonable, generally well tolerated, and inexpensive option for patients with advanced solid tumors

Gebbia V, Serretta V, Borsellino N, Valerio MR. Salvage therapy with oral metronomic cyclophosphamide and methotrexate for castration-refractory metastatic adenocarcinoma of the prostate resistant to docetaxel. *Urology* 2011 November;78(5):1125-30.

Abstract: OBJECTIVE: To investigate the activity and toxicity of metronomic chemotherapy with

low-dose oral cyclophosphamide (CTX) and methotrexate (MTX) in patients with metastatic CRPC that progresses after docetaxel. Patients with castration-resistant prostate cancer (CRPC) that progresses after docetaxel may benefit from receiving further chemotherapy. **METHODS:** Patients were treated with CTX 50 mg/d p.o. plus MTX 2.4 mg p.o. twice per week without rest periods. All patients received simultaneous luteinizing hormone-releasing hormone analogue. Prostate-specific antigen (PSA) response was defined as a 50% reduction on 2 evaluations at least 4 weeks apart. Objective response was measured according to the RECIST criteria. Pain relief was analyzed with the McGill-Melzack Pain Questionnaire. Simon's 2-stage design for phase II study was used. Time to progression and progression-free and overall survival were computed. Toxicity was recorded according to the CTC-NCCN criteria. **RESULTS:** A PSA decrease  $\geq 50\%$  was recorded in 15 of 58 evaluable patients (25%), and objective partial response in 3 (18%) and stable disease in 4 (24%) of 17 patients with measurable disease. Disease in 10 patients (59%) progressed. Pain intensity decreased in 16 (30%), increased in 18 (33%), and remained stable in 18 (33%) patients. Five patients discontinued narcotic analgesics for a mean duration of 12 weeks. Transitory grade 3 leukopenia was observed in 4 cases (7%), grade 3 thrombocytopenia in 2 (3%), and grade 2 anemia in 4 (7%). **CONCLUSION:** This study demonstrates the feasibility, activity, and tolerability of oral low-dose CTX and MTX given on a metronomic schedule in patients with CRPC progressing after docetaxel-based chemotherapy

Nelius T, Rinard K, Filleur S. Oral/metronomic cyclophosphamide-based chemotherapy as option for patients with castration-refractory prostate cancer: review of the literature. *Cancer Treat Rev* 2011 October;37(6):444-55.

**Abstract:** **PURPOSE:** Castration-refractory prostate cancer remains a therapeutic challenge even after introduction of docetaxel as first-line treatment. Castration-refractory prostate cancer cannot be cured by any available therapeutic option, and chemotherapy still needs to be considered palliative. The survival benefit is modest, and treating physicians are searching for alternative treatment options. Despite new drugs currently under investigation, some conventional and well known chemotherapeutic drugs are experiencing a renaissance. The development of anti-angiogenic approaches in cancer treatment has led to the development of metronomic dosing of conventional chemotherapeutic drugs including cyclophosphamide. The intention of this review is to evaluate the efficacy and toxicity of oral/metronomic cyclophosphamide in the treatment of patients with castration-refractory prostate cancer. **MATERIALS AND METHODS:** A comprehensive literature search was performed in different databases using various key words. Relevant articles and references between 1962 and 2010 were reviewed and analyzed for data regarding the association between oral cyclophosphamide treatment and prostate cancer. **RESULTS:** Oral cyclophosphamide is active in the treatment for castration-refractory prostate cancer even in patients treated with previous chemotherapy including docetaxel. It yields symptomatic and objective remissions. The side effects are usually grade 1-2 and easy to manage. Grade three to four side effects are less common. **CONCLUSIONS:** Oral cyclophosphamide treatment for patients with castration-refractory prostate cancer deserves more attention and validation, and warrants further testing of various treatment combinations. Given the fact that castration-refractory prostate cancer includes an extremely heterogeneous group of patients with variability of tumor growth rates, the combination of cyclophosphamide with other active agents such as angiogenesis inhibitors and immunomodulatory compounds need to be explored

Ladoire S, Eymard JC, Zanetta S et al. Metronomic oral cyclophosphamide prednisolone chemotherapy is an effective treatment for metastatic hormone-refractory prostate cancer after docetaxel failure. *Anticancer Res* 2010 October;30(10):4317-23.

**Abstract:** **BACKGROUND:** There is currently no standard of treatment for patients with hormone

refractory prostate cancer (HRPC) after failure of docetaxel-based chemotherapy. The purpose of this study was to assess the anticancer activity and tolerance of metronomic cyclophosphamide prednisolone combination in this setting. **PATIENTS AND METHODS:** From 2005 to 2010, patients with HRPC who failed at least docetaxel-based chemotherapy were proposed metronomic cyclophosphamide-prednisolone regimen, and were prospectively registered. Twenty-three patients received 50 mg cyclophosphamide and 10 mg prednisolone per os daily until disease progression. Treatment tolerance and efficacy on PSA decrease and pain were studied. **RESULTS:** Metronomic cyclophosphamide prednisolone was safe, well tolerated, and demonstrated interesting clinical activity, yielding a prostate specific antigen decrease by  $\geq 50\%$  in 26% of patients and decrease by  $\geq 30\%$  in 48% of patients, but also favorable palliative effects on pain in 43% of patients. The median progression-free survival was 6 months (95% CI: 4-8 months) and the median overall survival was 11 months (95% CI: 7-19 months). **CONCLUSION:** For this patient population, low dose metronomic cyclophosphamide prednisolone might be a viable alternative. Its convenient oral administration, low cost, and lack of toxicity justify further studies alone, or in combination with other agents in HRPC patients

Fontana A, Bocci G, Galli L et al. Metronomic cyclophosphamide in elderly patients with advanced, castration-resistant prostate cancer. *J Am Geriatr Soc* 2010 May;58(5):986-8.

Fontana A, Galli L, Fioravanti A et al. Clinical and pharmacodynamic evaluation of metronomic cyclophosphamide, celecoxib, and dexamethasone in advanced hormone-refractory prostate cancer. *Clin Cancer Res* 2009 August 1;15(15):4954-62.

**Abstract:** **PURPOSE:** The aims of the present study were to evaluate the clinical activity and the pharmacodynamic profile of the novel schedule of a single i.v. standard dose of cyclophosphamide (CTX) immediately followed by an oral metronomic CTX regimen with celecoxib (CXB) and dexamethasone (DEX) in advanced hormone-refractory prostate cancer patients. **EXPERIMENTAL DESIGN:** Twenty-eight patients (68% docetaxel-resistant) received 500 mg/m<sup>2</sup> CTX i.v. bolus on day 1 and, from day 2, 50 mg/day CTX p.o. plus 200 mg/twice a day CXB p.o. and 1 mg/day DEX p.o. until disease progression. Plasma vascular endothelial growth factor (VEGF) and thrombospondin-1 were detected by ELISA, and real-time reverse transcription-PCR of VEGF and thrombospondin-1 gene expression on peripheral blood mononuclear cell and of VE-cadherin (VE-C) in blood samples was done. **RESULTS:** A confirmed prostate-specific antigen decrease of  $>$  or  $=50\%$  from baseline was observed in 9 of 28 patients (32%). Median progression-free survival and overall survival were 3 months (95% confidence interval, 2.2-4.2 months) and 21 months (95% confidence interval, 12.4-29.4 months), respectively. Toxicity was mild and no grade 3 to 4 toxicities occurred. A significant relationship was found between plasma VEGF and prostate-specific antigen values ( $r = 0.4223$ ;  $P < 0.001$ ). VEGF levels significantly increased in nonresponders, whereas the responder patients maintained significantly lower levels of VE-C gene expression after the beginning of the treatment if compared with nonresponder ones. **CONCLUSION:** Metronomic CTX plus CXB and DEX showed favorable toxicity and activity profile in patients. VE-C gene expression and VEGF levels represent potentially useful pharmacodynamic markers for the clinical response

Nelius T, Klatter T, de RW, Haynes A, Filleur S. Clinical outcome of patients with docetaxel-resistant hormone-refractory prostate cancer treated with second-line cyclophosphamide-based metronomic chemotherapy. *Med Oncol* 2010 June;27(2):363-7.

**Abstract:** For patients with docetaxel-resistant hormone-refractory prostate cancer (HRPC) no standard chemotherapeutic treatment exists. In this study, we evaluate the efficacy of cyclophosphamide (CP)-based metronomic chemotherapy in this patient population. Patients with metastatic HRPC with disease progression under docetaxel-based chemotherapy were eligible. The

primary endpoint was prostate-specific antigen (PSA) response. Secondary endpoints were survival and toxicity. Low-dose CP (50 mg/d) and dexamethasone (1 mg/d) were administered orally in a metronomic manner. Treatment was continued until disease progression or intolerable side effects occurred. Seventeen patients were enrolled in this study. The median follow-up was 12 weeks (range: 4-60). Median age was 68 years (range: 42-85). Median PSA at study entry was 134 ng/ml (range: 46.0-6554). Nine patients had a PSA response (median 44.4%), four patients  $\geq$ 50% and five patients  $<$ 50%. Eight patients had a PSA progression. Overall survival was 24 months. Five patients reported a decrease in bone pain after 4 weeks' treatment. No grade 3 and 4 toxicities were noted. In this study, low-dose metronomically administered CP demonstrated efficacy as a second-line treatment in patients with docetaxel-resistant HRPC. The treatment was well tolerated and almost without toxicity. Further advantages of low-dose CP were its convenient oral administration, dosing schedule, low cost, and low-toxicity profile. These attributes in combination with immunoregulatory and antiangiogenic potentials make CP also a prime candidate for combination with other treatment regimens

Lord R, Nair S, Schache A et al. Low dose metronomic oral cyclophosphamide for hormone resistant prostate cancer: a phase II study. *J Urol* 2007 June;177(6):2136-40.

Abstract: **PURPOSE:** Cyclophosphamide is a bifunctional alkylating agent long associated with immune activation. Continuous, uninterrupted, low (so-called metronomic) doses of cyclophosphamide can lead to enhanced immunity against a variety of antigens possibly by targeting regulatory T cells and/or tumor angiogenesis. In this study we tested the observations from animal models and evaluated the safety and efficacy of continuous low dose oral cyclophosphamide in patients with hormone resistant prostate cancer. **MATERIALS AND METHODS:** A total of 80 patients were recruited during a 2-year period and 58 received at least 2 cycles (8 weeks) of 50 mg/m<sup>2</sup> oral cyclophosphamide to be included in the safety and intent to treat analysis. **RESULTS:** Metronomic cyclophosphamide was safe and well tolerated, and although lymphopenia (up to grade 3) was observed in a third of all patients, there were no clinical complications. The response rate was 34.5% inclusive of objective and prostate specific antigen (absolute reduction and reduction in prostate specific antigen velocity). The median duration of response was 7.5 months (range 3 to 18). **CONCLUSIONS:** Oral cyclophosphamide can be used on a metronomic basis safely in men with hormone resistant prostate cancer. The efficacy, low toxicity, low cost and ease of administration of cyclophosphamide justifies further studies in prostate cancer in combination with other agents

Glode LM, Barqawi A, Crighton F, Crawford ED, Kerbel R. Metronomic therapy with cyclophosphamide and dexamethasone for prostate carcinoma. *Cancer* 2003 October 15;98(8):1643-8.

Abstract: **BACKGROUND:** The current study was designed to evaluate the efficacy and toxicity of the continuous oral administration of a combination of cyclophosphamide (50 mg/day given in the morning) and dexamethasone (1 mg/day given in the evening) in patients with prostate specific antigen (PSA) progression despite single or multiagent hormone therapy and antiandrogen withdrawal. **METHODS:** The authors retrospectively evaluated the medical records of all patients with prostate carcinoma who were treated with dexamethasone and cyclophosphamide and who were unable to participate in Phase II drug trials or had failed previous chemotherapy regimens. **RESULTS:** Using clinical response guidelines set forth by the Prostate Specific Antigen Working Group, 29% of patients were found to have a  $\geq$  80% reduction in PSA, 39% were found to have a 50-79% reduction in PSA, 6% were found to have a  $<$  50% decrease in PSA, and 26% experienced disease progression while receiving treatment. The duration of response was 8 months (95% confidence interval [95% CI], 4-10 months). The duration of treatment was 9 months (95% CI, 6-14 months). The treatment was reported to be well tolerated with side effects being primarily bruising,

Cushingoid facies, and gastrointestinal distress. CONCLUSIONS: In the current study, low-dose dexamethasone and cyclophosphamide demonstrated efficacy as salvage therapy in the treatment of patients with hormone-refractory prostate carcinoma

Nicolini A, Mancini P, Ferrari P et al. Oral low-dose cyclophosphamide in metastatic hormone refractory prostate cancer (MHRPC). *Biomed Pharmacother* 2004 October;58(8):447-50.

Abstract: The chemotherapeutic approach to hormone-refractory metastatic prostate cancer (MHRPC) for a long time included only estramustine. Then, attempts have been made with other various agents as cyclophosphamide, vinblastine, etoposide, taxanes and carboplatinum. Although the new drugs and combinations have increased the response rate of MHRPC, they have had no impact on the natural history of MHRPC, which is about 1 year as median time of survival. After an occasional observation of prolonged response in a patient with MHRPC treated with a very well tolerated oral low-dose of cyclophosphamide, from February 1996 to October 2002, seven more patients with MHRPC and progressive disease were consecutively recruited. Response to treatment was evaluated by conventional radiological procedures and/or serial serum PSA measurements. The decline of PSA value was considered to assess the response consistent with the response guidelines from the prostate specific antigen-working group. All eight studied patients continuously received oral low dose cyclophosphamide until progression or the occurrence of significant toxicity. So far three patients (37.5%) progressed (PD), two (25%) showed PR and the three remaining SD. Response rate was 25%, and clinical benefit occurred in 62.5% of the studied patients. In the five patients with clinical benefit on cyclophosphamide median duration of clinical benefit, PR and SD were 9, 24+ and 8 months, respectively. In these five patients median overall survival times from cyclophosphamide and from the first regimen of chemotherapy were 17 and 33+ months respectively, while in the three patients with PD they were 4 and 13 months. The same interval times in patients with > or =50% decline of serum PSA were 29 and 50.5 months, while in those with <50% decline of the same marker, they were 13 and 32 months, respectively. Grade 2 or 3 neutropenia were observed in all the studied patients. In four (50%) of them pulmonary and urinary infections that were easily cured by the common antibiotics occurred. These data suggest that the metronomic use of cyclophosphamide, given alone, has similar or higher activity with lower toxicity than when administered with other active drugs. So it can be an useful option before or after the use of other single or combined potentially active chemotherapeutic agents

Bocci G, Francia G, Man S, Lawler J, Kerbel RS. Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. *Proc Natl Acad Sci U S A* 2003 October 28;100(22):12917-22.

Abstract: Chemotherapeutic drugs chronically administered to tumor-bearing mice, using a frequent schedule at doses substantially lower than the maximum tolerated dose (MTD) (i.e., metronomic dosing), can cause sustained and potent antiangiogenic effects by targeting the endothelial cells of newly growing tumor blood vessels. These effects appear to occur in the absence of an increase in the severity of side effects caused by destruction of other cell types normally sensitive to MTD chemotherapy, suggesting a marked and selective sensitivity of activated endothelial cells, the basis of which is unknown. Here we report that protracted exposure of endothelial cells in vitro to low concentrations of several different anticancer agents, including microtubule inhibitors and an alkylating agent, caused marked induction of gene and protein expression of TSP-1, a potent and endothelial-specific inhibitor of angiogenesis. Increases in circulating TSP-1 were also detected in the plasma of human tumor-bearing severe combined immunodeficient mice treated with metronomic low-dose cyclophosphamide. Most importantly, the antiangiogenic and antitumor effects of low-dose continuous cyclophosphamide were lost in TSP-1-null C57BL/6 mice, whereas, in contrast, these effects were retained by using a MTD schedule of the same drug. Taken together, the results

implicate TSP-1 as a secondary mediator of the antiangiogenic effects of at least some low-dose metronomic chemotherapy regimens

Man S, Bocci G, Francia G et al. Antitumor effects in mice of low-dose (metronomic) cyclophosphamide administered continuously through the drinking water. *Cancer Res* 2002 May 15;62(10):2731-5.

Abstract: A number of recent preclinical studies have sparked interest in the concept of exploiting conventional chemotherapeutic drugs as antiangiogenics. Such antiangiogenic activity is achieved or optimized by metronomic-dosing protocols in which the drug is given at comparatively low doses using a frequent schedule of administration (e.g., once to three times per week) with no breaks, particularly when combined with an endothelial cell-specific antiangiogenic drug. The use of p.o. chemotherapeutic drugs is particularly suitable for this type of treatment strategy. We tested one such drug, cyclophosphamide (CTX), in a protocol wherein the drug was administered to mice at low doses, of approximately 10-40 mg/kg on a daily basis through the drinking water. CTX is typically given p.o. to patients, but it has almost always been injected when treating preclinical mouse tumor models. We found p.o. CTX to be a safe and convenient treatment with significant antitumor efficacy. Growth delays were observed for human orthotopic breast or ectopic colon cancer xenografts in nude or SCID mice. Established PC3 human prostate tumor xenografts could be induced to almost fully regress, remaining virtually nonpalpable for > or =2 months of continuous therapy, after which tumors began to grow progressively. These re-emergent tumors were not found to be drug resistant when tested in new hosts, using the same treatment protocol. Regression of spontaneously arising, late-stage pancreatic islet cell carcinomas in Rip Tag transgenic mice was also observed. The effects of continuous p.o. CTX treatment were enhanced significantly in an orthotopic, metastatic breast cancer xenograft model when used in combination with an antivascular endothelial growth factor receptor-2 blocking antibody. Maximum tolerated dose levels established for other mouse strains proved highly toxic to SCID mice, whereas daily p.o. low-dose regimens of CTX were well tolerated. Taken together, the results demonstrate the feasibility of delivering CTX in a p.o. metronomic chemotherapy regimen, which proved safe, reasonably efficacious, and potentially applicable to chronic treatment. Such a regimen may be particularly well suited for integration with antiangiogenic drugs

## Selenate

Holmstrom A, Wu RT, Zeng H, Lei KY, Cheng WH. Nutritional and supranutritional levels of selenate differentially suppress prostate tumor growth in adult but not young nude mice. *J Nutr Biochem* 2012 September;23(9):1086-91.

Abstract: The inhibitory effect of oral methylseleninic acid or methylselenocysteine administration on cancer cell xenograft development in nude mice is well characterized; however, less is known about the efficacy of selenate and age on selenium chemoprevention. In this study, we tested whether selenate and duration on diets would regulate prostate cancer xenograft in nude mice. Thirty-nine homozygous NU/J nude mice were fed a selenium-deficient, Torula yeast basal diet alone (Se-) or supplemented with 0.15 (Se) or 1.0 (Se+) mg selenium/kg (as Na(2)SeO(4)) for 6 months in Experiment 1 and for 4 weeks in Experiment 2, followed by a 47-day PC-3 prostate cancer cell xenograft on the designated diet. In Experiment 1, the Se- diet enhanced the initial tumor development on days 11-17, whereas the Se+ diet suppressed tumor growth on days 35-47 in adult nude mice. Tumors grown in Se- mice were loosely packed and showed increased necrosis and inflammation as compared to those in Se and Se+ mice. In Experiment 2, dietary selenium did not affect tumor development or histopathology throughout the time course. In both experiments, postmortem plasma selenium concentrations in Se and Se+ mice were comparable and were twofold greater than those in Se- mice. Taken together, dietary selenate at

nutritional and supranutritional levels differentially inhibit tumor development in adult, but not young, nude mice engrafted with PC-3 prostate cancer cells

Corcoran NM, Hovens CM, Michael M, Rosenthal MA, Costello AJ. Open-label, phase I dose-escalation study of sodium selenate, a novel activator of PP2A, in patients with castration-resistant prostate cancer. *Br J Cancer* 2010 August 10;103(4):462-8.

Abstract: **BACKGROUND:** Angiogenesis is fundamental to the progression of many solid tumours including prostate cancer. Sodium selenate is a small, water-soluble, orally bioavailable activator of PP2A phosphatase with anti-angiogenic properties. **METHODS:** This was a dose-escalation phase I study in men with asymptomatic, chemotherapy-naïve, castration-resistant prostate cancer. The primary objective was to determine the maximum tolerated dose (MTD). Secondary objectives included establishing the safety, tolerability and pharmacokinetic profile. **RESULTS:** A total of 19 patients were enrolled. The MTD was 60 mg per day. Dose-limiting toxicity (fatigue and diarrhoea) was observed at 90 mg per day. The most frequently reported treatment-related adverse events across all treatment cohorts were nausea, diarrhoea, fatigue, muscle spasms, alopecia and nail disorders. No grade 4 toxicities were observed and there were no deaths on study. Linear pharmacokinetics was observed. One patient had a PSA response >50%. Median time to PSA progression (for non-responders) was 14.2 weeks. Mean PSA doubling time increased during the main treatment phase from 2.18 months before trial to 3.85 months. **CONCLUSION:** Sodium selenate is well tolerated at a dose of 60 mg per day with modest single-agent efficacy similar to other anti-angiogenic agents. Further trials in combination with conventional cytotoxic regimens are warranted

Corcoran NM, Najdovska M, Costello AJ. Inorganic selenium retards progression of experimental hormone refractory prostate cancer. *J Urol* 2004 February;171(2 Pt 1):907-10.

Abstract: **PURPOSE:** The development of hormone refractory prostate cancer marks the onset of the terminal phase of the disease. Despite the use of traditional chemotherapeutic drugs as well as many novel agents life expectancy is not significantly increased beyond palliative care alone. Selenium is a micronutrient that is incorporated into a number of essential enzymes and a minimum intake is necessary for the maintenance of health. In the last few years evidence has accumulated from case-control and limited randomized control data that supranutritional doses of selenium could inhibit the progression of prostate cancer. While much attention has focused on its use as a chemopreventive agent, its use as specific therapy has been limited. We hypothesized that dietary supplementation of selenium would inhibit the progression of hormone refractory prostate cancer in an experimental model. **MATERIALS AND METHODS:** We established orthotopic PC3 tumors in the prostates of 6-week-old male nude mice and fed them a baseline selenium replete diet (0.07 ppm), supplementing intake with different forms of selenium (sodium selenate, selenomethionine, methylselenocysteine and selenized yeast) at 2 different concentrations (0.3 and 3 ppm) in drinking water. **RESULTS:** Inorganic selenium (sodium selenate) significantly retarded the growth of primary prostatic tumors and the development of retroperitoneal lymph node metastases, which was associated with a decrease in angiogenesis. **CONCLUSIONS:** High dose dietary supplementation of inorganic selenium inhibits the progression of hormone refractory prostate cancer, which is due at least in part to a decrease in angiogenesis