

## Prostate Cancer

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**Abstract:** Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the prostate cancer.

[Smith MH. **Prostate Cancer**. *Cancer Biology* 2013;3(1):45-135]. (ISSN: 2150-1041). <http://www.cancerbio.net>. 2

**Keywords:** cancer; biology; life; disease; research; literature; prostate

### 1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

### Literatures

Afar, D. E., V. Bhaskar, et al. (2004). "Preclinical validation of anti-TMEFF2-auristatin E-conjugated antibodies in the treatment of prostate cancer." *Mol Cancer Ther* **3**(8): 921-32.

Current treatments for advanced stage, hormone-resistant prostate cancer are largely ineffective, leading to high patient mortality and morbidity. To fulfill this unmet medical need, we used global gene expression profiling to identify new potential antibody-drug conjugate (ADC) targets that showed maximal prostate cancer-specific expression. TMEFF2, a gene encoding a plasma membrane protein with two follistatin-like domains and one epidermal growth factor-like domain, had limited normal tissue distribution and was highly overexpressed in prostate cancer. Immunohistochemistry analysis using a specific monoclonal antibody (mAb) to human TMEFF2 showed significant protein expression in 74% of primary prostate cancers and 42% of metastatic lesions from lymph nodes and bone that represented both hormone-naive and hormone-resistant disease. To evaluate anti-TMEFF2 mAbs as potential ADCs, one mAb was conjugated to the cytotoxic agent auristatin E via a cathepsin B-sensitive valine-citrulline linker. This ADC, Pr1-vcMMAE, was used to treat male severe combined immunodeficient mice

bearing xenografted LNCaP and CWR22 prostate cancers expressing TMEFF2. Doses of 3 to 10 mg/kg of this specific ADC resulted in significant and sustained tumor growth inhibition, whereas an isotype control ADC had no significant effect. Similar efficacy and specificity was shown with huPr1-vcMMAE, a humanized anti-TMEFF2 ADC. No overt in vivo toxicity was observed with either murine or human ADC, despite significant cross-reactivity of anti-TMEFF2 mAb with the murine TMEFF2 protein, implying minimal toxicity to other body tissues. These data support the further evaluation and clinical testing of huPr1-vcMMAE as a novel therapeutic for the treatment of metastatic and hormone-resistant prostate cancer.

Ahonen, T. J., J. Xie, et al. (2003). "Inhibition of transcription factor Stat5 induces cell death of human prostate cancer cells." *J Biol Chem* **278**(29): 27287-92.

Identifying regulators of prostate cancer cell survival may lead to new therapeutic strategies for prostate cancer. We now report prevalent activation of transcription factor Stat5 in human prostate cancer and provide novel evidence that blocking activation of Stat5 in human prostate cancer cells leads to extensive cell death. Specifically, Stat5 was activated in 65% of human prostate cancer specimens examined based on nuclear location of tyrosine phosphorylated Stat5. Adenoviral gene delivery of a dominant-negative Stat5 mutant (DNStat5), but not wild-type Stat5, induced cell death of both the androgen-independent human prostate cancer cell line CWR22Rv and the androgen-sensitive LnCap cell line. Endogenous Stat5 was active in both CWR22Rv and LnCap cells. In contrast, only low levels of inactive Stat5 proteins were detected in the PC-3 cell line, which correlated with resistance to DNStat5-induced cell death. In CWR22Rv and LnCap cells, inhibition of Stat5 by expression of DNStat5 induced apoptotic cell death as judged from morphological changes, DNA

fragmentation, and caspase-3 activation with evidence of a caspase-9-dependent mechanism. We propose that blocking Stat5 function may represent a novel therapeutic approach for prostate cancer.

Amantana, A., C. A. London, et al. (2004). "X-linked inhibitor of apoptosis protein inhibition induces apoptosis and enhances chemotherapy sensitivity in human prostate cancer cells." *Mol Cancer Ther* 3(6): 699-707.

Androgen-insensitive prostate cancer cells are highly resistant to several chemotherapeutic drugs and are characterized by the appearance of apoptosis-resistant cells. In this study, we identified the critical role of X-linked inhibitor of apoptosis protein (XIAP), a potent antiapoptotic factor, in conferring chemotherapy resistance in an androgen-insensitive DU145 human prostate cancer cell line. Results reveal that DU145 cells were highly resistant to cisplatin, but this resistance was overridden when the cells were treated for a prolonged time (>96 hours) with cisplatin (IC(50) = 27.5 to 35.5 micromol/L). A decrease in levels of XIAP and Akt/phospho-Akt and an increase in caspase-3 activity were identified to be key factors in cisplatin sensitivity (40% to 55% decrease in cell viability) at later time points. In contrast, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) treatment caused a 40% to 50% decrease in cell viability within 6 hours (IC(50) = 135 to 145 ng/mL). However, increasing concentrations or prolonged treatment with TRAIL did not change drug potency. A significant increase in caspase-3 activity was observed with TRAIL treatment with no apparent change in XIAP levels. Specific inhibition of XIAP expression using an antisense XIAP phosphorodiamidate morpholino oligomer induced apoptosis and increased caspase-3 activity. Combination of cisplatin with XIAP antisense potentiated cisplatin sensitivity by decreasing the IC(50) from >200 micromol/L with cisplatin alone to 9 to 20 micromol/L and decreasing incubation time required for activity from 96 to 24 hours. Similarly, TRAIL in combination with XIAP antisense phosphorodiamidate morpholino oligomer enhanced TRAIL potency by 12- to 13-fold. In conclusion, abrogation of XIAP expression is essential for therapeutic apoptosis and enhanced chemotherapy sensitization in androgen-refractory prostate cancer cells.

Ast, G. (2003). "Drug-targeting strategies for prostate cancer." *Curr Pharm Des* 9(6): 455-66.

Prostate cancer is the most frequently diagnosed cancer in North American men and accounts for 10% of cancer-related deaths in men. Despite advances in early detection and aggressive

treatment of early disease, the overall mortality rate has not appear to have fallen, indicating that the current therapies are not beneficial for life expectancy and new strategies are required. Prostate cancer is a dynamic evolving process that develops in distinct steps, with each step liable to additional genetic hits that change the cancer cell phenotype and alter the patterns of gene expression. The molecular events in prostate cancer are beginning to be understood, including altered expression of tumor suppressor genes, pro- and anti-apoptotic genes, and oncogenes associated with the progression of the disease; and specific genes that are expressed predominantly or exclusively in prostate cells, prostate cancer cells, and prostate metastasis cells. These latter genes on the level of DNA, RNA and protein products are the targets of several new approaches to prostate cancer therapy and are the focus of this review.

Attard, G., C. Jameson, et al. (2009). "Hormone-sensitive prostate cancer: a case of ETS gene fusion heterogeneity." *J Clin Pathol* 62(4): 373-6.

Fusion of the hormone-regulated gene TMPRSS2 with ERG occurs in 50-70% of prostate cancers; fusions of ETV1 with one of several partners occur in approximately 10% of prostate cancers. These two translocations are mutually exclusive. The presence of subclasses of these chromosomal rearrangements may indicate worse prognosis, with the subclass 2+Edel, which has duplication of TMPRSS2:ERG fusion sequences, indicating particularly poor survival. However as this case shows, significant heterogeneity can exist with ERG and ETV1 rearrangements occurring in both prostate intra-epithelial neoplasia and cancer in the same prostatectomy specimen and with adjacent cancer areas containing a single copy, duplication and even triplication of the rearranged locus. As the majority of ETS gene fusions are hormone regulated, they could explain the pathogenesis underlying exquisitely hormone-sensitive prostate cancer. This is exemplified by the case presented here of a patient diagnosed in 1991 who remains asymptomatic and chemotherapy-naive after having long-lasting tumour responses to multiple lines of systemic hormonal treatments.

Banerjee, S., M. Hussain, et al. (2007). "In vitro and in vivo molecular evidence for better therapeutic efficacy of ABT-627 and taxotere combination in prostate cancer." *Cancer Res* 67(8): 3818-26.

Bone is the key metastatic site for prostate cancer. Endothelin 1 (ET-1) produced abundantly by prostate cancer cells binds to its receptor present on bone marrow stromal cells and favors osteoblastic response during bone metastases of prostate cancer. This suggests that interrupting ET-1 interaction with

its endothelin A (ET(A)) receptor could be useful for inhibiting prostate cancer bone metastasis and, as such, may enhance the therapeutic activity of docetaxel (Taxotere), the most commonly used drug for the treatment of metastatic prostate cancer. Therefore, the goal of our study was to obtain preclinical data supporting our hypothesis that the combined use of ET(A) receptor antagonist (ABT-627; Atrasentan) with Taxotere will be superior in inducing apoptosis in vitro and inhibiting tumor growth in vivo in a SCID-hu model of experimental bone metastasis induced by C4-2b prostate cancer cells. In vitro studies were done on a panel of prostate cancer cell lines to understand the molecular basis of combination therapy, and we found that the combination was more effective in the inhibition of cell viability and induction of apoptosis in LNCaP and C4-2b cells (androgen receptor positive) but not in PC-3 cells. These results were correlated with inactivation of Akt/nuclear factor-kappaB and its target genes. For in vivo studies, the therapeutic regimen was initiated when the tumor began showing signs of growth and treatment was continued for 5 weeks. Tumor volume and serum prostate-specific antigen were used as terminal index to evaluate the therapeutic advantage of combination therapy relative to a single regimen and untreated control. At termination, we found a 90% reduction in tumor volume by combination treatment relative to the untreated control group. Most importantly, the antitumor activity was associated with the down-regulation of molecular markers in tumor tissues that were similar to those observed in vitro.

Bao, B. Y., J. Yao, et al. (2006). "1alpha, 25-dihydroxyvitamin D3 suppresses interleukin-8-mediated prostate cancer cell angiogenesis." *Carcinogenesis* **27**(9): 1883-93.

Angiogenesis is an essential step in initial tumor development and metastasis. Consequently, compounds that inhibit angiogenesis would be useful in treating cancer. A variety of antitumor effects mediated by 1alpha, 25-dihydroxyvitamin D3 (1,25-VD) have been reported, one of which is anti-angiogenesis; however, detailed mechanisms remain unclear. We have demonstrated that 1,25-VD inhibits prostate cancer (PCa) cell-induced human umbilical vein endothelial cell migration and tube formation, two critical steps involved in the angiogenesis. An angiogenesis factor, interleukin-8 (IL-8), secreted from PCa cell was suppressed by 1,25-VD at both mRNA and protein levels. Mechanistic dissection found that 1,25-VD inhibits NF-kappaB signal, one of the most important IL-8 upstream regulators. The 1,25-VD-mediated NF-kappaB signal reduction was shown to result from the blocking of nuclear

translocation of p65, a subunit of the NF-kappaB complex, and was followed by attenuation of the NF-kappaB complex binding to DNA. The role of IL-8 in PCa progression was further examined by PCa tissue microarray analyses. We found that IL-8 expression was elevated during PCa progression, which suggests that IL-8 may play a role in tumor progression mediated through its stimulation on angiogenesis. These findings indicate that 1,25-VD could prevent PCa progression by interrupting IL-8 signaling, which is required in tumor angiogenesis, and thus applying vitamin D in PCa treatment may be beneficial for controlling disease progression.

Baron, V., G. De Gregorio, et al. (2003). "Inhibition of Egr-1 expression reverses transformation of prostate cancer cells in vitro and in vivo." *Oncogene* **22**(27): 4194-204.

Transcription factor early growth response-1 (Egr-1) is a crucial regulator of cell growth, differentiation and survival. Several observations suggest that Egr-1 is growth promoting in prostate cancer cells and that blocking its function may impede cancer progression. To test this hypothesis, we developed phosphorothioate antisense oligonucleotides that efficiently inhibit Egr-1 expression without altering the expression of other family members Egr-2, Egr-3 and Egr-4. In TRAMP mouse-derived prostate cancer cell lines, our optimal antisense oligonucleotide decreased the expression of the Egr-1 target gene transforming growth factor-beta1 whereas a control oligonucleotide had no effect, indicating that the antisense blocked Egr-1 function as a transcription factor. The antisense oligonucleotide deregulated cell cycle progression and decreased proliferation of the three TRAMP cell lines by an average of 54+/-3%. Both colony formation and growth in soft agar were inhibited by the antisense oligonucleotide. When TRAMP mice were treated systemically for 10 weeks, the incidence of palpable tumors at 32 weeks of age in untreated mice or mice injected with the control scramble oligonucleotide was 87%, whereas incidence of tumors in antisense-Egr-1-treated mice was significantly reduced to 37% (P=0.026). Thus, Egr-1 plays a functional role in the transformed phenotype and may represent a valid target for prostate cancer therapy.

Barton, B. E., T. F. Murphy, et al. (2004). "Novel single-stranded oligonucleotides that inhibit signal transducer and activator of transcription 3 induce apoptosis in vitro and in vivo in prostate cancer cell lines." *Mol Cancer Ther* **3**(10): 1183-91.

Signal transducers and activators of transcription (STAT) were originally discovered as components of cytokine signal transduction pathways.

Persistent activation of one of these transcription factors, STAT3, is a feature of many malignancies, including hormone-resistant prostate cancer. In this regard, malignant cells expressing persistently activated STAT3 become dependent on it for survival, thus rendering STAT3 a potential molecular target for therapy of hormone-resistant prostate cancer. Previously, we reported that antisense oligonucleotides specific for STAT3 were better at inducing apoptosis than inhibitors of JAK1 or JAK2, the upstream activating kinases of STAT3. Here, we report that novel single-stranded oligonucleotides, which putatively block STAT3-DNA binding, were better at inducing hormone-resistant prostate cancer apoptosis than antisense STAT3 oligonucleotides. We observed that the novel STAT3-inhibiting oligonucleotides induced apoptosis by a mitochondrial-dependent pathway involving the activation of caspase-3. Prostate cell lines not expressing persistently activated STAT3 did not become apoptotic after treatment with these same oligonucleotides. Scrambled-sequence control oligonucleotides had none of the effects of the active sequence oligonucleotides on any variable measured. Furthermore, the novel STAT3-inhibiting oligonucleotides, but not scrambled-sequence control oligonucleotide, significantly reduced the volume of s.c. DU145 tumors in vivo. Histologic examination of the tumors revealed no infiltrate of mononuclear or granulocytic cells, which would be indicative of evocation of a nonspecific immune response by the oligonucleotides. We conclude that single-stranded oligonucleotides based on the binding sequences of STAT3 are an additional strategy to design inhibitors for this molecular target and that these inhibitors should be useful as experimental therapeutics for hormone-resistant prostate cancer.

Bastian, P. J., G. S. Palapattu, et al. (2008). "CpG island hypermethylation profile in the serum of men with clinically localized and hormone refractory metastatic prostate cancer." *J Urol* **179**(2): 529-34; discussion 534-5.

**PURPOSE:** We have noted that hypermethylation at GSTP1 in the preoperative serum of men with localized prostate cancer predicts early prostate specific antigen failure following surgical treatment. In this study we investigated the hypermethylation profile of several genes in the serum of men with localized and hormone refractory prostate cancer. **MATERIALS AND METHODS:** We assayed the serum of 192 men with clinically localized prostate cancer and 18 with hormone refractory metastatic disease. A total of 35 serum samples from patients with negative prostate biopsy served as a negative control. CpG Island hypermethylation status

of certain genes was assessed, including MDR1, EDNRB, CD44, NEP, PTGS2, RASSF1A, RAR-beta and ESR1. The results of hypermethylation at GSTP1 were included from a previous study. **RESULTS:** CpG island hypermethylation at MDR1 was positive in 38.2% of cases without PSA recurrence and in 16.1% of those with biochemical recurrence after radical prostatectomy. DNA hypermethylation at the remaining 7 gene loci was not detected in the serum of patients with localized prostate cancer. In serum from metastatic prostate cancer cases CpG island hypermethylation was detected at MDR1 in 15 (83.3%), EDNRB in 9 (50%), RAR-beta in 7 (38.9%), GSTP1 in 5 (27.8%) and NEP or RASSF1A in 3 (16.7%). CpG island hypermethylation at CD44, PTGS2 or ESR was not detected in any samples. All histologically normal cases were negative for CpG island hypermethylation. **CONCLUSIONS:** DNA hypermethylation at MDR1 was detected in cases of localized prostate cancer. CpG island hypermethylation at several gene loci was detected in men with advanced disease. No single gene was consistently observed to be hypermethylated in men with hormone refractory disease. These results suggest that the CpG island hypermethylation status of a defined panel of genes may be a useful biomarker in men with hormone refractory prostate cancer.

Bellezza, I., A. Tucci, et al. (2008). "2-Chloroadenosine and human prostate cancer cells." *Anticancer Agents Med Chem* **8**(7): 783-9.

Cladribine, i.e.2-deoxy-Chloroadenosine is currently in use as chemotherapeutic agent in chronic lymphoid malignancies and pediatric acute myelogenous leukemia whereas the structurally related counterpart, 2-Chloroadenosine, has been less studied. Nevertheless, 2-Chloroadenosine has been shown to be capable of inducing apoptosis in several cell lines by acting either via adenosine receptors or via uptake that is followed by metabolic transformations leading to nucleotide analogues, i.e. antimetabolites effective in the treatment of a variety of malignancies. Triphosphate nucleoside analogues show specificity for cell in S-phase, inhibit DNA synthesis and kill the cells by mechanisms still largely unknown. 2-Chloroadenosine, at low micromolar concentration, acts as a metabolic precursor of an S-phase specific nucleoside analogue in human prostate cancer PC3 cells and inhibits DNA synthesis thereby leading to accumulation of cells in the S-phase. However, although responsible for the acquisition of resistance, the adenosine derivative is capable of sensitising the cells to the action of other antineoplastic agents and the ability of nucleoside analogues to trigger cell cycle arrest can be exploited to maximize cytotoxicity in combination with cell

cycle checkpoint disruptors. 2-Chloroadenosine, in combination with Docetaxel, known to improve the survival of hormone-refractory prostate cancer patients, further decreases in vitro PC3 cell proliferation and invasiveness. Moreover, 2-Chloroadenosine is capable of modulating PAR-1 and IL-23 gene expression suggesting a modulation of cancer metastasis and immune system activity. The present review summarizes research performed in our laboratory to propose a novel role for 2-Chloroadenosine as an anticancer agent.

Benitez, D. A., M. A. Hermoso, et al. (2009). "Regulation of cell survival by resveratrol involves inhibition of NF kappa B-regulated gene expression in prostate cancer cells." *Prostate* **69**(10): 1045-54.

**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.

Berezovska, O. P., A. B. Glinskii, et al. (2006). "Essential role for activation of the Polycomb group (PcG) protein chromatin silencing pathway in metastatic prostate cancer." *Cell Cycle* **5**(16): 1886-901.

The Polycomb group (PcG) gene BMI1 is required for the proliferation and self-renewal of normal and leukemic stem cells. Overexpression of Bmi1 oncogene causes neoplastic transformation of

lymphocytes and plays essential role in pathogenesis of myeloid leukemia. Another PcG protein, Ezh2, was implicated in metastatic prostate and breast cancers, suggesting that PcG pathway activation is relevant for epithelial malignancies. Whether an oncogenic role of the BMI1 and PcG pathway activation may be extended beyond the leukemia and may affect progression of solid tumors as well remains unknown. Here we demonstrate that activation of the BMI1 oncogene-associated PcG pathway plays an essential role in metastatic prostate cancer, thus mechanistically linking the pathogenesis of leukemia, self-renewal of stem cells, and prostate cancer metastasis. To characterize the functional status of the PcG pathway in metastatic prostate cancer, we utilized advanced cell- and whole animal-imaging technologies, gene and protein expression profiling, stable siRNA-gene targeting, and tissue microarray (TMA) analysis in relevant experimental and clinical settings. We demonstrate that in multiple experimental models of metastatic prostate cancer both BMI1 and Ezh2 genes are amplified and gene amplification is associated with increased expression of corresponding mRNAs and proteins. For the first time, we provide images of human prostate carcinoma metastasis precursor cells isolated from blood and shown to overexpress both BMI1 and Ezh2 oncoproteins. Consistent with the PcG pathway activation hypothesis, increased BMI1 and Ezh2 expression in metastatic cancer cells is associated with elevated levels of H2AubK119 and H3metK27 histones. Quantitative immunofluorescence colocalization analysis and expression profiling experiments documented increased BMI1 and Ezh2 expression in clinical prostate carcinoma samples and demonstrated that high levels of BMI1 and Ezh2 expression are associated with markedly increased likelihood of therapy failure and disease relapse after radical prostatectomy. Gene-silencing analysis reveals that activation of the PcG pathway is mechanistically linked with highly malignant behavior of human prostate carcinoma cells and is essential for in vivo growth and metastasis of human prostate cancer. We conclude that the results of experimental and clinical analyses indicate the important biological role of the PcG pathway activation in metastatic prostate cancer. Our work suggests that the PcG pathway activation is a common oncogenic event in pathogenesis of metastatic solid tumors and provides justification for development of small molecule inhibitors of the PcG chromatin silencing pathway as a novel therapeutic modality for treatment of metastatic prostate cancer.

Bhuiyan, M. M., Y. Li, et al. (2006). "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* **66**(20): 10064-72.

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.

Biroccio, A. and C. Leonetti (2004). "Telomerase as a new target for the treatment of hormone-refractory prostate cancer." *Endocr Relat Cancer* **11**(3): 407-21.

Prostate cancer is the leading cause of cancer-related deaths in men. Androgen ablation is the mainstay of treatment for advanced prostate cancer. This therapy is very effective in androgen-dependent cancer; however, these cancers eventually become androgen independent, rendering anti-androgen therapy ineffective. The exploration of novel modalities of treatment is therefore essential to

improve the prognosis of this neoplasia. Telomeres are specialized heterochromatin structures that act as protective caps at the ends of chromosomes. Telomere maintenance in the majority of tumor cells is achieved by telomerase, a reverse transcriptase enzyme that catalyzes the synthesis of further telomeric DNA. Telomerase is detected in the majority of prostate cancers, but not in normal or benign prostatic hyperplasia tissue. Moreover, the human telomerase reverse transcriptase (hTERT) gene, the catalytic subunit of telomerase, is regulated by androgens as well as by different oncogenes including Her-2, Ras, c-Myc and Bcl-2, which seem to play an important role in prostate cancer progression. Thus, telomerase may represent a very good candidate for targeted therapy in prostate tumors. To inhibit telomere maintenance by telomerase, approaches that directly target either telomerase and telomeres or the telomerase regulatory mechanisms have been used. Moreover, strategies targeting telomerase-positive cells as a means to directly kill the tumor cells have been tested. This review summarizes the most promising results achieved by anti-telomerase strategy in different solid tumors. Most of the telomerase-associated therapies described here have proved very promising for the treatment of prostate cancer. On the basis of the good results obtained and considering the multigenic defects of human tumors, including prostate cancer, the combination of anti-telomerase strategies with conventional drugs and/or molecules capable of interfering with oncogenic pathways could efficiently improve the response of this neoplasia.

Brambilla, C., M. Folini, et al. (2004). "Oligomer-mediated modulation of hTERT alternative splicing induces telomerase inhibition and cell growth decline in human prostate cancer cells." *Cell Mol Life Sci* **61**(14): 1764-74.

The expression of telomerase in human cells is strictly controlled by multiple mechanisms including transcription and alternative splicing of telomerase reverse transcriptase (hTERT). In this study, we demonstrated the possibility of modulating the hTERT splicing pattern in DU145 human prostate carcinoma cells through the use of 2'-O-methyl-RNA phosphorothioate oligonucleotides targeting the splicing site located between intron 5 and exon 6 in the hTERT pre-mRNA. An 18-h oligonucleotide exposure induced a decrease in the full-length hTERT transcript and a concomitant increase in the alternatively spliced transcripts, which resulted in significant inhibition of telomerase catalytic activity. Moreover, exposure to the R7 oligomer (which induced the most pronounced modulation of the hTERT splicing pattern and the greatest telomerase inhibition) caused a marked reduction in DU145 cell

growth and the induction of apoptosis starting 2 days after treatment. Such data support the concept that down-regulation of hTERT expression can cause short-term effects on tumour cell growth, which are telomere-shortening independent.

Burri, R. J., R. G. Stock, et al. (2008). "Association of single nucleotide polymorphisms in SOD2, XRCC1 and XRCC3 with susceptibility for the development of adverse effects resulting from radiotherapy for prostate cancer." *Radiat Res* **170**(1): 49-59.

The objective of this study was to determine whether an association exists between certain single nucleotide polymorphisms (SNPs), which have previously been linked with adverse normal tissue effects resulting from radiotherapy, and the development of radiation injury resulting from radiotherapy for prostate cancer. A total of 135 consecutive patients with clinically localized prostate cancer and a minimum of 1 year of follow-up who had been treated with radiation therapy, either brachytherapy alone or in combination with external-beam radiotherapy, with or without hormone therapy, were genotyped for SNPs in SOD2, XRCC1 and XRCC3. Three common late tissue toxicities were investigated: late rectal bleeding, urinary morbidity, and erectile dysfunction. Patients with the XRCC1 rs25489 G/A (Arg280His) genotype were more likely to develop erectile dysfunction after irradiation than patients who had the G/G genotype (67% compared to 24%; P=0.048). In addition, patients who had the SOD2 rs4880 T/C (Val16Ala) genotype exhibited a significant increase in grade 2 late rectal bleeding compared to patients who had either the C/C or T/T genotype for this SNP (8% compared to 0%; P=0.02). Finally, patients with the combination of the SOD2 rs4880 C/T genotype and XRCC3 rs861539 T/C (Thr241Met) genotype experienced a significant increase in grade 2 late rectal bleeding compared to patients without this particular genotypic arrangement (14% compared to 1%; P=0.002). These results suggest that SNPs in the SOD2, XRCC1 and XRCC3 genes are associated with the development of late radiation injury in patients treated with radiation therapy for prostate adenocarcinoma.

Carbone, G. M., S. Napoli, et al. (2004). "Triplex DNA-mediated downregulation of Ets2 expression results in growth inhibition and apoptosis in human prostate cancer cells." *Nucleic Acids Res* **32**(14): 4358-67.

Ets2 is a member of the Ets family of transcription factors that in humans comprise 25 distinct members. Various Ets-domain transcription factors have been implicated in cancer development. Ets2 is expressed in prostate and breast cancer cells

and is thought to have a role in promoting growth and survival in these cell types. However, a definitive role and the mechanisms whereby Ets2 acts in cancer cells are still unclear. Structural and functional similarities as well as overlapping DNA binding specificities complicate the identification of the specific roles of the various Ets factors. In this study, we used a triplex-forming oligonucleotide (TFO) to selectively inhibit Ets2 transcription in prostate cancer cells. We had previously shown that the Ets2-targeting TFO, which was directed to a unique purine-rich sequence critical for Ets2 promoter activity, acted with a high degree of sequence-specificity and target selectivity. TFO-mediated downregulation of Ets2 in prostate cancer cells induced important phenotypic changes, including inhibition of anchorage-dependent and anchorage-independent growth, cell cycle alterations and induction of apoptotic cell death. Expression of Ets2 under the control of a heterologous promoter abolished the anti-proliferative effects of the TFO in both short- and long-term assays, suggesting that these effects were a direct result of downregulation of Ets2 transcription and confirming target selectivity of the TFO. Furthermore, normal human fibroblasts, which expressed low levels of Ets2, were not affected by the Ets2-targeting TFO. Downregulation of Ets2 in prostate cancer cells was associated with reduced levels of the anti-apoptotic protein bcl-x(L) and growth regulatory factors cyclin D1 and c-myc. These data revealed a specific role of this transcription factor in promoting growth and survival of prostate cancer cells. Furthermore, the activity and selectivity of the Ets2-targeting TFO suggest that it might represent a valid approach to prostate cancer therapy.

Carey, A. M., R. Pramanik, et al. (2007). "Ras-MEK-ERK signaling cascade regulates androgen receptor element-inducible gene transcription and DNA synthesis in prostate cancer cells." *Int J Cancer* **121**(3): 520-7.

Treatment of prostate cancer (CaP) patients frequently involves androgen ablation, but resistance often develops and androgen-insensitive tumors emerge. The molecular basis for the development of refractory CaP that grows in an androgen-independent manner is poorly understood, but alterations in growth factor signaling pathways are likely to be involved. We examined the growth factor modulation of androgen-receptor element (ARE)-inducible luciferase reporter gene activity and consequent DNA synthesis as a measure of proliferative growth in androgen-dependent LNCaP or androgen-independent PC3 or DU145 CaP cells. The synthetic androgen R1881 stimulated ARE-inducible reporter gene activity and prostate-specific antigen expression in LNCaP cells and the MEK/ERK inhibitor U0126 or the anti-

androgen bicalutamide (casodex) prevented both of these responses. Activated V12-Ha-Ras expression in LNCaP cells also stimulated ARE-inducible gene transcription, and U0126 or the farnesyltransferase inhibitor FTI-277 but not bicalutamide blocked this. ARE-inducible reporter gene activity was elevated already in PC3 cells, and ERK was constitutively activated in serum-starved LNCaP or DU145 cells. U0126 inhibited each of these responses and also inhibited DNA synthesis in all 3 CaP cell lines. These results demonstrate that chronic stimulation of the Ras-MEK-ERK signaling pathway can sustain ARE-inducible gene transcription and growth of CaP cells, and suggests that components of this pathway may offer targets for cancer therapy.

Carey, J. L., L. M. Sasur, et al. (2004). "Mutually antagonistic effects of androgen and activin in the regulation of prostate cancer cell growth." *Mol Endocrinol* **18**(3): 696-707.

Activin, a member of the TGFbeta superfamily, is expressed in the prostate and inhibits growth. We demonstrate that the effects of activin and androgen on regulation of prostate cancer cell growth are mutually antagonistic. In the absence of androgen, activin induced apoptosis in the androgen-dependent human prostate cancer cell line LNCaP, an effect suppressed by androgen administration. Although activin by itself did not alter the cell cycle distribution, it potently suppressed androgen-induced progression of cells into S-phase of the cell cycle and thus inhibited androgen-stimulated growth of LNCaP cells. Expression changes in cell cycle regulatory proteins such as Rb, E2F-1, and p27 demonstrated a strong correlation with the mutually antagonistic growth regulatory effects of activin and androgen. The inhibitory effect of activin on growth was independent of serine, serine, valine, serine motif phosphorylation of Smad3. Despite their antagonistic effect on growth, activin and androgen costimulated the expression of prostate-specific antigen through a Smad3-mediated mechanism. These observations indicate the existence of a complex cross talk between activin and androgen signaling in regulation of gene expression and growth of the prostate.

Cesaretti, J. A., R. G. Stock, et al. (2005). "ATM sequence variants are predictive of adverse radiotherapy response among patients treated for prostate cancer." *Int J Radiat Oncol Biol Phys* **61**(1): 196-202.

**PURPOSE:** To examine whether the presence of sequence variants in the ATM (mutated in ataxia-telangiectasia) gene is predictive for the development of radiation-induced adverse responses resulting from (125)I prostate brachytherapy for early-stage prostate

cancer. **MATERIALS AND METHODS:** Thirty-seven patients with a minimum of 1-year follow-up who underwent (125)I prostate brachytherapy of early-stage prostate cancer were screened for DNA sequence variations in all 62 coding exons of the ATM gene using denaturing high-performance liquid chromatography. The clinical course and postimplant dosimetry for each genetically characterized patient were obtained from a database of 2,020 patients implanted at Mount Sinai Hospital after 1990. **RESULTS:** Twenty-one ATM sequence alterations located within exons, or in short intronic regions flanking each exon, were found in 16 of the 37 patients screened. For this group, 10 of 16 (63%) exhibited at least one form of adverse response. In contrast, of the 21 patients who did not harbor an ATM sequence variation, only 3 of 21 (14%) manifested radiation-induced adverse responses ( $p = 0.005$ ). Nine of the patients with sequence alterations specifically possessed missense mutations, which encode for amino acid substitutions and are therefore more likely to possess functional importance. For this group, 7 of 9 (78%) exhibited at least one form of adverse response. In contrast, of the 28 patients who did not have a missense alteration, only 6 of 28 (21%) manifested any form of adverse response to the radiotherapy ( $p = 0.004$ ). Of the patients with missense variants, 5 of 9 (56%) exhibited late rectal bleeding vs. 1 of 28 (4%) without such alterations ( $p = 0.002$ ). Of those patients who were at risk for developing erectile dysfunction, 5 of 8 (63%) patients with missense mutations developed prospectively evaluated erectile dysfunction as opposed to 2 of 20 (10%) without these sequence alterations ( $p = 0.009$ ). **CONCLUSIONS:** Possession of sequence variants in the ATM gene, particularly those that encode for an amino acid substitution, is predictive for the development of adverse radiotherapy responses among patients treated with (125)I prostate brachytherapy.

Chen, T. C., M. F. Holick, et al. (2003). "Evaluation of vitamin D analogs as therapeutic agents for prostate cancer." *Recent Results Cancer Res* **164**: 273-88.

Prostate cancer cells contain specific receptors (VDR) for 1 $\alpha$ ,25-dihydroxyvitamin D (1 $\alpha$ ,25(OH)<sub>2</sub>D), which is known to inhibit the proliferation and invasiveness of these cells. These findings support the use of 1 $\alpha$ ,25(OH)<sub>2</sub>D for prostate cancer therapy. However, because 1 $\alpha$ ,25(OH)<sub>2</sub>D can cause hypercalcemia, analogs of 1 $\alpha$ ,25(OH)<sub>2</sub>D that are less calcemic but which exhibit potent antiproliferative activity would be attractive as therapeutic agents. We studied four vitamin D compounds: 25-hydroxyvitaminD3 [25(OH)D<sub>3</sub>], which is converted to

1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in prostate cells, and three analogs of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>: EB1089, 19-nor-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> and hexafluoro-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (F6-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>). 19-nor-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> has been shown to be less calcemic than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in clinical trials. F6-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> has been shown to be 100-fold more active than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> and to be longer-lasting in inhibiting keratinocyte proliferation in vitro. EB1089 has been shown to be less calcemic than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in rats implanted with Leydig cell tumors. For 25(OH)<sub>2</sub>D<sub>3</sub>, 19-nor-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> and F6-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>, we studied the in vitro effects and compared their activity to 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> on cellular proliferation by 3H-thymidine incorporation assay. In addition, we studied transactivation of the VDR in the presence of 25(OH)<sub>2</sub>D<sub>3</sub> and 19-nor-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> in prostate cells. For EB1089, we compared its inhibition of prostate cancer metastasis to that induced by 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in vivo in the rat Dunning MAT LyLu prostate cancer model. We found that 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> and 19-nor-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> caused similar dose-dependent inhibition in 3H-thymidine incorporation into DNA in prostate cells and behaved similarly in the CAT reporter gene transactivation assay in PC-3/VDR cells. F6-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> is 10- to 50-fold more active than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in 3H-thymidine incorporation into DNA in the primary cultured prostate cells. Likewise, 25(OH)<sub>2</sub>D<sub>3</sub> had comparable antiproliferative activity to 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>. In the rat model, tumor volumes and the number of metastases in the lungs were significantly reduced by both 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (10.4  $\pm$  2.81 tumor foci) and EB1089 (7.7  $\pm$  1.29 tumor foci) compared to controls (22.7  $\pm$  1.98 tumor foci). Although serum calcium levels were significantly elevated in both 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>- and EB1089-treated rats, EB1089 was significantly less calcemic than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (12.59  $\pm$  0.21 mg/dl versus 14.47  $\pm$  0.46 mg/dl; 1 microg/kg; p < 0.001). In conclusion, our data indicate that 25(OH)<sub>2</sub>D<sub>3</sub> and the three 1 $\alpha$ ,25(OH)<sub>2</sub>D analogs represent two different solutions to the problem of hypercalcemia associated with vitamin D-based prostate cancer therapies: 25(OH)<sub>2</sub>D<sub>3</sub> requires the presence of 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase, whereas 19-nor-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub>, F6-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> and EB1089 do not. These compounds may be good candidates for human clinical trials in prostate cancer.

Chi, K. N. and M. E. Gleave (2004). "Antisense approaches in prostate cancer." *Expert Opin Biol Ther* 4(6): 927-36.

Patients with hormone refractory prostate cancer have limited treatment options and new

therapies are urgently needed. Advances in the understanding of the molecular mechanisms implicated in prostate cancer progression have identified many potential therapeutic gene targets that are involved in apoptosis, growth factors, cell signalling and the androgen receptor (AR). Antisense oligonucleotides are short sequences of synthetic modified DNA that are designed to be complementary to a selected gene's mRNA and thereby specifically inhibit expression of that gene. The antisense approach continues to hold promise as a therapeutic modality to target genes involved in cancer progression, especially those in which the gene products are not amenable to small molecule inhibition or antibodies. The current status and future direction of a number of antisense oligonucleotides targeting several genes, including BCL-2, BCL-XL, clusterin, the inhibitors of apoptosis (IAP) family, MDM2, protein kinase C- $\alpha$ , c-raf, insulin-like growth factor binding proteins and the AR, that have potential clinical use in prostate cancer are reviewed.

Cho, K. S., J. S. Lee, et al. (2008). "Gene amplification and mutation analysis of epidermal growth factor receptor in hormone refractory prostate cancer." *Prostate* 68(8): 803-8.

**BACKGROUND:** Amplification and mutation of the epidermal growth factor receptor (EGFR) and Her-2 genes were analyzed in both hormone sensitive and hormone refractory prostate cancer (HRPC). **METHODS:** Gene amplifications of EGFR and Her-2 were analyzed by fluorescence in situ hybridization (FISH) with direct sequencing. Studies were performed on a total of 10 patients; tissues were sampled at the time of initial diagnosis and after the conversion to HRPC (a total of 20 tissue samples). Direct sequencing was performed on exons 18-24 of EGFR and exons 19 and 20 of Her-2. Amplification and mutation were compared with clinicopathologic features. **RESULTS:** Gene amplification of EGFR was observed in 6 (30%) out of 20 samples. A total of six EGFR mutations in exons 18 and 19 were detected in three pairs of tissues (three patients). One patient, with hormone refractory status, had a novel deletion mutation in EGFR exon 19. EGFR mutations were associated with the acinar type of prostate cancer but were not associated with the ductal type. No significant correlation was found between mutation change and hormone sensitive or refractory status. However, the time to convert to HRPC was significantly shorter in the patients with a mutation in the EGFR gene (P = 0.017). There were no Her-2 gene amplifications or mutations found in any of the samples. **CONCLUSIONS:** EGFR gene mutation and amplification occurred frequently in advanced prostate cancer cases. EGFR mutations do

not appear to play a significant role in the hormone refractory pathway but are associated with prognosis.

Choi, Y. H., E. O. Im, et al. (2003). "Apoptosis and modulation of cell cycle control by synthetic derivatives of ursodeoxycholic acid and chenodeoxycholic acid in human prostate cancer cells." *Cancer Lett* **199**(2): 157-67.

The effects of synthetic derivatives of ursodeoxycholic acid (UDCA), HS-1183, and chenodeoxycholic acid (CDCA), HS-1199 and HS-1200, on the proliferation of human prostate carcinoma PC-3 cells were investigated. Whereas CDCA and UDCA had no effects on the growth of cells in a concentration range we have tested, HS-1199 and HS-1200 completely inhibited the cell proliferation, and HS-1183 showed a weak inhibitory activity. This proliferation-inhibitory effect of the synthetic bile acid derivatives was due to the induction of apoptosis, which was confirmed by observing DNA fragmentation, chromatin condensation and cleavage of PARP. Flow cytometric analysis also revealed that the synthetic bile acid derivatives arrested the cell cycle progression at the G1 phase, which effects were associated with inhibition of phosphorylation of pRB and enhanced binding of pRB and E2F-1. They also suppressed Cdk2 and cyclin E-dependent kinase activities without changes of their expressions. Furthermore, the synthetic bile acids increased the levels of Cdk inhibitor, p21WAF1/CIP1, expression and activated the reporter construct of p21WAF1/CIP1 promoter in p53-independent manner, and p21WAF1/CIP1 proteins induced by the synthetic bile acid derivatives were associated with Cdk2 and proliferating cell nuclear antigen. These distinctive features suggest that it is possible to create the new drugs useful for cancer therapy from the synthetic bile acid derivatives as lead compounds.

Christensen, M. J., E. T. Nartey, et al. (2007). "High selenium reduces NF-kappaB-regulated gene expression in uninduced human prostate cancer cells." *Nutr Cancer* **58**(2): 197-204.

Nuclear factor kappa B (NF-kappaB) induces expression of antiapoptotic and pro-inflammatory genes and is constitutively activated in prostate cancer. We tested the hypothesis that a biologically and physiologically relevant form and concentration of selenium (Se) may alter NF-kappa B activation in early prostate cancer cells in the absence of exogenously added inducers of the NF-kappaB pathway. LNCaP cells were cultured in medium without added tumor necrosis factor alpha or lipopolysaccharide but with methylseleninic acid added to provide final concentrations of Se of 30 nM-7.6 microM. Compared to 50 nM Se, treatment with

7.6 microM Se virtually eliminated NF-kappaB binding to its DNA response element and reduced transcription rates and mRNA levels by half for NF-kappaB-regulated genes. There were no differences due to Se in tyrosine phosphorylation, inhibitor of kappa B alpha (I kappa B alpha) levels, or NF-kappaB translocation from cytosol to nucleus. The observation in these basal, unstimulated cells of altered NF-kappaB binding to DNA in the absence of effects on the NF-kappaB activation pathway suggests an interaction of Se with the NF-kappaB protein or an effect on recruitment of NF-kappaB coactivators or corepressors. Inhibition of transcription factor binding and anti-apoptotic gene expression may be one mechanism for the chemopreventive effects of Se against prostate cancer.

Chung, L. W., C. L. Hsieh, et al. (2003). "New targets for therapy in prostate cancer: modulation of stromal-epithelial interactions." *Urology* **62**(5 Suppl 1): 44-54.

Changes in genomic and phenotypic expression of progressing prostate tumors and their stroma occur in a dynamic fashion based on bidirectional signaling from stromal-epithelial interactions. These interactions may underlie the ability of prostate cancer cells to survive and proliferate in the prostate and bone. By investigating the phenotypic and genotypic changes of stromal cells adjacent to cancer cells and the reciprocal changes of cancer cells, novel molecular markers may be developed to diagnose cancer earlier before pathologic appearance of cancer cells at the primary site. Attacking epithelial and stromal elements together is a unique approach to both localized and metastatic prostate cancer therapy. Co-targeting both tumor cells and stroma requires identifying a reliable tumor and tissue-specific cis-DNA element, such as osteocalcin (OC) promoter. OC expression is elevated in prostate tumor cells and in prostate and bone stromal cells interdigitating with both localized and metastatic prostate epithelium. We have previously designed an adenovirus-based therapeutic gene vehicle and demonstrated that a replication-competent adenoviral vector (Ad vector) is highly efficient in blocking the growth of cancer cells in culture, including cells without androgen receptor as well as cells that do or do not make prostate-specific antigen. In vivo, intravenous administration of an Ad-OC vector was effective against preexisting human prostate cancer subcutaneous and bone xenografts. The addition of vitamin D(3) enhanced further viral replication at target sites. Co-targeting tumor cells and stroma using systemic Ad vector is a viable and promising option for treatment of both localized and metastatic prostate cancer.

Chuu, C. P., R. A. Hiipakka, et al. (2006). "Inhibition of tumor growth and progression of LNCaP prostate cancer cells in athymic mice by androgen and liver X receptor agonist." *Cancer Res* **66**(13): 6482-6.

Androgen-dependent human LNCaP 104-S tumor xenografts progressed to androgen-independent relapsed tumors (104-Rrel) in athymic mice after castration. The growth of 104-Rrel tumors was suppressed by testosterone. However, 104-Rrel tumors adapted to androgen and regrew as androgen-stimulated 104-Radp tumors. Androgen receptor expression in tumors and serum prostate-specific antigen increased during progression from 104-S to 104-Rrel but decreased during transition from 104-Rrel to 104-Radp. Expression of genes related to liver X receptor (LXR) signaling changed during progression. LXRalpha, LXRbeta, ATP-binding cassette transporter A1 (ABCA1), and sterol 27-hydroxylase decreased during progression from 104-S to 104-Rrel. These coordinated changes in LXR signaling in mice during progression are consistent with our previous findings that reduction of ABCA1 gene expression stimulates proliferation of LNCaP cells. To test if attenuation of LXR signaling may enhance prostate cancer progression from an androgen-dependent state to an androgen-independent state, castrated mice carrying 104-S tumors were given the synthetic LXR agonist T0901317 by gavage. T0901317 delayed progression from 104-S to 104-Rrel tumors. Based on our in vivo model, androgen is beneficial for the treatment of androgen-independent androgen receptor-rich prostate cancer and modulation of LXR signaling may be a potentially useful therapy for prostate cancer.

Culig, Z., B. Comuzzi, et al. (2004). "Expression and function of androgen receptor coactivators in prostate cancer." *J Steroid Biochem Mol Biol* **92**(4): 265-71.

Human androgen receptor (AR) associates with coactivator or corepressor proteins that modulate its activation in the presence of ligand. Early studies on AR coactivators in carcinoma of the prostate were hampered because of lack of respective antibodies. Investigations at mRNA level revealed that most benign and malignant prostate cells express common coactivators. AR coactivators SRC-1 and TIF-2 are up-regulated in tissue specimens obtained from patients who failed prostate cancer endocrine therapy. Increased expression of these coactivators is associated with enhanced activation of the AR by the adrenal androgen dehydroepiandrosterone. Similar association between AR coactivator expression and high prostate cancer grade and stage was reported for RAC-3 (SRC-3). The transcriptional integrator CBP was detected in clinical specimens representing organ-confined prostate cancer, lymph node metastases and

tumour cell lines. Agonistic effect of the nonsteroidal antiandrogen hydroxyflutamide was strongly potentiated in prostate cells transfected with CBP cDNA. A functional homologue of CBP, p300, is implicated in ligand-independent AR activation by interleukin-6. The AR coactivator Tip60, which is up-regulated by androgen ablation, is recruited to the promoter of the prostate-specific antigen gene in the absence of androgen in androgen-independent prostate cancer sublines. It was proposed that the cofactor ARA70 is a specific enhancer of AR action. However, research from other laboratories has demonstrated interaction between ARA70 and other steroid receptors. Although in some cases dominant-negative coactivator mutants inhibited proliferation of prostate cancer cells in vitro, confirmation from in vivo tumour models is missing. In summary, several abnormalities in AR coactivator expression and function are associated with prostate cancer progression.

Curigliano, G., G. Pelosi, et al. (2007). "Absence of epidermal growth factor receptor gene mutations in patients with hormone refractory prostate cancer not responding to gefitinib." *Prostate* **67**(6): 603-4.

**BACKGROUND:** Mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene in human cancers are associated with increased sensitivity to anilinoquinazoline EGFR inhibitors. To our knowledge no data have been reported on EGFR gene mutations in hormone refractory prostate cancer (HRPC). **METHODS:** Between March 2003 and December 2004, 23 patients with HRPC received 250 mg oral gefitinib daily in addition to antiandrogen plus luteinizing hormone-releasing hormone (LH-RH) analog for at least 2 months or until disease progression. Patients with unresected prostate cancer prospectively underwent trans-rectal biopsy of primary tumor (before starting gefitinib treatment). **RESULTS:** None of the patients demonstrated PSA or objective response to gefitinib. We sequenced exons 18-21 of the EGFR TK domain from genomic DNA isolated from 8 HRPC patients. No patient showed EGFR TK domain mutations. **CONCLUSIONS:** Our results show EGFR mutations did not occur in these patients suggesting that gefitinib is unlikely to be effective in patients with tumors not harboring specific EGFR TK domain.

Damaraju, S., D. Murray, et al. (2006). "Association of DNA repair and steroid metabolism gene polymorphisms with clinical late toxicity in patients treated with conformal radiotherapy for prostate cancer." *Clin Cancer Res* **12**(8): 2545-54.

**OBJECTIVE:** To explore the possible relationship between single nucleotide polymorphisms

(SNP) in candidate genes encoding DNA damage recognition/repair/response and steroid metabolism proteins with respect to clinical radiation toxicity in a retrospective cohort of patients previously treated with three-dimensional conformal radiotherapy (3-DCRT) for prostate cancer. EXPERIMENTAL DESIGN: One hundred twenty-four patients with prostate cancer underwent 3-DCRT at our institution between September 1996 and December 2000. Of these, 83 consented for follow-up of blood sampling and SNP analysis. Twenty-eight patients were documented as having experienced grade  $\geq 2$  late bladder or rectal toxicity (scoring system of Radiation Therapy Oncology Group) on at least one follow-up visit. We analyzed 49 SNPs in BRCA1, BRCA2, ESR1, XRCC1, XRCC2, XRCC3, NBN, RAD51, RAD52, LIG4, ATM, BCL2, TGFB1, MSH6, ERCC2, XPF, NR3C1, CYP1A1, CYP2C9, CYP2C19, CYP3A5, CYP2D6, CYP11B2, and CYP17A1 genes using the Pyrosequencing technique. RESULTS: Significant univariate associations with late rectal or bladder toxicity (grade  $\geq 2$ ) were found for XRCC3 (A>G 5' untranslated region NT 4541), LIG4 (T>C Asp(568)Asp), MLH1 (C>T, Val(219)Ile), CYP2D6\*4 (G>A splicing defect), mean rectal and bladder dose, dose to 30% of rectum or bladder, and age <60 years. On Cox multivariate analysis, significant associations with toxicity were found for LIG4 (T>C, Asp(568)Asp), ERCC2 (G>A, Asp(711)Asp), CYP2D6\*4 (G>A, splicing defect), mean bladder dose >60 Gy, and dose to 30% of rectal volume >75 Gy. CONCLUSIONS: In this study, we identified SNPs in LIG4, ERCC2, and CYP2D6 genes as putative markers to predict individuals at risk for complications arising from radiation therapy in prostate cancer.

Dandekar, D. S. and B. L. Lokeshwar (2004). "Inhibition of cyclooxygenase (COX)-2 expression by Tet-inducible COX-2 antisense cDNA in hormone-refractory prostate cancer significantly slows tumor growth and improves efficacy of chemotherapeutic drugs." *Clin Cancer Res* **10**(23): 8037-47.

**PURPOSE:** Overexpression of the proinflammatory enzyme cyclooxygenase (COX)-2 is associated with the progression of various malignancies; the role of COX-2 in prostate cancer is less clear. The significance of COX-2 in prostate cancer growth and response to chemotherapy was investigated in an androgen-refractory prostate cancer cell line using a Tet-inducible antisense COX-2 expression system. EXPERIMENTAL DESIGN: An antisense COX-2 cDNA construct under the control of a doxycycline-inducible promoter was transfected into a prostate cancer cell line, PC-3ML. Modulations of cell growth, apoptosis, and chemosensitivity in the

presence or absence of doxycycline were analyzed. Tumor incidence, growth rate, and response to two cytotoxic drugs, COL-3 [chemically modified tetracycline-3-(6-demethyl-6-deoxy-4-dedimethylamino-tetracycline)] and Taxotere (docetaxel), were investigated in tumor xenografts. Apoptotic incidences and tumor microvessel density in tumors were determined by immunohistochemistry. RESULTS: Conditional suppression of COX-2 in PC-3ML caused reduced cell proliferation, decreased levels of phosphorylated AKT, G(0)-G(1) arrest, and increased apoptosis and caspase-3 activity. Suppression of COX-2 increased Bax protein and decreased Bcl-x(L) protein in vitro. COX-2 antisense-expressing PC-3ML tumors showed a 57% growth delay compared with nontransfected or vector controls. Oral administration of COL-3 (40 mg/kg, oral gavage) or Taxotere (2.3 mg/kg, intraperitoneally; 3x per week) in tumor-bearing mice further slowed tumor growth (65% and approximately 94%, respectively). Compared with the control group, the occurrence of apoptosis in antisense COX-2 tumors was eight times higher, and the tumor microvessel density was three times lower. CONCLUSIONS: These results provide direct evidence that constitutive expression of COX-2 in prostate cancer has both angiogenic and cytoprotective functions. Suppression of tumor cell COX-2 is sufficient to enhance chemotherapy response in prostate cancer.

Dasgupta, S., L. M. Wasson, et al. (2009). "Novel gene C17orf37 in 17q12 amplicon promotes migration and invasion of prostate cancer cells." *Oncogene* **28**(32): 2860-72.

C17orf37/MGC14832, a novel gene located on human chromosome 17q12 in the ERBB2 amplicon, is abundantly expressed in breast cancer. C17orf37 expression has been reported to positively correlate with grade and stage of cancer progression; however the functional significance of C17orf37 overexpression in cancer biology is not known. Here, we show that C17orf37 is highly expressed in prostate cancer cell lines and tumors, compared to minimal expression in normal prostate cells and tissues. Cellular localization studies by confocal and total internal reflection fluorescence microscopy revealed predominant expression of C17orf37 in the cytosol with intense staining in the membrane of prostate cancer cells. RNA-interference-mediated downregulation of C17orf37 resulted in decreased migration and invasion of DU-145 prostate cancer cells, and suppressed the DNA-binding activity of nuclear factor-kappaB (NF-kappaB) transcription factor resulting in reduced expression of downstream target genes matrix metalloproteinase 9, urokinase plasminogen activator and vascular endothelial growth

factor. Phosphorylation of PKB/Akt was also reduced upon C17orf37 downregulation, suggesting C17orf37 acts as a signaling molecule that increases invasive potential of prostate cancer cells by NF-kappaB-mediated downstream target genes. Our data strongly suggest C17orf37 overexpression in prostate cancer functionally enhances migration and invasion of tumor cells, and is an important target for cancer therapy.

Davis, D. A., S. H. Sarkar, et al. (2006). "Increased therapeutic potential of an experimental anti-mitotic inhibitor SB715992 by genistein in PC-3 human prostate cancer cell line." *BMC Cancer* 6: 22.

**BACKGROUND:** Kinesin spindle proteins (KSP) are motor proteins that play an essential role in mitotic spindle formation. HsEg5, a KSP, is responsible for the formation of the bipolar spindle, which is critical for proper cell division during mitosis. The function of HsEg5 provides a novel target for the manipulation of the cell cycle and the induction of apoptosis. SB715992, an experimental KSP inhibitor, has been shown to perturb bipolar spindle formation, thus making it an excellent candidate for anti-cancer agent. Our major objective was a) to investigate the cell growth inhibitory effects of SB715992 on PC-3 human prostate cancer cell line, b) to investigate whether the growth inhibitory effects of SB715992 could be enhanced when combined with genistein, a naturally occurring isoflavone and, c) to determine gene expression profile to establish molecular mechanism of action of SB715992. **METHODS:** PC-3 cells were treated with varying concentration of SB715992, 30 microM of genistein, and SB715992 plus 30 microM of genistein. After treatments, PC-3 cells were assayed for cell proliferation, induction of apoptosis, and alteration in gene and protein expression using cell inhibition assay, apoptosis assay, microarray analysis, real-time RT-PCR, and Western Blot analysis. **RESULTS:** SB715992 inhibited cell proliferation and induced apoptosis in PC-3 cells. SB715992 was found to regulate the expression of genes related to the control of cell proliferation, cell cycle, cell signaling pathways, and apoptosis. In addition, our results showed that combination treatment with SB715992 and genistein caused significantly greater cell growth inhibition and induction of apoptosis compared to the effects of either agent alone. **CONCLUSION:** Our results clearly show that SB715992 is a potent anti-tumor agent whose therapeutic effects could be enhanced by genistein. Hence, we believe that SB715992 could be a novel agent for the treatment of prostate cancer with greater success when combined with a non-toxic natural agent like genistein.

Davis, J. N., K. J. Wojno, et al. (2006). "Elevated E2F1 inhibits transcription of the androgen receptor in metastatic hormone-resistant prostate cancer." *Cancer Res* 66(24): 11897-906.

Activation of E2F transcription factors, through disruption of the retinoblastoma (Rb) tumor-suppressor gene, is a key event in the development of many human cancers. Previously, we showed that homozygous deletion of Rb in a prostate tissue recombination model exhibits increased E2F activity, activation of E2F-target genes, and increased susceptibility to hormonal carcinogenesis. In this study, we examined the expression of E2F1 in 667 prostate tissue cores and compared it with the expression of the androgen receptor (AR), a marker of prostate epithelial differentiation, using tissue microarray analysis. We show that E2F1 expression is low in benign and localized prostate cancer, modestly elevated in metastatic lymph nodes from hormone-naive patients, and significantly elevated in metastatic tissues from hormone-resistant prostate cancer patients ( $P = 0.0006$ ). In contrast, strong AR expression was detected in benign prostate (83%), localized prostate cancer (100%), and lymph node metastasis (80%), but decreased to 40% in metastatic hormone-resistant prostate cancer ( $P = 0.004$ ). Semiquantitative reverse transcription-PCR analysis showed elevated E2F1 mRNA levels and increased levels of the E2F-target genes dihydrofolate reductase and proliferating cell nuclear antigen in metastatic hormone-independent prostate cancer cases compared with benign tissues. To identify a role of E2F1 in hormone-independent prostate cancer, we examined whether E2F1 can regulate AR expression. We show that exogenous expression of E2F1 significantly inhibited AR mRNA and AR protein levels in prostate epithelial cells. E2F1 also inhibited an AR promoter-luciferase construct that was dependent on the transactivation domain of E2F1. Furthermore, using chromatin immunoprecipitation assays, we show that E2F1 and the pocket protein family members p107 and p130 bind to the AR promoter *in vivo*. Taken together, these results show that elevated E2F1, through its ability to repress AR transcription, may contribute to the progression of hormone-independent prostate cancer.

Davis-Searles, P. R., Y. Nakanishi, et al. (2005). "Milk thistle and prostate cancer: differential effects of pure flavonolignans from *Silybum marianum* on antiproliferative end points in human prostate carcinoma cells." *Cancer Res* 65(10): 4448-57.

Extracts from the seeds of milk thistle, *Silybum marianum*, are known commonly as silibinin and silymarin and possess anticancer actions on human prostate carcinoma *in vitro* and *in vivo*. Seven

distinct flavonolignan compounds and a flavonoid have been isolated from commercial silymarin extracts. Most notably, two pairs of diastereomers, silybin A and silybin B and isosilybin A and isosilybin B, are among these compounds. In contrast, silibinin is composed only of a 1:1 mixture of silybin A and silybin B. With these isomers now isolated in quantities sufficient for biological studies, each pure compound was assessed for antiproliferative activities against LNCaP, DU145, and PC3 human prostate carcinoma cell lines. Isosilybin B was the most consistently potent suppressor of cell growth relative to either the other pure constituents or the commercial extracts. Isosilybin A and isosilybin B were also the most effective suppressors of prostate-specific antigen secretion by androgen-dependent LNCaP cells. Silymarin and silibinin were shown for the first time to suppress the activity of the DNA topoisomerase II $\alpha$  gene promoter in DU145 cells and, among the pure compounds, isosilybin B was again the most effective. These findings are significant in that isosilybin B composes no more than 5% of silymarin and is absent from silibinin. Whereas several other more abundant flavonolignans do ultimately influence the same end points at higher exposure concentrations, these findings are suggestive that extracts enriched for isosilybin B, or isosilybin B alone, might possess improved potency in prostate cancer prevention and treatment.

Dehm, S. M. and D. J. Tindall (2006). "Ligand-independent androgen receptor activity is activation function-2-independent and resistant to antiandrogens in androgen refractory prostate cancer cells." *J Biol Chem* **281**(38): 27882-93.

Androgen ablation inhibits androgen receptor (AR) activity and is as an effective treatment for advanced prostate cancer (PCa). Invariably, PCa relapses in a form resistant to further hormonal manipulations. Although this stage of the disease is androgen-refractory, or androgen depletion-independent (ADI), most tumors remain AR-dependent through aberrant mechanisms of AR activation. We employed the LNCaP/C4-2 model of PCa progression to study AR activity in androgen-dependent and ADI PCa cells. In this report, we show that the AR is transcriptionally inactive in androgen-dependent LNCaP cells in the absence of androgens. However, in ADI C4-2 cells, the AR displays a high level of constitutive, androgen-independent transcriptional activity. To study the mechanisms of ligand-dependent and ligand-independent AR activation in these AR-expressing cells, we generated a reporter system based on swapping the DNA binding domain of the AR with the DNA binding domain of the yeast Gal4 transcription factor. In androgen-

dependent PCa cells, the well characterized C-terminal AR activation function-2 (AF-2) domain was critical for strong, ligand-dependent activity. Conversely, in ADI PCa cells, constitutive, ligand-independent AR activity was AF-2-independent but instead dependent on N-terminal AR domains. Importantly, the ligand- and AF-2-independent mode of AR activation observed in ADI PCa cells was completely resistant to the antiandrogen, bicalutamide. Our data thus demonstrate that the AR can inappropriately activate transcription in ADI PCa cells via mechanisms that are resistant to castration and AR antagonism, the two modes of androgen ablation used to treat advanced PCa.

Dehm, S. M. and D. J. Tindall (2007). "Androgen receptor structural and functional elements: role and regulation in prostate cancer." *Mol Endocrinol* **21**(12): 2855-63.

The androgen receptor (AR) is a nuclear receptor transcription factor that mediates the cellular actions of androgens, the male sex steroids. Androgen-dependent tissues, such as the prostate, rely on androgen action for their development as well as their maintenance in adulthood. This requirement is exploited during systemic therapy of prostate cancer, which is initially an androgen-dependent disease. Indeed, androgen ablation, which prevents the production or blocks the action of androgens, inhibits prostate cancer growth. Invariably, the disease recurs with a phenotype resistant to further hormonal manipulations. However, this so-called androgen depletion-independent prostate cancer remains dependent on a functional AR for growth. Many studies have focused on the mechanistic and structural basis of AR activation with the important goal of understanding how the AR is activated at this stage of the disease. In this review, we summarize how these studies have revealed important functional domains in the AR protein and have provided initial clues to their role in prostate cancer development and progression. A comprehensive understanding of the role and functional relationships between these AR domains could lead to the development of novel AR-directed therapies for prostate cancer.

Diaw, L., M. Roth, et al. (2005). "Characteristics of a human prostate stromal cell line related to its use in a stromal-epithelial coculture model for the study of cancer chemoprevention." *In Vitro Cell Dev Biol Anim* **41**(5-6): 142-8.

An immortalized human prostate stromal cell line (PS30) was previously established using recombinant retrovirus encoding human papillomavirus 16 gene products. In this study, we further characterize this stromal cell line for its

potential use in a stromal-epithelial coculture model for prostate cancer prevention. Using reverse transcriptase-polymerase chain reaction, enzyme-linked immunosorbent assay, and immunocytochemistry, we examined expression of androgen receptor (AR), vitamin D receptor (VDR), prostate-specific antigen (PSA), transforming growth factor-beta (TGF-beta), and insulin-like growth factors (IGF) families and their receptors, metalloproteinases (MMP) MMP-2 and MMP-9, as well as the cells' ability to respond to the synthetic androgen R1881. The PS30 stromal cells do not express PSA, confirming their stromal origin. They are positive for both AR messenger ribonucleic acid (mRNA) and protein; however, they do not respond to growth stimulation by the synthetic androgen R1881. The PS30 cells express mRNA for VDR, TGF-betas, IGFs and their receptors, as well as the MMPs. Moreover, they produce significant amounts of TGF-beta1, TGF-beta2, IGFBP-3, and MMP-2 proteins. Our observations confirm the use of PS30 for the study of stromal-epithelial interactions in the modulation of prostate carcinogenesis.

Du, Y. F., Y. Shi, et al. (2008). "Establishment of CXCR4-small interfering RNA retrovirus vector driven by human prostate-specific antigen promoter and its biological effects on prostate cancer in vitro and in vivo." *J Cancer Res Clin Oncol* **134**(11): 1255-64.

**PURPOSE:** CXC chemokine receptor-4 (CXCR4) is closely involved in bone metastasis of prostate cancer, and CXCR4 levels are frequently increased in prostate cancer cells and tissues. In the present study, its biological effects on prostate cancer in vitro and in vivo and feasibility to be a therapy target were investigated using a RNA interfering retrovirus vector targeting CXCR4 gene driven by human prostate-specific antigen promoter (pPSA). **METHODS:** We established a pPSA-siCXCR4 retrovirus vector and transfected prostate cancer cell PC-3m, LNCaP and breast cancer cell MCF-7, respectively. The expression of CXCR4 mRNA and protein was detected by RT-PCR and western blot, and the ability of adhesion, migration, invasion of prostate cancer cells was assessed using Transwell chamber. A metastasizing model using BALB/cA mice with human bone tissue implantation was established too, and transfected prostate cancer cells were via caudal vein. Survival time of mice suffering bone metastatic tumor as well as the weight and volume of these tumors were recorded and analyzed. **RESULTS:** The expression of CXCR4 mRNA and protein in androgen-responsive LNCaP cells was blocked by the pPSA-siCXCR4 vector, but it could not work in non androgen-responsive PC-3m cell and

breast cancer cell MCF-7. The results of experiments in vitro also showed that the adhesion, transendothelial migration and invasive ability of transfected LNCaP cells were impaired, while there was no change in PC-3m and MCF-7 cells after transfection. pPSA-siCXCR4 represented a similar inhibitory effect in fluorescent bone metastasis model of LNCaP cells compared with PC-3m cells. **CONCLUSION:** These results suggest that the downstream siRNA controlled by PSA promoter in retrovirus system can express selectively in androgen-responsive prostate cancer in vitro and in vivo, and CXCR4 plays an important role in prostate cancer metastasis. We believe that the pPSA-siCXCR4 retrovirus vector is a potential choice in gene therapy for androgen-responsive prostate cancer.

Duan, H., H. J. Zhang, et al. (2003). "MnSOD up-regulates maspin tumor suppressor gene expression in human breast and prostate cancer cells." *Antioxid Redox Signal* **5**(5): 677-88.

Manganese superoxide dismutase (MnSOD) is an antioxidant enzyme with tumor suppressor activity; however, the molecular mechanisms of MnSOD antitumor effects remain unclear. We hypothesized that MnSOD activity in cancer cells might cause downstream changes in the expression of other tumor suppressor genes. To determine whether maspin, a tumor suppressor gene that inhibits breast cancer cell invasion and metastasis, might be a target of MnSOD, we forced MnSOD expression in several human breast and prostate cancer cell lines by adenovirus-mediated gene transfer and measured maspin mRNA expression. Forced expression of MnSOD caused maspin mRNA to accumulate in a dose-dependent manner in both human breast and prostate cancer cells. Normal p53 was not necessary to mediate the effect of MnSOD because MnSOD up-regulated maspin in cells that harbor wild-type p53 and in cells that harbor mutant p53. Moreover, the effects of MnSOD on maspin were not due to demethylation of the maspin promoter. Analyses of maspin promoter activity, transcriptional run-on, and mRNA stability showed that maspin mRNA stability was the major mechanism for maspin up-regulation by MnSOD. Our findings identify a mechanism underlying MnSOD antitumor effects and provide evidence to support MnSOD as a genetic therapy in the treatment of human breast and prostate cancers.

Dubovsky, J. A. and D. G. McNeel (2007). "Inducible expression of a prostate cancer-testis antigen, SSX-2, following treatment with a DNA methylation inhibitor." *Prostate* **67**(16): 1781-90.

**BACKGROUND:** Active immunotherapies are one approach being developed as novel treatments

for prostate cancer. Critical to the success of these therapies is the identification of appropriate target antigens. We have been seeking to identify immunologically recognized proteins, cancer-testis antigens (CTA) in particular, in patients with prostate cancer that would be rational target antigens. METHODS: Using a previously reported panel of 29 different CTA, we used sera from 98 patients with prostate cancer and 50 healthy male blood donor controls to detect CTA-specific IgG. We then further evaluated the expression of one antigen, SSX-2, in prostate cancer cell lines and tissues. RESULTS: We identified IgG specific for NY-ESO-1, LAGE-1, NFX-2, and SSX-2 in at least 1/98 individuals with prostate cancer. We demonstrated that SSX-2 is a prostate CTA, and its expression is associated with metastatic prostate cancer. In addition, we report that the treatment of at least two human prostate cancer cell lines with the DNA methylation inhibitor 5-aza-2'-deoxycytidine induced the expression of SSX-2. In contrast, treatment of a normal prostate epithelial cell line (RWPE-1) with 5-aza-2'-deoxycytidine did not induce SSX-2 expression. CONCLUSIONS: Our findings suggest that SSX-2 could be further pursued as an immunotherapeutic target in prostate cancer, and that treatment with 5-aza-2'-deoxycytidine could be exploited to modulate antigen expression in combination with immunotherapeutic approaches.

Edwards, J., N. S. Krishna, et al. (2003). "Gene amplifications associated with the development of hormone-resistant prostate cancer." *Clin Cancer Res* **9**(14): 5271-81.

PURPOSE: Hormone resistance remains a significant clinical problem in prostate cancer with few therapeutic options. Research into mechanisms of hormone resistance is essential. EXPERIMENTAL DESIGN: We analyzed 38 paired (prehormone/posthormone resistance) prostate cancer samples using the Vysis GenoSensor. Archival microdissected tumor DNA was extracted, amplified, labeled, and hybridized to Amplionc I DNA microarrays containing 57 oncogenes. RESULTS: Genetic instability increased during progression from hormone-sensitive to hormone-resistant cancer ( $P = 0.008$ ). Amplification frequencies of 15 genes (TERC, MYBL3, HRAS, PI3KCA, JUNB, LAMC2, RAF1, MYC, GARP, SAS, FGFR1, PGY1, MYCL1, MYB, FGR) increased by >10% during hormone escape. Receptor tyrosine kinases were amplified in 73% of cases; this was unrelated to development of hormone resistance. However, downstream receptor tyrosine kinase signaling pathways showed increased amplification rates in resistant tumors for the mitogen-activated protein kinase (FGR/Src-2, HRAS, and RAF1;  $P = 0.005$ ) and phosphatidylinositol 3'-kinase

pathways (FGR/Src-2, PI3K, and Akt;  $P = 0.046$ ). Transcription factors regulated by these pathways were also more frequently amplified after escape (MYC family: 21% before versus 63% after,  $P = 0.027$ ; MYB family: 26% before versus 53% after,  $P = 0.18$ ). CONCLUSIONS: Development of clinical hormone escape is linked to phosphatidylinositol 3'-kinase and mitogen-activated protein kinase pathways. These pathways may function independently of the androgen receptor or via androgen receptor activation by phosphorylation, providing novel therapeutic targets.

Ellinger, J., S. C. Muller, et al. (2008). "Mitochondrial DNA in serum of patients with prostate cancer: a predictor of biochemical recurrence after prostatectomy." *BJU Int* **102**(5): 628-32.

OBJECTIVE: To investigate the role of circulating mitochondrial DNA (mtDNA) in patients with localized prostate cancer, as recent reports show that patients with advanced cancer have increased levels of mtDNA. PATIENTS AND METHODS: DNA was isolated from the serum of 100 patients with prostate cancer and 18 with benign prostate hyperplasia (BPH). A quantitative real-time polymerase chain reaction was used to amplify 79 bp and 230 bp fragments of the mitochondrial 16s-RNA gene, the short fragment representing total mtDNA, including mtDNA truncated by apoptosis, and the long fragment representing mostly mtDNA from other cell death entities. mtDNA integrity was defined as the ratio of long to short mtDNA fragments. RESULTS: The short and long mtDNA levels, and mtDNA integrity, were similar in patients with BPH and cancer ( $P = 0.940, 0.211$  and  $0.441$ , respectively), and were not correlated with clinical or pathological variables, e.g. age, prostate-specific antigen (PSA) level, cT stage, pT stage, seminal vesicle infiltration, lymph node invasion, or Gleason score ( $P = 0.075$  to  $0.961$ ). However, patients with high levels of short mtDNA (>75th percentile) had a greater risk of PSA progression and this variable was the strongest predictor of PSA recurrence in a multivariate Cox analysis ( $P = 0.023$ ; hazard ratio 0.31; 95% confidence interval 0.113-0.851). CONCLUSION: Circulating mtDNA levels did not distinguish between patients with prostate cancer or BPH. However, there was a significant increase in short mtDNA fragments in patients with early PSA recurrence after radical prostatectomy.

Ettinger, S. L., R. Sobel, et al. (2004). "Dysregulation of sterol response element-binding proteins and downstream effectors in prostate cancer during progression to androgen independence." *Cancer Res* **64**(6): 2212-21.

Androgen ablation, the most common therapeutic treatment used for advanced prostate cancer, triggers the apoptotic regression of prostate tumors. However, remissions are temporary because surviving prostate cancer cells adapt to the androgen-deprived environment and form androgen-independent (AI) tumors. We hypothesize that adaptive responses of surviving tumor cells result from dysregulated gene expression of key cell survival pathways. Therefore, we examined temporal alterations to gene expression profiles in prostate cancer during progression to androgen independence at several time points using the LNCaP xenograft tumor model. Two key genes, sterol response element-binding protein (SREBP)-1 and -2 (SREBP-1a,-1c, and -2), were consistently dysregulated. These genes are known to coordinately control the expression of the groups of enzymes responsible for lipid and cholesterol synthesis. Northern blots revealed modest increased expression of SREBP-1a, -1c, and -2 after castration, and at androgen independence (day 21-28), the expression levels of both SREBP-1a and -1c were significantly greater than precastrate levels. Changes in SREBP-1 and -2 protein expression were observed by Western analysis. SREBP-1 68-kDa protein levels were maintained throughout progression, however, SREBP-2 68-kDa protein expression increased after castration and during progression (3-fold). SREBPs are transcriptional regulators of over 20 functionally related enzymes that coordinately control the metabolic pathways of lipogenesis and cholesterol synthesis, some of which were likewise dysregulated during progression to androgen independence. RNA levels of acyl-CoA-binding protein/diazepam-binding inhibitor and fatty acid synthase decreased significantly after castration, and then, during progression, increased to levels greater than or equal to precastrate levels. Expression of farnesyl diphosphate synthase did not decrease after castration but did increase significantly during progression to androgen independence. Levels of SREBP cleavage-activating protein, a regulator of SREBP transcriptional activity, decreased after castration and increased significantly at androgen independence. In clinical prostate cancer specimens from patients with varying grades of disease, the stained tissue sections showed high levels of SREBP-1 protein compared with noncancerous prostate tissue. After hormone withdrawal therapy, tumor levels of SREBP-1 decreased significantly after 6 weeks. AI tumors expressed significantly higher levels of SREBP-1. In summary, the LNCaP xenograft model of human prostate cancer as well as clinical specimens of prostate cancer demonstrated an up-regulation of SREBPs and their downstream effector genes during progression to androgen independence. As the AI

phenotype emerges, enzymes critical for lipogenesis and cholesterol synthesis are activated and likely contribute significantly to cell survival of AI prostate cancer.

Febbo, P. G. (2009). "Genomic approaches to outcome prediction in prostate cancer." *Cancer* **115**(13 Suppl): 3046-57.

Prostate cancer remains a common cause of cancer death in men. Applications of emerging genomic technologies to high-quality prostate cancer models and patient samples in multiple contexts have made significant contributions to our molecular understanding of the development and progression of prostate cancer. Genomic analysis of DNA, RNA, and protein alterations allows for the global assessment of this disease and provides the molecular framework to improve risk classification, outcome prediction, and development of targeted therapies. In this review, the author focused on highlighting recent work in genomics and its role in evaluating molecular modifiers of prostate cancer risk and behavior and the development of predictive models that anticipate the risk of developing prostate cancer, prostate cancer progression, and the response of prostate cancer to therapy. This framework has the exciting potential to be predictive and to provide personalized and individual treatment to the large number of men diagnosed with prostate cancer each year. *Cancer* 2009;115(13 suppl):3046-57. (c) 2009 American Cancer Society.

Flanagan, J. N., S. Zheng, et al. (2009). "Evaluation of 19-nor-2alpha-(3-hydroxypropyl)-1alpha,25-dihydroxyvitamin D3 as a therapeutic agent for androgen-dependent prostate cancer." *Anticancer Res* **29**(9): 3547-53.

The high incidence of prostate cancer and lack of an effective, long-term treatment for metastatic disease highlights the need for more potent non-calcemic vitamin D analogs as potential alternative or combinational prostate cancer therapies. Among the analogs, 19-nor-1alpha,25-dihydroxyvitamin D2 (19-nor-1alpha,25(OH)2D2) known as paricalcitol or Zempler, has less calcemic effects and an equipotential activity as 1alpha,25-dihydroxyvitamin D3 (1alpha,25(OH)2D3) in several in vivo and in vitro systems. It was recently demonstrated that a modified analog of paricalcitol, 19-nor-2alpha-(3-hydroxypropyl)-1alpha,25-dihydroxyvitamin D3 (MART-10) compared to 1alpha,25(OH)2D3 was more effective in inhibiting proliferation of an immortalized normal prostate cell line (PZ-HPV-7) (1,000-fold) and invasion of PC-3 prostate cancer cells (10-fold). In this study, the effects of MART-10 and 1alpha,25(OH)2D3 on proliferation, vitamin D

receptor transactivation, vitamin D-binding protein (DBP) binding, CYP24A1 (24-OHase) substrate hydroxylation kinetics, and induction of CYP24A1 gene expression were compared in an androgen-dependent prostate cancer cell model, LNCaP. The results demonstrated that MART-10 was 1,000-fold more active than 1 $\alpha$ ,25(OH) $_2$ D $_3$  in inhibiting LNCaP cell proliferation. MART-10 was more active than 1 $\alpha$ ,25(OH) $_2$ D $_3$  in up-regulating a vitamin D receptor-responsive Luciferase construct and inducing CYP24A1 gene expression in LNCaP prostate cancer cells. In addition, MART-10 has a lower affinity for DBP and less substrate degradation by CYP24A1 compared to 1 $\alpha$ ,25(OH) $_2$ D $_3$ , indicating that MART-10 has more bioavailability and a longer half-life. Thus, these data suggest that MART-10 may be a potential candidate as a therapeutic agent for prostate cancer, especially for patients who fail in conventional therapies.

Freytag, S. O., H. Stricker, et al. (2003). "Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer." *Cancer Res* **63**(21): 7497-506.

The primary study objective was to determine the safety of intraprostatic administration of a replication-competent, oncolytic adenovirus containing a cytosine deaminase (CD)/herpes simplex virus thymidine kinase (HSV-1 TK) fusion gene concomitant with increasing durations of 5-fluorocytosine and valganciclovir prodrug therapy and conventional-dose three-dimensional conformal radiation therapy (3D-CRT) in patients with newly diagnosed, intermediate- to high-risk prostate cancer. Secondary objectives were to determine the persistence of therapeutic transgene expression in the prostate and to examine early posttreatment response. Fifteen patients in five cohorts received a single intraprostatic injection of 10(12) viral particles of the replication-competent Ad5-CD/TKrep adenovirus on day 1. Two days later, patients were administered 5-fluorocytosine and valganciclovir prodrug therapy for 1 (cohorts 1-3), 2 (cohort 4), or 3 (cohort 5) weeks along with 70-74 Gy 3D-CRT. Sextant needle biopsy of the prostate was obtained at 2 (cohort 1), 3 (cohort 2), and 4 (cohort 3) weeks for determination of the persistence of transgene expression. There were no dose-limiting toxicities and no significant treatment-related adverse events. Ninety-four percent of the adverse events observed were mild to moderate and self-limiting. Acute urinary and gastrointestinal toxicities were similar to those expected for conventional-dose 3D-CRT. Therapeutic transgene

expression was found to persist in the prostate for up to 3 weeks after the adenovirus injection. As expected for patients receiving definitive radiation therapy, all patients experienced significant declines in prostate-specific antigen (PSA). The mean PSA half-life in patients administered more than 1 week of prodrug therapy was significantly shorter than that of patients receiving prodrugs for only 1 week (0.6 versus 2.0 months;  $P < 0.02$ ) and markedly shorter than that reported previously for patients treated with conventional-dose 3D-CRT alone (2.4 months). With a median follow-up of only 9 months, 5 of 10 (50%) patients not treated with androgen-deprivation therapy achieved a serum PSA  $< \text{or} = 0.5$  ng/ml. The results demonstrate that replication-competent adenovirus-mediated double-suicide gene therapy can be combined safely with conventional-dose 3D-CRT in patients with intermediate- to high-risk prostate cancer. The shorter than expected PSA half-life in patients receiving more than 1 week of prodrug therapy may suggest a possible interaction between the oncolytic adenovirus and/or double-suicide gene therapies and radiation therapy.

Fukushima, M., Y. Hattori, et al. (2007). "Combination of non-viral connexin 43 gene therapy and docetaxel inhibits the growth of human prostate cancer in mice." *Int J Oncol* **30**(1): 225-31.

Docetaxel (DTX) is used for the treatment of advanced hormone refractory prostate cancer. Connexin 43 (Cx43) is a tumor suppressor gene, and transfection of the Cx43 gene increases sensitivity to several chemotherapeutic agents. The objective of this study was to evaluate the effectiveness of combination therapy of Cx43-expressing plasmid DNA (pCMV-Cx43) and DTX both in vitro and in vivo using a non-viral vector in human prostate cancer PC-3 cells. Transfection of pCMV-Cx43 into the cells neither inhibited tumor growth nor increased gap junctional intercellular communication; however, combination therapy of pCMV-Cx43 and DTX significantly inhibited cell growth. Forced expression of Cx43 in the cells induced apoptotic cells by down-regulation of Bcl-2 expression and significantly more up-regulation of caspase-3 activity than either treatment alone. The combination of repeated intratumoral injection of pCMV-Cx43 (10 microg/tumor) with non-viral vector and a single intravenous injection of DTX (15 mg/kg) was compared with a repeated injection of Cx43 alone and a single injection of DTX alone on PC-3 tumor xenografts. Significant antitumoral effects were observed in mice receiving combined treatment, compared with DTX alone. The data presented here provide a rational strategy for treating patients with advanced hormone refractory prostate cancer.

Galsky, M. and W. K. Kelly (2003). "The development of differentiation agents for the treatment of prostate cancer." *Semin Oncol* **30**(5): 689-97.

Given their novel mechanisms of action and relatively favorable toxicity profiles, differentiation agents have been the focus of much investigation in the field of oncology. Among the most well studied of these agents in prostate cancer have been the retinoids, vitamin D, peroxisome-proliferator-activated receptor gamma (PPARgamma) ligands, and, most recently, the histone deacetylase (HDAC) inhibitors. While the clinical activity of these agents has been limited, several obstacles to the development of these novel drugs have become apparent. A lack of validated measures of outcome and uncertainty regarding the appropriate disease states in which to test these agents have led to difficulty in trial design. Furthermore, a better understanding of the biologic targets and genes manipulated by these therapies is required such that more potent and selective drugs may be developed. By overcoming these obstacles, the full potential of this therapeutic class may be realized.

Garcia-Hernandez Mde, L., A. Gray, et al. (2007). "In vivo effects of vaccination with six-transmembrane epithelial antigen of the prostate: a candidate antigen for treating prostate cancer." *Cancer Res* **67**(3): 1344-51.

Immunotherapy may provide an alternative treatment for cancer patients, especially when tumors overexpress antigens that can be recognized by immune cells. The identification of markers and therapeutic targets that are up-regulated in prostate cancer has been important to design new potential treatments for prostate cancer. Among them, the recently identified six-transmembrane epithelial antigen of the prostate (STEAP) is considered attractive due to its overexpression in human prostate cancer tissues. Our study constitutes the first assessment of the in vivo effectiveness of STEAP-based vaccination in prophylactic and therapeutic mouse models. Two delivery systems, cDNA delivered by gene gun and Venezuelan equine encephalitis virus-like replicon particles (VRP), both encoding mouse STEAP (mSTEAP) and three vaccination strategies were used. Our results show that mSTEAP-based vaccination was able to induce a specific CD8 T-cell response against a newly defined mSTEAP epitope that prolonged the overall survival rate in tumor-challenged mice very significantly. This was achieved without any development of autoimmunity. Surprisingly, CD4 T cells that produced IFN-gamma, tumor necrosis factor-alpha (TNF-alpha), and interleukin-2 (IL-2) played the main role in tumor rejection in our model as shown by using CD4- and CD8-deficient mice. In addition, the

presence of high IL-12 levels in the tumor environment was associated with a favorable antitumor response. Finally, the therapeutic effect of STEAP vaccination was also assessed and induced a modest but significant delay in growth of established, 31 day old tumors. Taken together, our data suggest that vaccination against mSTEAP is a viable option to delay tumor growth.

Garcia-Hernandez Mde, L., A. Gray, et al. (2008). "Prostate stem cell antigen vaccination induces a long-term protective immune response against prostate cancer in the absence of autoimmunity." *Cancer Res* **68**(3): 861-9.

Prostate stem cell antigen (PSCA) is an attractive antigen to target using therapeutic vaccines because of its overexpression in prostate cancer, especially in metastatic tissues, and its limited expression in other organs. Our studies offer the first evidence that a PSCA-based vaccine can induce long-term protection against prostate cancer development in prostate cancer-prone transgenic adenocarcinoma mouse prostate (TRAMP) mice. Eight-week-old TRAMP mice displaying prostate intraepithelial neoplasia were vaccinated with a heterologous prime/boost strategy consisting of gene gun-delivered PSCA-cDNA followed by Venezuelan equine encephalitis virus replicons encoding PSCA. Our results show the induction of an immune response against a newly defined PSCA epitope that is mediated primarily by CD8 T cells. The prostates of PSCA-vaccinated mice were infiltrated by CD4-positive, CD8-positive, CD11b-positive, and CD11c-positive cells. Vaccination induced MHC class I expression and cytokine production [IFN-gamma, tumor necrosis factor-alpha, interleukin 2 (IL-2), IL-4, and IL-5] within prostate tumors. This tumor microenvironment correlated with low Gleason scores and weak PSCA staining on tumor cells present in hyperplastic zones and in areas that contained focal and well-differentiated adenocarcinomas. PSCA-vaccinated TRAMP mice had a 90% survival rate at 12 months of age. In contrast, all control mice had succumbed to prostate cancer or had heavy tumor loads. Crucially, this long-term protective immune response was not associated with any measurable induction of autoimmunity. The possibility of inducing long-term protection against prostate cancer by vaccination at the earliest signs of its development has the potential to cause a dramatic paradigm shift in the treatment of this disease.

Ghadersohi, A., D. Pan, et al. (2007). "Prostate-derived Ets transcription factor (PDEF) downregulates survivin expression and inhibits breast cancer cell

growth in vitro and xenograft tumor formation in vivo." Breast Cancer Res Treat **102**(1): 19-30.

Previous studies using immunohistochemistry suggest that loss of the expression of the prostate-derived Ets transcription factor (PDEF) is a strong indicator for cancer cell malignancy. However, the underlying mechanism for this has not been well elucidated. We determined the role of PDEF in breast cancer cell growth and tumor formation using a series of experiments including Western blotting, promoter-luciferase reporter assay, RNA interference technology and a mouse xenograft model. We also determined the relationship between PDEF expression in human breast tumor specimen and cancer patient survivability. These studies revealed that PDEF expression is inversely associated with survivin expression and breast cancer cell xenograft tumor formation. PDEF-specific shRNA-mediated silencing of PDEF expression resulted in the upregulation of survivin expression in MCF-7 cells, which was associated with increased cell growth and resistance to drug-induced DNA fragmentation (apoptosis). In contrast, survivin-specific siRNA-mediated silencing of survivin expression decreased MCF-7 cell growth. Ectopic expression of PDEF inhibited both survivin promoter activity and endogenous survivin expression. Importantly, shRNA-mediated silencing of PDEF expression in MCF-7 breast cancer cells enhanced survivin expression and xenograft tumor formation in vivo. Furthermore, loss of PDEF expression in breast cancer tissues tends to be associated with unfavorable prognosis. These studies provide new information for the role of PDEF and survivin in breast cancer cell growth and tumor formation.

Ghosh, R., G. E. Garcia, et al. (2007). "Regulation of Cox-2 by cyclic AMP response element binding protein in prostate cancer: potential role for nexrutine." Neoplasia **9**(11): 893-9.

We recently showed that Nexrutine, a Phellodendron amurense bark extract, suppresses proliferation of prostate cancer cell lines and tumor development in the transgenic adenocarcinoma of mouse prostate (TRAMP) model. Our data also indicate that the anti-proliferative effects of Nexrutine are mediated in part by Akt and Cyclic AMP response element binding protein (CREB). Cyclooxygenase (Cox-2), a pro-inflammatory mediator, is a CREB target that induces prostaglandin E(2) (PGE(2)) and suppresses apoptosis. Treatment of LNCaP cells with Nexrutine reduced tumor necrosis factor alpha-induced enzymatic as well as promoter activities of Cox-2. Nexrutine also reduced the expression and promoter activity of Cox-2 in PC-3 cells that express high constitutive levels of Cox-2.

Deletion analysis coupled with mutational analysis of the Cox-2 promoter identified CRE as being sufficient for mediating Nexrutine response. Immunohistochemical analysis of human prostate tumors show increased expression of CREB and DNA binding activity in high-grade tumors (three-fold higher in human prostate tumors compared to normal prostate; P = .01). We have identified CREB-mediated activation of Cox-2 as a potential signaling pathway in prostate cancer which can be blocked with a nontoxic, cost-effective dietary supplement like Nexrutine, demonstrating a prospective for development of Nexrutine for prostate cancer management.

Gleave, M. and B. Jansen (2003). "Clusterin and IGFbps as antisense targets in prostate cancer." Ann N Y Acad Sci **1002**: 95-104.

The primary hurdle to improved survival of advanced prostate cancer is our failure to prevent or treat the tumor's progression to its lethal and untreatable stage of androgen independence. Novel treatment modalities designed to prevent androgen-independent progression including prostate cancer metastasis are required. Accelerated identification and characterization of cancer-relevant molecular targets has sparked considerable interest in the development of new generations of anticancer agents that specifically inhibit a progression-relevant target. Antisense oligonucleotides, short synthetic stretches of chemically modified DNA capable of specifically hybridizing to the mRNA of a chosen cancer-relevant target gene, promise to show enhanced specificity for malignant cells with a favorable side-effect profile due to well-defined and tailored modes of action. Although not all of the challenges have been met to date, emerging clinical evidence supports the premise that antisense oligonucleotides stand a realistic chance of emerging as major partners of rationally designed anticancer regimens. The rationale and status of antisense targeting of the treatment resistance factor clusterin and of insulin-like growth factor binding protein (IGFBP) 2 and 5 are discussed.

Gleave, M., C. Nelson, et al. (2003). "Antisense targets to enhance hormone and cytotoxic therapies in advanced prostate cancer." Curr Drug Targets **4**(3): 209-21.

The main obstacle to improved survival of advanced prostate cancer is our failure to prevent or treat its progression to its lethal and untreatable stage of androgen independence. New therapeutic agents designed to prevent androgen-independent progression are required. Accelerated identification and characterization of cancer-relevant molecular targets has sparked considerable interest in the development of new generations of anti-cancer agents that

specifically inhibit a progression-relevant target. Antisense oligonucleotides, short synthetic stretches of chemically modified DNA capable of specifically hybridizing to the mRNA of a chosen cancer-relevant target gene, promise to show enhanced specificity for malignant cells with a more favorable side-effect profile due to well-defined and tailored modes of action. Although not all of the challenges have been met to date, emerging clinical evidence supports the premise that antisense oligonucleotides stand a realistic chance of emerging as major partners of rationally designed anti-cancer regimens. The status of antisense targeting of several genes, including bcl-2, bcl-xL, clusterin, androgen receptor and IGF2R, relevant to prostate and other cancers, are reviewed.

Gotoh, A., T. Shirakawa, et al. (2003). "The growth inhibitory effect of p21 adenovirus on androgen-dependent and -independent human prostate cancer cells." *BJU Int* **92**(3): 314-8.

**OBJECTIVE:** To assess the potential of p21 as a gene therapy treatment for prostate cancer, by introducing p21 into both androgen-dependent (AD) and -independent (AI) human prostate cancer cell lines via a recombinant adenoviral vector, Ad5CMV-p21, carrying human p21 cDNA. **MATERIALS AND METHODS:** The LNCaP, DU145 and PC-3 human prostate cancer cell lines were cultured and infected with Ad5CMV-p21. Cell growth, cell-cycle progression and tumorigenicity were then assessed by thymidine incorporation into cellular DNA, and cell number, flow cytometry, and tumour growth after inoculating the cells into nude mice. **RESULTS:** Growth was inhibited in Ad5CMV-p21 viral-infected AD and AI prostate cancer cells. The effects were dose-dependent, regardless of the androgen status of the cell lines. Flow cytometric analysis showed that Ad5CMV-p21 arrested cell-cycle progression at G1/S with no appreciable effect on the levels of apoptotic cells. The tumorigenicity of cancer cells infected with Ad5CMV-p21 was greatly reduced in athymic mice. **CONCLUSIONS:** These results suggest that Ad5CMV-p21 may be a new therapeutic agent for human prostate cancer gene therapy.

Griewe, G. L., R. C. Dean, et al. (2003). "p53 Immunostaining guided laser capture microdissection (p53-LCM) defines the presence of p53 gene mutations in focal regions of primary prostate cancer positive for p53 protein." *Prostate Cancer Prostatic Dis* **6**(4): 281-5.

**OBJECTIVES:** A wide range of p53 mutations (5-65%), detected by various methods, has been reported in primary prostate cancers (CaP). IHC staining of radical prostatectomy specimens shows marked heterogeneity of focally distributed p53-

positive cells. However, a significant relationship between the focal staining of p53 and cancer recurrence after radical prostatectomy has been noted. Increased frequency of p53 mutations has been generally observed in advanced stage CaP and metastatic prostate cancer cell lines. The significance of focal p53 immunostaining in primary CaP remains uncertain with respect to the p53 gene mutation or tumor progression. The goal of this study was to evaluate p53 gene mutations in focal regions of primary prostate cancers positive by p53 immunostaining. **METHODS:** Whole-mount prostates from men with clinically organ-confined prostate cancer were immunostained for p53 protein. Laser capture microdissection (LCM) was used to harvest p53 positive cells from areas of tumor and prostatic intraepithelial neoplasia and benign gland. DNA from microdissected cells were amplified for p53 exons 5-8 by polymerase chain reaction (PCR) and analyzed for mutations by single strand conformation polymorphism and DNA sequencing. Mutation analysis of the p53 gene exons 5-8 was performed in the p53 immunostaining positive focal regions (1+ to 4+) of whole-mount prostate sections from 16 patients. **RESULTS:** Of 16 patients with p53 IHC positive tumors, 11 (69%) had p53 gene mutations as determined by DNA sequence analysis. However, randomly microdissected tumor cells from 4 of 18 patients (22%) negative for p53 IHC also demonstrated mutations in the p53 gene. A significant fraction of prostate tumors with focally positive immunostaining for p53 have been confirmed to contain mutations in the p53 gene. **CONCLUSIONS:** p53 immunostaining guided LCM combined with DNA-based analyses emphasizes the presence of focal p53 mutations in primary prostate cancers and underscores the significance of previous observations showing a correlation between focal p53 immunostaining in primary CaP and cancer recurrence after radical prostatectomy.

Guan, M., X. Zhou, et al. (2006). "Aberrant methylation and deacetylation of deleted in liver cancer-1 gene in prostate cancer: potential clinical applications." *Clin Cancer Res* **12**(5): 1412-9.

**PURPOSE:** The deleted in liver cancer-1 (DLC-1) gene that encodes a Rho GTPase-activating protein with tumor suppressor function is located on chromosome 8p21-22, a region frequently deleted in prostate carcinomas. This study was designed to determine whether DLC-1 is deregulated in prostate carcinomas and to assess the contribution of DLC-1 alterations to prostate carcinogenesis. **EXPERIMENTAL DESIGN:** Primary prostate carcinomas, prostate carcinoma cell lines, benign prostatic hyperplasias, and normal prostatic tissues

were examined for detection of functional and structural alterations of the DLC-1 gene by real-time PCR, methylation-specific PCR, and Southern and Western blots. RESULTS: Down-regulation or loss of DLC-1 mRNA expression was detected in 10 of 27 (37%) prostate carcinomas, 3 of 5 (60%) prostate carcinoma cell lines, and 5 of 21 (24%) benign prostatic hyperplasias. DLC-1 promoter methylation was identified in 13 of 27 (48%) prostate carcinomas and 2 matching normal tissues and in 15 of 21 (71%) benign prostatic hyperplasias but was absent in 10 normal prostatic tissues from noncancerous individuals. Genomic deletions were found in only 3 prostate carcinomas and 1 benign prostatic hyperplasia. DLC-1 protein was not detected in 8 of 27 (30%) prostate carcinomas and 11 of 21 (52%) benign prostatic hyperplasias. Methylation of DLC-1 correlated with age in prostate carcinoma patients ( $P = 0.006$ ) and with prostate-specific antigen blood levels in benign prostatic hyperplasia patients ( $P = 0.029$ ). Treatment of the three prostate carcinoma cell lines (PC-3, LNCaP, and 22Rv1) expressing a low level of DLC-1 transcripts with inhibitors of DNA methyltransferase or histone deacetylase increased DLC-1 expression. CONCLUSIONS: These results show that the transcriptional silencing of DLC-1 by two epigenetic mechanisms is common and may be involved in the pathogenesis of prostate carcinomas and benign prostatic hyperplasias and could have potential clinical application in the early detection and gene therapy of prostate cancer.

Guo, N., J. J. Ye, et al. (2003). "The role of insulin-like growth factor-II in cancer growth and progression evidenced by the use of ribozymes and prostate cancer progression models." *Growth Horm IGF Res* **13**(1): 44-53.

Towards understanding the IGF system during cancer growth and progression, progressive prostate cancer models, such as SV40 large T antigen immortalized human prostate epithelial cells (P69, M2182, M2205, and M12) and LNCaP sublines (C4, C4-2, and C4-2B4), were used. IGF-II mRNA levels progressively increase as prostate cancer cells become more tumorigenic and metastatic, suggesting that IGF-II contributes in part to prostate cancer progression. The role of IGF-II in cancer cell growth was evaluated in LNCaP, PC3, and M12 prostate cancer cell lines and MCF-7 breast cancer cell line by ribozyme/antisense strategies which were previously shown to suppress endogenous IGF-II expression and cell growth in PC-3 cells [Xu et al., *Endocrinol* **140** (1999) 2134]. Retroviral mediated transient expression of IGF-II-specific ribozyme (RZ) caused extensive cell death. In stably cloned cell lines, both RZ and mutant ribozyme (MRZ) inhibited cancer cell growth,

suggesting that antisense effects of MRZ may be sufficient for cell growth inhibition. These results confirm an important role of IGF-II in cancer cell growth and progression, and support further development of gene therapy targeting IGF-II.

Gurel, B., T. Iwata, et al. (2008). "Molecular alterations in prostate cancer as diagnostic, prognostic, and therapeutic targets." *Adv Anat Pathol* **15**(6): 319-31.

Prostatic adenocarcinoma is extremely common in Western nations, representing the second leading cause of cancer death in American men. The recent application of increasingly sophisticated molecular approaches to the study of prostate cancer in this "postgenomic" era has resulted in a rapid increase in the identification of somatic genome alterations and germline heritable risk factors in this disease. These findings are leading to a new understanding of the pathogenesis of prostate cancer and to the generation of new targets for diagnosis, prognosis, and prediction of therapeutic response. Although we are still in the very early phase of clinical development, some of the molecular alterations identified in prostate cancer are being translated into clinical practice. The purpose of this review is to update the practicing surgical pathologist, and residents-in-training in pathology, regarding recent findings in the molecular pathobiology of prostate cancer. We will highlight some of the somatic molecular alterations associated with prostate cancer development and progression, with a focus on newer discoveries. In addition, recent studies in which new molecular diagnostic approaches have been applied in the clinic will be discussed.

Gust, K. M., M. D. Hofer, et al. (2009). "RHAMM (CD168) is overexpressed at the protein level and may constitute an immunogenic antigen in advanced prostate cancer disease." *Neoplasia* **11**(9): 956-63.

Localized prostate cancer (CaP) can be cured using several strategies. However, the need to identify active substances in advanced tumor stages is tremendous, as the outcome in such cases is still disappointing. One approach is to deliver human tumor antigen-targeted therapy, which is recognized by T cells or antibodies. We used data mining of the Cancer Immunome Database (CID), which comprises potential immunologic targets identified by serological screening of expression libraries. Candidate antigens were screened by DNA microarrays. Genes were then validated at the protein level by tissue microarrays, representing various stages of CaP disease. Of 43 targets identified by CID, 10 showed an overexpression on the complementary DNA array in CaP metastases. The RHAMM (CD168)

gene, earlier identified by our group as an immunogenic antigen in acute and chronic leukemia, also showed highly significant overexpression in CaP metastases compared with localized disease and benign prostatic hyperplasia. At the protein level, RHAMM was highest in metastatic tissue samples and significantly higher in neoplastic localized disease compared with benign tissue. High RHAMM expression was associated with clinical parameters known to be linked to better clinical outcome. Patients with high RHAMM expression in the primaries had a significantly lower risk of biochemical failure. The number of viable cells in cell cultures was reduced in blocking experiments using hormone-sensitive and hormone-insensitive metastatic CaP cell lines. Acknowledging the proven immunogenic effects of RHAMM in leukemia, this antigen is intriguing as a therapeutic target in far-advanced CaP.

Handayani, R., L. Rice, et al. (2006). "Soy isoflavones alter expression of genes associated with cancer progression, including interleukin-8, in androgen-independent PC-3 human prostate cancer cells." *J Nutr* **136**(1): 75-82.

High consumption of soy isoflavones in Asian diets has been correlated with a lower incidence of clinically important cases of prostate cancer. The chemopreventive properties of these diets may result from an interaction of several types of isoflavones, including genistein and daidzein. The present study investigated the effects of a soy isoflavone concentrate (ISF) on growth and gene expression profiles of PC-3 human prostate cancer cells. Trypan blue exclusion and [<sup>3</sup>H]-thymidine incorporation assays showed that ISF decreased cell viability and caused a dose-dependent inhibition of DNA synthesis, respectively, with 50% inhibition (IC<sub>50</sub>) of DNA synthesis at 52 mg/L (P = 0.05). The glucoside conjugates of genistein and daidzein in ISF were converted to bioactive free aglycones in cell culture in association with the inhibition of DNA synthesis. Flow cytometry and Western immunoblot analyses showed that ISF at 200 mg/L caused an accumulation of cells in the G<sub>2</sub>/M phase of the cell cycle (P < 0.05) and decreased cyclin A by 20% (P < 0.05), respectively. The effect of ISF on the gene expression profile of PC-3 cells was analyzed using Affymetrix oligonucleotide DNA microarrays that interrogate approximately 17,000 human genes. Of the 75 genes altered by ISF, 28 were upregulated and 47 were downregulated (P < 0.05). Further analysis showed that IL-8, matrix metalloproteinase 13, inhibin beta A, follistatin, and fibronectin mRNA levels were significantly reduced, whereas the expression of p21(CIP1), a major cell cycle inhibitory protein, was increased. The effects of ISF on the expression of IL-8 and p21(CIP1) mRNA

and protein were validated at high and low ISF concentrations. Our data show that ISF inhibits the growth of PC-3 cells through modulation of cell cycle progression and the expression of genes involved in cell cycle regulation, metastasis, and angiogenesis.

Hara, T., J. Kouno, et al. (2005). "Possible role of adaptive mutation in resistance to antiandrogen in prostate cancer cells." *Prostate* **65**(3): 268-75.

**BACKGROUND:** Some mutations of androgen receptor (AR) confer resistance to antiandrogen to prostate cancer (PC) cells. Previously we reported that LNCaP-cxD2 cells established from androgen-dependent LNCaP-FGC PC cells as an antiandrogen bicalutamide-resistant subline harbor W741C/L mutation in the AR gene. In this report, we examined one possible mechanism of the resistance. **METHODS:** Change in the gene expression and the protein levels relevant to mutagenesis in LNCaP-FGC cells during bicalutamide-treatment was assessed. The AR sequence of bicalutamide-resistant LNCaP-cxD2 cells was compared with that of parental LNCaP-FGC cells. **RESULTS:** The expression of DNA polymerases (Pol) switched from high-fidelity subset to error-prone subset, and DNA mismatch repair proteins (MMR) were down-regulated. The rate of multiple mutations in the AR gene was higher in LNCaP-cxD2 cells than LNCaP-FGC cells. **CONCLUSIONS:** These results suggest the hypermutational state might occur in LNCaP-FGC cells during bicalutamide-treatment, which might create the W741C/L mutant AR leading to bicalutamide-resistance.

Hattori, Y. and Y. Maitani (2004). "Enhanced in vitro DNA transfection efficiency by novel folate-linked nanoparticles in human prostate cancer and oral cancer." *J Control Release* **97**(1): 173-83.

Novel folate-linked, cationic nanoparticles (NPs) were developed and evaluated for potential use for gene delivery to human oral cancer (KB cells) and human prostate cancer (LNCaP cells), which abundantly expressed folate binding proteins. Folate-polyethylenglycol-distearoylphosphatidylethanolamine conjugate (f-PEG-DSPE) was incorporated in NPs composed of 3([N-(N',N'-dimethylaminoethane)-carbonyl] cholesterol (DC-Chol) and Tween 80. NP-0.3FT, -1FT and -1FLT, which contain 0.3 and 1 mol% f-PEG2000-DSPE, and 1 mol% f-PEG5000-DSPE, respectively, showed about 100-200 nm in size. The NP/plasmid DNA complex (nanoplex) remained in an injectable size (230-340 nm) and slightly increased its size in serum. The association of NP-1FT with KB cells was enhanced by f-PEG2000-DSPE and was blocked by co-incubation with free folic acid in

medium. In transfection activity, the NP-1FT, but not NP-1FLT, showed high activity into KB and LNCaP cells in the presence of serum. The NP-0.3FT also showed high activity into LNCaP cells, but not KB cells. In RT-PCR analysis, KB cells strongly expressed folate receptors mRNA, but LNCaP cells did not. In contrast, LNCaP cells expressed mRNA of prostate-specific membrane antigen (PSMA), which interacts with the folate substrate. Uptake mechanism of folate-linked NPs in LNCaP cells may be different from that in KB cells. This is the first report that folate-linked NPs selectively deliver the DNA to LNCaP cells, suggesting that such NPs are potentially targeted vectors to prostate cancer for gene delivery.

Hattori, Y. and Y. Maitani (2005). "Folate-linked nanoparticle-mediated suicide gene therapy in human prostate cancer and nasopharyngeal cancer with herpes simplex virus thymidine kinase." *Cancer Gene Ther* **12**(10): 796-809.

For targeted gene delivery to human prostate cancer LNCaP and PC-3 cells and nasopharyngeal cancer KB cells, we developed a folate-linked nanoparticle (NP-F), and evaluated the potential of NP-F-mediated suicide gene therapy in the cells and xenografts with herpes simplex virus thymidine kinase (HSV-tk) and connexin 43 (Cx43). An NP-F-plasmid DNA complex (NP-F nanoplex) showed high DNA transfection efficiency in KB, LNCaP and PC-3 cells. Cell growth inhibition in the presence of ganciclovir (GCV) was enhanced with HSV-tk and Cx43 genes in LNCaP cells. In suicide gene therapy, the tumor growths of KB and LNCaP xenografts were significantly inhibited when an NP-F nanoplex of the HSV-tk gene, and HSV-tk and Cx43 genes, respectively, was injected intratumorally and GCV was administered intraperitoneally. These findings suggested that the NP-F is a potential target vector in prostate and nasopharyngeal cancer for suicide gene therapy.

Hattori, Y. and Y. Maitani (2007). "DNA/Lipid complex incorporated with fibronectin to cell adhesion enhances transfection efficiency in prostate cancer cells and xenografts." *Biol Pharm Bull* **30**(3): 603-7.

Previously we have described the development and applications of lipid-based nanoparticles for gene delivery vector. In an attempt to improve transfection efficiency using the cell adhesion of extracellular matrix (ECM) to DNA/lipid complex (nanoplex), the mRNA expression of integrin  $\alpha 2\beta 1$  and CD44 in prostate cancer cells was detected as adhesion molecules for fibronectin (Fn), collagen I (Col) and laminin (Lam) using a commercially available cDNA array (GEArray) system. These ECM proteins could enhance DNA

transfection activity in cells when coated on the nanoplex. Among the ECM proteins, Fn-coating nanoplexes significantly increased transfection activity 2-fold in prostate cancer PC-3 cells, and exhibited higher DNA transfection activities to PC-3 xenografts, compared with commercially available cationic polymer in vivo jetPEI. These results indicated that Fn-coating nanoplexes could facilitate efficient transfection of prostate tumor cells.

Herman-Antosiewicz, A., H. Xiao, et al. (2007). "Induction of p21 protein protects against sulforaphane-induced mitotic arrest in LNCaP human prostate cancer cell line." *Mol Cancer Ther* **6**(5): 1673-81.

Previous studies have indicated that d,l-sulforaphane (SFN), a synthetic cancer chemopreventive analogue of cruciferous vegetable-derived isomer (-)-1-isothiocyanato-(4R)-(methylsulfinyl)-butane, activates a checkpoint kinase 2 (Chk2)-dependent G(2)-M phase cell cycle arrest in p53-deficient human prostate cancer cells. Because p53 is a downstream target of Chk2 kinase and known to regulate G(2)-M transition by transcriptional regulation of cyclin-dependent kinase (Cdk) inhibitor p21(Cip1/Waf1) (p21), the present study was undertaken to determine the role of p21 in SFN-induced cell cycle arrest using wild-type p53-expressing cell line LNCaP. The SFN treatment caused a modest increase in S phase fraction and a marked increase in G(2)-M fraction in LNCaP cells in a concentration- and time-dependent manner. The SFN-induced S phase arrest correlated with a reduction in protein levels of cyclin D1, cyclin E, Cdk4, and Cdk6, whereas activation of the G(2)-M checkpoint was accompanied by induction of cyclin B1 and down-regulation of Cdk1 and Cdc25C protein levels. The SFN-treated LNCaP cells were also arrested in mitosis as revealed by immunofluorescence microscopy and increased Ser(10) phosphorylation of histone H3, a sensitive marker for mitotic cells. The SFN treatment increased activating phosphorylation of Chk2 (Thr(68)) that was accompanied by induction of p53 and p21. The SFN-induced mitotic arrest was statistically significantly increased by small interfering RNA-based knockdown of p21. However, p21 protein knockdown did not have any appreciable effect on SFN-induced cytoplasmic histone-associated DNA fragmentation (apoptosis). In conclusion, the present study indicates that induction of p21 protects against SFN-induced mitotic arrest in LNCaP cells.

Hernandez, I., L. A. Maddison, et al. (2003). "Prostate-specific expression of p53(R172L) differentially regulates p21, Bax, and mdm2 to inhibit

prostate cancer progression and prolong survival." *Mol Cancer Res* 1(14): 1036-47.

Loss of heterozygosity or mutation at the p53 tumor suppressor gene locus is frequently associated with advanced human prostate cancer. Hence, replacement p53 gene therapy may prove to be efficacious for this disease. While many mutations result in p53 molecules with oncogenic properties, other variants may possess wild-type properties with increased tumor suppressor activity. We have chosen to investigate the activity of a naturally occurring variant p53 molecule, p53(R172L), carrying an arginine-to-leucine mutation at codon 172. We demonstrate that p53(R172L) can differentially activate expression of genes involved in cell cycle control and apoptosis in vitro. Transgenic mice expressing a subphysiological level of a p53(R172L) minigene (PB-p53(R172L)) in the prostate epithelium were generated and bred to the transgenic adenocarcinoma mouse prostate (TRAMP) model of prostate cancer. While PB-p53(R172L) transgenic mice developed normally with no detectable prostate gland phenotype, we observed a significant increase in the apoptotic index in the prostate glands of TRAMP x PB-p53(R172L) F1 mice. We noted an increase in the expression of Bax in the bigenic mice concomitant with the reduced incidence and rate of tumor growth and increased survival. While low-level expression of the p53(R172L) variant had no obvious influence on normal prostate tissue, it was able to significantly inhibit prostate cancer progression in the context of a genetically predisposed model system. This suggests that additional tumor-related events specifically influence the ability of the variant p53(R172L) molecule to inhibit tumor growth. These studies support gene therapy strategies employing specific p53 variants.

Hessels, D., G. W. Verhaegh, et al. (2004). "Applicability of biomarkers in the early diagnosis of prostate cancer." *Expert Rev Mol Diagn* 4(4): 513-26.

Early diagnosis of prostate cancer can increase the curative success rate for this disease. Although serum prostate-specific antigen measurement is regarded as the best conventional tumor marker available, there is little doubt that it has great limitations. The threshold above which biopsies are indicated has now decreased to a serum prostate-specific antigen value of 3 ng/ml, which results in a negative biopsy rate of 70-80%. This can readily be explained by the fact that prostate-specific antigen is not specific for prostate cancer. Clinicians need more sensitive tools to help diagnose prostate cancer and monitor progression of the disease. Molecular oncology is playing an increasing role in the fields of diagnosis and therapy for prostate cancer and has

already been instrumental in elucidating many of the basic mechanisms underlying the development and progression of this disease. The identification of new prostate cancer-specific genes, such as DD3PCA3, would represent a considerable advance in the improvement of diagnostic tests for prostate cancer. This could subsequently lead to a reduction of the number of unnecessary biopsies.

Ho, S. M., Y. K. Leung, et al. (2006). "Estrogens and antiestrogens as etiological factors and therapeutics for prostate cancer." *Ann N Y Acad Sci* 1089: 177-93.

Mounting evidence supports a key role played by estrogen or estrogen in synergy with an androgen, in the pathogenesis of prostate cancer (PCa). New experimental data suggest that this process could begin as early as prenatal life. During adulthood, estrogen carcinogenicity is believed to be mediated by the combined effects of hormone-induced, unscheduled cell proliferation and bioactivation of estrogens to genotoxic carcinogens. Increased bioavailability of estrogen through age-dependent increases in conversion from androgen could also be a contributing factor. Individual variations and race-/ethnic-based differences in circulating or locally formed estrogens or in tissue estrogen responsiveness may explain differential PCa risk among individuals or different populations. Estrogen receptor (ER)-alpha and ER-beta are the main mediators of estrogen action in the prostate. However, ER-beta is the first ER subtype expressed in the fetal prostate. During cancer development, ER-beta expression is first lost as tumors progress into high grade in the primary site. Yet, its reexpression occurs in all metastatic cases of PCa. A change in cytosine methylation in a regulatory CpG island located in the proximal promoter of ER-beta may constitute an "on/off" switch for reversible regulation of ER-beta expression. A variety of estrogenic/antiestrogenic/selective estrogen receptor modulator (SERM)-like compounds have been shown to use non-ERE pathways, such as tethering of ER-beta to NF-kappaB binding proteins, Sp2, or Ap1 for gene transactivation. These findings open new avenues for drug design that now focuses on developing a new generation of estrogen-based PCa therapies with maximal proapoptotic action but few or no side effects.

Hodgson, M. C., I. Astapova, et al. (2007). "Activity of androgen receptor antagonist bicalutamide in prostate cancer cells is independent of NCoR and SMRT corepressors." *Cancer Res* 67(17): 8388-95.

The mechanisms by which androgen receptor (AR) antagonists inhibit AR activity, and how their antagonist activity may be abrogated in prostate

cancer that progresses after androgen deprivation therapy, are not clear. Recent studies show that AR antagonists (including the clinically used drug bicalutamide) can enhance AR recruitment of corepressor proteins [nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid receptors (SMRT)] and that loss of corepressors may enhance agonist activity and be a mechanism of antagonist failure. We first show that the agonist activities of weak androgens and an AR antagonist (cyproterone acetate) are still dependent on the AR NH(2)/COOH-terminal interaction and are enhanced by steroid receptor coactivator (SRC)-1, whereas the bicalutamide-liganded AR did not undergo a detectable NH(2)/COOH-terminal interaction and was not coactivated by SRC-1. However, both the isolated AR NH(2) terminus and the bicalutamide-liganded AR could interact with the SRC-1 glutamine-rich domain that mediates AR NH(2)-terminal binding. To determine whether bicalutamide agonist activity was being suppressed by NCoR recruitment, we used small interfering RNA to deplete NCoR in CV1 cells and both NCoR and SMRT in LNCaP prostate cancer cells. Depletion of these corepressors enhanced dihydrotestosterone-stimulated AR activity on a reporter gene and on the endogenous AR-regulated PSA gene in LNCaP cells but did not reveal any detectable bicalutamide agonist activity. Taken together, these results indicate that bicalutamide lacks agonist activity and functions as an AR antagonist due to ineffective recruitment of coactivator proteins and that enhanced coactivator recruitment, rather than loss of corepressors, may be a mechanism contributing to bicalutamide resistance.

Hoffmann, M. J., R. Engers, et al. (2007). "Expression changes in EZH2, but not in BMI-1, SIRT1, DNMT1 or DNMT3B are associated with DNA methylation changes in prostate cancer." *Cancer Biol Ther* 6(9): 1403-12.

The polycomb proteins BMI-1, EZH2, and SIRT1 are characteristic components of the PRC1, PRC2, and PRC4 repressor complexes, respectively, that modify chromatin. Moreover, EZH2 may influence DNA methylation by direct interaction with DNA methyltransferases. EZH2 expression increases during prostate cancer progression, whereas BMI-1 and SIRT1 are not well investigated. Like EZH2 expression, DNA methylation alterations escalate in higher stage prostate cancers, raising the question whether these epigenetic changes are related. Expression of EZH2, BMI-1, SIRT1, and the DNA methyltransferases DNMT1 and DNMT3B measured by qRT-PCR in 47 primary prostate cancers was compared to APC, ASC, GSTP1, RARB2, and RASSF1A hypermethylation and LINE-1

hypomethylation. SIRT1 and DNMT3B were overexpressed in cancerous over benign tissues, whereas BMI-1 was rather downregulated and DNMT1 significantly diminished. Nevertheless, cancers with higher DNMT1 and BMI-1 expression had worse clinical characteristics, as did those with elevated EZH2. In particular, above median DNMT1 expression predicted a worse prognosis. EZH2 and SIRT1 overexpression were well correlated with increased MKI67. Immunohistochemistry confirmed limited EZH2 and heterogeneous DNMT3B overexpression and explained the decrease in BMI-1 by pronounced heterogeneity among tumor cells. EZH2 overexpression, specifically among all factors investigated, was associated with more frequent hypermethylation, in particular of GSTP1 and RARB2, and also with LINE-1 hypomethylation. Our data reveal complex changes in the composition of polycomb repressor complexes in prostate cancer. Heterogeneously expressed BMI-1 and slightly increased EZH2 may characterize less malignant cancers, whereas more aggressive cases express both at higher levels. SIRT1 appears to be generally increased in prostate cancers. Intriguingly, our data suggest a direct influence of increased EZH2 on altered DNA methylation patterns in prostate cancer.

Hoque, M. O., O. Topaloglu, et al. (2005). "Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects." *J Clin Oncol* 23(27): 6569-75.

**PURPOSE:** Aberrant promoter hypermethylation of several known or putative tumor suppressor genes occurs frequently during the pathogenesis of prostate cancers and is a promising marker for cancer detection. We sought to develop a test for prostate cancer based on a quantitative methylation-specific polymerase chain reaction (QMSP) of multiple genes in urine sediment DNA. **PATIENTS AND METHODS:** We tested urine sediment DNA for aberrant methylation of nine gene promoters (p16INK4a, p14(ARF), MGMT, GSTP1, RARBeta2, CDH1 [E-cadherin], TIMP3, Rassf1A, and APC) from 52 patients with prostate cancer and 21 matched primary tumors by quantitative fluorogenic real-time polymerase chain reaction. We also analyzed urine sediments from 91 age-matched individuals without any history of genitourinary malignancy as controls. **RESULTS:** Promoter hypermethylation of at least one of the genes studied was detected in urine samples from all 52 prostate cancer patients. Urine samples from the 91 controls without evidence of genitourinary cancer revealed no methylation of the p16, ARF, MGMT, and GSTP1 gene promoters, whereas methylation of RARBeta2, TIMP3, CDH1,

Rassf1A, and APC was detected at low levels. CONCLUSION: Overall, methylation found in urine samples matched the methylation status in the primary tumor. A combination of only four genes (p16, ARF, MGMT, and GSTP1) would theoretically allow us to detect 87% of prostate cancers with 100% specificity. Our data support further development of the noninvasive QMSP assay in urine DNA for early detection and surveillance of prostate cancer.

Horvath, L. G., S. M. Henshall, et al. (2004). "Loss of BMP2, Smad8, and Smad4 expression in prostate cancer progression." *Prostate* 59(3): 234-42.

BACKGROUND: The role of the bone morphogenetic protein (BMP) pathway in prostate cancer (PC) is unclear. This study aimed to characterize aspects of the BMP pathway in PC by assessing BMP2, Smad8, and Smad4 expression in normal, hyperplastic, and malignant prostate tissue, and to correlate findings with progression to PC. METHODS: Radical prostatectomy (RP) specimens from 74 patients with clinically localized PC (median follow-up 51 months, range 15-152), 44 benign prostatic hypertrophy (BPH) lesions, and 4 normal prostates (NPs) were assessed for BMP2, Smad8, and Smad4 expression using immunohistochemistry. RESULTS: Both BMP2 ( $P < 0.001$ ) and nuclear Smad4 ( $P < 0.0001$ ) expression were significantly decreased in PC compared to benign prostate tissue. Nuclear Smad8 was present in normal/benign prostate tissue but absent in PC and adjacent hyperplasia. Furthermore, loss of BMP2 ( $P < 0.001$ ) and decreased nuclear Smad4 ( $P = 0.05$ ) expression correlated with increasing Gleason score. CONCLUSIONS: These data suggest that decreased BMP2, nuclear smad8 and nuclear Smad4 expression are associated with the progression to PC, and in particular loss of BMP2 and Smad4 are related to progression to a more aggressive phenotype.

Hu, C. K., S. McCall, et al. (2006). "Loss of heterozygosity of M6P/IGF2R gene is an early event in the development of prostate cancer." *Prostate Cancer Prostatic Dis* 9(1): 62-7.

BACKGROUND: The genetic events leading to initiation and/or progression of prostate cancer are not well characterized. The gene coding for the mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) has recently been identified as a tumor suppressor in several types of cancer. The purpose of the present study is to determine whether the M6P/IGF2R gene is inactivated in human prostate cancer, and if so, whether this is an early or late transformational event. METHODS: In total, 43 patients with prostate cancer treated by radical prostatectomy, with archival material available for

analysis, were assessed for loss of heterozygosity (LOH) in the M6P/IGF2R gene using six different gene-specific nucleotide polymorphisms. Regions of tumor, normal prostate and premalignant high-grade prostate intraepithelial neoplasia (PIN) were identified and cells were excised by laser capture microdissection (LCM). DNA segments were amplified using polymerase chain reaction (PCR). RESULTS: The M6P/IGF2R gene was polymorphic in 83.7% (36/43) of patients, and 41.7% (15/36) of these informative patients had LOH in the tumor tissue. In 11/15 patients with LOH in malignant tissue, high-grade PIN could be identified, and 63.6% (7/11) also had LOH in this premalignant tissue. CONCLUSIONS: This study is the first to find that the M6P/IGF2R gene is inactivated in prostate cancer. LOH in premalignant tissue as well suggests that mutation in the M6P/IGF2R gene is an early event in the development of prostate cancer, supporting the conclusion that it functions as a tumor suppressor gene in this disease.

Huang, S. P., C. Y. Huang, et al. (2007). "Prognostic significance of p53 and X-ray repair cross-complementing group 1 polymorphisms on prostate-specific antigen recurrence in prostate cancer post radical prostatectomy." *Clin Cancer Res* 13(22 Pt 1): 6632-8.

PURPOSE: The tumor suppressor p53 and DNA repair gene X-ray repair cross-complementing group 1 (XRCC1) are thought to play important roles on prostate cancer susceptibility and tumor development. We investigated the potential prognostic roles of p53 (codon 72) and XRCC1 (codons 194, 280, and 399) polymorphisms in clinical localized prostate cancer after radical prostatectomy. EXPERIMENTAL DESIGN: A total of 126 clinical localized prostate cancer patients undergoing curative radical prostatectomy at the Kaohsiung Medical University Hospital and Kaohsiung Veterans General Hospital were included in this study. The p53 codon 72 and XRCC1 codons 194, 280 and 399 polymorphisms were determined by the PCR-RFLP method. Their prognostic significance on prostate-specific antigen (PSA) recurrence were assessed using the Kaplan-Meier analysis and Cox regression model. RESULTS: The p53 codon 72 Arg/Arg genotype was associated with increased PSA recurrence risk compared with the Arg/Pro and Pro/Pro genotypes, although the difference did not reach significance (30.3% versus 20.4%,  $P = 0.247$ ). Of these three XRCC1 polymorphisms, the codon 399 Arg/Gln + Gln/Gln genotypes were significantly associated with higher risk of PSA recurrence after radical prostatectomy compared with the Arg/Arg genotype (34.0% versus 15.1%,  $P = 0.013$ ) and poorer PSA-free

survival (log-rank test,  $P = 0.0056$ ). After considering for other covariates in a Cox proportional hazard model, the XRCC1 Arg/Gln and Gln/Gln genotypes (hazard ratio, 4.73; 95% confidence interval, 1.61-13.92;  $P = 0.005$ ) and high Gleason score (Gleason score, 8-10; hazard ratio, 5.58; 95% confidence interval, 1.58-19.71;  $P = 0.008$ ) were still independent predictors of poor PSA-free survival after radical prostatectomy. The similar significant results were not found in XRCC1 codons 194 and 280. CONCLUSIONS: Our results suggest that the XRCC1 codon 399 polymorphism may be a prognostic factor for PSA recurrence after radical prostatectomy.

Huang, Y. W., L. S. Wang, et al. (2006). "Molecular mechanisms of (-)-gossypol-induced apoptosis in human prostate cancer cells." *Anticancer Res* **26**(3A): 1925-33.

**BACKGROUND:** Gossypol, a natural compound present in cottonseeds, displays antiproliferative and pro-apoptotic effects against various cancer cells. The (-)-gossypol enantiomer is a more potent inhibitor of cancer cell growth. Here, the molecular mechanisms of apoptosis induced by (-)-gossypol were studied in human prostate cancer cells. **MATERIALS AND METHODS:** After the prostate cancer cell DU-145 had been treated with (-)-gossypol, the trypan blue exclusion assay and DNA fragment end-labeling assay were used to stain the dead cells and to detect DNA laddering, respectively. The effects of (-)-gossypol on the expression of apoptotic-regulated gene markers in both death receptor- and mitochondria-mediated apoptotic pathways, such as the Bcl-2 family and caspase, etc., were detected by RT-PCR and Western blot analysis. To further investigate the apoptotic pathways induced by (-)-gossypol, different caspase inhibitors were used to block caspase activities and cell viability was detected by the CellTiter 96 AQueous assay in DU-145 cells. **RESULTS:** At a 5-10 microM dose-level, (-)-gossypol significantly enhanced apoptosis measured by DNA fragmentation. (-)-Gossypol caused apoptosis in DU-145 cells through the down-regulation of Bcl-2 and Bcl-xL and the up-regulation of Bax at the mRNA and protein levels. (-)-Gossypol also activated caspases-3, -8 and -9 and increased PARP [poly (ADP-ribose) polymerase] cleavage. Furthermore, (-)-gossypol-induced apoptosis might be due to an increase in CAD (caspase-activated deoxyribonuclease) proteins and a decrease in ICAD (inhibitor of CAD) proteins. By using caspase inhibitors, (-)-gossypol caused apoptosis via the caspase-dependent pathways. **CONCLUSION:** Our results indicated that the apoptotic processes caused by (-)-gossypol are mediated by the regulation of the Bcl-2 and caspase families in human prostate cancer

cells. Our data also suggested that (-)-gossypol may have chemotherapeutic benefits for prostate cancer patients.

Huizen, I. V., G. Wu, et al. (2005). "Establishment of a serum tumor marker for preclinical trials of mouse prostate cancer models." *Clin Cancer Res* **11**(21): 7911-9.

Current prostate cancer research in both basic and preclinical trial studies employ genetically engineered mouse models. However, unlike in human prostate cancer patients, rodents have no counterpart of prostatic-specific antigen (PSA) for monitoring prostate cancer initiation and progression. In this study, we established a mouse serum tumor marker from a mouse homologue of human prostate secretory protein of 94 amino acids (PSP94). Immunohistochemistry studies on different histologic grades from both transgenic and knock-in mouse prostate cancer models showed the down-regulation of tissue PSP94 expression ( $P < 0.001$ ), the same as for PSA and PSP94 in humans. The presence of mouse serum PSP94 was shown by affinity column and immunoprecipitation purification using a polyclonal mouse PSP94 antibody. A competitive ELISA protocol was established to quantify serum PSP94 levels with a sensitivity of 1 ng/mL. Quantified serum levels of mouse PSP94 ranged from 49.84 ng/mL in wild-type mice to 113.86, 400.45, and 930.90 ng/mL in mouse prostatic intraepithelial neoplasia with microinvasion, well differentiated, moderately differentiated, and poorly differentiated prostate cancer genetically engineered prostate cancer mice, respectively ( $P < 0.01$ ,  $n = 68$ ). This increase in serum PSP94 is also well correlated with age and tumor weight. Through longitudinal monitoring of serum PSP94 levels of castrated mice (androgen ablation therapy), we found a correlation between responsiveness/refractory prostate tissues and serum PSP94 levels. The utility of mouse serum PSP94 as a marker in hormone therapy was further confirmed by three-dimensional ultrasound imaging. The establishment of the first rodent prostate cancer serum biomarker will greatly facilitate both basic and preclinical research on human prostate cancer.

Hummerich, J., G. Werle-Schneider, et al. (2006). "Constitutive mRNA expression of DNA repair-related genes as a biomarker for clinical radioresistance: A pilot study in prostate cancer patients receiving radiotherapy." *Int J Radiat Biol* **82**(8): 593-604.

**PURPOSE:** Repair of radiation-induced DNA damage is believed to play a critical role in the development of adverse reactions in radiotherapy patients. Constitutive mRNA expression of repair

genes was investigated in such patients to analyze whether expression patterns are predictive for therapy-related acute side effects. **MATERIALS AND METHODS:** Prostate cancer patients (n = 406) receiving intensity-modulated radiotherapy were recruited in a prospective epidemiological study. Adverse effects were monitored during therapy using common toxicity criteria. For expression analyses, samples from 58 patients were selected according to their observed grade of clinical side effects to radiotherapy. Expression profiles were generated from peripheral blood lymphocytes using customized cDNA-arrays which carried probes for 143 DNA repair or repair-related genes. In addition, expression of selected genes was confirmed by quantitative real-time reverse transcription PCR (RT-PCR). Constitutive mRNA expression profiles were analyzed for predicting acute clinical radiosensitivity or radio-resistance. **RESULTS:** Cluster analysis identified 19 differentially expressed genes. Many of these genes are involved in DNA double strand break repair. Expression levels of these genes differed up to 7-fold from the mean of all patients whereas expression levels of housekeeping genes varied only up to 2-fold. High expression of the identified genes was associated with a lack of clinical radiation sensitivity thus indicating radio-resistance. **CONCLUSIONS:** Constitutive expression of DNA repair-related genes may affect the development of acute side effects in radiotherapy patients, and high expression levels of these genes seem to support protection from adverse reactions.

Iczkowski, K. A., A. L. Omara-Opyene, et al. (2004). "Metafectene is superior to lipofectamine in the transfection of G(s) alpha prostate cancer cells." *Mol Biotechnol* **28**(2): 97-103.

Transfection efficiency of the novel reagent metafectene has not been compared with that of lipofectamine in the published English literature. We used these agents to transfect two prostate cancer cell lines, PC3 and G(s) alpha, with a deoxyribonucleic acid (DNA) expression vector that generates double-stranded ribonucleic acid (RNA) for RNA interference (RNAi). Cotransfection of the green fluorescent protein (GFP) reporter gene revealed that the mean (+/- standard deviation) transfection efficiencies with lipofectamine were 5.8+/-0.4% for PC3 cells and 3.6+/-1.5% for G(s) alpha cells. Mean transfection efficiency with metafectene declined to 0.1+/-0% for PC3 cells but improved to 54.6+/-5.5% for G(s) alpha cells. With G(s) alpha cells, metafectene transfection of GFP plasmid alone yielded 46.9% positive cells, and cotransfection with CD44v9 expression vector yielded 45.9% positive cells. The visual impact of the transfected RNAi construct was detectable at the

protein level 4 to 6 d posttransfection and was more dramatic after using metafectene than after using lipofectamine. Thus, in vitro, metafectene transfection efficiency was sufficient to allow us to assess the functional significance of our RNAi construct, suggesting metafectene as an excellent candidate for RNAi-mediated anticancer gene therapy.

Igawa, T., F. F. Lin, et al. (2003). "Suppression of LNCaP prostate cancer xenograft tumors by a prostate-specific protein tyrosine phosphatase, prostatic acid phosphatase." *Prostate* **55**(4): 247-58.

**BACKGROUND:** Although the molecular mechanism of androgen-independent prostate cancer growth and progression has been gradually elucidated, there is limited effective treatment for this prevalent disease. Human prostatic acid phosphatase (PACp), a major protein tyrosine phosphatase in prostate epithelium, plays a critical role in regulating the growth of prostate cancer cells. In prostate carcinomas, the expression of cellular PACp decreases. To explore directly the possible therapeutic potential of cellular PACp, we investigated the suppression effect of PACp by utilizing cDNA direct intratumoral administration in androgen-independent LNCaP xenograft tumors. **METHODS:** An androgen-independent LNCaP cell model (C-33 and C-81 cells) and stable subclones of PACp cDNA-transfected C-81 cells (LNCaP-23 and LNCaP-34 cells) were used for the experiments. We examined the growth property and expression of PACp and c-ErbB-2 of these different LNCaP cells in vitro and in vivo. We subsequently investigated the growth suppression effect of PACp cDNA intratumoral injection in pre-established C-81 xenograft tumors, and analyzed the expression of PACp, prostate-specific antigen (PSA), proliferating cell nuclear antigen (PCNA), and c-ErbB-2 in the tumors by immunohistochemistry and Western blotting. **RESULTS:** The different LNCaP cells exhibited different growth property and tumorigenicity, both in cell culture and xenograft. Biochemical characterizations revealed that the level of cellular PACp correlated negatively with the growth property of different LNCaP cells, while the level of tyrophosphorylated c-ErbB-2 had an inverse correlation with cellular PACp. The single intratumoral administration of the wild type PACp cDNA showed a significant suppression effect on C-81 xenograft tumor growth, compared to vector alone-injected control (P<0.05). In the tumors injected with this PACp cDNA, the PACp expression was detected 1 week (wk) after injection, but was undetectable at 6 wk, which inversely correlated with the level of tyrophosphorylated c-ErbB-2 and the degree of cell proliferation indicated by PCNA staining. **CONCLUSIONS:** Our results clearly demonstrated

that cellular PAcP has a suppression effect on the growth of androgen-independent LNCaP xenograft tumors. This effect occurs at least partly through the dephosphorylation of c-ErbB-2 by PAcP, the prostate-specific protein tyrosine phosphatase. The data indicates that human PAcP could be utilized in the corrective gene therapy for a subgroup of androgen-independent human prostate cancer cells that lack cellular PAcP expression.

Ikeda, N., H. Uemura, et al. (2003). "Combination treatment with 1alpha,25-dihydroxyvitamin D3 and 9-cis-retinoic acid directly inhibits human telomerase reverse transcriptase transcription in prostate cancer cells." *Mol Cancer Ther* 2(8): 739-46.

The vitamin D(3) receptor, which is the nuclear receptor for 1alpha,25-dihydroxyvitamin D(3) (VD(3)), forms a heterodimer with the retinoid X receptor (RXR), which is the nuclear receptor for 9-cis-retinoic acid (9-cis-RA). The heterodimer binds to a specific response element consisting of two directly repeated pairs of motifs, AGGTGA, spaced by three nucleotides [direct repeat (DR) 3] and modulates the expression of VD(3)-responsive genes. Telomerase activity, which is seen in most immortal cells and germ cells, is a complex of enzymes that maintain the length of telomeres. One of the major components of human telomerase, human telomerase reverse transcriptase (hTERT), is the catalytic subunit, and the expression of hTERT might correlate most strongly with telomerase activity. We found that the sequence of 5'-AGTTCATGGAGTTCA-3' (DR3') is similar to that of DR3 in the promoter region of hTERT. Our results showed that the combination of VD(3) and 9-cis-RA inhibited telomerase activity through direct interaction of the heterodimer of vitamin D(3) receptor and RXR with the DR3' sequence in the hTERT promoter as well as the combination of VD(3) and selective RXR ligand did. Also, in vivo data showed that the growth of xenografts in nude mice was inhibited by VD(3) and 9-cis-RA. The results of the present study provide evidence on the molecular mechanism of the inhibition of cell growth by these agents, and they could be novel therapeutic agents for prostate cancer.

Ikegami, S., T. Tadakuma, et al. (2005). "Selective gene therapy for prostate cancer cells using liposomes conjugated with IgM type monoclonal antibody against prostate-specific membrane antigen." *Hum Cell* 18(1): 17-23.

Prostate cancer cells express prostate-specific membrane antigen (PSMA). We developed an IgM type monoclonal antibody against PSMA. The antibody was coupled to poly-L-lysine and thereafter this conjugate was mixed with cationic liposomes

containing plasmid DNA. The antibody-liposome complex was tested whether it could deliver the gene of interest selectively to the PSMA positive cells. As assessed by beta-galactosidase reporter gene, the transfection efficiency was 13.2% with anti-PSMA-liposome complex as compared to 4% with control IgM liposome complex. In contrast, no such differences were observed in PSMA negative PC-3, DU145 and T24 cells. Furthermore, in the suicide gene therapy in vitro with thymidine kinase gene plus ganciclovir system, anti-PSMA liposome complex demonstrated a selective growth inhibitory effect on PSMA positive LNCaP cells but not on PSMA negative cell lines.

Ikegami, S., K. Yamakami, et al. (2006). "Targeting gene therapy for prostate cancer cells by liposomes complexed with anti-prostate-specific membrane antigen monoclonal antibody." *Hum Gene Ther* 17(10): 997-1005.

Prostate-specific membrane antigen (PSMA) is a membrane-bound antigen expressed on the surface of prostate cancer cells, and this paper describes the use of an antibody against PSMA for targeting gene therapy. We coupled anti-PSMA monoclonal antibody with poly-L-lysine and then incubated it with plasmids. These complexes were then transfected with cationic liposomes into cells. The transfection efficiency of anti-PSMA-liposome complex was higher than that of normal IgG-liposome complex in PSMA-positive LNCaP cells. Furthermore, anti-PSMA-liposome complex containing a suicide gene, thymidine kinase, demonstrated a selective growth-inhibitory effect on LNCaP cells in vitro, but did not exert a significant effect on PSMA-negative cells. In an in vivo xenograft model of LNCaP cells in nu/nu mice, we administered the complexes via the tail vein. Judging on the basis of both 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal) staining and luciferase assay findings, a significant enrichment of plasmid DNA was observed in LNCaP xenografts with anti-PSMA-liposome complex in comparison with normal IgG-liposome complex. However, the distribution of plasmid DNA did not change substantially in any other organs including the liver, kidney, lung, and spleen. Moreover, in suicide gene therapy, anti-PSMA-liposome complex exerted a significant inhibitory effect on the growth of LNCaP xenograft, in contrast to normal IgG-liposome complex.

Inoue, T., E. S. Leman, et al. (2008). "The potential role of purine-rich element binding protein (PUR) alpha as a novel treatment target for hormone-refractory prostate cancer." *Prostate* 68(10): 1048-56.

**BACKGROUND:** Hormonal therapy for advanced prostate cancer is typically effective at first, but almost all men suffer refractory disease which often is life threatening. The nuclear matrix comprises not only of the structural elements of the nucleus, but is associated with many components of the molecular machinery. Our aim is to find novel targets for the treatment of hormone-refractory prostate cancer (HRPC) by focusing on the composition of the nuclear matrix proteins (NMPs). **METHODS:** LN96 cells were established at our Institution after long-term culturing of LNCaP cells under androgen deprived conditions. The composition of NMPs of LNCaP cells and LN96 cells were analyzed by two-dimensional (2D) electrophoresis and spots differentially expressed were investigated by mass spectrometry for identification. Among the spots identified, we analyzed the potential functional role of the identified proteins in prostate cancer cells by establishing stable overexpressed cells. **RESULTS:** We found that purine-rich element binding protein (PUR)alpha was significantly repressed not only in NMPs but also in total protein and mRNA levels of LN96 cells in comparison to LNCaP cells under the same steroid deprived conditions. Moreover, PURalpha was decreased in its expression both at the protein and mRNA levels in the androgen-independent prostate cancer cell lines, PC3 and DU145 in comparison to LNCaP cells. Stably overexpressing PURalpha in PC3 and DU145 cells negatively regulates cell proliferation, resulting in decreases in PCNA expression. **CONCLUSION:** Further dissection of the role of PURalpha in cell growth regulation may reveal a novel target for HRPC.

Inoue, T., A. Maeno, et al. (2009). "Purine-rich element binding protein (PUR) alpha induces endoplasmic reticulum stress response, and cell differentiation pathways in prostate cancer cells." *Prostate* **69**(8): 861-73.

**BACKGROUND:** Following androgen ablation treatment for advanced prostate cancer, almost all men relapse after a period of initial response to therapy, which eventually is life threatening. We have previously found that purine-rich element binding protein, PURalpha, was significantly repressed in androgen-independent prostate cancer cell lines in comparison to an androgen-dependent line. Moreover, over-expressing PURalpha in androgen-independent prostate cancer cells attenuated their cell proliferation. The aim of the studies described here was to uncover some of the mechanisms by which over-expression of PURalpha attenuates cell proliferation. **METHODS:** A set of common genes induced by over-expressing PURalpha both in PC3 and LNCaP cells was analyzed by DNA

microarray. The results were then validated utilizing quantitative reverse transcription-PCR. Using a 5.3-kb region of the PSA promoter containing androgen response elements, the participation of PURalpha in androgen regulated gene expression was determined. **RESULTS:** Genes involved in stress response and cell differentiation were induced in cells over-expressing PURalpha. Some of the genes that are targets of androgen regulation are also induced. Most strikingly, ectopic expression of PURalpha induced transcriptional activity of the 5.3-kb PSA promoter containing androgen response elements, without androgen stimulation. **CONCLUSION:** Based upon the consideration that some of the genes involved in cell stress and differentiation are also regulated by androgens our data suggest that PURalpha shares some common pathways regulated by the androgen receptor. These findings suggest that regulation of PURalpha expression in prostate cancer cells may serve as a therapeutic target for hormone refractory prostate cancer.

Isharwal, S., M. C. Miller, et al. (2008). "Prognostic value of Her-2/neu and DNA index for progression, metastasis and prostate cancer-specific death in men with long-term follow-up after radical prostatectomy." *Int J Cancer* **123**(11): 2636-43.

Abnormal DNA content in tumor cells represents large scale chromosomal alterations and reflects later changes of genetic instability. Her-2/neu oncogene is amplified in 20-30% of breast and ovarian cancer patients and is associated with poor prognosis. Therefore, we evaluated prognostic value of Her-2/neu expression and DNA content measurements in 252 clinically localized PCa patients with long-term follow-up after radical prostatectomy for progression, metastasis and PCa-specific death. Her-2/neu expression was determined by immunohistochemistry and DNA content measurements employed Feulgen-stained cancer nuclei captured using static image cytometry system. Cox proportional hazard regression and Kaplan-Meier plots were used to identify significant prognostic factors for progression, metastasis and PCa-specific death. The proportions of Her-2/neu positive and high %DNA index tumors significantly increased from nonprogressor to progressors without metastasis to progressors with metastasis ( $p < 0.0001$ ;  $<0.0001$ ). Further, the proportions of Her-2/neu positive and high %DNA index tumors significantly increased from patients who died from another cause without progression to those who died from another cause with progression to those died with PCa-specific death ( $p = 0.027$ ;  $<0.0001$ ). Her-2/neu expression and %DNA index were significant prognosticators for progression ( $p \leq 0.001$ ), metastasis ( $p \leq 0.01$ ) PCa-specific

death ( $p \leq 0.04$ ) in univariate analyses. Multivariately, Her-2/neu expression and %DNA index were also significant for progression ( $p = 0.001$ ), metastasis ( $p = 0.001$ ) and PCa-specific death ( $p = 0.02$ ). When all other clinicopathologic information is available, the increment in concordance index by addition of either Her-2/neu or DNA index was approximately 2% and of both biomarkers was approximately 3% for progression, metastasis and PCa-specific death free survival models. Therefore, patients with Her-2/neu positive and high %DNA index are at a higher risk for disease progression, metastasis and PCa-specific death. Further, Her-2/neu expression and %DNA index may be used with clinicopathologic parameters for prediction of long-term prognosis in PCa.

Ishiguro, H., H. Uemura, et al. (2003). "55 kDa nuclear matrix protein (nmt55) mRNA is expressed in human prostate cancer tissue and is associated with the androgen receptor." *Int J Cancer* **105**(1): 26-32.

Most prostate cancer grows in a hormone-dependent manner. Most patients, however, show hormone-independent growth after several years of hormone therapy. The mechanism of hormone-refractory prostate cancer remains unknown. It is important, therefore, to identify gene(s) related to prostate cancer that are up- or downregulated. We studied differences in gene expression in pairs of prostate cancer and normal prostate tissue utilizing the differential display method. Expression of the identified gene was examined by RT-PCR and real time quantitative PCR (TaqMan-PCR) using 26 pairs of human prostate cancer and normal tissues. We identified a specific upregulated gene encoding a 55 kDa nuclear matrix protein (nmt55) in human prostate cancer. nmt55 gene expression in human prostate cancer tissue was higher (20/26 cases) than that in normal prostate tissue. Moreover, the relationship between nmt55 and androgen receptor (AR) expression showed a positive correlation. In another experiment, transcriptional activity of the prostate specific antigen (PSA) promoter was upregulated by nmt55 in 293 cells. nmt55 showed high expression in prostate cancer compared to normal tissue and its expression showed a positive correlation with AR expression. The PSA promoter was activated by nmt55 expression. These results suggest the possibility that nmt55 expression is related to hormone-dependency or -independence associated with the AR.

Jiang, J., Y. Sugimoto, et al. (2004). "The inhibitory effects of gossypol on human prostate cancer cells-PC3 are associated with transforming growth factor

beta1 (TGFbeta1) signal transduction pathway." *Anticancer Res* **24**(1): 91-100.

BACKGROUND: Racemic gossypol [(+/-)-GP], a naturally occurring polyphenolic yellow pigment present in cottonseed products, inhibits in vitro proliferation of Dunning prostate cancer cells (MAT-LyLu), human prostate cancer cells derived from a bone marrow metastasis (PC3), MCF-7 and primary cultured human prostate cells. (+/-)-GP also has the ability to inhibit the metastasis of lung and lymph nodes of the androgen-independent rodent prostate cancer cell line, MAT-LyLu, after implantation into Copenhagen rats. MATERIALS AND METHODS: The effects of (+/-)-GP on the proliferation of human prostate cancer PC3 cells were determined by thymidine incorporation assay and doubling-time (DT) determination. The mechanisms of action of (+/-)-GP on the proliferation of PC3 cells were determined by RT-PCR analysis, ELISA assay and Western blot analysis. RESULTS: The results show that (+/-)-GP caused reductions in DNA synthesis and prolonged the DTs in PC3 cells. RT-PCR and ELISA results show that (+/-)-GP elevate the mRNA expression and protein secretion of transforming growth factor beta1 (TGFbeta1) in PC3 cells. Consistent with these findings, (+/-)-GP has been shown to decrease the cyclin D1 mRNA expression and protein expression in PC3 cells. Furthermore, the growth inhibition of PC3 cells by conditioned media collected from the (+/-)-GP-treated-PC3 cells was completely reversed by addition of 25 microg/ml of mouse monoclonal anti-TGFbeta1, -beta2, -beta3 antibody, suggesting the involvement of TGFbeta1 in (+/-)-GP-induced growth inhibition of PC3 cells. CONCLUSION: These results indicate that the inhibitory effects of (+/-)-GP on the proliferation of human prostate cancer PC3 cells are associated with induction of TGFbeta1, which in turn influences the expression of the cell cycle-regulatory protein, cyclin D1, in prostate cancer cells.

Jin, F., X. Liu, et al. (2005). "Activation of nuclear factor-kappaB contributes to induction of death receptors and apoptosis by the synthetic retinoid CD437 in DU145 human prostate cancer cells." *Cancer Res* **65**(14): 6354-63.

Activation of the transcription factor, nuclear factor-kappaB (NF-kappaB), results in up-regulation of not only antiapoptotic genes but also proapoptotic genes, including death receptor 4 (DR4) and death receptor 5 (DR5). Therefore, NF-kappaB activation either suppresses or promotes apoptosis depending on the type of stimulus or cell context. We showed previously that the synthetic retinoid, 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437), effectively induces

apoptosis particularly in androgen-independent prostate carcinoma cells. This effect was associated with the ability of CD437 to induce the expression of DR4 and DR5. In the present study, we examined the hypothesis that NF-kappaB activation plays a role in CD437-induced death receptor expression and apoptosis. Treatment of DU145 cells with CD437 resulted in a rapid decrease ( $>$  or  $=$  3 hours) of IkappaB $\alpha$ , which was accompanied by increased translocation of the NF-kappaB subunit p65 from the cytoplasm to the nucleus and increased NF-kappaB DNA-binding activity ( $>$  or  $=$  4 hours). The NF-kappaB inhibitor, helenalin, inhibited CD437-induced IkappaB $\alpha$  reduction and p65 nuclear translocation. Accordingly, it also abrogated CD437-induced up-regulation of DR4, activation of caspase-8 and caspase-3, and increased DNA fragmentation. Overexpression of an IkappaB $\alpha$  dominant-negative mutant blocked not only CD437-induced p65 nuclear translocation but also DR4 up-regulation, caspase activation, and DNA fragmentation. CD437 was unable to decrease IkappaB $\alpha$  protein levels and up-regulate DR4 expression in CD437-resistant DU145 cells. Moreover, knockdown of Fas-associated death domain, caspase-8, and DR4, respectively, suppressed CD437-induced apoptosis. Collectively, these results indicate that CD437 activates NF-kappaB via decreasing IkappaB $\alpha$  protein and thereby induces DR4 expression and subsequent apoptosis in DU145 cells.

Karpf, A. R., S. Bai, et al. (2009). "Increased expression of androgen receptor coregulator MAGE-11 in prostate cancer by DNA hypomethylation and cyclic AMP." *Mol Cancer Res* 7(4): 523-35.

Melanoma antigen gene protein-A11 (MAGE-11) of the MAGE family of cancer germ-line antigens increases androgen receptor (AR) transcriptional activity through its interaction with the AR NH(2)-terminal FXXLF motif. The present study investigated the regulatory mechanisms that control MAGE-11 expression during androgen deprivation therapy and prostate cancer progression. Studies include the CWR22 xenograft model of human prostate cancer, clinical specimens of benign and malignant prostate, and prostate cancer cell lines. MAGE-11 mRNA levels increased 100- to 1,500-fold during androgen deprivation therapy and prostate cancer progression, with highest levels in the castration-recurrent CWR22 xenograft and clinical specimens of castration-recurrent prostate cancer. Pyrosequencing of genomic DNA from prostate cancer specimens and cell lines indicated the increase in MAGE-11 resulted from DNA hypomethylation of a CpG island in the 5' promoter of the MAGE-11 gene. Sodium bisulfite sequencing of genomic DNA

from benign and malignant prostate tumors and prostate cancer cell lines revealed DNA hypomethylation at individual CpG sites at the transcription start site were most critical for MAGE-11 expression. Cyclic AMP (cAMP) also increased MAGE-11 expression and AR transcriptional activity in prostate cancer cell lines. However, cAMP did not alter DNA methylation of the promoter and its effects were inhibited by extensive DNA methylation in the MAGE-11 promoter region. Increased expression of the AR coregulator MAGE-11 through promoter DNA hypomethylation and cAMP provides a novel mechanism for increased AR signaling in castration-recurrent prostate cancer.

Kawada, M., H. Inoue, et al. (2007). "Highly tumorigenic human androgen receptor-positive prostate cancer cells overexpress angiogenin." *Cancer Sci* 98(3): 350-6.

We have recently established a highly tumorigenic cell line, LNCaP-CR, derived from human androgen-dependent prostate cancer LNCaP cells. In the present study, we examined the genes responsible for the high tumorigenicity of LNCaP-CR cells. The cDNA microarray analysis and protein array of secreted factors indicated angiogenin (ANG), an angiogenic factor, as a candidate gene. Reverse transcription-polymerase chain reaction and immunoassay confirmed that LNCaP-CR cells expressed high levels of ANG but not vascular endothelial growth factor (VEGF), compared with the parental LNCaP cells. We also proved that another tumorigenic androgen receptor-positive prostate cancer cell line, 22Rv1, secretes higher levels of ANG than VEGF. To assess the contribution of ANG to the highly tumorigenic phenotype, we transfected the ANG gene into LNCaP cells in order to overexpress ANG, and also transfected ANG small interfering RNA-expressing constructs into LNCaP-CR cells to downregulate ANG. Overexpression of ANG in LNCaP cells did not affect their growth in vitro, but it significantly enhanced tumorigenicity and angiogenesis in vivo. In contrast, knockdown of ANG expression in LNCaP-CR cells also did not affect the growth in vitro, but it led to a significant decrease in tumorigenicity and angiogenesis. Taken together, ANG is one of the genes responsible for the high tumorigenicity of LNCaP-CR cells. Thus, our results support the idea that ANG is an attractive target for cancer therapy and show that LNCaP-CR cells are useful for studying ANG action and experimental therapeutic approaches targeting ANG.

Kong, D., Y. Li, et al. (2007). "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* 67(7): 3310-9.

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.

Kopper, L. and J. Timar (2005). "Genomics of prostate cancer: is there anything to "translate"?" *Pathol Oncol Res* 11(4): 197-203.

This review provides an up-dated collection of data concerning the genetic and epigenetic changes during development, growth and progression of prostate cancer. Hereditary and susceptibility factors have a long list, similarly to the expression of single genes connected to various cell functions. It was a hope that covering a large set of genes, array technologies would clarify very rapidly the role of genetics in malignant diseases, offering targets for molecular diagnostics and therapy. The power of high-throughput techniques for the detection and global analysis of gene expression is unquestionable, interesting, astonishing as well as puzzling data have already been obtained. However, the standardization

of the procedures is still missing and the reproducibility is rather low in many instances. Moreover, the different array methods can select different gene expression profiles, which makes the decision rather difficult. Another important question is, coming again from the array technologies, how far the genotype (the gene profiles or fingerprints) can reflect the actual phenotype in a highly complex and readily changing disease as cancer. Proteomics will provide a closer look to this seemingly unanswerable problem. We are at the beginning of the exploration of the behavior of cancer cells in order to apply a more effective therapy based on a more reliable set of diagnostic and prognostic informations.

Kosari, F., J. M. Munz, et al. (2008). "Identification of prognostic biomarkers for prostate cancer." *Clin Cancer Res* 14(6): 1734-43.

**PURPOSE:** This paper describes a process for the identification of genes that can report on the aggressiveness of prostate tumors and thereby add to the information provided by current pathologic analysis. **MATERIALS AND METHODS:** Expression profiling data from over 100 laser capture microdissection derived samples from nonneoplastic epithelium; Gleason patterns 3, 4, and 5 and node metastasis prostate cancer were used to identify genes at abnormally high levels in only some tumors. These variably overexpressed genes were stratified by their association with aggressive phenotypes and were subsequently filtered to exclude genes with redundant expression patterns. Selected genes were validated in a case-control study in which cases (systemic progression within 5 years) and controls (no systemic progression at 7 years of follow-up) were matched for all clinical and pathologic criteria from time of prostatectomy (n = 175). Both cases and controls, therefore, could have nodal invasion or seminal vesicle involvement at the time of initial treatment. **RESULTS:** A number of candidate variably overexpressed genes selected for their association with aggressive prostate cancer phenotype were evaluated in the case control study. The most prominent candidates were SSTR1 and genes related to proliferation, including TOP2A. **CONCLUSIONS:** The process described here identified genes that add information not available from current clinical measures and can improve the prognosis of prostate cancer.

Krishnan, A. V., R. Shinghal, et al. (2004). "Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays." *Prostate* 59(3): 243-51.

**BACKGROUND:** 1,25-dihydroxyvitamin D(3) [1,25(OH)2D3] exerts growth inhibitory, pro-

differentiating, and pro-apoptotic effects on prostate cells. To better understand the molecular mechanisms underlying these actions, we employed cDNA microarrays to study 1,25(OH)<sub>2</sub>D<sub>3</sub>-regulated gene expression in the LNCaP human prostate cancer cells. METHODS: mRNA isolated from LNCaP cells treated with vehicle or 50 nM 1,25(OH)<sub>2</sub>D<sub>3</sub> for various lengths of time were hybridized to microarrays carrying approximately 23,000 genes. Some of the putative target genes revealed by the microarray analysis were verified by real-time PCR assays. RESULTS: 1,25(OH)<sub>2</sub>D<sub>3</sub> most substantially increased the expression of the insulin-like growth factor binding protein-3 (IGFBP-3) gene. Our analysis also revealed several novel 1,25(OH)<sub>2</sub>D<sub>3</sub>-responsive genes. Interestingly, some of the key genes regulated by 1,25(OH)<sub>2</sub>D<sub>3</sub> are also androgen-responsive genes. 1,25(OH)<sub>2</sub>D<sub>3</sub> also down-regulated genes that mediate androgen catabolism. CONCLUSIONS: The putative 1,25(OH)<sub>2</sub>D<sub>3</sub> target genes appear to be involved in a variety of cellular functions including growth regulation, differentiation, membrane transport, cell-cell and cell-matrix interactions, DNA repair, and inhibition of metastasis. The up-regulation of IGFBP-3 gene has been shown to be crucial in 1,25(OH)<sub>2</sub>D<sub>3</sub>-mediated inhibition of LNCaP cell growth. 1,25(OH)<sub>2</sub>D<sub>3</sub> regulation of androgen-responsive genes as well as genes involved in androgen catabolism suggests that there are interactions between 1,25(OH)<sub>2</sub>D<sub>3</sub> and androgen signaling pathways in LNCaP cells. Further studies on the role of these genes and others in mediating the anti-cancer effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> may lead to better approaches to the prevention and treatment of prostate cancer.

Kucukzeybek, Y., M. K. Gul, et al. (2008). "Enhancement of docetaxel-induced cytotoxicity and apoptosis by all-trans retinoic acid (ATRA) through downregulation of survivin (BIRC5), MCL-1 and LTbeta-R in hormone- and drug resistant prostate cancer cell line, DU-145." *J Exp Clin Cancer Res* **27**: 37.

**BACKGROUND:** The management of hormone-refractory prostate cancer (HRPC) still remains as an important challenge of daily oncology practice. Docetaxel has proved to be a first line treatment choice. All-trans retinoic acid (ATRA) could potentially inhibit the growth of prostate cancer cells in vitro and its combination with various anticancer agents results in increased cytotoxicity. Based on these data, our aim was to examine the synergistic/additive cytotoxic and apoptotic effects of combination of docetaxel and ATRA, in hormone- and drug refractory human DU-145 prostate cancer cells. Furthermore, we have searched for the underlying mechanisms of apoptosis by demonstrating apoptosis-

related genes. METHODS: XTT cell proliferation assay was used for showing cytotoxicity. For verifying apoptosis, both DNA Fragmentation by ELISA assay and caspase 3/7 activity measurement were used. For detecting the mechanism of apoptosis induced by docetaxel-ATRA combination, OligoGeArray which consists of 112 apoptosis related genes was used. RESULTS: Our results revealed that docetaxel and ATRA were synergistically cytotoxic and apoptotic in DU-145 cells, in a dose- and time dependent manner. It was also shown by our studies that apoptosis was induced in DU-145 prostate carcinoma cells with significant cytotoxicity, no matter which agent applied first. We have found out that docetaxel-ATRA combination significantly downregulates survivin (BIRC5), myeloid cell leukemia-1 (MCL-1) and lymphotoxin beta-receptor (LTbetaR) genes, which all three have pivotal roles in regulation of apoptosis and cell cycle progression. CONCLUSION: In conclusion, we strongly suggest that docetaxel and ATRA combination is a good candidate for this challenging era of daily oncologic practice. Also, the combination of docetaxel and ATRA might allow a reduction in docetaxel doses and by this way may diminish docetaxel adverse effects while maintaining the therapeutic effect in patients with HRPc.

Lai, J. C., L. Benimetskaya, et al. (2003). "G3139 (oblimersen) may inhibit prostate cancer cell growth in a partially bis-CpG-dependent non-antisense manner." *Mol Cancer Ther* **2**(10): 1031-43.

G3139 is an 18-mer phosphorothioate oligodeoxyribonucleotide, which is targeted to the initiation codon region of the bcl-2mRNA. Although treatment of PC3 prostate cancer cells with G3139, which contains two CpG motifs, causes a dramatic decrease in bcl-2 protein expression after 3 days, it did not result in significant cellular apoptosis, as it does in many other cell lines. The absence of apoptosis was demonstrated by the absence of pro-caspase 3 cleavage products and of Annexin V cell surface expression. In addition, ATP production and the mitochondrial membrane potential DeltaPsim were preserved. Despite this, G3139 significantly inhibited the rate of cellular proliferation in complete media and blocked cloning in soft agar. G4232, a variant of G3139 that down-regulates bcl-2 expression to the same extent but has both CpG cytidines C5 methylated, was only minimally antiproliferative. A series of mismatched G3139-related oligomers were synthesized that could also substantially down-regulate bcl-2 protein expression, but only if the CpG motifs were preserved, demonstrating the presence of additional non-antisense mechanisms. G3139 caused production of reactive oxygen species in growth-arrested cells and oxidation of nuclear guanosine to 8-

hydroxy-2'-deoxyguanosine, as determined by 1F7 monoclonal antibody staining. Bromodeoxyuridine incorporation studies demonstrated that G3139 induced a G1-S entry block and an intra-S-phase block in PC3 cells that persisted as long as 3 days. This finding coincides with the observation that expression of several proteins encoded by S-phase genes, including c-myc and poly(ADP-ribose) polymerase, were significantly reduced. These results illustrate the complexity of the mechanism of action of G3139 in PC3 cells.

Langsenlehner, T., U. Langsenlehner, et al. (2008). "The Glu228Ala polymorphism in the ligand binding domain of death receptor 4 is associated with increased risk for prostate cancer metastases." *Prostate* **68**(3): 264-8.

**BACKGROUND:** Death receptor 4, encoded by the TNFRSF10A gene, is an important mediator of apoptosis and its dysfunction may be related to cancer development and distant tumor spread. A single nucleotide polymorphism in TNFRSF10A (Glu228Ala, rs20576) within a conserved region of the extracellular cysteine-rich domain of death receptor 4 has been associated with an increased risk for a variety of tumor entities. Aim of the present study was to evaluate the role of the TNFRSF10A polymorphism in metastatic progression of prostate cancer after radiation therapy. **METHODS:** We carried out a prospective study including 702 prostate cancer patients from the Austrian PROCAGENE (Prostate Cancer Genetics) study. Development of metastases was examined in regular follow-up investigations. TNFRSF10A genotypes were determined by a 5'-nuclease assay (TaqMan). **RESULTS:** Within a median follow-up time of 10 months (range 0-60 months), 24 (3.4%) patients developed metastases. In a Cox regression model including age at diagnosis and risk group as potential confounders, carriage of an 228Ala allele was associated with a relative risk of 2.47 (95% CI 1.10-5.54; P=0.028) for metastases. TNFRSF10A genotypes were not associated with tumor stage, grade, risk group or age at diagnosis. **CONCLUSION:** We conclude that the TNFRSF10A Glu228Ala polymorphism may be a novel independent risk factor for prostate cancer metastases after radiation therapy.

Lebedeva, I. V., D. Sarkar, et al. (2003). "Bcl-2 and Bcl-x(L) differentially protect human prostate cancer cells from induction of apoptosis by melanoma differentiation associated gene-7, mda-7/IL-24." *Oncogene* **22**(54): 8758-73.

Subtraction hybridization identified melanoma differentiation associated gene-7, mda-7, in the context of terminally differentiated human

melanoma cells. Based on its structure, cytokine-like properties and proposed mode of action, mda-7 has now been classified as IL-24. When expressed by means of a replication-incompetent adenovirus, Ad.mda-7 induces apoptosis in a broad range of cancer cells, without inducing harmful effects in normal fibroblast or epithelial cells. These unique properties of mda-7/IL-24 suggest that this gene will prove beneficial for cancer gene therapy. We now demonstrate that Ad.mda-7 decreases viability by induction of apoptosis in hormone-responsive (LNCaP) and hormone-independent (DU-145 and PC-3) human prostate carcinomas, without altering growth or survival in early-passage normal human prostate epithelial cells (HuPEC). Ad.mda-7 causes G(2)/M arrest and apoptosis in LNCaP (p53-wildtype), DU-145 (p53 mutant, Bax-negative) and PC-3 (p53-negative) prostate carcinomas, but not in HuPEC. Apoptosis induction correlated with changes in the ratio of pro- to antiapoptotic Bcl-2 protein family members. A potential functional role for changes in bcl-2 family gene expression in Ad.mda-7-induced apoptosis was suggested by the finding that forced overexpression of bcl-x(L) or bcl-2 differentially diminished the apoptotic effect of Ad.mda-7 in prostate carcinomas. These results confirm that induction of apoptosis by the mda-7/IL-24 gene in prostate cancer cells is Bax- and p53-independent and is mediated by mitochondrial pathways involving bcl-2 family gene members. The mda-7/IL-24 gene represents a new class of cancer-specific apoptosis-inducing genes with obvious potential for the targeted gene-based therapy of human prostate cancer.

Lehmann, B. D., J. A. McCubrey, et al. (2007). "A dominant role for p53-dependent cellular senescence in radiosensitization of human prostate cancer cells." *Cell Cycle* **6**(5): 595-605.

Because p53 inactivation may limit the effectiveness of radiation therapy for localized prostate cancer, it is important to understand how this gene regulates clonogenic survival after an exposure to ionizing radiation. Here, we show that premature cellular senescence is the principal mode of cell death accounting for the radiosensitivity of human prostate cancer cell lines retaining p53 function. Alternative stress response pathways controlled by this tumor suppressor, including cell cycle arrest, DNA damage repair, mitotic catastrophe and apoptosis, contributed significantly less to radiation-induced clonogenic death. Using a dominant negative C-terminal fragment of p53, we present the first evidence that a complete loss of endogenous p53 function is sufficient to limit the irradiation-induced senescence and clonogenic death of prostate cancer cells. Conversely, inheritance

of wild-type p53 by prostate cancer cells lacking a functional allele of this gene (i.e., DU145) significantly increases clonogenic death through p53-dependent cellular senescence and apoptotic pathways. Our data provide evidence that mutations of even one p53 allele may be sufficient to alter their clonogenic fate. In addition, they support the idea that the p53 pathway can be used as a specific target for enhancing the radiosensitivity of prostate cancer cells. Activation of p53 by the drug nutlin-3 is shown to be an effective radiosensitizer of prostate cancer cells retaining functional alleles of p53 and this effect was entirely attributable to an increased induction of p53-dependent cellular senescence.

Lexander, H., C. Palmberg, et al. (2006). "Correlation of protein expression, Gleason score and DNA ploidy in prostate cancer." *Proteomics* **6**(15): 4370-80.

The prognosis of prostate cancer correlates with tumor differentiation. Gleason score and DNA ploidy are two prognostic factors that correlate with prognosis. We analyzed differences in protein expression in prostate cancer of high and low aggressiveness according to these measures. From 35 prostatectomy specimens, 29 cancer samples and 10 benign samples were harvested by scraping cells from cut surfaces. DNA ploidy was assessed by image cytometry. Protein preparations from cell suspensions were examined by 2-DE. Protein spots that differed quantitatively between sample groups were identified by MS fingerprinting of tryptic fragments and MS/MS sequence analysis. We found 39 protein spots with expression levels that were raised or lowered in correlation with Gleason score and/or DNA ploidy pattern (31 overexpressed in high-malignant cancer, 8 underexpressed). Of these, 30 were identified by MS. Among overexpressed proteins were heat-shock, structural and membrane proteins and enzymes involved in gene silencing, protein synthesis/degradation, mitochondrial protein import (metaxin 2), detoxification (GST-pi) and energy metabolism. Stroma-associated proteins were generally underexpressed. The protein expression of prostate cancer correlates with tumor differentiation. Potential prognostic markers may be found among proteins that are differentially expressed and the clinical value of these should be validated.

Li, L. C., P. R. Carroll, et al. (2005). "Epigenetic changes in prostate cancer: implication for diagnosis and treatment." *J Natl Cancer Inst* **97**(2): 103-15.

Prostate cancer is the most common noncutaneous malignancy and the second leading cause of cancer death among men in the United States. DNA methylation and histone modifications are important epigenetic mechanisms of gene regulation

and play essential roles both independently and cooperatively in tumor initiation and progression. Aberrant epigenetic events such as DNA hypo- and hypermethylation and altered histone acetylation have both been observed in prostate cancer, in which they affect a large number of genes. Although the list of aberrantly epigenetically regulated genes continues to grow, only a few genes have, so far, given promising results as potential tumor biomarkers for early diagnosis and risk assessment of prostate cancer. Thus, large-scale screening of aberrant epigenetic events such as DNA hypermethylation is needed to identify prostate cancer-specific epigenetic fingerprints. The reversibility of epigenetic aberrations has made them attractive targets for cancer treatment with modulators that demethylate DNA and inhibit histone deacetylases, leading to reactivation of silenced genes. More studies into the mechanism and consequence of demethylation are required before the cancer epigenome can be safely manipulated with therapeutics as a treatment modality. In this review, we examine the current literature on epigenetic changes in prostate cancer and discuss the clinical potential of cancer epigenetics for the diagnosis and treatment of this disease.

Li, P., H. Lee, et al. (2003). "AKT-independent protection of prostate cancer cells from apoptosis mediated through complex formation between the androgen receptor and FKHR." *Mol Cell Biol* **23**(1): 104-18.

Recent studies suggested that the protection of cell apoptosis by AKT involves phosphorylation and inhibition of FKHR and related FOXO forkhead transcription factors and that androgens provide an AKT-independent cell survival signal in prostate cancer cells. Here, we report receptor-dependent repression of FKHR function by androgens in prostate cancer cells. Transcriptional analysis demonstrated that activation of the androgen receptor caused an inhibition of both wild-type FKHR and a mutant in which all three known AKT sites were mutated to alanines, showing that the repression is AKT independent. In vivo and in vitro coprecipitation studies demonstrated that the repression is mediated through protein-protein interaction between FKHR and the androgen receptor. Mapping analysis localized the interacting domains to the carboxyl terminus between amino acids 350 and 655 of FKHR and to the amino-terminal A/B region and the ligand binding domain of the receptor. Further analysis demonstrated that the activated androgen receptor blocked FKHR's DNA binding activity and impaired its ability to induce Fas ligand expression and prostate cancer cell apoptosis and cell cycle arrest. These studies identify a new mechanism for androgen-mediated prostate

cancer cell survival that appears to be independent of the activity of the receptor on androgen response element-mediated transcription and establish FKHR and related FOXO forkhead proteins as important nuclear targets for both AKT-dependent and -independent survival signals in prostate cancer cells.

Li, X., Y. P. Zhang, et al. (2005). "Gene therapy for prostate cancer by controlling adenovirus E1a and E4 gene expression with PSES enhancer." *Cancer Res* **65**(5): 1941-51.

PSES is a chimeric enhancer containing enhancer elements from prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) genes that are prevalently expressed in androgen-independent prostate cancers. PSES shows strong activity equivalent to cytomegalovirus (CMV) promoter, specifically in PSA/PSMA-positive prostate cancer cells, the major cell types in prostate cancer in the absence of androgen. We developed a recombinant adenovirus (AdE4PSESE1a) by placing adenoviral E1a and E4 genes under the control of the bidirectional enhancer PSES and enhanced green fluorescent protein gene for the purpose of intratumoral virus tracking under the control of CMV promoter. Because of PSES being very weak in nonprostatic cells, including HEK293 and HER911 that are frequently used to produce recombinant adenovirus, AdE4PSESE1a can only be produced in the HER911E4 cell line which expresses both E1 and E4 genes. AdE4PSESE1a showed similar viral replication and tumor cell killing activities to wild-type adenovirus in PSA/PSMA-positive prostate cancer cells. The viral replication and tumor cell killing activities were dramatically attenuated in PSA/PSMA-negative cells. To test whether AdE4PSESE1a could be used to target prostate tumors in vivo, CWR22rv s.c. tumors were induced in nude mice and treated with AdE4PSESE1a via intratumoral and tail vein injection. Compared to tumors treated with control virus, the growth of CWR22rv tumors was dramatically inhibited by AdE4PSESE1a via tail vein injection or intratumoral injection. These data show that adenoviral replication can be tightly controlled in a novel fashion by controlling adenoviral E1a and E4 genes simultaneously with a single enhancer.

Li, Y., X. Hong, et al. (2005). "Gene expression profiling revealed novel molecular targets of docetaxel and estramustine combination treatment in prostate cancer cells." *Mol Cancer Ther* **4**(3): 389-98.

Both docetaxel and estramustine are antimicrotubule agents with antitumor activity in various cancers including prostate cancer. Clinical trials for docetaxel and estramustine combination

treatment have suggested improved antitumor activity in hormone-refractory prostate cancer. However, the molecular mechanisms involved in the combination treatment with docetaxel and estramustine have not been fully elucidated. In order to establish such molecular mechanisms in both hormone insensitive (PC-3) and sensitive (LNCaP) prostate cancer cells, gene expression profiles of docetaxel- and estramustine-treated prostate cancer cells were obtained by using Affymetrix Human Genome U133A Array. Total RNA from PC-3 and LNCaP cells untreated and treated with 2 nmol/L docetaxel, 4 micromol/L estramustine, or 1 nmol/L docetaxel plus 2 micromol/L estramustine for 6, 36, and 72 hours was subjected to microarray analysis. Real-time PCR and Western blot analysis were conducted to confirm the microarray data. Clustering analysis based on biological function showed that docetaxel and estramustine combination treatment down-regulated some genes that are known to regulate cell proliferation, transcription, translation, and oncogenesis. In contrast, docetaxel and estramustine combination treatment up-regulated some genes related to induction of apoptosis, cell cycle arrest, and tumor suppression. Docetaxel and estramustine also showed differential effects on gene expression between mono- and combination treatment. Combination treatment with docetaxel and estramustine caused alternations of a large number of genes, many of which may contribute to the molecular mechanisms by which docetaxel and estramustine inhibit the growth of prostate cancer cells. These results provide novel molecular targets of docetaxel and estramustine combination treatment in prostate cancer cells. This information could be utilized for further mechanistic research and for devising optimized therapeutic strategies against prostate cancer.

Liao, X., S. Tang, et al. (2005). "Small-interfering RNA-induced androgen receptor silencing leads to apoptotic cell death in prostate cancer." *Mol Cancer Ther* **4**(4): 505-15.

Prostate cancer is the second leading cause of cancer death in the United States and, thus far, there has been no effective therapy for the treatment of hormone-refractory disease. Recently, the androgen receptor (AR) has been shown to play a critical role in the development and progression of the disease. In this report, we showed that knocking down the AR protein level by a small interfering RNA (siRNA) approach resulted in a significant apoptotic cell death as evidenced by an increased annexin V binding, reduced mitochondrial potential, caspase-3/6 activation, and DFF45 and poly(ADP-ribose) polymerase cleavage. The apoptotic response was

specifically observed in those siRNA-transfected cells that harbor a native AR gene. No cell death was found in the AR-null prostate cancer cell PC-3 or its subline that has been reconstituted with an exogenous AR gene, as well as two breast cancer cell lines that are AR positive. Moreover, in parallel with the siRNA-induced AR silencing, the antiapoptotic protein Bcl-xL was significantly reduced, which might account for the apoptotic cell death because ectopic enforced expression of Bcl-xL protein partially inhibited apoptosis after AR silencing. Taken together, our data showed that knocking down the AR protein level in prostate cancer cells leads to apoptosis by disrupting the Bcl-xL-mediated survival signal downstream of AR-dependent survival pathway.

Lin, C. C., H. C. Wu, et al. (2003). "CYP17 gene promoter allelic variant is not associated with prostate cancer." *Urol Oncol* **21**(4): 262-5.

Prostate cancer is the most common urological malignancy in Taiwan. The formation of prostate cancer has been reported to be associated with androgen. Two key steps in the sex steroid synthesis are mediated by the enzyme cytochrome p450c17alpha which is encoded in the CYP17 gene. Our aim was to investigate whether a polymorphism of CYP17 gene could be used as a genetic marker for associating prostate cancer. In this study, we compared the frequency of the C/T polymorphism of CYP17 gene 5'-UTR promoter region between 93 patients with prostate cancer and 121 healthy male volunteers (age, >60 years). The result revealed no significant association between the CYP17 genotype and prostate cancer ( $P = .781$ ). Therefore, CYP17 C/T polymorphism is not a valid genetic marker for prostate cancer. Although a possible interaction between CYP17 gene C/T polymorphism and SP-1 transcription factor has been reported in the literature, we did not find any evidence for this the difference among clinical staging, pathological grading, or responsiveness to hormonal therapy in prostate cancer.

Lin, D., A. Watahiki, et al. (2008). "ASAP1, a gene at 8q24, is associated with prostate cancer metastasis." *Cancer Res* **68**(11): 4352-9.

Metastatic prostate cancer is a terminal disease, and the development of reliable prognostic tools and more effective therapy is critically important for improved disease survival and management. This study was aimed at identifying genes that are differentially expressed in metastatic and nonmetastatic prostate cancer cells and, as such, could be critical in the development of metastasis. Long-SAGE analysis was used to compare a transplantable human metastatic prostate cancer subline, PCa1-met, with a nonmetastatic counterpart, PCa2. Both sublines

were developed from a patient's prostate cancer specimen via subrenal capsule grafting and subsequent orthotopic implantation into SCID mice. Among various differentially expressed genes identified, ASAP1, an 8q24 gene encoding an ADP-ribosylation factor GTPase-activating protein not previously associated with prostate cancer, was up-regulated in the metastatic subline as confirmed by quantitative real-time PCR. Immunohistochemistry of xenograft sections showed that cytoplasmic ASAP1 protein staining was absent or weak in benign tissue, significantly stronger in nonmetastatic PCa2 tissue, and strongest in PCa1-met tissue. In clinical specimens, ASAP1 protein staining was elevated in 80% of primary prostate cancers and substantially higher in metastatic lesions compared with benign prostate tissue. Moreover, additional ASAP1 gene copies were detected in 58% of the primary prostate cancer specimens. Small interfering RNA-induced reduction of ASAP1 protein expression markedly suppressed *in vitro* PC-3 cell migration (approximately 50%) and Matrigel invasion (approximately 67%). This study suggests that the ASAP1 gene plays a role in prostate cancer metastasis and may represent a therapeutic target and/or biomarker for metastatic disease.

Lin, J. C., S. Y. Chang, et al. (2005). "The association of Id-1, MIF and GSTpi with acquired drug resistance in hormone independent prostate cancer cells." *Oncol Rep* **13**(5): 983-8.

Most patients with prostate cancer respond to androgen ablation therapy, but the tumor eventually progresses to an androgen-independent stage with multiple anti-cancer drug resistance. Novel therapeutic strategies for hormone independent prostate cancer need to be developed. The genes for Id-1, MIF and GSTpi were up-regulated in drug resistant cells of hormone independent prostate cancer cells using cDNA microarray gene expression analysis. In this study we aimed to investigate whether constitutive overexpression of these candidate genes in cells can affect cellular resistance to chemotherapeutic agents. The mRNA expression was determined by reverse transcriptase-polymerase chain reaction, and the Id-1, MIF and GSTpi protein contents in these cell lines were measured by Western blotting. Susceptibility of the cells to chemotherapeutic agents including doxorubicin, paclitaxel and cyclophosphamide was determined by microculture tetrazolium bromide assay. The analysis of apoptosis was assessed by annexin V assay. The cytotoxicity assay results demonstrated that cells overexpressing the Id-1 gene increased in their resistance to doxorubicin, paclitaxel and cyclophosphamide. MIF expression can drive cells to increase in their resistance to paclitaxel, and

GSTpi expression confers drug resistance to doxorubicin and cyclophosphamide. The induction of *mdr1* gene expression was noted in up-regulation of MIF and GSTpi. Our findings suggest that overproduction of the Id-1, MIF and GSTpi proteins and coinduction of *mdr-1* play an important role in the development of acquired drug resistance to various drugs of prostate cancer cells.

Liu, Z., M. Marquez, et al. (2008). "Incubation with somatostatin, 5-aza decitabine and trichostatin up-regulates somatostatin receptor expression in prostate cancer cells." *Oncol Rep* **20**(1): 151-4.

Somatostatin (SMS), binds to its specific receptors (SSTRs) and transduces growth inhibitory, anti-secretory and apoptotic signals. Several human cancers express SSTRs, including prostate cancer, and therefore SMS is of interest for anti-cancer therapy. DNA methylation and histone modifications are involved in normal cell development, gene imprinting and human carcinogenesis. Reversing DNA methylation is an attractive therapeutic possibility, since epigenetic modifications change gene expression without changing the gene function. DNA methylation inhibitors such as 5-aza-2'-deoxycytidine (5'-aza, decitabine) have been used to treat several types of haematological malignancies. Histone deacetylase inhibitors such as trichostatin (TSA), are a new class of 'targeted anti-cancer agents'. TSA and decitabine can induce growth arrest, apoptosis or terminal differentiation in a variety of solid and haematological cancers in advanced disease patients. In the present study, the LNCaP cell line (prostate cancer) was incubated with SMS or Somadex (an SMS polymer conjugate) for three days, 1 nM per day, and the untreated cells were the negative control. For DNA demethylation, cells were grown in the presence of 2.5 microM 5-aza for 120 h, and re-fed with 5-aza-containing fresh medium at day 3. The total incubation time with 5-aza was 120 h. TSA at 1.0 microM was added into the cultured cells for 24 h. The combined treatment of 5-aza and TSA was performed by incubating the cells with 5-aza for 120 h followed by a 24-h exposure to TSA. Using cDNA obtained from these cell lines, the difference in the expression level of SSTR mRNA transcripts before and after 5-aza and TSA treatments was analyzed by RT-PCR. An increased induction of mRNA expression of the five SSTR subtypes was observed in the LNCaP cells when incubated with SMS/Somadex (dose-dependent). The inhibition of DNA methylation and histone acetylation resulted in the up-regulation of SSTR5 mRNA expression. The results demonstrate a positive feedback loop between SMS and its receptors. This regulation pathway may enhance the anti-tumor activity of somatostatin. To benefit from this effect in

a clinical setting, the dose, dose frequency and pan affinity of the SMS derivative are important factors. The epigenetic manipulation with DNA methylation or histone deacetylase inhibitors, combined with SMS, may offer a novel alternative for the treatment of advanced prostate cancer.

Lu, Y., X. Zhang, et al. (2009). "Adenoviral-mediated pHyde gene transfer and cisplatin additively inhibit human prostate cancer growth by enhancing apoptosis." *Prostate* **69**(3): 234-48.

**BACKGROUND:** A novel gene, rat pHyde, has been cloned by us recently. The rat pHyde was shown by the same group to have growth inhibitory effects on human prostate cancer through the induction of apoptosis. **METHODS:** In this report, a human homologue, hpHyde of the rat pHyde, was cloned by cDNA libraries screening. The database search and in situ hybridization were used to map the genomic loci of hpHyde in human chromosome. The anti-prostate cancer effects of pHyde in conjunction with chemotherapy agent were analyzed by in vitro and in vivo assays using adenoviral vector expressing pHyde (AdRSVpHyde) in combination with DNA damaging chemotherapeutic agent, cisplatin, and docetaxel, respectively. **RESULTS:** Database search and FISH analysis consistently indicated that hpHyde gene localizes at human chromosome 2q14. Protein sequence analysis suggests that hpHyde may be a plasma membrane protein. hpHyde is differentially expressed in various normal human tissues and organs, suggesting that hpHyde may play roles in development and differentiation. Growth suppression and induction of apoptosis were additively greater in DU145 human prostate cancer cells treated with AdRSVpHyde and cisplatin than either agent alone both in vitro and in vivo. Moreover, AdRSVpHyde and docetaxel also have a similar additively inhibitory effect on DU145 cell growth. **CONCLUSIONS:** A novel gene hpHyde, the human homologue of rat pHyde, has been cloned and its genomic location in the human chromosome has been identified. Our results support the potential use of pHyde for prostate cancer gene therapy coupled with chemotherapy to improve therapeutic index.

Lucia, M. S. and K. C. Torkko (2004). "Inflammation as a target for prostate cancer chemoprevention: pathological and laboratory rationale." *J Urol* **171**(2 Pt 2): S30-4; discussion S35.

**PURPOSE:** We review the literature addressing a potential causal role for chronic or recurrent inflammation or infection in the development of prostate cancer. **MATERIALS AND METHODS:** A literature search was conducted using MEDLINE to identify articles on chronic

inflammation as a risk factor for cancer, particularly prostate cancer. RESULTS: A causal role for chronic or recurrent inflammation or infection in the development of prostate cancer has yet to be proven. Inflammation may contribute to carcinogenesis by 1 or more of several potentially interrelated mechanisms, including 1) the elaboration of cytokines and growth factors that favor tumor cell growth, 2) induction of cyclooxygenase-2 in macrophages and epithelial cells, and 3) generation of mutagenic reactive oxygen and nitrogen species. Chronic inflammation in the form of stromal and epithelial infiltrates of lymphocytes and histiocytes is extremely common in the peripheral zone of the prostate where most cancers arise. Although differences in histology and terminology exist for these inflammatory and atrophic lesions, as a group they often display evidence of epithelial proliferation. Heterogeneous expression of the GSTP1 gene in such lesions has been proposed as evidence for susceptibility to oxidative damage, thereby providing fertile ground for carcinogenesis. CONCLUSIONS: Although the cumulative evidence demonstrates that chronic inflammation may be a legitimate target for chemopreventive efforts, more study is needed to prove its etiological role in prostate cancer.

Luo, Q., Z. Li, et al. (2009). "Lycium barbarum polysaccharides induce apoptosis in human prostate cancer cells and inhibits prostate cancer growth in a xenograft mouse model of human prostate cancer." *J Med Food* **12**(4): 695-703.

Lycium barbarum polysaccharides (LBPs) are important functional constituents in red-colored fruits of *L. barbarum* (Guo Qi Zi, a well-known traditional Chinese medicinal plant commonly known as Goji berry or wolfberry). The influence of LBP on human prostate cancer cells was systematically investigated in vitro and in vivo. The in vitro effects of LBP on two cell lines (PC-3 and DU-145) were examined by using trypan blue exclusion staining, single-cell gel electrophoresis, flow cytometry, terminal dUTP nick-end labeling assay, and immunohistochemical assay (assessment of Bcl-2 and Bax expression). The in vivo effect of LBP on PC-3 cells was assessed in the nude mouse xenograft tumor model. The in vitro results showed that LBP can dose- and time-dependently inhibit the growth of both PC-3 and DU-145 cells. LBP caused the breakage of DNA strands of PC-3 and DU-145 cells; the tail frequency and tail length were significantly higher than that of control cells. LBP also markedly induced PC-3 and DU-145 cell apoptosis, with the highest apoptosis rates at 41.5% and 35.5%, respectively. The ratio of Bcl-2/Bax protein expression following LBP treatments decreased significantly with a dose-effect

relationship, which suggested that LBP can regulate the expression of Bcl-2 and Bax to induce apoptosis of PC-3 and DU-145 cells. The in vivo experimental results indicate that LBP might significantly inhibit PC-3 tumor growth in nude mice. Both the tumor volume and weight of the LBP treatment group were significantly lower than those of the control group.

Lynch, R. L., B. W. Konicek, et al. (2005). "The progression of LNCaP human prostate cancer cells to androgen independence involves decreased FOXO3a expression and reduced p27KIP1 promoter transactivation." *Mol Cancer Res* **3**(3): 163-9.

The progression of human prostate cancer from the initial androgen-dependent phase to androgen independence involves diminished apoptosis and a release from the cell cycle block triggered by androgen ablation therapy. FOXO transcription factors play a central role in promoting expression of proapoptotic and cell cycle regulatory genes (e.g., FasL and p27KIP1). Reduced FOXO function might, therefore, play a role in androgen-independent progression of human prostate cancer. Herein, we show that FOXO function is compromised in androgen-independent prostate cancer cells (LNAI) versus androgen-dependent LNCaP cells. The FOXO3a protein, the most highly expressed FOXO family member in prostate cancer cells, is hyperphosphorylated in LNAI cells. FOXO3a expression is also markedly reduced in these androgen-independent LNAI cells when compared with parental LNCaP cells. Together, reduced FOXO3a expression coupled to FOXO3a hyperphosphorylation would suppress FOXO transcriptional activity. Accordingly, activity of the FOXO-responsive p27KIP1 promoter is reduced 60% in these LNAI cells when compared with LNCaP cells. Moreover, mutation of a conserved FOXO response element suppresses p27KIP1 promoter activity, substantiating a regulatory role for this FOXO response element in p27KIP1 promoter transactivation. Finally, we show that the activity of a distinct FOXO-responsive promoter, the 3X-IRS promoter, is also reduced in LNAI cells. Collectively, these data show that reduced FOXO3a expression coupled to increased FOXO3a phosphorylation coincide with reduced FOXO-responsive promoter activity in androgen-independent LNAI cells when compared with androgen-dependent LNCaP cells. To the extent that this model reflects human disease, these data suggest that FOXO function may be compromised with androgen-independent progression of human prostate cancer.

Majid, S., N. Kikuno, et al. (2008). "Genistein induces the p21WAF1/CIP1 and p16INK4a tumor suppressor

genes in prostate cancer cells by epigenetic mechanisms involving active chromatin modification." *Cancer Res* **68**(8): 2736-44.

Genistein (4',5,7-trihydroxyisoflavone) is the most abundant isoflavone found in the soybean. The effects of genistein on various cancer cell lines have been extensively studied but the precise molecular mechanisms are not known. We report here the epigenetic mechanism of the action of genistein on androgen-sensitive (LNCaP) and androgen-insensitive (DuPro) human prostate cancer cell lines. Genistein induced the expression of tumor suppressor genes p21 (WAF1/CIP1/KIP1) and p16 (INK4a) with a concomitant decrease in cyclins. There was a G(0)-G(1) cell cycle arrest in LNCaP cells and a G(2)-M arrest in DuPro cells after genistein treatment. Genistein also induced apoptosis in DuPro cells. DNA methylation analysis revealed the absence of p21 promoter methylation in both cell lines. The effect of genistein on chromatin remodeling has not been previously reported. We found that genistein increased acetylated histones 3, 4, and H3/K4 at the p21 and p16 transcription start sites. Furthermore, we found that genistein treatment also increased the expression of histone acetyl transferases that function in transcriptional activation. This is the first report on epigenetic regulation of various genes by genistein through chromatin remodeling in prostate cancer. Altogether, our data provide new insights into the epigenetic mechanism of the action of genistein that may contribute to the chemopreventive activity of this dietary isoflavone and have important implications for epigenetic therapy.

Maki, H. E., O. R. Saramaki, et al. (2007). "Overexpression and gene amplification of BAG-1L in hormone-refractory prostate cancer." *J Pathol* **212**(4): 395-401.

BAG-1L (Bcl-2-associated anthanogene 1) has been found to interact with androgen receptor (AR), and has been suggested to be involved in the development of prostate cancer. In order to determine the presence of genetic and/or expression alterations of BAG-1L in prostate cancer, we analysed human prostate cancer cell lines and xenografts as well as patient samples of untreated, hormone-naive, and hormone-refractory prostate carcinomas for sequence variations using denaturing high-performance liquid chromatography (DHPLC), for gene copy number using fluorescence in situ hybridization (FISH), and for expression using both quantitative RT-PCR and immunostaining. Only one sequence variation was found in all 37 cell lines and xenografts analysed. BAG-1 gene amplification was detected in two xenografts. In addition, gene amplification was found in 6 of 81 (7.4%) hormone-refractory clinical tumours,

whereas no amplification was found in any of the 130 untreated tumours analysed. Additionally, gain of the BAG-1 gene was observed in 27.2% of the hormone-refractory tumours and in 18.5% of the untreated carcinomas. In a set of 263 patient samples, BAG-1L protein expression was significantly higher in hormone-refractory tumours than in primary tumours ( $p = 0.002$ ). Altogether, these data suggest that amplification and overexpression of BAG-1L may be involved in the progression of prostate cancer.

Maki, H. E., K. K. Waltering, et al. (2006). "Screening of genetic and expression alterations of SRC1 gene in prostate cancer." *Prostate* **66**(13): 1391-8.

**BACKGROUND:** Genetic alterations of the SRC1 gene have not been thoroughly studied in prostate cancer. **MATERIALS AND METHODS:** Five prostate cancer cell lines and 32 xenografts were screened for mutations and gene copy number alterations. Subsequently, frequencies of detected sequence variations were further analyzed in 44 clinical prostate cancers, 6 benign prostate hyperplasias, and 48 normal controls. Finally, the protein expression of SRC1 in 254 clinical prostate tumors was investigated. **RESULTS:** Three non-recurrent sequence variations, and one single nucleotide polymorphism in the coding region of SRC1, as well as one case of SRC1 gene amplification were found. The protein expression of SRC1 was higher in androgen ablation resistant than untreated prostate carcinomas, but the difference was not statistically significant ( $P = 0.0796$ ). **CONCLUSIONS:** Genetic alterations of SRC1 are rare in prostate cancer. The nuclear protein accumulation of SRC1 seems to be mildly increased in androgen ablation resistant prostate cancers. .

Mammi, C., G. V. Frajese, et al. (2006). "PAC1-R null isoform expression in human prostate cancer tissue." *Prostate* **66**(5): 514-21.

**BACKGROUND:** PACAP is a member of the VIP/GHRH family of neuropeptides and has important effects on prostate cell proliferation. Here we analyze the expression and localization of PACAP and its specific receptor variants (PAC(1)-R) in tissues collected from patients undergoing prostate biopsy and surgery for benign prostatic hyperplasia (BPH) and prostate cancer (PCa). **METHODS:** Reverse transcriptase (RT)-polymerase chain reaction (PCR), DNA sequencing, and immunohistochemistry. **RESULTS:** PACAP and PAC(1)-R were localized by immunohistochemistry in the prostate tissue. While in healthy and BPH tissues PAC(1)-R positive staining is present in all the epithelial cells lining the lumen of the acini and in some stromal cells (mostly in the apical portion of the cells), in PCa tissues, anti-

PAC(1)-R antibody stained the apical portion of the cells. We provide evidence that PAC(1)-R null and SV(1)/SV(2) variants are all present in normal and hyperplastic tissues, while in PCa tissue PAC(1)-R null is the most relevant receptor variant expressed. CONCLUSIONS: Our data demonstrates that the PAC(1)-R null variant is the most relevant isoform expressed in human PCa tissue being suggestively related with the events determining the outcome of prostate cancer.

Marandola, P., A. Bonghi, et al. (2004). "Molecular biology and the staging of prostate cancer." *Ann N Y Acad Sci* **1028**: 294-312.

Cancer of the prostate is still controlled or cured by surgery, radiotherapy, and hormone therapy. The present criteria of complication and prediction are criticized more and more for not being sufficiently reliable, due to the high heterogeneity of prostatic cells. The continuing discoveries of intra- and extracellular mechanisms of the molecular informational network, which allow the continuity or discontinuity of the cell's life, are also related to prostate cancer. The role of androgen receptors is now under close scrutiny, in the light of the knowledge of regulatory genes and their molecular expression. In the near future, a complete study of prostate cancer's DNA is certainly envisaged. Looking forward to the extraordinary applications of molecular biology in this field, this article is aimed at establishing a clear link between the conventional ways of interpreting the clinical expression of prostate cancer and the oncoming applications of genomics and proteomics.

Maria McCrohan, A., C. Morrissey, et al. (2006). "Effects of the dual 5 alpha-reductase inhibitor dutasteride on apoptosis in primary cultures of prostate cancer epithelial cells and cell lines." *Cancer* **106**(12): 2743-52.

BACKGROUND: The profound reduction in serum dihydrotestosterone (DHT) observed with the dual 5 alpha-reductase inhibitor (5ARI) dutasteride makes it an attractive agent for prostate cancer therapy. The objective of the current study was to determine whether dutasteride would induce apoptosis in a range of prostate epithelial cell lines and primary cultures. METHODS: Both human prostate androgen-sensitive cell lines (PwR-1E, PNT-2, LNCaP, and PC3[AR2]) and an androgen-independent cell line (PC-3) were grown to confluence. Primary epithelial cells extracted from fresh prostate cancer radical prostatectomy specimens also were grown to confluence under optimal conditions. Total cellular protein was extracted to confirm cytokeratin 18 and antihuman alpha-methylacyl-CoA racemase (AMACR) expression of the primary cells. Apoptosis

was assessed by propidium iodide DNA staining and flow cytometry after 24 hours of culture in from 0 microM to 10 microM of dutasteride. RESULTS: Dutasteride induced a dose-dependent increase in apoptosis in the androgen-sensitive prostate cell lines PwR-1E, PNT-2, and LNCaP and in the androgen receptor-expressing PC3(AR2) cell line. However, there was no significant apoptosis noted in the parental PC-3 cells. Of 16 primary epithelial cultures that were treated, 7 cultures were induced to undergo apoptosis, and 9 cultures were unresponsive. All primary cultures were positive for cytokeratin 18 expression, confirming their epithelial phenotype. Responder epithelial cells were positive for AMACR expression. CONCLUSIONS: The results of the current study confirmed that dutasteride differentially induced apoptosis in a subset of prostate cell lines and primary prostate epithelial cells. Understanding the cellular phenotype may indicate susceptible cells.

Marrocco, D. L., W. D. Tilley, et al. (2007). "Suberoylanilide hydroxamic acid (vorinostat) represses androgen receptor expression and acts synergistically with an androgen receptor antagonist to inhibit prostate cancer cell proliferation." *Mol Cancer Ther* **6**(1): 51-60.

Growth of prostate cancer cells is initially dependent on androgens, and androgen ablation therapy is used to control tumor growth. Unfortunately, resistance to androgen ablation therapy inevitably occurs, and there is an urgent need for better treatments for advanced prostate cancer. Histone deacetylase inhibitors, such as suberoylanilide hydroxamic acid (SAHA; vorinostat), are promising agents for the treatment of a range of malignancies, including prostate cancer. SAHA inhibited growth of the androgen-responsive LNCaP prostate cancer cell line at low micromolar concentrations and induced caspase-dependent apoptosis associated with chromatin condensation, DNA fragmentation, and mitochondrial membrane depolarization at higher concentrations ( $\geq 5$   $\mu\text{mol/L}$ ). Gene profiling and immunoblot analyses showed a decrease in androgen receptor (AR) mRNA and protein in LNCaP cells cultured with SAHA compared with control cells, with a corresponding decrease in levels of the AR-regulated gene, prostate-specific antigen. Culture of LNCaP cells in steroid-free medium markedly sensitized the cells to SAHA. Moreover, a combination of low, subeffective doses of SAHA and the AR antagonist bicalutamide resulted in a synergistic reduction in cell proliferation and increase in caspase-dependent cell death. Addition of exogenous androgen prevented the induction of cell death, indicating that suppression of androgen signaling was required for synergy. At the

subeffective concentrations, these agents had no effect, alone or in combination, on proliferation or death of AR-negative PC-3 prostate cancer cells. Our findings indicate that SAHA is effective in targeting the AR signaling axis and that androgen deprivation sensitizes prostate cancer cells to SAHA. Consequently, combinatorial treatments that target different components of the AR pathway may afford a more effective strategy to control the growth of prostate cancer cells.

Martiniello-Wilks, R., X. Y. Wang, et al. (2004). "Purine nucleoside phosphorylase and fludarabine phosphate gene-directed enzyme prodrug therapy suppresses primary tumour growth and pseudo-metastases in a mouse model of prostate cancer." *J Gene Med* 6(12): 1343-57.

Gene-directed enzyme prodrug therapy based on the E. coli purine nucleoside phosphorylase (PNP) gene produces efficient tumour cell killing. PNP converts adenosine analogs into toxic metabolites that diffuse across cell membranes to kill neighbouring untransduced cells (PNP-GDEPT). Interference with DNA, RNA and protein synthesis kills dividing and non-dividing cells, an important consideration for slow-growing prostate tumours. This study examined the impact of administering PNP-GDEPT into orthotopically grown RM1 prostate cancers (PCas) on the growth of lung pseudo-metastases of immunocompetent mice. C57BL/6 mice bearing orthotopic RM1 PCas received a single intraprostatic injection of OAdV220 (10(10) particles), a recombinant ovine adenovirus containing the PNP gene controlled by the Rous Sarcoma virus promoter, followed by fludarabine phosphate (approximately 600 mg/m(2)/day) administered intraperitoneally (ip) once daily for 5 days. Pseudo-metastases were induced 2 days after intraprostatic vector administration by tail-vein injection of untransduced RM1 cells. Mice given PNP-GDEPT showed a significant reduction both in prostate volume (approximately 50%) and in lung colony counts (approximately 60%). Apoptosis was increased two-fold in GDEPT-treated prostates compared with controls ( $P < 0.01$ ), but was absent in the lungs. Staining for proliferating cell nuclear antigen (PCNA) indicated that proliferation of both RM1 prostate tumours ( $P < 0.01$ ) and lung colonies ( $P < 0.01$ ) was significantly suppressed after GDEPT. Although prostate tumour immune cell infiltration did not differ significantly between treatments, immunostaining for Thy-1.2 (CD90) showed that GDEPT promoted Thy-1.2(+) cell infiltration into the prostate tumour site. This study showed that a single course of PNP-GDEPT significantly suppressed local PCa growth

and reduced lung colony formation in the aggressive RM1 tumour model.

McCabe, M. T., J. A. Low, et al. (2006). "Inhibition of DNA methyltransferase activity prevents tumorigenesis in a mouse model of prostate cancer." *Cancer Res* 66(1): 385-92.

Transcriptional silencing of tumor suppressor genes by DNA methylation plays an important role in tumorigenesis. These aberrant epigenetic modifications may be mediated in part by elevated DNA methyltransferase levels. DNA methyltransferase 1 (DNMT1), in particular, is overexpressed in many tumor types. Recently, we showed that Dnmt1 is transcriptionally regulated by E2F transcription factors and that retinoblastoma protein (pRb) inactivation induces Dnmt1. Based on these observations, we investigated regulation of Dnmt1 by polyomavirus oncogenes, which potently inhibit the pRb pocket protein family. Infection of primary human prostate epithelial cells with BK polyomavirus dramatically induced Dnmt1 transcription following large T antigen (TAg) translation and E2F activation. For in vivo study of Dnmt1 regulation, we used the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, which expresses the SV40 polyomavirus early region, including TAg, under control of a prostate-specific promoter. Analysis of TRAMP prostate lesions revealed greatly elevated Dnmt1 mRNA and protein levels beginning in prostatic intraepithelial neoplasia and continuing through advanced prostate cancer and metastasis. Interestingly, when TRAMP mice were treated in a chemopreventive manner with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-aza), 0 of 14 mice developed prostate cancer at 24 weeks of age, whereas 7 of 13 (54%) control-treated mice developed poorly differentiated prostate cancer. Treatment with 5-aza also prevented the development of lymph node metastases and dramatically extended survival compared with control-treated mice. Taken together, these data suggest that Dnmt1 is rapidly activated by pRb pathway inactivation, and that DNA methyltransferase activity is required for malignant transformation and tumorigenesis.

McCarthy, H. O., J. A. Coulter, et al. (2007). "Human osteocalcin: a strong promoter for nitric oxide synthase gene therapy, with specificity for hormone refractory prostate cancer." *J Gene Med* 9(6): 511-20.

BACKGROUND: Gene therapy has been identified as a promising treatment strategy for hormone refractory prostate cancer (HRPC). We report, for the first time, the use of the human osteocalcin (hOC) promoter to control inducible nitric

oxide synthase (iNOS) transgene expression in HRPC. METHODS: Human prostate carcinoma cells (PC3, DU145, LNCaP), colon cancer cells (HT29) and human microvascular endothelial cells (HMEC-1) were transfected in vitro with constitutively driven CMV/iNOS or hOC/iNOS plasmid DNA by cationic lipid vector. End points of these experiments were Western blotting, NO(.) generation using the Greiss test to measure accumulated nitrite, and clonogenic assay. RESULTS: Transfection of the hOC/iNOS plasmid increased iNOS protein and total nitrite levels in PC3 and DU145 cells, but not LNCaP or HT29. Transfection with CMV/iNOS or hOC/iNOS resulted in no additional cytotoxicity in androgen-dependent LNCaP cells or in the non-prostate cell lines. However, transfection with either construct resulted in a greatly reduced cell survival (to 10-20%) in the androgen-independent PC3 and DU145 cell lines. CONCLUSIONS: Utilising the tumour-type specific properties of the hOC promoter in tandem with the iNOS gene, we have demonstrated target cell specificity, and transgene activation, in the androgen-independent prostate cancer cell lines (PC3 and DU145), an effect absent in normal and androgen-dependent cells. Furthermore, the levels of NO(.) generated are comparable with those seen generated with constitutively (CMV)-driven iNOS. The data obtained from this study provide a basis for future development of hOC/iNOS gene therapy.

Meyer, A., B. Wilhelm, et al. (2007). "ATM missense variant P1054R predisposes to prostate cancer." *Radiother Oncol* **83**(3): 283-8.

**BACKGROUND:** Prostate cancer is associated with defective DNA strand break repair after DNA damage leading to genetic instability and prostate cancer progression. The ATM (ataxia-telangiectasia mutated) gene product is known to play an important role in cell cycle regulation and maintenance of genomic integrity. We investigated whether the prevalence of the ATM missense substitution P1054R is increased in a hospital-based series of prostate cancer patients and whether carriers are at increased risk for treatment-related side effects. **MATERIALS AND METHODS:** A consecutive series of 261 patients treated for early-stage prostate cancer with I-125 brachytherapy (permanent seed implantation) between 10/2000 and 04/2006 at our institution and a comparison group of 460 male control individuals were screened for the presence of the P1054R variant. Outcome of therapy regarding morbidity was assessed prospectively and compared between carriers vs. non-carriers with the International Prostate Symptom Score (IPSS), a Quality-of-Life-index (QoL) and the International Index of Erectile Function (IIEF-15) with its subgroups (IIEF-5 and

EF). **RESULTS:** The proportion of carriers of the P1054R variant was significantly higher among prostate cancer patients than in the general population (25 out of 261 vs. 22 out of 460; OR 2.1; 95% CI 1.2-3.8,  $p < 0.01$ ). A subgroup of the carriers additionally harboured the ATM missense variant F858L that was associated with a similar risk (OR=2.2; 95% CI 1.1-4.6;  $p = 0.03$ ). After a mean follow-up of 18 months there were no statistically significant differences regarding IPSS ( $p = 0.48$ ), QoL ( $p = 0.61$ ), IIEF-15 score ( $p = 0.78$ ), IIEF-5 score ( $p = 0.83$ ), and EF score ( $p = 0.80$ ), respectively. **CONCLUSIONS:** The ATM missense variant P1054R confers an about twofold increased risk for prostate cancer in our series. The subgroup of patients with the second-site variant F858L is not at significantly higher risk. After 18 months, there was no evidence for an increased adverse radiotherapy response in P1054R carriers.

Miller, A. M., V. Ozenci, et al. (2005). "Immune monitoring in a phase I trial of a PSA DNA vaccine in patients with hormone-refractory prostate cancer." *J Immunother* **28**(4): 389-95.

Prostate cancer remains a leading cause of cancer illness and death among men in Europe. No curative treatment exists when the disease has spread beyond the prostate. Immunotherapy with DNA vaccines has emerged as a potential therapeutic approach for the induction of antitumor specific cytotoxic T lymphocytes. In this study six patients with hormone-refractory prostate cancer were monitored for their ability to mount PSA-specific cellular responses after receiving a pVAX/PSA DNA vaccine (patients 1-3, 100 microg; patients 7-9, 900 microg) with recombinant GM-CSF and IL-2 as adjuvants. IFN $\gamma$  ELISPOT showed that naturally processed PSA protein and PSA peptides are recognized by T cells in the blood of some prostate cancer patients after a PSA DNA vaccine. Analysis of other cytokines showed the production of IL-4 and IL-6 but importantly did not show an increase in the number of IL-10-producing cells after vaccination in any of the patients. The authors conclude that a pVAX/PSA DNA vaccine can induce PSA-specific cellular immune responses in patients with hormone-refractory prostate cancer, thus emphasizing the potential for PSA as a target molecule for the immunotherapy of prostate cancer.

Miltyk, W., C. N. Craciunescu, et al. (2003). "Lack of significant genotoxicity of purified soy isoflavones (genistein, daidzein, and glycitein) in 20 patients with prostate cancer." *Am J Clin Nutr* **77**(4): 875-82.

**BACKGROUND:** Genistein may be useful in the prevention or treatment of prostate cancer; however, it causes genetic damage in cultured human

cells. **OBJECTIVE:** The objective was to assess the potential genotoxicity of a purified soy unconjugated isoflavone mixture in men with prostate cancer. **DESIGN:** Twenty patients with prostate cancer were treated with 300 mg genistein/d for 28 d and then with 600 mg/d for another 56 d. In peripheral lymphocytes, DNA strand breaks were assessed as nuclear tail moment, chromosomal damage was assessed as micronucleus frequency (MF), and translocations of the MLL gene (11q23) were assessed by using fluorescence in situ hybridization. Values are also reported for 6 healthy men. The studies were performed under Investigational New Drug application no. 54 137 at a tertiary referral academic medical center. **RESULTS:** No changes in group average or individual nuclear tail moment and MF were observed. We observed a single elevated MF value in one subject that exceeded a clinical threshold set before we initiated the study. A significant decrease in average COMET tail moment was observed on day 28 relative to day 0. We detected no genistein-induced rearrangements of the MLL gene in the 3 subjects we studied with this technique. MF increased significantly in lymphocytes exposed in vitro to unconjugated genistein at concentrations  $>$  or  $=$  100 micromol/L. Total genistein never exceeded a peak concentration of 27.1 micro mol/L, and unconjugated genistein never exceeded a peak concentration of 0.32 micromol/L. **CONCLUSION:** Although isoflavones are capable of inducing genetic damage in vitro, a similar effect was not observed in subjects treated with a purified soy unconjugated isoflavone mixture.

Minelli, A., I. Bellezza, et al. (2005). "Inhibition of cell growth and induction of apoptosis in human prostate cancer cell lines by 6-aminoquinolone WM13." *Oncol Rep* **13**(6): 1113-20.

Fluoroquinolones affect the proliferation and apoptotic cell death of several human malignancies. Therefore, we investigated whether new 6-aminoquinolone derivatives, initially synthesized as anti-HIV agents, could affect the proliferation and apoptotic cell death of human prostate cancer cell lines. PC3 and LNCaP cell lines were used as models of androgen-resistant and androgen-responsive prostate cancer, and proliferation of PC3 and LNCaP cells was strongly inhibited by 6-aminoquinolone WM13. Cytotoxicity, which was more pronounced in LNCaP, was accompanied by morphological changes, DNA damage, arrest at the S/G(2)/M phase of the cell cycle, and an increase of the sub-G(1) population. Molecular mechanism underlying WM13-induced cell death involved caspase-8 and -3 and modulation of the expression of apoptotic genes, as well as cleavage of poly-ADP ribose polymerase. Cell death following the

treatment of human prostate cancer cell lines with WM13 can be attributed to apoptosis which, depending on the cell line, proceeds through different pathways.

Mittal, R. D., D. Mishra, et al. (2007). "Role of an androgen receptor gene polymorphism in development of hormone refractory prostate cancer in Indian population." *Asian Pac J Cancer Prev* **8**(2): 275-8.

**BACKGROUND:** Androgen receptors play critical roles in the development of primary as well as advanced hormone-refractory prostate cancers. Since the growth of prostate cancer is androgen-sensitive, metastatic disease has been treated by hormonal therapy in the form of androgen ablation. Prostate cancer cells rely on androgen receptor (AR) for proliferation and survival. **AIM:** To evaluate the prognostic significance of androgen receptor polymorphism in patients under hormonal therapy in any form. **METHODS:** Complete follow up data were available for 87 patients out of 130 patients enrolled for study. DNA was extracted from blood samples using salting out method and then subjected to PCR Genscan for CAG and GGN genotyping. The mean follow up was 10.12 $\pm$ 8.83 months. **RESULTS:** Out of 87 patients, 64 experienced clinical as well as biochemical recurrence. The overall hormone refractory rates were 73.4% after one year. We observed a significant shorter median CAG repeats in HRPC patients (20 vs 22). The hazard ratio for HRPCs with the  $<$  or  $=$ 20 CAG repeat genotype was 0.602 (0.33-1.08,  $p=0.09$ ). Kaplan-Meier analysis showed that HRPC rates were not significantly associated with CAG repeat ( $p=0.06$ ) but a trend was observed with short CAG repeats. No significant association was observed with AR-GGN repeats. **CONCLUSIONS:** A trend for association of AR-CAG repeats with HRPC patients in north Indian population was observed, suggesting this to be a prognostic factor for determining the therapeutic regimen.

Miyagi, T., O. Hori, et al. (2002). "Antitumor effect of reduction of 150-kDa oxygen-regulated protein expression on human prostate cancer cells." *Int J Urol* **9**(10): 577-85.

**BACKGROUND:** The 150-kDa oxygen-regulated protein ORP150, a new member of the heat shock protein family that functions as a molecular chaperone in the endoplasmic reticulum, was found to increase in infiltrating cancer cells. Since enhancement of ORP150 expression and the presence of vascular endothelial growth factor (VEGF) in human prostate cancer glands were immunohistochemically demonstrated, we examined whether transduced antisense ORP150 cDNA can reduce angiogenicity and tumorigenicity through

suppression of VEGF secretion. **METHODS:** Human prostate cancer specimens were immunohistochemically stained with fluorescein isothiocyanate (FITC) for ORP150 or vascular endothelial growth factor (VEGF). An adenovirus vector (Ad) carrying antisense ORP150 cDNA (AdCA-Antisense ORP150) was constructed and infected to prostate cancer DU145 cells. Expression of ORP150 in the cells was analyzed with western blotting and secretion of VEGF into the supernatant with an enzyme-linked immunosorbent assay (ELISA). Angiogenicity was evaluated by chorioallantoic membrane (CAM) assay. A nude mouse xenograft model was used to examine tumorigenicity. **RESULTS:** Immunohistochemical study proved that the expression of ORP150 and VEGF was enhanced in the cytoplasm of prostate cancer cells. The Ad showed 100% transduction efficiency and minimum cytotoxicity when the cells were infected at a multiplicity of infection (MOI) of 20 for 24 h. Expression of ORP150 was substantially reduced by the antisense treatment. Secretion of VEGF into the culture supernatant was reduced to 30%. Consequently, the CAM assay showed relatively low angiogenicity, while marked suppression of tumor formation was observed in the xenograft model. **CONCLUSION:** Adenoviral-mediated antisense ORP150 cDNA transfer is well worth considering as an option for prostate cancer gene therapy.

Miyake, H., I. Hara, et al. (2005). "Antisense oligodeoxynucleotide therapy targeting clusterin gene for prostate cancer: Vancouver experience from discovery to clinic." *Int J Urol* **12**(9): 785-94.

**BACKGROUND:** The objective of this study was to review our experience in the development of antisense (AS) oligodeoxynucleotide (ODN) therapy for prostate cancer targeting antiapoptotic gene, clusterin. **METHODS:** We initially summarized our data demonstrating that clusterin could be an optimal therapeutic target for prostate cancer, then presented the process of developing AS ODN therapy using several preclinical animal models. Finally, the preliminary data of the recently completed phase I clinical trial using AS clusterin ODN as well as the future prospects of this therapy are discussed. **RESULTS:** Expression of clusterin was highly up-regulated after androgen withdrawal and during progression to androgen-independence, but low or absent in untreated tissues in both prostate cancer animal model systems and human clinical specimens. Introduction of the clusterin gene into human prostate cancer cells confers resistance to several therapeutic stimuli, including androgen ablation, chemotherapy and radiation. AS ODN targeting the translation initiation site of the clusterin gene markedly inhibited

clusterin expression in prostate cancer cells in a dose-dependent and sequence-specific manner. Systemic treatment with AS clusterin ODN enhanced the effects of several conventional therapies through the effective induction of apoptosis in prostate cancer xenograft models. Based on these findings, a phase I clinical trial was completed using AS clusterin ODN incorporating 2'-O-(2-methoxy)ethyl-gapmer backbone (OGX-011), showing up to 90% suppression of clusterin in prostate cancer. **CONCLUSIONS:** The data described above identified clusterin as an antiapoptotic gene up-regulated in an adaptive cell survival manner following various cell death triggers that helps confer a phenotype resistant to therapeutic stimuli. Inhibition of clusterin expression using AS ODN technology enhances apoptosis induced by several conventional treatments, resulting in the delay of AI progression and improved survival. Clinical trials using AS ODN confirm potent suppression of clusterin expression and phase II studies will begin in early 2005.

Mizokami, A., E. Koh, et al. (2004). "The adrenal androgen androstenediol is present in prostate cancer tissue after androgen deprivation therapy and activates mutated androgen receptor." *Cancer Res* **64**(2): 765-71.

Despite an initial response to androgen deprivation therapy, prostate cancer (PCa) progresses eventually from an androgen-dependent to an androgen-independent phenotype. One of the mechanisms of relapse is antiandrogen withdrawal phenomenon caused by mutation of 877th amino acid of androgen receptor (AR). In the present study, we established a method to measure the concentration of androstenediol (adiol) in prostate tissue. We found that adiol maintains a high concentration in PCa tissue even after androgen deprivation therapy. Furthermore, adiol is a stronger activator of mutant AR in LNCaP PCa cells and induces more cell proliferation, prostate-specific antigen (PSA) mRNA expression, and PSA promoter than dihydrotestosterone (DHT). Because antiandrogen, bicalutamide, blocked adiol activity in LNCaP cells, it was suggested that adiol effect was mediated through AR. However, high concentration of bicalutamide was necessary to block completely adiol activity. These effects were specific to LNCaP cells because adiol had less effect in PC-3 PCa cells transfected with wild-type AR than DHT and had similar effect in PC-3 cells transfected with mutant AR. The mechanism that adiol activates mutant AR in LNCaP cells did not result from the increased affinity to mutant AR or from AR's association with coactivator ARA70. However, low concentration of adiol induced more AR nuclear translocation than DHT in LNCaP cells and not PC-3

cells transfected with AR. These results indicate that adiol may cause the progression of PCa even after hormone therapy.

Molinie, B. and P. Georgel (2009). "Genetic and epigenetic regulations of prostate cancer by genistein." *Drug News Perspect* **22**(5): 247-54.

The regulatory mechanisms governing the expression of specific genes associated with prostate cancer cannot all be explained by monitoring DNA sequences and potential mutations. This suggests the involvement of additional modes of regulating gene expression, such as epigenetics. The contribution of epigenetic events, although fully recognized, remains to be fully understood. There is increasing evidence for the regulatory roles of both DNA methylation and the composition and conformation of chromatin. These epigenetic changes can be modulated by molecules that are part of our daily diet. Genistein, an isoflavonoid present in soy products, has been shown to have anticancer effects. Epidemiological studies have indicated a link between a low occurrence of prostate cancer and a genistein-rich diet. This review summarizes the current knowledge about genistein-modulated genetic and epigenetic effects on both the gene expression and biological pathways associated with prostate cancer.

Monge, A., M. Jagla, et al. (2006). "Unfaithfulness and promiscuity of a mutant androgen receptor in a hormone-refractory prostate cancer." *Cell Mol Life Sci* **63**(4): 487-97.

Missense mutations in the androgen receptor (AR) contribute to the failure of hormonal therapy for prostate cancer (PCa), but the underlying molecular bases remain uncharacterized. Here, we describe a new AR variant found in a hormone-refractory metastatic PCa, in which threonine 575 in the DNA binding domain, and threonine 877 in the ligand-binding domain, were both replaced by an alanine. Using gene reporter assays, we demonstrate that the T575A mutation weakened transcriptional activity from promoters containing AR-specific responsive elements, while activity from promoters with AR-non-specific elements was enhanced. Data from gel shift experiments revealed a preferential binding of the T575A mutant to AR-non-specific motifs. We demonstrate that the two mutations T575A and T877A cooperate to confer new functional properties on the AR, and that the mutant AR functions simultaneously as a promiscuous AR due to the T877A mutation, and an unfaithful AR due to the T575A mutation.

Moon, H. S., H. Lim, et al. (2009). "Benzylidihydroxyoctenone, a novel anticancer agent, induces apoptosis via mitochondrial-mediated

pathway in androgen-sensitive LNCaP prostate cancer cells." *Bioorg Med Chem Lett* **19**(3): 742-4.

This study was aimed to evaluate detailed mechanisms on the apoptotic induction of benzylidihydroxyoctenone, a novel compound isolated from *Streptomyces* sp. KACC91015, in androgen-sensitive LNCaP prostate cancer cells. Benzylidihydroxyoctenone, designated as F3-2-5 in the current study, caused accumulation of apoptotic sub-G(1) phase in the flow cytometric analysis using propidium iodide staining. Moreover, the typical apoptotic DNA fragmentation of the LNCaP cells treated with 30 microM of F3-2-5 was confirmed using the TUNEL assay. This apoptotic induction of F3-2-5 in the LNCaP cells was associated with the cytochrome c release from mitochondria to cytosol, and the activation of procaspase-8, -9, and -3, as well as the specific proteolytic cleavage of poly(ADP-ribose) polymerase (PARP). In addition, F3-2-5 treatment caused the down-regulation of the antiapoptotic protein, such as Bcl-2 and Bcl-X(L), but the proapoptotic protein, such as Bax, was not influenced. To investigate whether apoptotic induction by F3-2-5 is also due to the down-regulation of androgen receptor (AR), Western blot analysis and quantitative RT-PCR were conducted in F3-2-5-treated LNCaP prostate cancer cells. We found that F3-2-5 significantly inhibited the expression levels of AR and prostate-specific antigen (PSA) proteins in a time-dependent manner, as well as F3-2-5 abrogated the up-regulation of AR and PSA genes with and without DHT. Therefore, F3-2-5 has been shown to be an androgen antagonist, suggesting that F3-2-5 could be a potent agent for the treatment of both androgen-dependent and hormone-refractory prostate cancer.

Moriyama-Gonda, N., H. Shiina, et al. (2008). "Rationale and clinical implication of combined chemotherapy with cisplatin and oestrogen in prostate cancer: primary evidence based on methylation analysis of oestrogen receptor-alpha." *BJU Int* **101**(4): 485-91.

**OBJECTIVE:** To determine whether oestrogen enhances platinum sensitivity, and if promoter CpG methylation of the oestrogen receptor-alpha (ER-alpha) gene determines the potential of cisplatin-induced apoptosis in prostate cancer, as the high-mobility group 1 (HMG1) preferentially binds to cisplatin-modified DNA and is up-regulated after oestrogen treatment in breast cancer cell line MCF-7. **MATERIALS AND METHODS:** The study comprised prostate cancer cell lines (LNCaP and PC-3), 156 pathologically confirmed 156 radical prostatectomy samples and eight hormone-refractory prostate cancer (HRPC) samples (from needle biopsy). Expression of HMG1 in cell lines was analysed by

Western blotting or differential reverse-transcription-polymerase chain reaction (PCR). The methylation status of ER-alpha was analysed by methylation-specific PCR using bisulphite DNA as a template or bisulphite DNA sequencing. RESULTS: In LNCaP cells, treatment with oestrogen increased HMG1 expression and co-treatment with cisplatin and oestrogen reduced cell viability by accelerating apoptosis, compared with cisplatin alone. However, in PC-3, oestrogen did not up-regulate HMG1 or accelerate the cisplatin-induced apoptosis. Although ER-beta was expressed in both LNCaP and PC-3, ER-alpha was expressed only in LNCaP. Bisulphite DNA sequencing of the ER-alpha promoter showed partial methylation in LNCaP but complete methylation in PC-3. ER-alpha AS transfection diminished the cisplatin-induced apoptosis in oestrogen-treated LNCaP cells. In clinical samples there was ER-alpha hypermethylation in 40% of prostate cancers this correlated with Gleason score (GS, 31% for GS < 7, 50% for GS = 7 and 56% for GS > 7). In addition, five of eight HRPC samples showed ER-alpha hypermethylation. CONCLUSION: These findings suggest that HMG1 induction as an enhancer of platinum sensitivity is mediated through interaction between oestrogen and ER-alpha. As CpG hypermethylation of the ER-alpha promoter is a frequent event in aggressive prostate cancer, negative conversion of ER-alpha methylation is essential to achieve the most beneficial effect when combined chemotherapy of cisplatin with oestrogen is used in patients with prostate cancer.

Mujoo, K., M. Watanabe, et al. (2005). "Increased sensitivity of a metastatic model of prostate cancer to a novel tetravalent platinum analog." *Prostate* **62**(1): 91-100.

BACKGROUND: DACH-Ac-Pt [(1R,2R-diaminocyclohexane)-(trans-diacetato)-(dichloro)-platinum(IV)] is a novel cisplatin (CDDP) analog, and we have evaluated its potential activity in human prostate cancers. METHODS: Cytotoxic, biochemical pharmacologic, cell cycle, and Western blot evaluations were conducted with platinum agents to assess the role of p53 genotype and androgen-dependence status on cellular response. RESULTS: CDDP and DACH-Ac-Pt were equiactive against mutant p53 and androgen-independent DU-145 or PC-3 tumor cells. In wild-type p53 cells, CDDP was threefold more potent against androgen-dependent LNCaP than isogenic androgen-independent LNCaP-LN3 cells. However, the analog was equipotent in these two wild-type p53 tumor models. The greater potency of DACH-Ac-Pt than CDDP in wild-type p53 cells was not due to increased cellular drug uptake or increased adduct levels, but correlated with a lower

tolerance to DNA damage. The analog also activated the p53-p21(WAF1/CIP1) signal transduction pathway more efficiently in LNCaP and LNCaP-LN3 cells, and this induced G(1)-phase cell-cycle arrest. CDDP, in contrast, activated this pathway efficiently in LNCaP cells only. In addition, and compared to CDDP, DACH-Ac-Pt was more effective in inducing Bax and increasing the Bax/Bcl-2 ratios in both the tumor models. CONCLUSIONS: DACH-Ac-Pt is highly effective against wild-type p53 LNCaP and its LN3 variant, and this activity is androgen-independent. The differential induction of p21(WAF1/CIP1) and increase in Bax/Bcl-2 ratios with CDDP and DACH-Ac-Pt in LNCaP-LN3 cells appear to be linked to the relative activity of the two agents against this model.

Mukhopadhyay, U. K., A. M. Senderowicz, et al. (2005). "RNA silencing of checkpoint regulators sensitizes p53-defective prostate cancer cells to chemotherapy while sparing normal cells." *Cancer Res* **65**(7): 2872-81.

p53 is frequently mutated in patients with prostate cancer, especially in those with advanced disease. Therefore, the selective elimination of p53 mutant cells will likely have an impact in the treatment of prostate cancer. Because p53 has important roles in cell cycle checkpoints, it has been anticipated that modulation of checkpoint pathways should sensitize p53-defective cells to chemotherapy while sparing normal cells. To test this idea, we knocked down ataxia telangiectasia mutated (ATM) gene by RNA interference in prostate cancer cell lines and in normal human diploid fibroblasts IMR90. ATM knockdown in p53-defective PC3 prostate cancer cells accelerated their cell cycle transition, increased both E2F activity and proliferating cell nuclear antigen expression, and compromised cell cycle checkpoints, which are normally induced by DNA damage. Consequently, PC3 cells were sensitized to the killing effects of the DNA-damaging drug doxorubicin. Combining ATM knockdown with the Chk1 inhibitor UCN-01 further increased doxorubicin sensitivity in these cells. In contrast, the same strategy did not sensitize either IMR90 or LNCaP prostate cancer cells, both of which have normal p53. However, IMR90 and LNCaP cells became more sensitive to doxorubicin or doxorubicin plus UCN-01 when both p53 and ATM functions were suppressed. In addition, knockdown of the G(2) checkpoint regulators ATR and Chk1 also sensitized PC3 cells to doxorubicin and increased the expression of the E2F target gene PCNA. Together, our data support the concept of selective elimination of p53 mutant cells by combining DNA damage with checkpoint inhibitors

and suggest a novel mechanistic insight into how such treatment may selectively kill tumor cells.

Murthy, S., I. U. Agoulnik, et al. (2005). "Androgen receptor signaling and vitamin D receptor action in prostate cancer cells." *Prostate* **64**(4): 362-72.

**BACKGROUND:** 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibits the growth of prostate cancer cells; previous reports suggest that 1,25(OH)<sub>2</sub>D<sub>3</sub> actions in LNCaP prostate cancer cells are androgen dependent. This is due in part to the observation that the androgen receptor (AR) antagonist, Casodex, modestly inhibits LNCaP cell growth, but reduces the greater growth inhibition induced by 1,25(OH)<sub>2</sub>D<sub>3</sub> to the level of Casodex alone. Because androgen ablation therapy is used for metastatic prostate cancer, we sought to better characterize this androgen dependence. **METHODS:** We have assessed the requirement for endogenous androgens in 1,25(OH)<sub>2</sub>D<sub>3</sub> mediated growth inhibition of AR+ prostate cancer cell lines. We have also sought the mechanism for anti-androgen mediated reversal of 1,25(OH)<sub>2</sub>D<sub>3</sub> dependent growth inhibition in LNCaP cells. **RESULTS:** Although 1,25(OH)<sub>2</sub>D<sub>3</sub> does not inhibit the growth of LNCaP cells grown in medium lacking androgens, we find that growth of androgen independent derivatives of LNCaP cells is inhibited by 1,25(OH)<sub>2</sub>D<sub>3</sub>. Despite this independence, Casodex treatment reduced the response of these cells to 1,25(OH)<sub>2</sub>D<sub>3</sub> suggesting a unique function for Casodex-bound AR. Because Casodex does not directly inhibit the transcriptional activity of the vitamin D receptor (VDR) we sought a common primary target of VDR and AR action whose VDR dependent transcription could be repressed by Casodex. We report that AS3 (APRIN), a novel gene required for androgen dependent growth arrest, is a primary target for 1,25(OH)<sub>2</sub>D<sub>3</sub> and androgens. Moreover, Casodex reduces induction of AS3 by 1,25(OH)<sub>2</sub>D<sub>3</sub> suggesting that it is a candidate for the Casodex effect. Analysis of functional interactions between AR and VDR in other AR containing prostate cancer cells lines (PC-3 AR, LAPC-4, and 22Rv1) revealed that Casodex reversal was unique to LNCaP derived cells. **CONCLUSION:** Anti-androgen mediated reversal of 1,25(OH)<sub>2</sub>D<sub>3</sub> dependent growth inhibition is limited to LNCaP derived prostate cancer cell lines. Moreover, the growth of androgen independent derivatives of LNCaP cells in medium depleted of androgens is strongly inhibited by 1,25D. Therefore, most forms of androgen ablation should not eliminate the utility of VDR agonist treatment in most prostate cancers.

Narayanan, B. A., M. S. Condon, et al. (2003). "Suppression of N-methyl-N-nitrosourea/testosterone-induced rat prostate cancer growth by celecoxib:

effects on cyclooxygenase-2, cell cycle regulation, and apoptosis mechanism(s)." *Clin Cancer Res* **9**(9): 3503-13.

**PURPOSE:** This study was aimed at examining the mechanisms underlying the chemopreventive effect of celecoxib against prostate cancer. We focused our attention on events at the cellular level to show the ability of celecoxib to inhibit prostate cancer growth, by inducing cell cycle arrest and apoptosis. Moreover, we attempted to demonstrate the expression of genes involved in the downstream events related to cyclooxygenase-2 (COX-2) regulation and apoptosis. **EXPERIMENTAL DESIGN:** To determine the level of COX-2 expression, we used paraffin-embedded tumor tissue sections and cancer cells (I-26) derived from N-methyl-N-nitroso-urea/testosterone-induced rat dorsolateral prostate, and we used immunofluorescence detection and Western blot analyses with anti-COX-2 monoclonal antibodies. We conducted clonogenic cell survival assays to demonstrate cell growth inhibition at very low doses of celecoxib. Flow cytometric analysis demonstrated the effects on the cell cycle. Reverse transcription-PCR and Western blot analyses were performed to show the effect of celecoxib on the downstream events of COX-2 and apoptosis-related targets. **RESULTS:** The summary of our findings indicates that (a). these cells from chemically induced rat prostate tumors express COX-2 at both the mRNA and the protein level; (b). celecoxib significantly reduces COX-2 expression in these cancer cells; and (c). celecoxib induces cell cycle arrest at the G(1)-S phase transition point and modifies cell cycle regulatory proteins such as cyclin D1, retinoblastoma (Rb), and phosphorylated Rb, cyclin E, p27(KIP1), and p21(WAF1/CIP1). Furthermore, celecoxib inhibits DNA synthesis and induces apoptosis. Most importantly, celecoxib-induced apoptosis was associated with down-regulation of COX-2, nuclear factor kappaBp65, and with activation of peroxisome proliferator-activated receptor gamma, apoptosis activating factor-1, and caspase-3. **CONCLUSION:** Results from the present study clearly indicate that celecoxib exerts its anticancer effect partly through COX-2-independent mechanisms in addition to the known primary function of COX-2 inhibition.

Nasu, Y., T. Saika, et al. (2007). "Suicide gene therapy with adenoviral delivery of HSV-tK gene for patients with local recurrence of prostate cancer after hormonal therapy." *Mol Ther* **15**(4): 834-40.

We conducted a Phase I study of in situ herpes simplex virus thymidine kinase (HSV-tk) plus ganciclovir (GCV) gene therapy, which was approved by the Japanese government as the first prostate

cancer gene therapy trial. Major inclusion criteria were local recurrence of prostate cancer after hormonal therapy and no metastasis. Adv.HSV-tk was injected directly into the prostate in escalating doses from 10(9) to 10(10) infection units, followed by intravenous administration of GCV for 14 days. Eight patients received nine courses of this gene therapy. The detection of vector DNA in blood/urine was only transient and no remarkable adverse events were observed in any patient. With regard to clinical response, significant prolongation of the median serum prostate-specific antigen (PSA) doubling time from 2.9 to 6.2 months ( $P = 0.041$ ) was detected. In five patients (six injections), a clear decrease of PSA values was observed. One patient showed repeated clinical response after repeated injections. Serum cytokine analysis showed no notable changes after treatment. Fluorescence-activated cell sorting analysis also showed no influence on phenotypic distribution in peripheral blood samples, except for an increasing trend of CD8(+)/HLA-DR(+) after therapy. This study confirmed the safety profile and possibility of clinical response at the surrogate marker level in a clinical trial of HSV-tk gene therapy for hormone-refractory prostate cancer.

Ng, S. S., G. R. MacPherson, et al. (2004). "Antitumor effects of thalidomide analogs in human prostate cancer xenografts implanted in immunodeficient mice." *Clin Cancer Res* **10**(12 Pt 1): 4192-7.

**PURPOSE:** Thalidomide has demonstrated clinical activity in various malignancies including androgen-independent prostate cancer. The development of novel thalidomide analogs with better activity/toxicity profiles is an ongoing research effort. Our laboratory previously reported the in vitro antiangiogenic activity of the N-substituted thalidomide analog CPS11 and the tetrafluorinated analogs CPS45 and CPS49. The current study evaluated the therapeutic potential of these analogs in the treatment of prostate cancer in vivo. **EXPERIMENTAL DESIGN:** Severely combined immunodeficient mice bearing s.c. human prostate cancer (PC3 or 22Rv1) xenografts were treated with the analogs at their maximum tolerated doses. Tumors were then excised and processed for ELISA and CD31 immunostaining to determine the levels of various angiogenic factors and microvessel density (MVD), respectively. **RESULTS:** CPS11, CPS45, and CPS49 induced prominent and modest growth inhibition in PC3 and 22Rv1 tumors, respectively. Thalidomide had no effect on tumor growth in either xenograft. Vascular endothelial growth factor and basic fibroblast growth factor levels were not significantly altered by any of the thalidomide analogs or thalidomide in both PC3 and 22Rv1 tumors. CPS45,

CPS49, and thalidomide significantly reduced PC3 tumor platelet-derived growth factor (PDGF)-AA levels by 58-82% ( $P < 0.05$ ). Interestingly, treatment with the analogs and thalidomide resulted in differential down-regulation ( $\geq 1.5$ -fold) of genes encoding PDGF and PDGF receptor isoforms as determined by DNA microarray analysis. Intratumoral MVD of 22Rv1 xenografts was significantly decreased by CPS45 and CPS49. CPS49 also reduced MVD in PC3 xenografts. **CONCLUSIONS:** Thalidomide analogs CPS11 and 49 are promising anti-cancer agents. PDGF signaling pathway may be a potential target for these thalidomide analogs. Detailed microarray and functional analyses are under way with the aim of elucidating the molecular mechanism(s) of action of these thalidomide analogs.

Nguyen, K. H., P. Hachem, et al. (2005). "Adenoviral-E2F-1 radiosensitizes p53wild-type and p53null human prostate cancer cells." *Int J Radiat Oncol Biol Phys* **63**(1): 238-46.

**PURPOSE:** E2F-1 is a transcription factor that enhances the radiosensitivity of various cell lines by inducing apoptosis. However, there are conflicting data concerning whether this enhancement is mediated via p53 dependent pathways. Additionally, the role of E2F-1 in the response of human prostate cancer to radiation has not been well characterized. In this study, we investigated the effect of Adenoviral-E2F-1 (Ad-E2F-1) on the radiosensitivity of p53wild-type (LNCaP) and p53null (PC3) prostate cancer cell lines. **METHODS AND MATERIALS:** LNCaP and PC3 cells were transduced with Ad-E2F-1, Adenoviral-Luciferase (Ad-Luc) control vector, or Adenoviral-p53 (Ad-p53). Expression of E2F-1 and p53 was examined by Western blot analysis. Annexin V and caspase 3 + 7 assays were performed to estimate the levels of apoptosis. Clonogenic survival assays were used to determine overall cell death. Statistical significance was determined by analysis of variance, using the Bonferroni method to correct for multiple comparisons. **RESULTS:** Western blot analysis confirmed the efficacy of transductions with Ad-E2F-1 and Ad-p53. Ad-E2F-1 transduction significantly enhanced apoptosis and decreased clonogenic survival in both cell lines. These effects were compounded by the addition of RT. Although E2F-1-mediated radiosensitization was independent of p53 status, this effect was more pronounced in p53wild-type LNCaP cells. When PC3 cells were treated with Ad-p53 in combination with RT and Ad-E2F-1, there was at least an additive reduction in clonogenic survival. **CONCLUSIONS:** Our results suggest that Ad-E2F-1 significantly enhances the response of p53wild-type and p53null prostate cancer cells to radiation therapy, although radiosensitization is more pronounced in the

presence of p53. Ad-E2F-1 may be a useful adjunct to radiation therapy in the treatment of prostate cancer.

Nwachukwu, J. C., P. Mita, et al. (2009). "Genome-wide impact of androgen receptor trapped clone-27 loss on androgen-regulated transcription in prostate cancer cells." *Cancer Res* **69**(7): 3140-7.

The androgen receptor (AR) directs diverse biological processes through interaction with coregulators such as AR trapped clone-27 (ART-27). Our results show that ART-27 is recruited to AR-binding sites by chromatin immunoprecipitation analysis. In addition, the effect of ART-27 on genome-wide transcription was examined. The studies indicate that loss of ART-27 enhances expression of many androgen-regulated genes, suggesting that ART-27 inhibits gene expression. Surprisingly, classes of genes that are up-regulated upon ART-27 depletion include regulators of DNA damage checkpoint and cell cycle progression, suggesting that ART-27 functions to keep expression levels of these genes low. Consistent with this idea, stable reduction of ART-27 by short-hairpin RNA enhances LNCaP cell proliferation compared with control cells. The effect of ART-27 loss was also examined in response to the antiandrogen bicalutamide. Unexpectedly, cells treated with ART-27 siRNA no longer exhibited gene repression in response to bicalutamide. To examine ART-27 loss in prostate cancer progression, immunohistochemistry was conducted on a tissue array containing samples from primary tumors of individuals who were clinically followed and later shown to have either recurrent or nonrecurrent disease. Comparison of ART-27 and AR staining indicated that nuclear ART-27 expression was lost in the majority of AR-positive recurrent prostate cancers. Our studies show that reduction of ART-27 protein levels in prostate cancer may facilitate antiandrogen-resistant disease.

Oh, J. E., K. S. So, et al. (2006). "Induction of apoptotic cell death by a ceramide analog in PC-3 prostate cancer cells." *Arch Pharm Res* **29**(12): 1140-6.

Ceramide analogs are potential chemotherapeutic agents. We report that a ceramide analog induces apoptosis in human prostate cancer cells. The ceramide analog induced cell death through an apoptotic mechanism, which was demonstrated by DNA fragmentation, the cleavage of poly ADP ribose polymerase (PARP), and a loss of membrane asymmetry. Treating the cells with ceramide analog resulted in the release of various proapoptotic mitochondrial proteins including cytochrome c and Smac/DIBLO into the cytosol, and a decrease in the mitochondrial membrane potential. In addition, the

ceramide analog decreased the phospho-Akt and phospho-Bad levels. The expression of the antiapoptotic Bcl-2 decreased slightly with increasing Bax to Bcl-2 ratio. These results suggest that the ceramide analog induces apoptosis by regulating multiple signaling pathways that involve the mitochondrial pathway.

O'Mahony, O. A., M. P. Steinkamp, et al. (2008). "Profiling human androgen receptor mutations reveals treatment effects in a mouse model of prostate cancer." *Mol Cancer Res* **6**(11): 1691-701.

Gain-of-function mutations in the androgen receptor (AR) are found in prostate cancer and are implicated in the failure of hormone therapy. Most studies have emphasized the ligand-binding domain (LBD) where mutations can create promiscuous receptors, but mutations in the NH(2)-terminal transactivation domain have also been found. To assess AR alteration as a mechanism of treatment resistance, a mouse model (h/mAR-TRAMP) was used in which the murine AR coding region is replaced by human sequence and prostate cancer initiated by a transgenic oncogene. Mice received either no treatment, androgen depletion by castration, or treatment with antiandrogens, and 20 AR transcripts were sequenced per end-stage tumor. All tumors expressed several mutant alleles, although most mutations were low frequency. Some mutations that occurred multiple times within the population were differentially located dependent on treatment. Mutations in castrated or antiandrogen-treated mice were widely dispersed but with a prominent cluster in the LBD (amino acids 736-771), whereas changes in intact mice centered near the NH(2)-terminal polymorphic glutamine tract. Functional characterization of selected LBD mutant alleles showed diverse effects on AR activity, with about half of the mutations reducing transactivation *in vitro*. One receptor, AR-R753Q, behaved in a cell- and promoter-dependent manner, although as a germ-line mutation it causes androgen insensitivity syndrome. This suggests that alleles that are loss of function during development may still activate a subset of AR targets to become gain of function in tumorigenesis. Mutant ARs may thus use multiple mechanisms to evade cancer treatment.

Padua, M. B. and P. J. Hansen (2009). "Changes in expression of cell-cycle-related genes in PC-3 prostate cancer cells caused by ovine uterine serpin." *J Cell Biochem* **107**(6): 1182-8.

The hormonal-regulated serpin, ovine uterine serpin (OvUS), also called uterine milk protein (UTMP), inhibits proliferation of lymphocytes and prostate cancer (PC-3) cells by blocking cell-cycle

progression. The present aim was to identify cell-cycle-related genes regulated by OvUS in PC-3 cells using the quantitative human cell-cycle RT(2) Profiler PCR array. Cells were cultured +/-200 microg/ml recombinant OvUS (rOvUS) for 12 and 24 h. At 12 h, rOvUS increased expression of three genes related to cell-cycle checkpoints and arrest (CDKN1A, CDKN2B, and CCNG2). Also, 14 genes were down-regulated including genes involved in progression through S (MCM3, MCM5, PCNA), M (CDC2, CKS2, CCNH, BIRC5, MAD2L1, MAD2L2), G(1) (CDK4, CUL1, CDKN3) and DNA damage checkpoint and repair genes RAD1 and RBPP8. At 24 h, rOvUS decreased expression of 16 genes related to regulation and progression through M (BIRC5, CCNB1, CKS2, CDK5RAP1, CDC20, E2F4, MAD2L2) and G(1) (CDK4, CDKN3, TFDP2), DNA damage checkpoints and repair (RAD17, BRCA1, BCCIP, KPNA2, RAD1). Also, rOvUS down-regulated the cell proliferation marker gene MKI67, which is absent in cells at G(0). Results showed that OvUS blocks cell-cycle progression through upregulation of cell-cycle checkpoint and arrest genes and down-regulation of genes involved in cell-cycle progression.

Pakneshan, P., M. Szyf, et al. (2005). "Hypomethylation of urokinase (uPA) promoter in breast and prostate cancer: prognostic and therapeutic implications." *Curr Cancer Drug Targets* 5(7): 471-88.

Methylation of CpG islands of tumor suppressor genes, growth factors, and hormone receptors among other genes causes epigenetic changes in chromatin structure without altering DNA sequence to regulate transcription of these genes. This epigenetic regulation of gene expression plays an important role in the process of tumor invasion, growth and metastasis in malignancies. In hormone dependent malignancies such as breast and prostate cancer, sex steroids play an important role in the process of tumor initiation and progression. These malignancies are often initiated as a less aggressive hormone-responsive type that gradually progresses to become highly invasive and hormone-insensitive. At the early stages, cells lose a functional hormone receptor due to mutations, blockage of signaling pathway or hormone receptor gene silencing. This transition of cancer cells causes them to become refractory to the standard hormone therapies. In later stages, important factors like growth factors, cytokines and proteases promote tumor growth, invasion and metastases. The most commonly implicated protease in these processes is urokinase type plasminogen activator (uPA), which is known to be expressed in a number of malignancies including breast and prostate cancer and is directly associated with the higher

invasive and metastatic potential of malignancies. In this chapter, we will review DNA methylation as the underlying molecular mechanism regulating uPA gene expression and its potential diagnostic, prognostic and therapeutic implication.

Pandini, G., M. Genua, et al. (2007). "17beta-estradiol up-regulates the insulin-like growth factor receptor through a nongenotropic pathway in prostate cancer cells." *Cancer Res* 67(18): 8932-41.

Prostate carcinomas frequently express estrogen receptors (ER), irrespective of androgen receptor (AR) expression; however, the role of ERs and estrogens in prostate cancer is controversial. We found that 17beta-estradiol (E(2)) is able to markedly up-regulate insulin-like growth factor (IGF)-I receptor (IGF-IR) mRNA and protein expression in both AR-positive (LNCaP cells) and AR-negative (PC-3 cells) prostate cancer cells. This effect occurs not only via ERalpha but also via ERbeta stimulation and is specific for IGF-IR because it does not involve the cognate insulin receptor. IGF-IR up-regulation is associated with increased IGF-IR phosphorylation and with increased mitogenic and motogenic activities in response to IGF-I. IGF-IR up-regulation by E(2) does not require ER binding to DNA and is poorly sensitive to antiestrogen blockade, whereas it is associated with the activation of cytosolic kinase cascades involving Src, extracellular signal-regulated kinase (ERK)-1/2, and, to a lesser extent, phosphatidylinositol 3-kinase and is sensitive to the inhibition of these kinases. In conclusion, our data indicate that estrogens may contribute to IGF system deregulation in prostate cancer through the activation of a nongenotropic pathway. Estrogens may have a role, therefore, in tumor progression to androgen independence. Inhibition of the IGF-IR or the Src-ERK pathway should be considered, therefore, as an adjuvant therapy in prostate cancer.

Pandini, G., M. Genua, et al. (2009). "Sex steroids upregulate the IGF-1R in prostate cancer cells through a nongenotropic pathway." *Ann N Y Acad Sci* 1155: 263-7.

Most types of prostate cancer (PCa) are usually initially responsive to androgenic regulation and, therefore, to androgen ablation therapy. However, in several patients tumors may progress to androgen resistance and be poorly responsive to any therapy. Many factors may account for this progression to androgen independence, including increased responsiveness to estrogens and peptide growth factors. The role of estrogens in androgen independence has been suggested by the observation that both primary and metastatic PCa express the estrogen receptor (ER-beta), a recently discovered ER

subtype. On the other hand, peptide growth factors, like IGF-1, IGF-2, and the insulin-like growth factor receptor (IGF-1R), may play a role in regulating growth, survival, and invasion of PCa cells. Here, we show that both androgens and estrogens markedly upregulate the IGF-1R expression in PCa cells by activating a nongenotropic pathway and sensitizing cells to the biological effects of IGF-1. This effect is specific for IGF-1R because it does not involve the highly homologous insulin receptor. IGF-1R upregulation is caused by increased mRNA transcription. However, it does not require steroid receptor binding to DNA, but involves AR and ER binding to c-Src and subsequent activation of ERK1/2 and other cytoplasmatic kinases, which eventually stimulate IGF-1R promoter activity. In conclusion, our data indicate that both androgens and estrogens contribute to IGF system deregulation in PCa and may play a role in tumor progression to androgen independence. Inhibition of the IGF-1R or the Src-ERK pathway should be considered, therefore, as an adjuvant therapy in PCa.

Papadopoulou, E., E. Davilas, et al. (2006). "Cell-free DNA and RNA in plasma as a new molecular marker for prostate and breast cancer." *Ann N Y Acad Sci* **1075**: 235-43.

In this study, we examined several molecular markers in prostate and breast cancer patients and in normal individuals. The markers tested were: variations in the quantity of plasma DNA, glutathione-S-transferase P1 gene (GSTP1), Ras association domain family 1A (RASSF1A), and ataxia telangiectasia mutated (ATM) methylation status in plasma, carcinoembryonic antigen (CEA) and prostate-specific membrane antigen (PSMA) mRNA in peripheral blood mononuclear cells (PBMC) and plasma samples from prostate cancer patients. DNA quantification in plasma was performed using real-time PCR (RT-PCR). We assessed the methylation status of GSTP1 in plasma DNA using methylation-specific PCR (MSP) assay, while the methylation status of RASSF1A and ATM genes was examined by the MethyLight technology. RT-PCR analysis was used for the detection of mRNA, PSMA, and CEA. In 58.3% of newly diagnosed prostate cancer patients and 26.7% of prostate cancer patients under therapy, plasma DNA levels were increased. Additionally, 48.5% of breast cancer patients showed plasma DNA levels above the cutoff limit. GSTP1 Promotor hypermethylation was detectable in 75% of plasma samples obtained from patients with newly diagnosed prostate cancer and in 36.8% of patients under therapy, whereas 26% and 14% of the breast cancer patients tested were positive for RASSF1A and ATM methylation, respectively. The combination of DNA

load and promotor methylation status identified 88% of prostate cancer patients and 54% of breast cancer patients. This study shows that free-circulating DNA can be detected in cancer patients compared with disease-free individuals, and suggests a new, noninvasive approach for early detection of cancer.

Paradowska, A., I. Fenic, et al. (2009). "Aberrant epigenetic modifications in the CTCF binding domain of the IGF2/H19 gene in prostate cancer compared with benign prostate hyperplasia." *Int J Oncol* **35**(1): 87-96.

Expression of the imprinted genes insulin-like growth factor 2 (IGF2) and H19 depends on the methylation pattern of their common imprinting control region (ICR) located on chromosome 11p15. As the somatic imprinting pattern may be lost during tumorigenesis due to epigenetic alterations, in the present study, we analyzed the DNA methylation and histone modifications in the differentially methylated region (DMR) of IGF2/H19 in benign prostate hyperplasia (BPH) and prostate carcinoma (PCa). Sodium bisulfite sequencing was performed on frozen tissue collected after radical prostatectomy. Thirty tumors and 17 non-cancerous tissue samples were analyzed. Histological diagnosis was, in addition, confirmed by amplification of the epithelial tumor marker alpha-methylacyl coenzyme-A racemase. Chromatin immunoprecipitation assay (ChIP) was carried out on sonicated chromatin from fresh tissue samples from 10 PCa, 10 BPH using antibodies against trimethyl histone H3K9, dimethyl histone H3K9, trimethyl H3K27 and acetyl H3K9. The methylation pattern of 17 CpGs within 227 bp of the H19 fragment was characterized from each DNA sample. All (BPH) samples demonstrated >80% methylation of CpGs. In contrast, we found 41% of CpGs methylated in 9 out of 30 PCa specimens. We observed statistically significant differences in the methylation state between PCa and BPH groups, especially in the DMR of H19 ( $p < 0.0001$ ) and in the ICR ( $p = 0.0034$ ), which corresponds to CTCF binding domain. ChIP assay revealed that dimethyl H3K9 is associated with the ICR of IGF2/H19 in BPH, but not in PCa ( $p < 0.0001$ ). Our data demonstrate that DNA methylation and histone methylation analysis of the ICR within the DMR of IGF2/H19 provides important insights into early steps of carcinogenesis and, therefore, may contribute to improving diagnosis of PCa.

Pavlenko, M., A. K. Roos, et al. (2004). "A phase I trial of DNA vaccination with a plasmid expressing prostate-specific antigen in patients with hormone-refractory prostate cancer." *Br J Cancer* **91**(4): 688-94.

Prostate-specific antigen (PSA) is a serine protease secreted at low levels by normal luminal epithelial cells of the prostate and in significantly higher levels by prostate cancer cells. Therefore, PSA is a potential target for various immunotherapeutic approaches against prostate cancer. DNA vaccination has been investigated as immunotherapy for infectious diseases in patients and for specific treatment of cancer in certain animal models. In animal studies, we have demonstrated that vaccination with plasmid vector pVAX/PSA results in PSA-specific cellular response and protection against tumour challenge. The purpose of the trial was to evaluate the safety, feasibility and biological efficacy of pVAX/PSA vaccine in the clinic. A phase I trial of pVAX/PSA, together with cytokine granulocyte/macrophage-colony stimulating factor (GM-CSF) (Molgramostim) and IL-2 (Aldesleukin) as vaccine adjuvants, was carried out in patients with hormone-refractory prostate cancer. To evaluate the biologically active dose, the vaccine was administered during five cycles in doses of 100, 300 and 900 microg, with three patients in each cohort. Eight patients were evaluable. A PSA-specific cellular immune response, measured by IFN-gamma production against recombinant PSA protein, and a rise in anti-PSA IgG were detected in two of three patients after vaccination in the highest dose cohort. A decrease in the slope of PSA was observed in the two patients exhibiting IFN-gamma production to PSA. No adverse effects (WHO grade >2) were observed in any dose cohort. We demonstrate that DNA vaccination with a PSA-coding plasmid vector, given with GM-CSF and IL-2 to patients with prostate cancer, is safe and in doses of 900 microg the vaccine can induce cellular and humoral immune responses against PSA protein.

Peng, W., A. Verbitsky, et al. (2002). "Regulated expression of diphtheria toxin in prostate cancer cells." *Mol Ther* **6**(4): 537-45.

Despite their known potential for effectively killing cells, the therapeutic use of plant and bacterial toxins for the treatment of cancer has been slow to enter the clinical setting. This has been due in large part to the lack of gene regulatory elements that control expression of highly toxic genes in a sufficiently tight manner, such that the toxins are only expressed in specific target cells. "Leaky" promoters result in unwanted and harmful cell death. In this study, we tested a novel gene therapy strategy aimed at expressing diphtheria toxin (DT-A) in androgen-independent prostate cancer cells that express the protein BCL2. This strategy relies on both transcriptional regulation and inducibly regulated DNA recombination mediated by the site-directed F<sub>1</sub> recombinase to control expression of the toxin.

Adenoviruses are used to introduce the genetic elements required for this approach into cultured cells and xenografts. Administration of 4-hydroxytamoxifen, resulting in recombination and expression of the toxin, effectively kills the cancer cells. Our results suggest that following androgen ablation therapy for the treatment of prostate cancer, use of a regulated recombination system to target expression of DT-A to androgen-independent cancer cells would be an effective way to arrest the development of recurrent tumors.

Perry, A. S., R. Foley, et al. (2006). "The emerging roles of DNA methylation in the clinical management of prostate cancer." *Endocr Relat Cancer* **13**(2): 357-77.

Aberrant DNA methylation is one of the hallmarks of carcinogenesis and has been recognized in cancer cells for more than 20 years. The role of DNA methylation in malignant transformation of the prostate has been intensely studied, from its contribution to the early stages of tumour development to the advanced stages of androgen independence. The most significant advances have involved the discovery of numerous targets such as GSTP1, Ras-association domain family 1A (RASSF1A) and retinoic acid receptor beta2 (RARbeta2) that become inactivated through promoter hypermethylation during the course of disease initiation and progression. This has provided the basis for translational research into methylation biomarkers for early detection and prognosis of prostate cancer. Investigations into the causes of these methylation events have yielded little definitive data. Aberrant hypomethylation and how it impacts upon prostate cancer has been less well studied. Herein we discuss the major developments in the fields of prostate cancer and DNA methylation, and how this epigenetic modification can be harnessed to address some of the key issues impeding the successful clinical management of prostate cancer.

Pisters, L. L., C. A. Pettaway, et al. (2004). "Evidence that transfer of functional p53 protein results in increased apoptosis in prostate cancer." *Clin Cancer Res* **10**(8): 2587-93.

PURPOSE: INGN 201 (Ad-p53) is a replication-defective adenoviral vector that encodes a wild-type p53 gene driven by the cytomegalovirus promoter. INGN 201 has been shown to have antitumoral activity against human prostate cancer cell lines. This study was undertaken to determine the safety of INGN 201 in patients with locally advanced prostate cancer, to assess transgene expression, and to evaluate antitumoral activity. EXPERIMENTAL DESIGN: Our study included patients with clinical

stage T3, T1c-T2a with Gleason score 8-10 disease, or T2a-T2b with Gleason score 7 disease and a prostate-specific antigen level >10 ng/ml. INGN 201 was administered by intraprostatic injection under ultrasonographic guidance. One course of INGN 201 was defined as three separate INGN 201 administrations 2 weeks apart. Biopsies at baseline and 24 h after the first administration were assessed for p53 protein by immunohistochemical staining and for apoptosis by terminal deoxynucleotidyl transferase-mediated nick end labeling assay. RESULTS: A total of 38 courses of INGN 201 gene therapy were administered to 30 patients, of whom 26 underwent radical prostatectomy. There were no grade 3 or 4 adverse events related to INGN 201 administration. Of the 11 patients with negative baseline immunostaining for p53 protein, 10 had positive p53 immunostaining after the first administration of INGN 201, and 8 had an increase in apoptotic cells by terminal deoxynucleotidyl transferase-mediated nick end labeling staining. All 26 of the patients who underwent radical prostatectomy had significant residual viable prostate cancer, and 12 have experienced biochemical failure (median follow-up, 42 months). CONCLUSION: Intraprostatic INGN 201 gene therapy is safe and can reliably result in p53 protein production and apoptosis.

Ponguta, L. A., C. W. Gregory, et al. (2008). "Site-specific androgen receptor serine phosphorylation linked to epidermal growth factor-dependent growth of castration-recurrent prostate cancer." *J Biol Chem* **283**(30): 20989-1001.

The androgen receptor (AR) is required for prostate cancer development and contributes to tumor progression after remission in response to androgen deprivation therapy. Epidermal growth factor (EGF) increases AR transcriptional activity at low levels of androgen in the CWR-R1 prostate cancer cell line derived from the castration-recurrent CWR22 prostate cancer xenograft. Here we report that knockdown of AR decreases EGF stimulation of prostate cancer cell growth and demonstrate a mechanistic link between EGF and AR signaling. The EGF-induced increase in AR transcriptional activity is dependent on phosphorylation at mitogen-activated protein kinase consensus site Ser-515 in the AR NH(2)-terminal region and at protein kinase C consensus site Ser-578 in the AR DNA binding domain. Phosphorylation at these sites alters the nuclear-cytoplasmic shuttling of AR and AR interaction with the Ku-70/80 regulatory subunits of DNA-dependent protein kinase. Abolishing AR Ser-578 phosphorylation by introducing an S578A mutation eliminates the AR transcriptional response to EGF and increases both AR binding of Ku-70/80 and nuclear retention of AR

in association with hyperphosphorylation of AR Ser-515. The results support a model in which AR transcriptional activity increases castration-recurrent prostate cancer cell growth in response to EGF by site-specific serine phosphorylation that regulates nuclear-cytoplasmic shuttling through interactions with the Ku-70/80 regulatory complex.

Prtilo, A., F. S. Leach, et al. (2005). "Tissue microarray analysis of hMSH2 expression predicts outcome in men with prostate cancer." *J Urol* **174**(5): 1814-8; discussion 1818.

PURPOSE: Mismatch repair genes are responsible for the coordinated correction of misincorporated nucleotides formed during DNA replication. Mismatch repair expression is altered in a subset of prostate cancers (PCs) and a recent study suggested that time to biochemical recurrence following prostatectomy correlated with the degree of hMSH2 immunohistochemical staining. We compared hMSH2 expression and survival in clinically organ confined PC. MATERIALS AND METHODS: A prostate tissue microarray was constructed using 243 specimens from patients who underwent radical prostatectomy with extended lymph node dissection for clinically organ confined PC with up to 12 years of followup. Immunohistochemistry was performed with anti-human MSH2 monoclonal antibody. Three independent observers evaluated hMSH2 expression on a scale of 0 to 4. Low expression was defined as a score of less than 2 and high expression was defined as a score of 2 or higher. Statistical analysis used the Fisher exact test, and Goodman and Kruskal gamma coefficient. RESULTS: Higher Gleason score significantly correlated with higher hMSH2 expression ( $p < 0.0002$ ). Low hMSH2 expression correlated with increased overall, disease-free and biochemical disease-free survival (all  $p < 0.01$ ). Analysis comparing low vs high hMSH2 expression was significant with respect to overall ( $p = 0.0004$ ), disease-free ( $p = 0.005$ ) and biochemical disease-free ( $p = 0.0177$ ) survival. CONCLUSIONS: hMSH2 is differentially expressed in malignant prostate tissue and hMSH2 immunohistochemical staining intensity correlates with Gleason score, overall and disease-free survival. Taken together our results suggest that hMSH2 expression may be a useful prognostic biomarker for outcome in men with clinically organ confined PC.

Pulukuri, S. M., J. A. Knost, et al. (2009). "Small interfering RNA-directed knockdown of uracil DNA glycosylase induces apoptosis and sensitizes human prostate cancer cells to genotoxic stress." *Mol Cancer Res* **7**(8): 1285-93.

Uracil DNA glycosylase (UNG) is the primary enzyme responsible for removing uracil residues from DNA. Although a substantial body of evidence suggests that DNA damage plays a role in cancer cell apoptosis, the underlying mechanisms are poorly understood. In particular, very little is known about the role of base excision repair of misincorporated uracil in cell survival. To test the hypothesis that the repair of DNA damage associated with uracil misincorporation is critical for cancer cell survival, we used small interfering RNA (siRNA) to target the human UNG gene. In a dose-dependent and time-dependent manner, siRNA specifically inhibited UNG expression and modified the expression of several genes at both mRNA and protein levels. In LNCaP cells, p53, p21, and Bax protein levels increased, whereas Bcl2 levels decreased. In DU145 cells, p21 levels were elevated, although mutant p53 and Bax levels remained unchanged. In PC3 cells, UNG inhibition resulted in elevated p21 and Bax levels. In all three cell lines, UNG inhibition reduced cell proliferation, induced apoptosis, and increased cellular sensitivity to genotoxic stress. Furthermore, an *in vitro* cleavage experiment using uracil-containing double-stranded DNA as a template has shown that siRNA-mediated knockdown of UNG expression significantly reduced the uracil-excising activity of UNG in human prostate cancer cells, which was associated with DNA damage analyzed by comet assay. Taken together, these findings indicate that RNA interference-directed targeting of UNG is a convenient, novel tool for studying the biological role of UNG and raises the potential of its application for prostate cancer therapy.

Pulukuri, S. M. and J. S. Rao (2005). "Activation of p53/p21Waf1/Cip1 pathway by 5-aza-2'-deoxycytidine inhibits cell proliferation, induces pro-apoptotic genes and mitogen-activated protein kinases in human prostate cancer cells." *Int J Oncol* **26**(4): 863-71.

The tumor suppressor gene p53 plays an essential role in cell proliferation and apoptosis. Due to its relevance to cancer therapy, most studies have focused on the cellular consequences of p53 activation in relation to cytotoxic drugs. 5-aza-2'-deoxycytidine (5-aza-CdR) is widely used as an anti-cancer drug for the treatment of leukemia and solid tumors. However, the mechanism by which 5-aza-CdR exerts its anti-neoplastic activity remains unclear. Here, we address the role of p53 in regulating cellular responses to 5-aza-CdR treatment in human prostate cancer cells. We found that 5-aza-CdR induces p53 and p21Waf1/Cip1 expression associated with inhibition of cell proliferation in LNCaP cells (p53 wild-type), but not in DU145 cells (p53 mutant). By using pifithrin- $\alpha$ ,

a chemical inhibitor of p53, we confirmed that the increase in p21Waf1/Cip1 expression and inhibition of cell proliferation in LNCaP cells by 5-aza-CdR is p53-dependent. Also, the activation of p53 and p21Waf1/Cip1 pathway by 5-aza-CdR modified multiple gene expressions including apoptotic target genes and MAP kinases in LNCaP cells. 5-aza-CdR-induced apoptosis in LNCaP cells is assessed by DNA fragmentation analysis. Furthermore, knockdown of p53 by pU6-p53 siRNA vector suggests the involvement of MAP kinases in the process of 5-aza-CdR-mediated activation of p53 pathway to inhibit cell proliferation and induce apoptosis. Finally, the comet or SCGE assay and methylation-sensitive restriction analysis demonstrated that 5-aza-CdR induced p53 and p21Waf1/Cip1 expression as a consequence of DNA damage and independent of DNA demethylation. Our findings suggest that 5-aza-CdR induces anti-neoplastic activity primarily through the activation of p53 pathway in response to DNA damage and subsequently leads to inhibition of cell proliferation as well as induction of apoptosis. Therefore, our data indicate that p53 status in tumor cells may be critical for the clinical efficacy and toxicity of 5-aza-CdR.

Qin, H., C. Zhou, et al. (2005). "Specific antitumor immune response induced by a novel DNA vaccine composed of multiple CTL and T helper cell epitopes of prostate cancer associated antigens." *Immunol Lett* **99**(1): 85-93.

The loss of immunogenic epitopes by tumors has urged the development of vaccines against multiple epitopes. Recombinant DNA technologies have opened the possibility to develop multiepitope vaccines in a relatively rapid and efficient way. In this study, several DNA fragments encoding multiple cytotoxic T lymphocyte (CTL) and T helper (Th) cell epitopes were selected from human prostate-specific membrane antigen (hPSM), mouse prostatic acid phosphatase (mPAP), and human prostate-specific antigen (hPSA). These DNA fragments were ligated together to form a novel fusion gene, termed 3P gene. The 3P gene and human IgG Fc gene were inserted into pcDNA3.1 to construct a DNA vaccine designated psig-3P-Fc. Vaccination with psig-3P-Fc by gene gun inoculation induced strong antitumor response in a mouse tumor model, which significantly inhibited tumor growth and prolonged survival time of the tumor-bearing mice. *In vitro*, when lymphocytes were stimulated by psig-3P-Fc-transfected autologous peripheral blood mononuclear cells (PBMC), CTLs were induced which could specifically kill hPSM-, hPAP-, or hPSA-expressing tumor cells. These observations provide a new vaccine strategy for cancer therapy through concomitant enhancement of

antigen specific CD4(+) helper and CD8(+) cytotoxic T-cell responses against tumors.

Qiu, Q., F. Dudouit, et al. (2004). "Inhibition of cell signaling by the combi-nitrosourea FD137 in the androgen independent DU145 prostate cancer cell line." *Prostate* **59**(1): 13-21.

**BACKGROUND:** FD137, a nitrosourea appended to a quinazoline ring, was designed to simultaneously block epidermal growth factor receptor (EGFR)-mediated signaling and damage genomic DNA in refractory EGF-dependent prostate tumors. **METHODS:** The mixed inhibition of cell signaling and DNA damage by FD137 were determined by Western blotting, RT-PCR, flow cytometry, sulforhodamine B (SRB), and comet assay. **RESULTS:** FD137 and its metabolite FD110 induced a dose-dependent increase in inhibition of EGF-stimulated EGFR autophosphorylation and this translated into blockade of c-fos gene expression in DU145 cells. FD137 induced significant levels of DNA damage and showed 150-fold greater anti-proliferative activity than BCNU, a classical nitrosourea. In contrast to BCNU, complete inhibition of EGF-induced cell transition to S-phase was observed at concentrations of FD137 as low as 3 microM. **CONCLUSION:** FD137 could not only damage DNA, but also significantly block downstream EGFR-mediated signaling. The superior activity of FD137 may be imputable to the combined effect of its mixed EGFR/DNA targeting properties. This novel strategy may well represent a new approach to target nitrosoureas to EGFR-overexpressing carcinomas of the prostate.

Qu, Y., L. Zhang, et al. (2008). "Effects of DNazymes targeting Aurora kinase A on the growth of human prostate cancer." *Cancer Gene Ther* **15**(8): 517-25.

Aurora kinase A has been demonstrated to be involved in the malignant progression of many types of cancer including prostate cancer, we therefore hypothesized that Aurora kinase A might work as a valuable target for prostate cancer treatment. To test this hypothesis, we used DNzyme technology to inhibit Aurora kinase A expression and evaluated the effects of DNazymes as therapeutic agents to treat prostate cancer. In an in vitro cleavage assay, we found that a DNzyme (DZ2) targeting Aurora kinase A could effectively cleave Aurora kinase A mRNA. When transfected into the prostate cancer cell line PC3, DZ2 was found to strongly inhibit the expression of Aurora kinase A examined by western blot analysis, and thus suppressed cell growth, arrested the progression of cell cycle, induced cell apoptosis and attenuated cell migration, as measured by 3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay, flow cytometry and Boyden chamber assay. Through in vivo study, we also found that DZ2 could significantly inhibit the growth of human prostate cancer xenografts in nude mice. In conclusion, DZ2 could effectively attenuate malignant progression of prostate cancer both in vitro and in vivo, suggesting that DNzyme targeting Aurora kinase A may be used as a valuable therapy to treat prostate cancer.

Ragde, H., W. A. Cavanagh, et al. (2004). "Dendritic cell based vaccines: progress in immunotherapy studies for prostate cancer." *J Urol* **172**(6 Pt 2): 2532-8.

**PURPOSE:** No effective treatment is currently available for metastatic prostate cancer. Dendritic cell (DC) based cancer vaccine research has emerged from the laboratories to human clinical trials. We describe progress in the development of DC based prostate cancer vaccine. **MATERIALS AND METHODS:** The literature was reviewed for major contributions to a growing number of studies that demonstrate the potential of DC based immunotherapeutics for prostate cancer. Background topics relating to DC based immunotherapy theory and practice are also addressed. **RESULTS:** DCs have been recognized as the most efficient antigen presenting cells that have the capacity to initiate naive T cell response in vitro and in vivo. During their differentiation and maturation pathways, dendritic cells can efficiently capture, process and present antigens for T cell activation. These characteristics make DC an attractive choice as the cellular adjuvant for cancer vaccines. Advances in DC generation, loading, and maturation methodologies have made it possible to generate clinical grade vaccines for various human trials. More than 100 DC vaccine trials, including 7 studies of patients with advanced prostate cancer have been reported to date. These vaccines were generally well tolerated with no significant adverse toxicity reported. Clinical responders have been identified in these studies. **CONCLUSIONS:** The new prospects opened by DC based vaccines for prostate cancer are fascinating. When compared to conventional treatments, DC vaccinations have few side effects. Improvements in patient selection, vaccine delivery strategies, immune monitoring and vaccine manufacturing will be crucial in moving DC based prostate cancer vaccines closer to the clinics.

Rahman, M. M., H. Miyamoto, et al. (2003). "Inactivation of androgen receptor coregulator ARA55 inhibits androgen receptor activity and agonist effect of antiandrogens in prostate cancer cells." *Proc Natl Acad Sci U S A* **100**(9): 5124-9.

Antiandrogens given to antagonize androgen receptor (AR) activity gradually lose their efficacy as antagonists and eventually function as agonists to promote (instead of block) AR-mediated growth of prostate cancer cells. The mechanisms of how antiandrogens acquire this agonist activity during hormonal therapy are largely unknown. Here, we report that expression of a dominant-negative AR-associated protein 55 (dARA55) coregulator, inhibits AR transcriptional activity and reduces the agonist activity of antiandrogens. Inducibly expressed dARA55 inhibits prostate-specific antigen and cell growth in prostate cancer cells. Further dissection of the molecular mechanism shows dARA55 can selectively suppress endogenous AR-associated protein 55 (ARA55) enhanced AR transactivation by means of interruption of dimerization between ARA55 and ARA55. These results were confirmed by using RNA interference-mediated silencing of the ARA55 gene. These results therefore provide evidence that AR function could be suppressed without mutation or change in AR itself. Taken together, these findings not only demonstrate the important roles of the ARA55 coregulator in the AR-mediated growth of prostate cancer, they also may provide a critical target for developing therapeutic agents for the antiandrogen therapy that almost always fails in the treatment of hormone-refractory prostate cancer.

Rahman, M. M., H. Miyamoto, et al. (2003). "Reducing the agonist activity of antiandrogens by a dominant-negative androgen receptor coregulator ARA70 in prostate cancer cells." *J Biol Chem* **278**(22): 19619-26.

Although the progression of prostate cancer initially is dependent on androgens, tumor progression to an androgen-independent growth eventually occurs in most of patients treated with androgen ablation and/or antiandrogen therapy. After the initial response, antiandrogens lose their efficacy and eventually act as agonists to promote androgen receptor (AR)-mediated growth of prostate cancer cells. An aberrant regulation of AR activity, presumably by AR coregulators, may contribute to this acquired agonist activity of antiandrogens. Using an in vitro mutagenesis and a double-negative selection in yeast two-hybrid screening, we have identified a dominant-negative AR coregulator ARA70 (dARA70N), which can inhibit AR transcriptional activity by inactivating the normal function of ARA70 in the LNCaP cells. Whereas ARA70 in oligomeric form interacts with AR and enhances its transcriptional activity, dARA70N lacks AR interaction and might retain the ability to form a non-functional heteromer with ARA70 and interrupt AR transcriptional activity without a change in AR protein itself. The addition of dARA70N reduces the

agonist activity and rescues the normal function of antiandrogens in prostate cancer cells. RNA-interference-mediated silencing of ARA70 gene further confirms these observations. Taken together, these findings indicate that ARA70 may contribute to the acquired agonist activity of antiandrogens and plays an important role in making prostate cancer cells resistant to androgen ablation and/or antiandrogen therapy. ARA70 may, thus, be a critical target for developing therapeutic agents against AR-mediated progression of prostate cancer.

Ramachandran, K., G. Gopisetty, et al. (2009). "Methylation-mediated repression of GADD45alpha in prostate cancer and its role as a potential therapeutic target." *Cancer Res* **69**(4): 1527-35.

Defects in apoptotic pathway contribute to uncontrolled proliferation of cancer cells and confer resistance to chemotherapy. Growth arrest and DNA damage inducible, alpha (GADD45alpha) is up-regulated on docetaxel treatment and may contribute to docetaxel-mediated cytotoxicity. We examined the mechanism of regulation of GADD45alpha in prostate cancer cells and the effect of its up-regulation on sensitivity to docetaxel chemotherapy. Expression of GADD45alpha in PC3 cells was higher than that in Du145 and LNCaP cells (17- and 12-fold, respectively;  $P < 0.05$ ). Although the proximal promoter region was unmethylated in all three cell lines, methylation of a 4 CpG region upstream of the proximal promoter correlated inversely with gene expression levels. Methylation was reversed by treatment of Du145 and LNCaP cells with DNA methyltransferase inhibitors, leading to reactivation of GADD45alpha expression in these cells. The 5' 4 CpG region was also frequently methylated in prostate cancer tissues. Methylation of this region correlated inversely with gene expression in prostate cancer and benign prostate tissues. The methyl binding protein MeCP2 was associated with the methylated 4 CpGs in Du145 cells, and knockdown of MeCP2 in these cells (Du145 MeCP2(-)) led to a significantly increased expression of GADD45alpha (3-fold;  $P = 0.035$ ) without affecting the methylation status of the gene. Enhanced sensitivity to docetaxel was observed by up-regulation of GADD45alpha in Du145 cells by recombinant expression of GADD45alpha or pretreatment with 5-azacytidine. Our results show that GADD45alpha is epigenetically repressed and is a potential target for treatment of prostate cancer.

Ramachandran, K., L. Navarro, et al. (2007). "Methylation-mediated silencing of genes is not altered by selenium treatment of prostate cancer cells." *Anticancer Res* **27**(2): 921-5.

**BACKGROUND:** The role of selenium in reducing the risk of multiple cancers has been described in the literature. Although reports have described the antiproliferative and pro-apoptotic function of selenium by up-regulation of genes in these pathways, information is lacking on the target mechanisms of selenium on specific genes. This study examines whether selenium treatment alters the methylation status of epigenetically silenced genes in prostate cancer cells. **MATERIALS AND METHODS:** Methylation of glutathione sulfotransferase pi (GSTP1) and Ras associated family 1A (RASSF1A) genes was studied using methylation sensitive PCR (MS-PCR). Gene expression was studied using Reverse Transcriptase PCR and Western Blotting. **RESULTS AND CONCLUSION:** Treatment of prostate cancer cells with selenium did not alter the expression of genes that were silenced by DNA methylation. Furthermore, the methylation status of these genes remained unaltered after treatment with seleno-DL-methionine.

Rao, D. S., T. S. Hyun, et al. (2002). "Huntingtin-interacting protein 1 is overexpressed in prostate and colon cancer and is critical for cellular survival." *J Clin Invest* **110**(3): 351-60.

Huntingtin-interacting protein 1 (HIP1) is a cofactor in clathrin-mediated vesicle trafficking. It was first implicated in cancer biology as part of a chromosomal translocation in leukemia. Here we report that HIP1 is expressed in prostate and colon tumor cells, but not in corresponding benign epithelia. The relationship between HIP1 expression in primary prostate cancer and clinical outcomes was evaluated with tissue microarrays. HIP1 expression was significantly associated with prostate cancer progression and metastasis. Conversely, primary prostate cancers lacking HIP1 expression consistently showed no progression after radical prostatectomy. In addition, the expression of HIP1 was elevated in prostate tumors from the transgenic mouse model of prostate cancer (TRAMP). At the molecular level, expression of a dominant negative mutant of HIP1 led to caspase-9-dependent apoptosis, suggesting that HIP1 is a cellular survival factor. Thus, HIP1 may play a role in tumorigenesis by allowing the survival of precancerous or cancerous cells. HIP1 might accomplish this via regulation of clathrin-mediated trafficking, a fundamental cellular pathway that has not previously been associated with tumorigenesis. HIP1 represents a putative prognostic factor for prostate cancer and a potential therapy target in prostate as well as colon cancers.

Reddy, G. P., E. R. Barrack, et al. (2006). "Regulatory processes affecting androgen receptor expression,

stability, and function: potential targets to treat hormone-refractory prostate cancer." *J Cell Biochem* **98**(6): 1408-23.

Prostate cancer cells rely on androgen receptor (AR) for proliferation and survival. Therefore, curing prostate cancer will require elimination of AR. Although androgen is the natural ligand that activates AR, AR activity is also subject to regulation by growth factor/growth factor receptor-stimulated signaling pathways that control the cell cycle. Cell cycle regulatory proteins and protein kinases in signaling pathways affected by growth factors can lead to AR activation in the absence of androgen. While downstream signaling proteins such as cyclins, cyclin-dependent kinases (CDKs), and pRB can modulate AR activity, upstream signaling pathways involving protein kinases such as mitogen-activated protein kinases, protein kinase A, and protein kinase B/Akt can affect post-translational modification of AR to affect not only AR function but also AR stability. Calcium and calmodulin (CaM), essential for proliferation and viability of a number of cells, including prostate cancer cells, play an important role in AR expression, stability, and function. CaM affects AR partly by interacting directly with AR and partly by activating protein kinases such as Akt and DNA-PK that can phosphorylate AR. The ubiquitin/26S proteasome pathway responsible for timely destruction of cell cycle regulatory proteins whose levels impede cell cycle progression also induces AR expression by activating NF-kappaB, and promotes AR activity by participating in the assembly of an AR transcription complex. Maspin, a serine protease inhibitor that is known mostly for its role as a tumor suppressor can also regulate AR intracellular localization and function by competing with AR for binding to the chaperone protein Hsp90 and co-repressor HDAC1, respectively. This perspective reviews the experimental evidence implicating these diverse cellular processes in AR expression, stability, and/or function, and presents a rationale for disrupting these cellular processes as a viable option for the treatment of both the hormone-sensitive and the hormone-insensitive prostate cancer.

Richter, E., K. Masuda, et al. (2007). "A role for DNA methylation in regulating the growth suppressor PMEPA1 gene in prostate cancer." *Epigenetics* **2**(2): 100-9.

A cascade of epigenetic events contributes to the selective growth advantage of cancer cells during tumor progression. PMEPA1 gene is an androgen-inducible negative regulator of cell growth in the prostate epithelium. During prostate cancer progression PMEPA1 gene transcription is reduced or

lost prompting us to investigate the role of epigenetic events in this process. In LAPC4 cells harboring wild type androgen receptor decitabine (5-aza-2'-deoxycytidine) treatment resulted in increased expression of PMEPA1 along with other androgen-inducible genes, suggesting a role for DNA methylation in the repression of androgenic cell growth control signals in prostate cancer. In contrast, mutant androgen receptor expressing LNCaP cells were deficient in this response. Therefore, decitabine-induced expression of cell growth controlling genes such as NKX3.1 or PMEPA1 underlines the clinical applicability of decitabine in prostate tumors harboring wild type androgen receptor. Further analysis of DNA methylation within the PMEPA1 promoter downstream sequences suggests that methylation of SP1 binding sites may also contribute to the repression of PMEPA1 gene.

Ritter, M. A., K. W. Gilchrist, et al. (2002). "The role of p53 in radiation therapy outcomes for favorable-to-intermediate-risk prostate cancer." *Int J Radiat Oncol Biol Phys* 53(3): 574-80.

**PURPOSE:** Some prostate cancers may have molecular alterations that render them less responsive to radiation therapy; identification of these alterations before treatment might allow improved treatment optimization. This study investigated whether p53, a potential molecular determinant, could predict long-term radiation therapy outcome in a restricted group of relatively favorable-risk prostate cancer patients treated uniformly with irradiation alone. **METHODS AND MATERIALS:** This study included 53 patients previously treated with radiotherapy for favorable-to-intermediate-risk prostate cancer. These patients were selected for relatively low pretreatment PSAs (< or =21 ng/mL) and Gleason scores (< or =7) to decrease the likelihood of nonlocalized disease, because disease localization was necessary to examine the efficacy of localized radiation therapy. The status of p53 was immunohistochemically assessed in paraffin-embedded pretreatment biopsy specimens, along with appropriate controls. This marker was selected based upon a usable mutation prevalence in early-stage prostate cancer and its potential linkage with radiation response via cell cycle, DNA repair, and cell death pathways. Correlation between p53 mutation and clinical outcome was analyzed in univariate and multivariate fashion and included conventional prognosticators, such as stage, grade, and PSA. Freedom from biochemical failure was determined using American Society for Therapeutic Radiology and Oncology criteria. Limitations of prior studies were potentially avoided by requiring adequate posttreatment follow-up (median follow-up in nonfailing patients of 5.1 years), as well as

pretreatment PSA and Gleason scores that suggested localized disease, and uniformity of treatment. **RESULTS:** The total group of 53 favorable-to-intermediate-risk patients demonstrated an actuarial biochemical failure rate of 35% at 5 years. Forty percent of all specimens had a greater than 10% labeling index for p53 mutation, and actuarial biochemical control was found to strongly and independently correlate with p53 status. Patients with higher p53 labeling indices demonstrated significantly higher PSA failure rates ( $p < 0.001$ ). In contrast, p53 status did not correlate with pretreatment PSA, grade, or tumor stage. Similarly, pretreatment PSA (log-rank 0.22), Gleason score (log-rank 0.93), and T stage (log-rank 0.15) were not prognostic for outcome in this group of patients selected for their relatively favorable clinical characteristics. **CONCLUSIONS:** (1) p53 status in pretreatment biopsies strongly predicted for long-term biochemical control after radiation therapy in favorable-to-intermediate-risk prostate cancer patients. (2) If validated in other independent clinical data sets, p53 status should be considered as a stratification factor in future clinical trials and could be useful in guiding treatment. Abnormal p53 status might favor surgical management, aggressive dose escalation, or p53-targeted therapy.

Roos, A. K., A. King, et al. (2008). "DNA vaccination for prostate cancer." *Methods Mol Biol* 423: 463-72.

DNA-based cancer vaccines have been used successfully in mice to induce cytotoxic T lymphocytes (CTLs) specific for prostate antigens. Translation of a prostate-specific antigen (PSA) DNA vaccine into a phase I clinical trial demonstrated that PSA-specific immune responses could be induced but at a significantly lower level compared with those in mice. To enhance the efficacy of DNA vaccination against prostate cancer, we have explored and optimized intradermal electroporation as an effective way of delivering a PSA DNA vaccine. The results demonstrated that intradermal DNA vaccination using low amounts of DNA, followed by two sets of electrical pulses of different length and voltage, effectively induced PSA-specific T cells. Here we describe in detail how to perform intradermal DNA electroporation to induce high gene expression in skin and, more important, how to induce and analyze PSA-specific T cell responses.

Roos, A. K., S. Moreno, et al. (2006). "Enhancement of cellular immune response to a prostate cancer DNA vaccine by intradermal electroporation." *Mol Ther* 13(2): 320-7.

Recently it has become clear that more potent methods for DNA vaccine delivery need to be developed to enhance the efficacy of DNA vaccines.

In vivo electroporation has emerged as a potent method for DNA vaccine delivery. In a mouse model, we evaluated the CD8(+) T lymphocyte response to a prostate cancer DNA vaccine encoding prostate-specific antigen (PSA) after intradermal electroporation. A significantly increased gene expression (100- to 1000-fold) and higher levels of PSA-specific T cells, compared to DNA delivery without electroporation, was demonstrated. Interestingly, investigation of a panel of different electroporation conditions showed that only some conditions that induce high levels of gene expression additionally induced cellular immunity. This suggests that electroporation parameters should be carefully optimized, not only to enhance transfection efficiency, but also to enhance the immune response to the vaccine. This study demonstrates the applicability of intradermal electroporation as a delivery method for genetic cancer vaccines and other DNA vaccines relying on antigen-specific T cell induction.

Roos, A. K., M. Pavlenko, et al. (2005). "Induction of PSA-specific CTLs and anti-tumor immunity by a genetic prostate cancer vaccine." *Prostate* **62**(3): 217-23.

**BACKGROUND:** Prostate cancer is the most common malignancy in Swedish and American men. Effective curative treatment modalities are debilitating and available only for localized disease. As an immunotherapy approach, DNA encoding prostate-specific antigen (PSA), was used to immunize mice and induce PSA-specific cellular immunity. **METHODS:** A plasmid expressing PSA, alone or in combination with plasmids coding for GM-CSF and/or IL-2, was used for DNA immunization. Cr-release, intracellular IFN-gamma cytokine staining, and tumor challenge assays were used to evaluate the immune response. **RESULTS:** The DNA vaccine induces PSA-specific cytotoxic T lymphocytes (CTLs) and when co-injected with IL-2 and GM-CSF it protects four of five mice against a PSA-expressing tumor challenge. **CONCLUSIONS:** We demonstrate that immunization with a PSA DNA vaccine can evoke PSA-specific cellular immune responses. We also show, for the first time, that a PSA DNA vaccine can induce anti-tumor immunity in vivo.

Rosenbaum, E., M. O. Hoque, et al. (2005). "Promoter hypermethylation as an independent prognostic factor for relapse in patients with prostate cancer following radical prostatectomy." *Clin Cancer Res* **11**(23): 8321-5.

**PURPOSE:** To analyze the prognostic significance of six epigenetic biomarkers (APC, Cyclin D2, GSTP1, TIG1, Rassf1A, and RARbeta2 promoter hypermethylation) in a homogeneous group

of prostate cancer patients, following radical prostatectomy alone. **PATIENTS AND METHODS:** Biomarker analyses were done retrospectively on tumors from 74 prostate cancer patients all with a Gleason score of 3 + 4 = 7 and minimum follow-up period of 7 years. Using quantitative methylation-specific PCR, we analyzed six gene promoters in primary prostate tumor tissues. Time to any progression was the primary end point, and development of metastatic disease and/or death from prostate cancer was a secondary point. The association of clinicopathologic and biomolecular risk factors to recurrence was done using the log-rank test and Cox proportional hazards model for multivariate analysis. To identify independent prognostic factors, a stepwise selection method was used. **RESULTS:** At a median follow-up time of 9 years, 37 patients (50%) had evidence of recurrence: biochemical/prostate-specific antigen relapse, metastases, or death from prostate cancer. In the final multivariate analysis for time to progression (TTP), the significant factors were age > 60 [hazard ratio (HR), 0.4; 95% confidence interval (95% CI), 0.2-0.8; P = 0.01], hypermethylation of GSTP1 (HR, 0.23; 95% CI; 0.09-0.64; P = 0.004), and hypermethylation of APC (HR, 3.0; 95% CI, 1.42-6.32; P = 0.004). In another multivariate analysis, a profile of hypermethylation of APC and cyclin D2 hypermethylation was significant as well: if either any one was hypermethylated (HR, 1.84; 95% CI, 0.92-3.72; P = 0.09) or if both were hypermethylated (HR, 4.3; 95% CI, 1.52-12.33; P = 0.01). **CONCLUSIONS:** Methylation status of selected genes in the prostate cancer specimen may predict for time to recurrence in Gleason 3 + 4 = 7 patients undergoing prostatectomy. These results should be validated in a larger and unselected cohort.

Ross, R. W., W. K. Oh, et al. (2008). "Inherited variation in the androgen pathway is associated with the efficacy of androgen-deprivation therapy in men with prostate cancer." *J Clin Oncol* **26**(6): 842-7.

**PURPOSE:** Androgen-deprivation therapy (ADT) is the most common and effective systemic therapy for advanced prostate cancer. We hypothesized that germline genetic variation in the androgen axis would improve the efficacy of ADT. **PATIENTS AND METHODS:** A cohort of 529 men with advanced prostate cancer treated with ADT was genotyped for 129 DNA polymorphisms distributed across 20 genes involved in androgen metabolism. **RESULTS:** Three polymorphisms in separate genes (CYP19A1, HSD3B1, and HSD17B4) were significantly (P < .01) associated with time to progression (TTP) during ADT, remaining so in multivariate analyses and after correcting for the number of hypotheses tested. Individuals carrying

more than one of the polymorphisms associated with improved TTP demonstrated a better response to therapy than individuals carrying zero or one ( $P < .0001$ ). CONCLUSION: This report is the first to examine the influence of inherited variation in the androgen metabolic pathway on the efficacy of ADT, establishing the importance of pharmacogenomics on individual's response to this therapy. At least two potential clinical benefits may be realized from this study. The first is prognostic -genotyping patients at these loci may yield important information that could improve efficacy prediction. The second is therapeutic -these results shed light on the pathways that govern response to ADT. Drugs could be developed (or may already exist) to inhibit or augment these targets to improve ADT efficacy.

Rostad, K., M. Mannelqvist, et al. (2007). "ERG upregulation and related ETS transcription factors in prostate cancer." *Int J Oncol* **30**(1): 19-32.

The aim of this study was to identify and validate differentially expressed genes in matched pairs of benign and malignant prostate tissue. Samples included 29 histologically verified primary tumors and 23 benign controls. Microarray analysis was initially performed using a sequence verified set of 40,000 human cDNA clones. Among the genes most consistently and highly upregulated in prostate cancer was the ETS family transcription factor ERG (ETS related gene). This finding was validated in an expanded patient series (37 tumors and 38 benign samples) using DNA oligonucleotide microarray and real-time quantitative PCR assays. ERG was 20- to more than 100-fold overexpressed in prostate cancer compared with benign prostate tissue in more than 50% of patients according to quantitative PCR. Surprisingly, ERG mRNA levels were found to be significantly higher in the endothelial cell line, HUVEC, than in the prostate cell lines PC3, DU145 and LNCaP. In situ hybridization of prostate cancer tissue revealed that ERG was abundantly expressed in both prostate cancer cells and associated endothelial cells. The consistency and magnitude of ERG overexpression in prostate cancer appeared unique, but several related ETS transcription factors were also overexpressed in matched pairs of tumor and benign samples, whereas ETS2 was significantly underexpressed. Our findings support the hypothesis that ERG overexpression and related ETS transcription factors are important for early prostate carcinogenesis.

Roupret, M., V. Hupertan, et al. (2008). "Promoter hypermethylation in circulating blood cells identifies prostate cancer progression." *Int J Cancer* **122**(4): 952-6.

Promoter hypermethylation of circulating cell DNA has been advocated as a diagnostic marker for prostate cancer, but its prognostic use is currently unclear. To assess this role, we compared hypermethylation of circulating cell DNA from prostate cancer patients with (Group 1,  $n = 20$ ) and without (Group 2,  $n = 22$ ) disease progression and age-matched controls (benign prostatic hyperplasia, Group 3,  $n = 22$ ). We measured hypermethylation of 10 gene promoters in 2 sequential venous samples, obtained at diagnosis and during disease progression (median time, 15 months later). Matched time samples were obtained in the nonprogressing patients. We found that more hypermethylation was detected in the diagnostic sample from the patients with cancer than in controls for GSTP1, RASSF1 alpha, APC and RAR beta ( $p < 0.0001$ ). Patients undergoing disease progression had a significant increase in methylation levels of these 4 genes when compared to the other patients ( $p < 0.001$ ). Patients at risk of disease progression have higher detectable concentrations of circulating cell hypermethylation, than those without progression. The extent of this hypermethylation increases during disease progression and can be used to identify the extent and duration of treatment response in prostate cancer.

Rubin, M. A. and A. M. Chinnaiyan (2006). "Bioinformatics approach leads to the discovery of the TMPRSS2:ETS gene fusion in prostate cancer." *Lab Invest* **86**(11): 1099-102.

Recurrent chromosomal rearrangements have not been well characterized in common carcinomas. We describe the use of a novel bioinformatics approach to discover candidate oncogenic chromosomal aberrations on the basis of outlier gene expression called COPA (cancer outlier profile analysis). We demonstrate how this approach led to the identification of gene fusions of the 5'-untranslated region of TMPRSS2 (21q22.3), an androgen regulated gene, with the ETS transcription factor family members, either ERG (21q22.2), ETV1 (7p21.2), or ETV4(17q21). These novel gene fusions suggest a mechanism for overexpression of the ETS genes in the majority of prostate cancers identified through PSA screening. Considering the high incidence of prostate cancer and the high frequency of this gene fusion, the TMPRSS2-ETS gene fusions are the most common genetic aberration so far described in human malignancies. The clinical implications of this discovery are significant for diagnosis and potentially for the development of targeted therapy.

Ruiz, M., C. Pettaway, et al. (2004). "Activator protein 2alpha inhibits tumorigenicity and represses

vascular endothelial growth factor transcription in prostate cancer cells." *Cancer Res* **64**(2): 631-8.

Activator protein-2alpha (AP-2) is a transcription factor that regulates proliferation and differentiation in mammalian cells. We have shown previously that although AP-2 is expressed highly in normal prostatic epithelium, its expression is lost in high-grade prostatic intraepithelial neoplasia and prostate cancer, suggesting that loss of AP-2 plays a role in prostate cancer development. We demonstrate that forced AP-2 expression in the prostate cancer cell line LNCaP-LN3 (AP-2 negative) inhibited dramatically tumor incidence in nude mice. To identify the genes that might have been responsible for this effect, we used microchip expression array. We found several genes known to be involved in malignancy were deregulated, including the vascular endothelial growth factor (VEGF) gene. Because VEGF was down-regulated by 14.7-fold in the AP-2-transfected cells and because it is a major angiogenic factor in prostate cancer development and progression, we chose to examine the AP-2-VEGF interaction. Our evidence suggests that AP-2 repressed transcriptionally the VEGF promoter by competing with the transcriptional activator Sp3. Loss of AP-2 in prostate cancer cells reduced the AP-2:Sp3 ratio and activated VEGF expression. AP-2 acts as a tumor-suppressor gene in prostate cancer. Elucidating the molecular events resulting from loss of AP-2 in the prostate epithelium has implications for the understanding and prevention of the onset of prostate cancer.

Salah, Z., M. Maoz, et al. (2005). "Identification of a novel functional androgen response element within hPar1 promoter: implications to prostate cancer progression." *Faseb J* **19**(1): 62-72.

Human protease-activated receptor-1 (hPar1) plays a role in malignant and physiological invasion processes. We have identified a functional androgen response element (ARE) located in the hPar1 promoter upstream of the transcription start site at -1791 to -1777. Dihydrotestosterone treatment of the prostate cancer cell line LNCaP increased endogenous hPar1 mRNA levels, consistent with the threefold increase in promoter activity of hPar1-luciferase reporter construct. Specific binding of the hPar1-derived ARE to LNCaP nuclear extracts was demonstrated by electrophoretic mobility shift assay. This binding was abrogated by antiandrogen receptor (anti-AR) antibodies or excess cold oligonucleotide but not by a mutated oligonucleotide. Moreover, using chromatin immunoprecipitation assays, we confirm the *in vivo* interaction between the AR and ARE domain of the hPar1 promoter. In parallel, we show that hormone ablation therapy markedly reduces the

otherwise high hPar1 expression levels in prostate cancer biopsy specimens. We suggest that the hPar1 gene is regulated transcriptionally by androgens, representing one of several target genes effectively reduced during hormone ablation therapy. A major limitation of hormonal deprivation is that it causes only a temporary remission, and the cancer eventually reappears in a more malignant, androgen-independent form. hPar1 is also overexpressed in CL1 cells, an aggressively metastasizing, hormone-independent subclone of LNCaP, and in PC3 prostate adenocarcinoma lacking AR in a mechanism yet to be fully elucidated. These data may imply that hPar1 expression correlates with prostate cancer progression in androgen-dependent and -independent phases and therefore, provides an instrumental, therapeutic target for treatment in prostate cancer.

Sanford, D. C. and J. W. DeWille (2005). "C/EBPdelta is a downstream mediator of IL-6 induced growth inhibition of prostate cancer cells." *Prostate* **63**(2): 143-54.

**BACKGROUND:** Although a number of reports have investigated the effects of IL-6 family cytokines on prostate cell growth, there is limited information available identifying IL-6 inducible downstream effector genes and their function in growth control. Previous studies have demonstrated that IL-6 treatment results in the activation of signal transducer and activator of transcription3 (STAT3) in prostate cancer cells. The goal of this study was to investigate the influence of IL-6 treatment and activation of the Jak/STAT signal transduction pathway on C/EBPdelta gene expression and growth inhibition of human prostate cancer cells. **METHODS:** Expression of C/EBPdelta and STAT3 activation were assayed using Northern and Western blotting techniques. Proliferation was assessed by [<sup>3</sup>H] thymidine incorporation, flow cytometry, and colony formation analyses. The analysis of the transcriptional regulation of C/EBPdelta was performed using luciferase-reporter constructs. **RESULTS:** In this report, we demonstrate that IL-6 treatment induces STAT3 activation (pSTAT3), pSTAT3 binds to the human C/EBPdelta gene promoter and induces its expression. We also demonstrate that C/EBPdelta over-expression is capable of suppressing prostate cancer cell growth. **CONCLUSIONS:** These results demonstrate that C/EBPdelta gene expression is increased in IL-6 treated LNCaP cells. Increased C/EBPdelta gene expression plays an important role in IL-6/STAT3 mediated growth arrest of LNCaP prostate cancer cells. Ongoing studies are investigating the mechanism by which C/EBPdelta controls prostate cancer cell growth and the potential role of C/EBPdelta in the survival and chemo

resistance of prostate cancer metastasis. (c) 2004 Wiley-Liss, Inc.

Schlicht, M., B. Matysiak, et al. (2004). "Cross-species global and subset gene expression profiling identifies genes involved in prostate cancer response to selenium." *BMC Genomics* **5**(1): 58.

**BACKGROUND:** Gene expression technologies have the ability to generate vast amounts of data, yet there often resides only limited resources for subsequent validation studies. This necessitates the ability to perform sorting and prioritization of the output data. Previously described methodologies have used functional pathways or transcriptional regulatory grouping to sort genes for further study. In this paper we demonstrate a comparative genomics based method to leverage data from animal models to prioritize genes for validation. This approach allows one to develop a disease-based focus for the prioritization of gene data, a process that is essential for systems that lack significant functional pathway data yet have defined animal models. This method is made possible through the use of highly controlled spotted cDNA slide production and the use of comparative bioinformatics databases without the use of cross-species slide hybridizations. **RESULTS:** Using gene expression profiling we have demonstrated a similar whole transcriptome gene expression patterns in prostate cancer cells from human and rat prostate cancer cell lines both at baseline expression levels and after treatment with physiologic concentrations of the proposed chemopreventive agent Selenium. Using both the human PC3 and rat P4II prostate cancer cell lines have gone on to identify a subset of one hundred and fifty-four genes that demonstrate a similar level of differential expression to Selenium treatment in both species. Further analysis and data mining for two genes, the Insulin like Growth Factor Binding protein 3, and Retinoic X Receptor alpha, demonstrates an association with prostate cancer, functional pathway links, and protein-protein interactions that make these genes prime candidates for explaining the mechanism of Selenium's chemopreventive effect in prostate cancer. These genes are subsequently validated by western blots showing Selenium based induction and using tissue microarrays to demonstrate a significant association between downregulated protein expression and tumorigenesis, a process that is the reverse of what is seen in the presence of Selenium. **CONCLUSIONS:** Thus the outlined process demonstrates similar baseline and selenium induced gene expression profiles between rat and human prostate cancers, and provides a method for identifying testable functional pathways for the action

of Selenium's chemopreventive properties in prostate cancer.

Schlomm, T., A. Erbersdobler, et al. (2007). "Molecular staging of prostate cancer in the year 2007." *World J Urol* **25**(1): 19-30.

Numerous attempts towards improving patient management by molecular staging have been fruitless so far. No single molecular parameter is routinely analyzed in prostate cancer tissue. This may be partly due to genuine properties of prostate cancer that may make this tumor a difficult target. Furthermore, inherent logistical problems result in a shortage of prostate cancer tissue for research purposes. For the future, it can be hoped that the availability of more powerful molecular techniques in combination with better tissue archives will allow more rapid progress. Powerful DNA array and proteomics methods allow the systematic analysis of virtually all genes of a cancer on the DNA, RNA, and protein level. Although such approaches are sometimes labeled as "fishing expeditions," it cannot be totally disregarded that the simultaneous analysis of all genes has a high likelihood of identifying significant new information. In future, one of the major scientific challenges will be the validation of several potential biomarkers in large enough and clinically well-characterized patient cohorts. In particular, studies on needle core biopsies and hormone refractory cancers are imperatively needed for investigating the natural history of the disease or to discover potential predictive markers for radiation therapy and new therapeutic target genes to answer the clinically most important questions for optimal clinical decision making in prostate cancer patients: which patients will not require local therapy? If local therapy is needed, what is the treatment of choice? What medications should be given if metastases are present?

Schmidt, U., S. Fuessel, et al. (2006). "Quantitative multi-gene expression profiling of primary prostate cancer." *Prostate* **66**(14): 1521-34.

**BACKGROUND:** This study describes the evaluation of the expression patterns of prostate-related transcripts in 106 matched prostate tissues from prostatectomies as predictors for prostate cancer (PCa). **METHODS:** Quantitative PCR (QPCR) assays with site-specific hybridization probes were established for four housekeeping genes (GAPDH, HPRT, PBGD, TBP) and nine prostate-related genes (AibZIP, D-GPCR, EZH2, PCA3, PDEF, prostein, PSA, PSCA, TRPM8). **RESULTS:** The relative mRNA expression levels of AibZIP, D-GPCR, EZH2, PCA3, PDEF, PSA, TRPM8 (all  $P < 0.001$ ) and prostein ( $P = 0.019$ ) normalized to the TBP reference

gene were significantly higher in malignant compared to non-malignant prostate tissues. Employing receiver-operating characteristic (ROC) analyses, PCA3 was the best single tumor marker with the highest area-under-the-curve (AUC = 0.85). A multivariate logit model for the predictability of the tumor was developed, which employed the relative expression levels of EZH2, PCA3, prostein, and TRPM8 and yielded an AUC of 0.90. CONCLUSIONS: The transcript marker PCA3 is a powerful predictor of primary PCa but the inclusion of EZH2, prostein, and TRPM8 adds even more to the diagnostic power. The finding of a significantly higher mRNA expression of three different genes (prostein, PSA, TRPM8) in organ-confined tumors compared to non-organ-confined tumors as well as the multi-marker PCA prediction model developed in the retrospective model system on prostatectomies could be of clinical importance for diagnostic purposes, and should be verified in diagnostic biopsies.

Schulz, W. A. and M. J. Hoffmann (2009). "Epigenetic mechanisms in the biology of prostate cancer." *Semin Cancer Biol* **19**(3): 172-80.

Prostate cancer is one of the most frequent cancers in males in Western industrialized countries. Its course is highly variable, from indolent to highly lethal. Genetic changes vary accordingly, with chromosomal losses, gains and translocations, although often recurrent, differing between individual cases of the disease. In contrast, certain epigenetic changes are highly consistent, in particular hypermethylation of a specific set of genes, and others regularly associated with progression, such as global DNA hypomethylation, certain chromatin modifications and altered levels and composition of polycomb complexes. Although changes in polycombs and DNA methylation appear to both accompany the progression of prostate cancer, recent studies do not suggest that they cause one another. However, they may contribute to establishing and maintaining an aberrant differentiation potential of prostate cancer initiating cells. Global DNA hypomethylation in prostate cancer may relate to adaptative changes in several signaling pathways typical of this cancer type, including innate immunity responses. Similarly, adaptative changes in the expression and function of chromatin regulators required to diminish the dependency of prostate cancer cells on androgens may shape the epigenome, beyond individual genes regulated by the androgen receptor. Because of their crucial role, epigenetic alterations may become highly useful for diagnostics and therapy of prostate cancer.

Scott, S. L., J. D. Earle, et al. (2003). "Functional p53 increases prostate cancer cell survival after exposure

to fractionated doses of ionizing radiation." *Cancer Res* **63**(21): 7190-6.

External beam radiation therapy is an effective therapy for localized prostate cancer, although failures occur at high rates. One variable that may affect the radiosensitivity of prostate tumor cells is their p53 status because this gene controls radiation-induced cell cycle arrest, apoptosis, and the repair of DNA damage. Using a system in which p53 function was conditionally restored to p53-null PC3 prostate cancer cells by stable transfection with a human temperature-sensitive p53 mutant allele, we tested the hypothesis that functional p53 increases cell cycle arrest and contributes to increased clonogenic survival after ionizing radiation (IR) of prostate carcinoma cells. Cell cycle arrest and clonogenic survival in response to single and multiple daily exposures to clinically relevant 2-Gy doses of IR were examined. Whereas the temperature-sensitive p53 protein was activated by phosphorylation after IR exposure at both the restrictive and permissive temperatures, Cdkn1/p21 was only induced by functional p53 (at the permissive temperature). In the presence of functional p53, the maintenance of G(2) arrest was significantly longer ( $P < 0.01$ ), and a small increase in cell survival measured by clonogenic assay was seen after exposure to a single 2-Gy dose of IR. However, functional p53 significantly increased clonogenic survival ( $P < 0.01$ ) after exposure to daily doses of 2 Gy of IR and contributed to a more sustained G(2) arrest and increased G(1) arrest in response to the multifraction regimen. These studies implicate the presence of wild-type p53 with increased survival of prostate carcinoma cells after fractionated exposure to radiation. Additionally, the data provide evidence that wild-type p53 in prostate tumor cells may reduce the effectiveness of radiation therapy.

Segawa, N., M. Nakamura, et al. (2002). "Expression and somatic mutation on androgen receptor gene in prostate cancer." *Int J Urol* **9**(10): 545-53.

BACKGROUND: Lack of androgen receptor (AR) expression or mutation on the AR gene creates the tendency for androgen independence and progression of prostate cancer. However, the association between the progression and AR expression or mutations is still controversial. In this study, we evaluated the prognostic significance of AR expression and mutations in prostate cancers. METHODS: Forty-two prostate adenocarcinomas and three lymph node metastatic lesions sampled prior to hormonal therapy were included in this study; AR expression was analyzed immunohistochemically using an antibody against AR and the result was scored as the percentage of AR-positive tumor cells in the total tumor cells. Polymerase chain reaction-

single-strand conformational polymorphism (PCR-SSCP) analysis and DNA sequencing were used to detect AR mutations. RESULTS: Our study revealed the average AR expression in the prostate adenocarcinoma was 52.2 +/- 27.1%, which was significantly lower than that in the adjacent non-tumorous prostate tissue (68.3 +/- 18.3% in average) ( $P < 0.001$ ). A significant correlation was obtained between progression-free survival and AR expression ( $P < 0.01$ ). By SSCP analysis, three silent mutations (T649T, E709E and E711E) were detected in three separate prostate carcinomas. CONCLUSION: : We conclude that AR expression is a useful prognostic indicator for tumor progression. Androgen receptor mutation may be an uncommon molecular event in untreated prostate cancer in Japanese men.

Setlur, S. R., K. D. Mertz, et al. (2008). "Estrogen-dependent signaling in a molecularly distinct subclass of aggressive prostate cancer." *J Natl Cancer Inst* **100**(11): 815-25.

BACKGROUND: The majority of prostate cancers harbor gene fusions of the 5'-untranslated region of the androgen-regulated transmembrane protease serine 2 (TMPRSS2) promoter with erythroblast transformation-specific transcription factor family members. The common fusion between TMPRSS2 and v-ets erythroblastosis virus E26 oncogene homolog (avian) (ERG) is associated with a more aggressive clinical phenotype, implying the existence of a distinct subclass of prostate cancer defined by this fusion. METHODS: We used complementary DNA-mediated annealing, selection, ligation, and extension to determine the expression profiles of 6144 transcriptionally informative genes in archived biopsy samples from 455 prostate cancer patients in the Swedish Watchful Waiting cohort (1987-1999) and the United States-based Physicians' Health Study cohort (1983-2003). A gene expression signature for prostate cancers with the TMPRSS2-ERG fusion was determined using partitioning and classification models and used in computational functional analysis. Cell proliferation and TMPRSS2-ERG expression in androgen receptor-negative (NCI-H660) prostate cancer cells after treatment with vehicle or estrogenic compounds were assessed by viability assays and quantitative polymerase chain reaction, respectively. All statistical tests were two-sided. RESULTS: We identified an 87-gene expression signature that distinguishes TMPRSS2-ERG fusion prostate cancer as a discrete molecular entity (area under the curve = 0.80, 95% confidence interval [CI] = 0.792 to 0.81;  $P < .001$ ). Computational analysis suggested that this fusion signature was associated with estrogen receptor (ER) signaling. Viability of NCI-H660 cells decreased after

treatment with estrogen (viability normalized to day 0, estrogen vs vehicle at day 8, mean = 2.04 vs 3.40, difference = 1.36, 95% CI = 1.12 to 1.62) or ERbeta agonist (ERbeta agonist vs vehicle at day 8, mean = 1.86 vs 3.40, difference = 1.54, 95% CI = 1.39 to 1.69) but increased after ERalpha agonist treatment (ERalpha agonist vs vehicle at day 8, mean = 4.36 vs 3.40, difference = 0.96, 95% CI = 0.68 to 1.23). Similarly, expression of TMPRSS2-ERG decreased after ERbeta agonist treatment (fold change over internal control, ERbeta agonist vs vehicle at 24 hours, NCI-H660, mean = 0.57- vs 1.0-fold, difference = 0.43-fold, 95% CI = 0.29- to 0.57-fold) and increased after ERalpha agonist treatment (ERalpha agonist vs vehicle at 24 hours, mean = 5.63- vs 1.0-fold, difference = 4.63-fold, 95% CI = 4.34- to 4.92-fold). CONCLUSIONS: TMPRSS2-ERG fusion prostate cancer is a distinct molecular subclass. TMPRSS2-ERG expression is regulated by a novel ER-dependent mechanism.

Shah, R. B., M. Zhou, et al. (2002). "Comparison of the basal cell-specific markers, 34betaE12 and p63, in the diagnosis of prostate cancer." *Am J Surg Pathol* **26**(9): 1161-8.

The basal cell-specific cytokeratin antibody (34betaE12) is widely used to aid in the diagnosis of cancer in challenging prostate needle biopsies (NBX) and transurethral resections of the prostate (TURP). Because prostate carcinoma (PCa) lacks basal cells, the absence of basal cell as determined by 34betaE12 can aid in the confirmation of a histologically suspicious lesion. However, false-negative staining occurs because of patchy cytoplasmic staining, making a definitive diagnosis difficult. A recently identified basal cell marker p63, a p53 homologue, stains basal cell nuclei but not secretory cells. The aim of this study is to determine if the p63 antibody offers any clinically useful advantage over 34betaE12 in the diagnosis of challenging atypical prostate lesions. Ninety-four cases, comprised of 25 consecutive prostate NBX and 2 TURP with an atypical suspicious focus, 55 NBX cases of histologically unequivocal PCa and 12 TURP specimen removed for benign prostate hyperplasia, were stained with the monoclonal antibodies 34betaE12 and 4A4 anti-p63. Basal cell staining intensity, percentage basal cell-positive glands in benign, malignant, and atypical foci, and number of benign glands not staining were evaluated for 34betaE12 and p63 stains. A total of 67 prostate NBX cases, including one TURP, were diagnosed with PCa, 1 atypical small acinar proliferation, 10 benign, and 4 cases excluded because of lost tissue on step sections. None of the 67 PCa NBX cases demonstrated 34betaE12 or p63 immunoreactivity (100% specific). Whereas 57 of 108

(53%) prostate NBX cores from 78 cases demonstrated a similar percentage of basal cell staining for both antibodies, 45 of 108 (41%) NBX cores demonstrated a higher percentage of p63 basal cell staining in benign glands. Only 6 of 108 NBX (6%) cores had a higher percentage of basal cell staining with 34betaE12 (Wilcoxon signed rank test,  $p < 0.0001$ ). Lack of basal cell staining in more than two benign glands occurred in 25 of 108 (23%) and 10 of 108 (9%) prostate NBX cores stained with 34betaE12 and p63, respectively. In the vast majority of atypical cases, both 34betaE12 and p63 staining differences were not clinically significant, except in 2 of 27 (7%) cases p63 offered diagnostic utility beyond the 34betaE12 immunostain. p63 in these cases demonstrated discontinuous but strong staining in atypical glands and adjacent benign glands, whereas 34betaE12 failed to stain optimally in this critical area. For 12 TURP cases the mean percentage basal cell positivity in benign glands was 75% and 95% for 34betaE12 and p63, respectively ( $p = 0.006$ ). Lack of basal cell staining in more than two glands occurred in 12 of 12 (100%) and 2 of 12 (17%) TURP specimens stained with 34betaE12 and p63, respectively ( $p < 0.0001$ ). In summary, 34betaE12 and p63 are highly specific for basal cells and therefore are negative in areas of PCa. p63 is more sensitive than 34betaE12 in staining benign basal cells, particularly for TURP specimens, offering slight advantage over 34betaE12 in diagnostically challenging cases. p63 may be used as an alternative to 34betaE12 stain for difficult prostate lesions.

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

**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.

Sharifi, N., E. M. Hurt, et al. (2008). "Effects of manganese superoxide dismutase silencing on androgen receptor function and gene regulation: implications for castration-resistant prostate cancer." *Clin Cancer Res* **14**(19): 6073-80.

**PURPOSE:** Advanced prostate cancer is first treated with androgen deprivation therapy. However, tumors become resistant to and grow despite castrate levels of testosterone. Growth and proliferation of CRPC is mediated by gain-of-function changes in the AR and AR reactivation. Expression of manganese superoxide dismutase (SOD2), which regulates cellular ROS, is markedly down-regulated in CRPC when compared with hormone-responsive tumors. **EXPERIMENTAL DESIGN:** Here, we knocked down SOD2 expression in AR-expressing LNCaP prostate cancer cells and determined gene expression changes, transcription factor binding, and AR transcription activity in SOD2 knockdown cells. **RESULTS:** SOD2 knockdown results in an increase in ROS. Gene expression changes induced by SOD2 knockdown results in the up-regulation of genes that are also androgen responsive and 46% of genes up-regulated 2-fold by the androgen ligand R1881 are also up-regulated to the same extent with SOD2 knockdown. The induction of many of these genes with SOD2 knockdown, such as VEGFA and FKBP5, is reversible with the antioxidant N-acetylcysteine, suggesting that this mechanism is directly linked to ROS. Furthermore, an array for transcription factor DNA-binding activity shows that SOD2 knockdown induces DNA binding by several transcription factors, including AR. SOD2 knockdown-induced AR activation was confirmed by electrophoretic mobility shift assay and luciferase activity, and both were readily reversible with N-acetylcysteine. **CONCLUSIONS:** These findings show that down-regulation of SOD2 induces AR activity in a ROS-dependent manner, and suggest that there may be a role for antioxidant therapy in CRPC.

Sharma, A., C. E. Comstock, et al. (2007). "Retinoblastoma tumor suppressor status is a critical determinant of therapeutic response in prostate cancer cells." *Cancer Res* **67**(13): 6192-203.

The retinoblastoma tumor suppressor protein (RB), a critical mediator of cell cycle progression, is functionally inactivated in the majority of human cancers, including prostatic adenocarcinoma. The

importance of RB tumor suppressor function in this disease is evident because 25% to 50% of prostatic adenocarcinomas harbor aberrations in RB pathway. However, no previous studies challenged the consequence of RB inactivation on tumor cell proliferation or therapeutic response. Here, we show that RB depletion facilitates deregulation of specific E2F target genes, but does not confer a significant proliferative advantage in the presence of androgen. However, RB-deficient cells failed to elicit a cytostatic response (compared with RB proficient isogenic controls) when challenged with androgen ablation, AR antagonist, or combined androgen blockade. These data indicate that RB deficiency can facilitate bypass of first-line hormonal therapies used to treat prostate cancer. Given the established effect of RB on DNA damage checkpoints, these studies were then extended to determine the impact of RB depletion on the response to cytotoxic agents used to treat advanced disease. In this context, RB-deficient prostate cancer cells showed enhanced susceptibility to cell death induced by only a selected subset of cytotoxic agents (antimicrotubule agents and a topoisomerase inhibitor). Combined, these data indicate that RB depletion dramatically alters the cellular response to therapeutic intervention in prostate cancer cells and suggest that RB status could potentially be developed as a marker for effectively directing therapy.

Shen, L. and R. Pili (2008). "Posttranscription regulation of prostate cancer growth." *Cancer J* **14**(1): 46-53.

Advances in the understanding of the molecular mechanisms implicated in prostate cancer progression have allowed identification of many potential therapeutic gene targets that are involved in apoptosis, growth factors, cell signaling, and the androgen receptor. A critical factor responsible for the malignant progression of prostate cancer is the abnormal expression and function of specific proteins. From the transcription of mRNA to the translation of proteins and their function, several steps can be exploited as "drugable" targets. In this article we will review some of the key molecular targets and posttranscriptional strategies that are currently being tested both preclinically and clinically as targeted therapeutic approach for prostate cancer. Most of the targets mentioned in this review involve the prostate cancer signal transduction cascade, and their functions include prosurvival, antiapoptosis, and proangiogenesis. We will focus in particular on the emerging role of the "chromatin modifiers," histone deacetylase inhibitors, not only in transcriptional gene regulation but also in posttranscriptional protein

modifications as a tool for therapeutic intervention in prostate cancer.

Shimada, K., M. Nakamura, et al. (2003). "c-Jun NH2-terminal kinase-dependent Fas activation contributes to etoposide-induced apoptosis in p53-mutated prostate cancer cells." *Prostate* **55**(4): 265-80.

**BACKGROUND:** The death receptor, Fas, has recently been demonstrated to contribute the chemotherapeutic agents-induced apoptosis, however, the detail mechanisms have yet to be fully understood, especially in prostate cancer cells. **METHODS:** PC-3 and DU145 stably transfected with dominant negative form of Fas-associated death domain (FADD) or specific kinase of c-Jun NH2-terminal kinase (JNK) (mitogen-activated protein kinase kinase, MKK7) were selected in the presence of hygromycin B (Hyg B). Cell viability was examined by (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphonyl)-2H-tetra zolium, inner salt (MTS) assay or flowcytometric analysis using green fluorescent protein (GFP). Apoptosis was examined by DNA ladder, Western blotting analysis of cleaved caspases, or morphological analysis. The expression of Fas and JNK activation were investigated by Western blotting/flowcytometric analysis and in vitro kinase assay, respectively. **RESULTS:** Stimulation with etoposide significantly up-regulated Fas, and the death-inducing signaling complex (DISC) was formed in PC-3 and DU145. Stable transfection with dominant-negative FADD inhibited etoposide-induced apoptosis. In addition, stable transfection with dominant-negative MKK7, by which JNK activation was inhibited, canceled both the up-regulation of Fas and the formation of DISC by etoposide. Re-introduction of wild type p53 into PC-3 and DU145 completely suppressed these inhibitory effects. **CONCLUSIONS:** These results suggest that, in p53-mutated prostate cancer, JNK-initiated Fas-mediated apoptotic signals may play an important role in chemosensitivity.

Shukeir, N., P. Pakneshan, et al. (2006). "Alteration of the methylation status of tumor-promoting genes decreases prostate cancer cell invasiveness and tumorigenesis in vitro and in vivo." *Cancer Res* **66**(18): 9202-10.

We tested the hypothesis that cell invasiveness and tumorigenesis are driven by hypomethylation of genes involved in tumor progression. Highly invasive human prostate cancer cells PC-3 were treated with either the methyl donor S-adenosylmethionine (SAM) or methyl DNA-binding domain protein 2 antisense oligonucleotide (MBD2-AS). Both treatments resulted in a dose- and time-dependent inhibition of key genes, such as urokinase-

type plasminogen activator (uPA), matrix metalloproteinase-2 (MMP-2), and vascular endothelial growth factor expression to decrease tumor cell invasion in vitro. No change in the levels of expression of genes already known to be methylated in late-stage prostate cancer cells, such as glutathione S-transferase P1 and androgen receptor, was seen. Inoculation of PC-3 cells pretreated with SAM and MBD2-AS into the flank of male BALB/c nu/nu mice resulted in the development of tumors of significantly smaller volume compared with animals inoculated with PC-3 cells treated with vehicle alone or MBD2 scrambled oligonucleotide. Immunohistochemical analysis of tumors showed the ability of SAM and MBD2-AS to significantly decrease tumoral uPA and MMP-2 expression along with levels of angiogenesis and survival pathway signaling molecules. Bisulfite sequencing analysis of tumoral genomic DNA showed that inhibition of both uPA and MMP-2 expression was due to methylation of their 5' regulatory region. These studies support the hypothesis that DNA hypomethylation controls the activation of multiple tumor-promoting genes and provide valuable insight into developing novel therapeutic strategies against this common disease, which target the demethylation machinery.

Singh, L. S., M. Berk, et al. (2007). "Ovarian cancer G protein-coupled receptor 1, a new metastasis suppressor gene in prostate cancer." *J Natl Cancer Inst* **99**(17): 1313-27.

**BACKGROUND:** Metastasis is a process by which tumors spread from primary organs to other sites in the body and is the major cause of death for cancer patients. The ovarian cancer G protein-coupled receptor 1 (OGR1) gene has been shown to be expressed at lower levels in metastatic compared with primary prostate cancer tissues. **METHODS:** We used an orthotopic mouse metastasis model, in which we injected PC3 metastatic human prostate cancer cells stably transfected with empty vector (vector-PC3) or OGR1-expressing vector (OGR1-PC3) into the prostate lobes of athymic or NOD/SCID mice (n = 3-8 mice per group). Migration of PC3 cells transiently transfected with vector control or with OGR1- or GPR4 (a G protein-coupled receptor with the highest homology to OGR1)-expressing vectors was measured in vitro by Boyden chamber assays. G protein alpha-inhibitory subunit 1 (G alpha(i1)) expression after treatment with pertussis toxin (PTX) was measured using immunoblotting analysis. The inhibitory factor present in the conditioned medium was extracted using organic solvents and analyzed by mass spectrometry. **RESULTS:** In vivo, all 26 mice carrying tumors that were derived from vector-PC3 cells developed prostate cancer metastases (mean =

100%, 95% confidence interval [CI] = 83.97% to 100%) but few (4 of 32) mice carrying tumors derived from OGR1-expressing PC3 cells (mean = 12.50%, 95% CI = 4.08% to 29.93%) developed metastases. However, exogenous OGR1 overexpression had no effect on primary prostate tumor growth in vivo. In vitro, expression of OGR1, but not GPR4, inhibited cell migration (mean percentage of cells migrated, 30.2% versus 100%, difference = 69.8%, 95% CI = 63.0% to 75.9%; P<.001) via increased expression of G alpha(i1) and the secretion of a chloroform/methanol-extractable heat-insensitive factor into the conditioned medium through a PTX-sensitive pathway. **CONCLUSION:** OGR1 is a novel metastasis suppressor gene for prostate cancer. OGR1's constitutive activity via G alpha(i) contributes to its inhibitory effect on cell migration in vitro.

Sissung, T. M., R. Danesi, et al. (2008). "Association of the CYP1B1\*3 allele with survival in patients with prostate cancer receiving docetaxel." *Mol Cancer Ther* **7**(1): 19-26.

Using a single nucleotide polymorphism association study in 52 men with prostate cancer receiving docetaxel, we found that individuals carrying two copies of the CYP1B1\*3 polymorphic variant had a poor prognosis after docetaxel-based therapies compared with individuals carrying at least one copy of the CYP1B1\*1 allele (30.6 versus 12.8 months; P=0.0004). The association between CYP1B1\*3 and response to therapy was not observed in similar subjects receiving non-taxane-based therapy (P=0.18). The systemic clearance of docetaxel was also unrelated to CYP1B1 genotype status (P=0.39), indicating that the association of CYP1B1\*3 with clinical response is not due to docetaxel metabolism. To explain these results, we hypothesized that an indirect gene-drug interaction was interfering with the primary mechanism of action of docetaxel, tubulin polymerization. We therefore conducted tubulin polymerization experiments with taxanes in the presence or absence of certain CYP1B1 estrogen metabolites, which are known to bind to nucleophilic sites in proteins and DNA, that revealed the primary estrogen metabolite of CYP1B1, 4-hydroxyestradiol (4-OHE2), when oxidized to estradiol-3,4-quinone strongly inhibits tubulin polymerization. The 4-OHE2 is also formed more readily by the protein encoded by the CYP1B1\*3 allele, validating further our data in patients. Furthermore, estradiol-3,4-quinone reacted in vitro with docetaxel to form the 4-OHE2-docetaxel adduct. This pilot study provides evidence that CYP1B1\*3 may be an important marker for estimating docetaxel efficacy in patients with prostate cancer. This link is likely associated with CYP1B1\*3 genotype-dependent estrogen metabolism.

Smith, D. J., M. Jaggi, et al. (2006). "Metallothioneins and resistance to cisplatin and radiation in prostate cancer." *Urology* **67**(6): 1341-7.

**OBJECTIVES:** The metallothioneins (MTs) are a family of small molecular weight trace metal and free radical scavenging proteins well established to play a role in the resistance to chemotherapy and radiotherapy in human cancers. MT gene expression is upregulated in response to the presence of metal ions such as zinc. Because prostatic tissue has the greatest concentration of zinc in the human body, in this study we analyzed the effect of MT induction by zinc in prostate cancer (PCa). **METHODS:** The activation of MT gene expression in response to zinc treatment in LNCaP and C4-2 PCa cells was shown by Western blotting and DNA microarray analysis. Chemotherapy and radiation sensitivity assays of cells after treatment with cisplatin or radiation were performed in the presence, or absence, of 150 microM ZnSO<sub>4</sub>, and cell viability was measured after 72 hours by MTS viability and clonogenic and flow cytometry assays. The experiments were repeated three times and the data analyzed. **RESULTS:** Increasing concentrations of ZnSO<sub>4</sub> upregulated MT expression in a dose-dependent manner. Microarray analysis demonstrated a specific increase in MT expression. Cells treated with zinc demonstrated a significantly decreased sensitivity to cisplatin and radiotherapy compared with controls ( $P < 0.05$ ). **CONCLUSIONS:** Our data have confirmed that treatment of PCa with zinc causes an increase in MT expression, which is significantly associated with resistance to cisplatin chemotherapy and radiotherapy in PCa. Therapeutic targeting of MT may therefore provide a means to overcome resistance to radiotherapy and cisplatin chemotherapy in PCa.

Sobti, R. C., L. Gupta, et al. (2008). "Role of hormonal genes and risk of prostate cancer: gene-gene interactions in a North Indian population." *Cancer Genet Cytogenet* **185**(2): 78-85.

Prostate cancer represents a heterogeneous disease with varying degrees of aggressiveness, patterns of metastasis, and response to therapy. It arises from a complex etiology that involves both exogenous (diet, environment, etc.) and endogenous (hormonal and genetic) factors. The present study was performed to explore the role of various genotypes involved in steroid metabolism and synthesis in the causation of prostate cancer. Genetic polymorphism of the ER, CYP17, SRD5A2 (TA repeats), and PSA genes were analyzed in 157 cases of prostate cancer and 340 controls [170 healthy males and 170 patients of benign prostate hyperplasia (BPH)]. Mutant genotypes of ER and CYP17 showed 2- and 3- and 3.5-fold increased risk of prostate cancer, respectively,

as compared to BPH and healthy controls. Interaction of mutant (homozygous and heterozygous) alleles of CYP17 with TA (0/0) led to a twofold increased risk of prostate cancer. Risk was more than twofold with the combination of mutant alleles of ER and CYP17. The PSA gene polymorphism did not show any increased risk of prostate cancer. This indicates the role of mutant allele of ER and CYP17 in the development and progression of prostate cancer and rules out any increased risk with PSA polymorphism in the north Indian population.

Song, H. J., A. A. Sneddon, et al. (2006). "Induction of apoptosis and inhibition of NF-kappaB activation in human prostate cancer cells by the cis-9, trans-11 but not the trans-10, cis-12 isomer of conjugated linoleic acid." *Prostate* **66**(8): 839-46.

**BACKGROUND:** Conjugated linoleic acids (CLAs) have anti-tumorigenic properties in animal models and anti-proliferative effects on cancer cells in vitro. Previous studies have shown that the NF-kappaB pathway is involved regulating anti-apoptotic gene expression. The present study investigated the effects of CLAs (cis-9, trans-11, and trans-10, cis-12 isomers and a 50:50 mixture) on apoptosis and NF-kappaB activation in LNCaP cells. **METHODS:** Apoptosis was assessed by annexin V staining using flow cytometry. TNF-alpha-induced NF-kappaB activity was determined by gel shift and reporter gene assays in addition to monitoring IkappaBalpha phosphorylation. **RESULTS:** Only the CLA cis-9, trans-11 isomer significantly increased TNF-alpha-induced apoptosis (by 59%), which correlated with a reduction in NF-kappaB transcriptional activity (by 35%,  $P < 0.05$ ), NF-kappaB binding activity (by 15%,  $P < 0.05$ ), and phosphorylation of IkappaBalpha (by 36%,  $P < 0.01$ ). **CONCLUSIONS:** Our results may offer a mechanistic explanation for the reported inhibition of prostate tumor growth by CLAs in animal models of disease.

Sorensen, K. D., P. J. Wild, et al. (2009). "Genetic and epigenetic SLC18A2 silencing in prostate cancer is an independent adverse predictor of biochemical recurrence after radical prostatectomy." *Clin Cancer Res* **15**(4): 1400-10.

**PURPOSE:** This study investigates SLC18A2 (vesicular monoamine transporter 2) expression in prostate adenocarcinoma and examines its potential as a predictive marker for prostate cancer patient outcome after radical prostatectomy. **EXPERIMENTAL DESIGN:** Expression and single nucleotide polymorphism microarray analyses identified SLC18A2 as both down-regulated and subject to common loss-of-heterozygosity in prostate cancer. Down-regulated SLC18A2 expression was

validated on tissue microarrays containing benign and malignant prostate specimens from an independent patient group (n=738). Furthermore, SLC18A2 immunoreactivity in radical prostatectomy tumor specimens (n=506) was correlated to clinicopathologic characteristics and recurrence-free survival. The possibility of SLC18A2 silencing by aberrant DNA methylation in prostate cancer cells was investigated by bisulfite sequencing. RESULTS: Tissue microarray analysis revealed markedly lower cytoplasmic SLC18A2 staining in cancer compared with nonmalignant prostate tissue samples, confirming RNA expression profiling results. Furthermore, multivariate analysis identified cytoplasmic SLC18A2 immunoreactivity as a novel predictor of biochemical recurrence following prostatectomy (hazard ratio, 0.485; 95% confidence interval, 0.333-0.709; P<0.001) independent of prostate-specific antigen, Gleason score, tumor stage, and surgical margin status. SLC18A2 showed loss-of-heterozygosity in 23% of the tumors and was densely hypermethylated in 15 of 17 (88%) prostate cancer samples plus 6 of 6 prostate cancer cell lines. In contrast, SLC18A2 was unmethylated in 4 of 4 adjacent nonmalignant prostate and 3 of 5 benign prostatic hyperplasia tissue samples, whereas 2 of 5 benign prostatic hyperplasia samples had monoallelic hypermethylation. Methylation and histone deacetylase inhibitory agents rescued SLC18A2 expression in three prostate cancer cell lines. CONCLUSIONS: SLC18A2 silencing by DNA hypermethylation and/or allelic loss is a frequent event in prostate cancer and a novel independent predictor of biochemical recurrence after prostatectomy.

Stewart, J., J. Ware, et al. (2008). "Effects of selenomethionine on the gene expression profile of cloned human prostate cancer cells representing a phenotypic continuum of cancer progression." *Nutr Cancer* **60**(6): 826-36.

We previously characterized three cell clones that were derived by limiting dilution from a human prostate cancer cell line (LNCaP) representing a phenotypic continuum of cancer progression (1). The present study was undertaken to examine the effects of L-selenomethionine (SeM), a potential cancer chemopreventive agent, on the gene expression profile of the cultured cell clones. Following a three-day incubation period with SeM, total RNA was extracted, and the gene expression profile was evaluated using Affymetrix human HG U133A microarrays and analyzed by ViaLogy's (Altadena, CA) VMAS platform deploying quantum resonance interferometry (QRI) processing. The differentially expressed genes and corresponding biological processes were compared across the different treatments and cell

types. Whereas SeM significantly affected RNA-DNA metabolism and protein transport and metabolism in all of the cell types evaluated, significant effects of SeM on genes mainly involved in the pathways of cell cycle, growth, differentiation, and apoptosis were observed only in the cell clone with a more malignant phenotype.

Suga, T., M. Iwakawa, et al. (2008). "Influence of multiple genetic polymorphisms on genitourinary morbidity after carbon ion radiotherapy for prostate cancer." *Int J Radiat Oncol Biol Phys* **72**(3): 808-13.

PURPOSE: To investigate the genetic risk of late urinary morbidity after carbon ion radiotherapy in prostate cancer patients. METHODS AND MATERIALS: A total of 197 prostate cancer patients who had undergone carbon ion radiotherapy were evaluated for urinary morbidity. The distribution of patients with dysuria was as follows: Grade 0, 165; Grade 1, 28; and Grade 2, 4 patients. The patients were divided (2:1) consecutively into the training and test sets and then categorized into control (Grade 0) and case (Grade 1 or greater) groups. First, 450 single nucleotide polymorphisms (SNPs) in 118 candidate genes were genotyped in the training set. The associations between the SNP genotypes and urinary morbidity were assessed using Fisher's exact test. Then, various combinations of the markers were tested for their ability to maximize the area under the receiver operating characteristics (AUC-ROC) curve analysis results. Finally, the test set was validated for the selected markers. RESULTS: When the SNP markers in the SART1, ID3, EPDR1, PAH, and XRCC6 genes in the training set were subjected to AUC-ROC curve analysis, the AUC-ROC curve reached a maximum of 0.86. The AUC-ROC curve of these markers in the test set was 0.77. The SNPs in these five genes were defined as "risk genotypes." Approximately 90% of patients in the case group (Grade 1 or greater) had three or more risk genotypes. CONCLUSIONS: Our results have shown that patients with late urinary morbidity after carbon ion radiotherapy can be stratified according to the total number of risk genotypes they harbor.

Sun, Y., D. K. St Clair, et al. (2007). "The radiosensitization effect of parthenolide in prostate cancer cells is mediated by nuclear factor-kappaB inhibition and enhanced by the presence of PTEN." *Mol Cancer Ther* **6**(9): 2477-86.

Parthenolide has been shown to have anti-inflammatory and antitumor properties. However, whether and how parthenolide enhances tumor sensitivity to radiation therapy are unknown. In this study, we show that inhibition of the nuclear factor-kappaB (NF-kappaB) pathway is a common

mechanism for the radiosensitization effect of parthenolide in prostate cancer cells LNCaP, DU 145, and PC3. Parthenolide inhibits radiation-induced NF-kappaB DNA-binding activity and the expression of its downstream target *sod2*, the gene coding for an important antiapoptotic and antioxidant enzyme (manganese superoxide dismutase) in the three prostate cancer cells. Different susceptibilities to parthenolide's effect are observed in two radioresistant cancer cells, DU 145 and PC3, with DU 145 cells showing higher sensitivity. This differential susceptibility to parthenolide is due, in part, to the fact that in addition to NF-kappaB inhibition, parthenolide activates the phosphatidylinositol-3-kinase/Akt prosurvival pathway in both cell lines. However, the activated Akt in DU 145 cells is kept at a relatively low level compared with that in PC3 cells due to the presence of functional PTEN. Transfection of wild-type PTEN into PTEN-null cells, PC3, confers the enhanced radiosensitization effect of parthenolide in PTEN-expressing cells. When PTEN expression is knocked down in DU 145 cells, the cells become more resistant to parthenolide's effect. Taken together, these results suggest that parthenolide inhibits the NF-kappaB pathway and activates the phosphatidylinositol-3-kinase/Akt pathway in prostate cancer cells. The radiosensitization effect of parthenolide is due, in part, to the inhibition of the NF-kappaB pathway. The presence of PTEN enhances the radiosensitization effect of parthenolide, in part, by suppressing the absolute amount of activated p-Akt.

Suzuki, H., T. Ueda, et al. (2003). "Androgen receptor involvement in the progression of prostate cancer." *Endocr Relat Cancer* **10**(2): 209-16.

Since the growth of prostate cancer is androgen-sensitive, metastatic disease has been treated by hormonal therapy. Almost all prostate cancer patients initially respond to hormonal therapy, but the majority gradually develop resistance. The mechanism of the change in tumors from being androgen-responsive to androgen-unresponsive is generally explained by clonal selection, adaptation, an alternative pathway of signal transduction and androgen receptor (AR) involvement. Since androgen action is mediated by ARs, abnormalities in ARs are believed to play an important role in the progression of prostate cancer. Hyperactivated AR gene mutations have been detected in 20-30% of hormone-refractory tumors and functional analyses have demonstrated a wide responsiveness to estrogens, progesterone and anti-androgens as well as to androgens. The AR is highly amplified in 30% of patients with hormone-refractory prostate cancer that has been treated by castration without anti-androgens.

Immunohistochemical studies of ARs in hormone-refractory prostate cancer specimens have shown that AR protein is down-regulated. DNA hypermethylation of the AR promoter region leading to AR down-regulation has been identified in 30% of hormone-refractory prostate cancers. The AR N-terminal domain in the LNCaP cell line model is activated by interleukin-6 via mitogen-activated protein kinase and single transducers and activators of transcription 3. Epidemiological observations have shown that short CAG repeats are more frequently associated with higher transactivational function in the African-American population, which may explain racial differences in the incidence of prostate cancer. Among Japanese, a short CAG repeat appears to predict a response to hormonal therapy, indicating a positive prognostic value and good prognosis at the metastatic stage of prostate cancer. Several co-factors between ARs and the transcriptional complex have been cloned and reports indicate that steroid receptor co-activator 1 is correlated with the hormone-refractory progression of prostate cancer. Thus, ARs play an important role in the progression of prostate cancer. Based on the findings described above, genetic diagnosis and/or molecular-targeted therapy via AR pathways can be developed for hormone-refractory states.

Suzuki, K., H. Koike, et al. (2002). "Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3." *Int J Cancer* **99**(6): 846-52.

Genistein is a major component of soybean isoflavone and has multiple functions resulting in antitumor effects. Prostate cancer is 1 of the targets for the preventive role of genistein. We examined the effect of genistein on human prostate cancer (LNCaP and PC-3) cells. Proliferation of both cell lines was inhibited by genistein treatment in a dose-dependent manner. To obtain the gene expression profile of genistein in LNCaP cells, we performed cDNA microarray analysis. The expression of many genes, including apoptosis inhibitor (survivin), DNA topoisomerase II, cell division cycle 6 (CDC6) and mitogen-activated protein kinase 6 (MAPK 6), was downregulated. Expression levels were increased more than 2-fold in only 4 genes. The glutathione peroxidase (GPx)-1 gene expression level was the most upregulated. Quantitative real-time polymerase chain reaction revealed significant elevation of transcript levels of GPx-1 in both LNCaP and PC-3 cells. Upregulation of gene expression levels accompanied elevation of GPx enzyme activities. In contrast, no significant changes were observed in the gene expression levels and enzyme activities of the other antioxidant enzymes, superoxide dismutase and catalase. GPx activation might be one of the important

characteristics of the effects of genistein on prostate cancer cells.

Swamy, N., T. C. Chen, et al. (2004). "Inhibition of proliferation and induction of apoptosis by 25-hydroxyvitamin D<sub>3</sub>-3β-(2)-Bromoacetate, a nontoxic and vitamin D receptor-alkylating analog of 25-hydroxyvitamin D<sub>3</sub> in prostate cancer cells." *Clin Cancer Res* **10**(23): 8018-27.

The 25-hydroxyvitamin D<sub>3</sub> (25-OH-D<sub>3</sub>) is a nontoxic and low-affinity vitamin D receptor (VDR)-binding metabolic precursor of 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>]. We hypothesized that covalent attachment of a 25-OH-D<sub>3</sub> analog to the hormone-binding pocket of VDR might convert the latter into transcriptionally active holo-form, making 25-OH-D<sub>3</sub> biologically active. Furthermore, it might be possible to translate the nontoxic nature of 25-OH-D<sub>3</sub> into its analog. We showed earlier that 25-hydroxyvitamin D<sub>3</sub>-3-bromoacetate (25-OH-D<sub>3</sub>-3-BE) alkylated the hormone-binding pocket of VDR. In this communication we describe that 10<sup>-6</sup> mol/L of 25-OH-D<sub>3</sub>-3-BE inhibited the growth of keratinocytes, LNCaP, and LAPC-4 androgen-sensitive and PC-3 and DU145 androgen-refractory prostate cancer cells, and PZ-HPV-7 immortalized normal prostate cells with similar or stronger efficacy as 1,25(OH)<sub>2</sub>D<sub>3</sub>. But its effect was strongest in LNCaP, PC-3, LAPC-4, and DU145 cells. Furthermore, 25-OH-D<sub>3</sub>-3-BE was toxic to these prostate cancer cells and caused these cells to undergo apoptosis as shown by DNA-fragmentation and caspase-activation assays. In a reporter assay with COS-7 cells, transfected with a 1α,25-dihydroxyvitamin D<sub>3</sub>-24-hydroxylase (24-OHase)-construct and VDR-expression vector, 25-OH-D<sub>3</sub>-3-BE induced 24-OHase promoter activity. In a "pull down assay" with PC-3 cells, 25-OH-D<sub>3</sub>-3-BE induced strong interaction between VDR and general transcription factors, retinoid X receptor, and GRIP-1. Collectively, these results strongly suggested that the cellular effects of 25-OH-D<sub>3</sub>-3-BE were manifested via 1,25(OH)<sub>2</sub>D<sub>3</sub>/VDR signaling pathway. A toxicity study in CD-1 mice showed that 166 microg/kg of 25-OH-D<sub>3</sub>-3-BE did not raise serum-calcium beyond vehicle control. Collectively, these results strongly suggested that 25-OH-D<sub>3</sub>-3-BE has a strong potential as a therapeutic agent for androgen-sensitive and androgen-refractory prostate cancer.

Sweeney, C., L. Li, et al. (2004). "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* **10**(16): 5501-7.

**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.

Swinnen, J. V., H. Heemers, et al. (2004). "Androgens, lipogenesis and prostate cancer." *J Steroid Biochem Mol Biol* **92**(4): 273-9.

Both experimental and epidemiological data indicate that androgens are among the main factors controlling the development, maintenance and progression of prostate cancer. Identifying the genes that are regulated by androgens represents a major step towards the elucidation of the mechanisms underlying the impact of androgens on prostate cancer cell biology and is an attractive approach to find novel targets for prostate cancer therapy. Among the genes that have been identified thus far, several genes encode lipogenic enzymes. Studies aimed at the elucidation of the mechanisms underlying androgen regulation of lipogenic genes revealed that androgens

coordinately stimulate the expression of these genes through interference with the molecular mechanism controlling activation of sterol regulatory element-binding proteins (SREBPs), lipogenic transcription factors governing cellular lipid homeostasis. The resulting increase in lipogenesis serves the synthesis of key membrane components (phospholipids, cholesterol) and is a major hallmark of cancer cells. Pharmacologic inhibition of lipogenesis or RNA-interference-mediated down-regulation of key lipogenic genes induces apoptosis in cancer cell lines and reduces tumor growth in xenograft models. While increased lipogenesis is already found in the earliest stages of cancer development (PIN) and initially is androgen-responsive it persists or re-emerges with the development of androgen-independent cancer, indicating that lipogenesis is a fundamental aspect of prostate cancer cell biology and is a potential target for chemoprevention and for antineoplastic therapy in advanced prostate cancer.

Takahashi, Y., J. A. Lavigne, et al. (2004). "Using DNA microarray analyses to elucidate the effects of genistein in androgen-responsive prostate cancer cells: identification of novel targets." *Mol Carcinog* **41**(2): 108-119.

Many studies have correlated the consumption of soy-rich diets with a decreased risk of developing hormone-dependent cancers, including prostate cancer. Genistein is a candidate prostate cancer preventive phytochemical found at high levels in soybean and soy foods. To better understand the molecular mechanisms underlying the beneficial effects of genistein on prostate cancer prevention, we used a DNA microarray approach to examine the effects of genistein at concentrations in the physiologic range on global gene expression patterns in androgen-responsive cancer cells. Microarray analyses were performed on androgen-responsive LNCaP human prostate cancer cells exposed to 0, 1, 5, or 25 microM genistein. We found a concentration-dependent modulation of multiple cellular pathways that are important in prostate carcinogenesis. Interestingly, the androgen receptor (AR)-mediated pathways, in particular, appeared to be modulated by genistein at the lowest concentrations. Based on these results, we propose that the regulation of AR-mediated pathways is potentially the most relevant chemopreventive mechanism for genistein administered at physiologic levels.

Takeda, M., A. Mizokami, et al. (2007). "The establishment of two paclitaxel-resistant prostate cancer cell lines and the mechanisms of paclitaxel resistance with two cell lines." *Prostate* **67**(9): 955-67.

**BACKGROUND:** Although paclitaxel is used for hormone-resistant prostate cancer, relapse definitely occurs later. Details of the molecular mechanism responsible for paclitaxel-resistance remain unclear. **METHODS:** We established paclitaxel-resistant cells, DU145-TxR and PC-3-TxR from parent DU145 and PC-3. To characterize these cells, we examined cross-resistance to other anticancer drugs. Expression of several potential genes that had been related to drug-resistance was compared with parent cells by RT-PCR and Western blotting. Methylation analysis of multiple drug resistance (MDR1) promoter was carried out using bisulfite-modified DNA from cell lines. Knockdown experiments using small interfering RNA (siRNA) were also performed to confirm responsibility of drug-resistance. Finally, cDNA microarray was performed to quantify gene expression in PC-3 and PC-3-TxR cells. **RESULTS:** The IC(50) for paclitaxel in DU145-TxR and PC-3-TxR was 34.0- and 43.4-fold higher than that in both parent cells, respectively. Both cells showed cross-resistance to some drugs, but not to VP-16 and cisplatin. Methylation analysis revealed that methylated CpG sites of MDR1 promoter in DU145 and PC-3 cells were demethylated in DU145-TxR cells, but not in PC-3-TxR cells. Knockdown of P-glycoprotein (P-gp), which was up-regulated in resistant cells, by MDR-1 siRNA restored paclitaxel sensitivity in DU145-TxR but not in PC-3-TxR, indicating that up-regulation of P-gp was not always main cause of paclitaxel-resistance. Microarray analysis identified 201 (1.34%) up-regulated genes and 218 (1.45%) out of screened genes in PC-3-TxR. **CONCLUSIONS:** Our data will provide molecular mechanisms of paclitaxel-resistance and be useful for screening target genes to diagnose paclitaxel sensitivity.

Taplin, M. E., B. Rajeshkumar, et al. (2003). "Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663." *J Clin Oncol* **21**(14): 2673-8.

**PURPOSE:** The mechanisms responsible for prostate cancer androgen independence are diverse. Mutations of the androgen receptor (AR) gene that broaden ligand specificity have been implicated. Bone marrow specimens containing prostate tumor were obtained from men undergoing antiandrogen withdrawal for AR sequence analysis and clinical correlation. **MATERIALS and METHODS:** Eligible men enrolled on a trial of antiandrogen withdrawal had a minimum prostate-specific antigen (PSA) level of 5 ng/dL that was increasing on castration therapy including an antiandrogen. With informed consent, marrow biopsies were obtained to collect prostate tumor. Additional samples were obtained from men

enrolled on chemotherapy trials. AR cDNA or DNA was polymerase chain reaction-amplified, cloned, and sequenced. The AR CAG repeat length was recorded. RESULTS: One hundred eighty-four bone marrow biopsies were obtained, and 48 had prostate tumor detected by light microscopy. The ARs from these 48 samples were sequenced. Overall, five (10%) of 48 tumors had mutated ARs. AR point mutations were detected in the hormone-binding domain involved in transcription factor binding. Three mutations were novel in prostate cancer. One tumor sample had a CAG repeat length of 21, compared with germline length of 22 repeats. There was no association between detectability of AR mutations and antiandrogen withdrawal response or survival. CONCLUSION: These data suggest that AR mutations are present in approximately 10% of patients with prostate cancer who experience treatment failure with hormone therapy that included an antiandrogen. Mutations in the AR likely confer a growth advantage for a subset of progressive prostate cancers. Correlation of AR mutation with antiandrogen withdrawal response or survival could not be made.

Tekur, S. and S. M. Ho (2002). "Ribozyme-mediated downregulation of human metallothionein II(a) induces apoptosis in human prostate and ovarian cancer cell lines." *Mol Carcinog* **33**(1): 44-55.

Human metallothioneins (MTs) are low-molecular-weight, cysteine-rich, metal ion-binding proteins that constitute the majority of intracellular protein thiols. They are overexpressed in prostate and ovarian cancers and are believed to confer resistance to radiation and cytotoxic anticancer drugs. The aim of this study was to investigate the roles of MTs in prostate and ovarian cancer cells and their possible relationship with other cancer development and progression factors. The main problem in investigating the role of MT, however, is the absence of any known specific inhibitor. To this end, in a previous study, we had developed sequence-specific ribozymes (Rzs) targeting MT and had shown their in cellulo efficacy. Here we show that transient transfection of a vector carrying a hammerhead Rz (Rz4-9), designed to cleave class II MT, in the human prostate cancer cell line PC-3 and the ovarian cancer cell line SKOV-3 resulted in a dose-dependent attenuation of MT-II(a) transcripts and dramatic cell loss. Transient transfection with 2 microg of Rz4-9 vector DNA completely abolished MT-II(a) mRNA levels and induced a 94% and a 67% reduction in cell number in PC-3 cells and SKOV-3 cells, respectively. Fluorescence-activated cell sorting (FACS) showed that the Rz-induced cell loss probably was due to apoptosis, because it was associated with marked

increases in the hypodiploid cell population, reaching maximums of 52% and 64% in cultures of PC-3 and SKOV-3, respectively. Additionally, annexin V-propidium iodide double-staining, followed by FACS, confirmed that Rz4-9-induced cell death was due to apoptosis and showed a vector DNA-dependent increase in late apoptotic cell numbers that reached maximums of 80% and 42%, respectively, in PC-3 and SKOV-3 cell cultures transfected with the highest concentration of vector DNA. In parallel experiments, transfection with a vector containing the enzymatically inactive mutant Rz-3-3 or the empty vector was not effective in inducing similar responses. The Rz-induced loss of MT-II(a) mRNA-associated cell death in these cancer cell lines was attended by dose-dependent downregulation of the proto-oncogene c-myc and the apoptosis inhibitory mediator bcl-2, suggesting that these signaling pathways are involved in the process. In conclusion, our data indicate that MT-II(a) is an important cell-survival or anti-apoptotic factor for prostate and ovarian cancer cells and that downregulation of its expression via transgene expression of a sequence-specific Rz is a feasible target for cancer therapy.

Thelen, P., J. G. Scharf, et al. (2005). "Tectorigenin and other phytochemicals extracted from leopard lily *Belamcanda chinensis* affect new and established targets for therapies in prostate cancer." *Carcinogenesis* **26**(8): 1360-7.

Isoflavones have been shown to exert antiproliferative effects on cancer cells by steroid receptor signaling. In this study, we demonstrate the potential of plant constituents extracted from *Belamcanda chinensis* as anticancer drugs, which regulate the aberrant expression of genes relevant in proliferation, invasion, immortalization and apoptosis. LNCaP cells were treated with *B.chinensis* extract, tectorigenin or other isoflavones and mRNA expression was quantified by using real time RT-PCR. In addition, ELISA, TRAP assays and western blots were used to measure protein expression or activity. Male nude mice (n=18) were injected subcutaneously with LNCaP cells and were fed with extracts from *B.chinensis*, and tumor development was monitored versus a control animal group (n=18). Tectorigenin and several other phytochemicals downregulated PDEF, PSA and IGF-1 receptor mRNA expression in vitro. Furthermore, PSA secretion and IGF-1 receptor protein expression were diminished, and hTERT mRNA expression and telomerase activity decreased after tectorigenin treatments. However, TIMP-3 mRNA was upregulated on tectorigenin treatment. Growth of subcutaneous tumors in nude mice was delayed and diminished in animals fed with extracts from *B.chinensis*. The downregulation of PDEF, PSA,

hTERT and IGF-1 receptor gene expression by tectorigenin demonstrates the antiproliferative potential of these agents. The upregulation of TIMP-3 gene expression indicates a pro-apoptotic function of the drug and a reduction of the invasiveness of tumors. The animal experiments demonstrate that *B.chinensis* markedly inhibited the development of tumors in vivo. Thus, these compounds may be useful for the prevention or treatment of human prostate cancer.

Tischkowitz, M., N. Sabbaghian, et al. (2008). "Analysis of the gene coding for the BRCA2-interacting protein PALB2 in hereditary prostate cancer." *Prostate* **68**(6): 675-8.

**BACKGROUND:** The genetic basis of susceptibility to prostate cancer (PRCA) remains elusive. Mutations in BRCA2 have been associated with increased prostate cancer risk and account for around 2% of young onset (<56 years) prostate cancer cases. PALB2 is a recently identified breast cancer susceptibility gene whose protein is closely associated with BRCA2 and is essential for BRCA2 anchorage to nuclear structures. This functional relationship made PALB2 a candidate PRCA susceptibility gene. **METHODS:** We sequenced PALB2 in probands from 95 PRCA families, 77 of which had two or more cases of early onset PRCA (age at diagnosis <55 years), and the remaining 18 had one case of early onset PRCA and five or more total cases of PRCA. **RESULTS:** Two previously unreported variants, K18R and V925L were identified, neither of which is in a known PALB2 functional domain and both of which are unlikely to be pathogenic. No truncating mutations were identified. **CONCLUSIONS:** These results indicate that deleterious PALB2 mutations are unlikely to play a significant role in hereditary prostate cancer.

Todorova, K., I. Ignatova, et al. (2005). "Humoral immune response in prostate cancer patients after immunization with gene-based vaccines that encode for a protein that is proteasomally degraded." *Cancer Immun* **5**: 1.

Prostate-specific membrane antigen (PSMA), whose expression is upregulated in poorly differentiated, metastatic, and hormone refractory prostate cancer, could be targeted by gene-based vaccines. The aim of this study was to characterize the humoral immune response against PSMA in prostate carcinoma patients who have been vaccinated against PSMA with gene-based vaccines. Sera from prostate cancer patients who had been immunized repeatedly with plasmid DNA and a recombinant adenoviral vector, both carrying an expression cassette for human PSMA, and sera from healthy donors were tested for anti-PSMA antibodies by Western blot analysis and

immunofluorescence. PSMA-producing LNCaP cells, recombinant PSMA protein, and a specific antibody against PSMA were used as positive controls. Specific anti-PSMA antibodies were detected by both Western blot and immunofluorescence in the sera of patients who had been vaccinated against PSMA with plasmid and recombinant adenoviral vectors. The specificity of the anti-PSMA antibodies was confirmed by preincubation and blocking experiments. Positive reactions were detected in 86% of the vaccinated prostate cancer patients. Anti-PSMA antibodies were not detected either in the patients' sera prior to vaccination or in the sera from healthy men and women. These data demonstrate that PSMA, a specific marker for prostate cancer, is a target for humoral immune response induced by gene-based PSMA vaccination. Detection of anti-PSMA antibodies by immunoblot analysis and by indirect immunofluorescence could be used to monitor the vaccination effect.

Tomioka, A., M. Tanaka, et al. (2008). "Delivery of PTEN via a novel gene microcapsule sensitizes prostate cancer cells to irradiation." *Mol Cancer Ther* **7**(7): 1864-70.

The tumor suppressor gene MMAC/PTEN located on chromosome 10q23.3 has dual phosphatase activity in the phosphoinositide-3-kinase signaling pathway and inhibits Akt activation, a serine-threonine kinase, which is involved in proliferative and antiapoptotic pathways. Furthermore, MMAC/PTEN is frequently inactivated in a variety of tumors including prostate cancer. In this study, we generated a new type of gene transfer drug, GelaTen, which is a microsphere of cationized gelatin hydrogels incorporating PTEN plasmid DNA. Using our previously reported radiation-resistant PC3-Bcl-2 human prostate cancer cells (PTEN deleted), we examined the efficacy of GelaTen to force the expression of PTEN in vivo to inhibit tumor growth after intratumoral injection alone or with irradiation. Combinational therapy with GelaTen and irradiation improved both the in vitro and in vivo efficacy of growth inhibition compared with GelaTen or irradiation alone. These data show that GelaTen gene therapy, enabling radiosensitization, can potentially treat prostate cancers that have MMAC/PTEN gene alterations associated with radioresistance.

Tran, C., S. Ouk, et al. (2009). "Development of a second-generation antiandrogen for treatment of advanced prostate cancer." *Science* **324**(5928): 787-90.

Metastatic prostate cancer is treated with drugs that antagonize androgen action, but most patients progress to a more aggressive form of the

disease called castration-resistant prostate cancer, driven by elevated expression of the androgen receptor. Here we characterize the diarylthiohydantoin RD162 and MDV3100, two compounds optimized from a screen for nonsteroidal antiandrogens that retain activity in the setting of increased androgen receptor expression. Both compounds bind to the androgen receptor with greater relative affinity than the clinically used antiandrogen bicalutamide, reduce the efficiency of its nuclear translocation, and impair both DNA binding to androgen response elements and recruitment of coactivators. RD162 and MDV3100 are orally available and induce tumor regression in mouse models of castration-resistant human prostate cancer. Of the first 30 patients treated with MDV3100 in a Phase I/II clinical trial, 13 of 30 (43%) showed sustained declines (by >50%) in serum concentrations of prostate-specific antigen, a biomarker of prostate cancer. These compounds thus appear to be promising candidates for treatment of advanced prostate cancer.

Tricoli, J. V., M. Schoenfeldt, et al. (2004). "Detection of prostate cancer and predicting progression: current and future diagnostic markers." *Clin Cancer Res* **10**(12 Pt 1): 3943-53.

Carcinoma of the prostate is the second leading cause of male cancer-related death in the United States. Better indicators of prostate cancer presence and progression are needed to avoid unnecessary treatment, predict disease course, and develop more effective therapy. Numerous molecular markers have been described in human serum, urine, seminal fluid, and histological specimens that exhibit varying capacities to detect prostate cancer and predict disease course. However, to date, few of these markers have been adequately validated for clinical use. The purpose of this review is to examine the current status of these markers in prostate cancer and to assess the diagnostic potential for future markers from identified genes and molecules that display loss, mutation, or alteration in expression between tumor and normal prostate tissues. In this review we cite 91 molecular markers that display some level of correlation with prostate cancer presence, disease progression, cancer recurrence, prediction of response to therapy, and/or disease-free survival. We suggest criteria to consider when selecting a marker for further development as a clinical tool and discuss five examples of markers (chromogranin A, glutathione S-transferase pi 1, prostate stem cell antigen, prostate-specific membrane antigen, and telomerase reverse transcriptase) that fulfill some of these criteria. Finally, we discuss how to conduct evaluations of candidate prostate cancer markers and some of the issues involved in the validation process.

Trofimova, I., A. Dimtchev, et al. (2002). "Gene therapy for prostate cancer by targeting poly(ADP-ribose) polymerase." *Cancer Res* **62**(23): 6879-83.

Poly(ADP-ribose) polymerase (PARP) has strong affinity for DNA strand breaks and cycles on and off the DNA ends to allow DNA repair. A DNA-binding domain of PARP (PARP-DBD) acts as a dominant-negative mutant by binding to DNA strand breaks irreversibly and sensitizing mammalian cells to DNA-damaging agents. Therefore, expression of PARP-DBD in prostate carcinoma cells offers a strategy to achieve sensitization to genotoxic treatments. Toward this end, we developed recombinant plasmids expressing the PARP-DBD under the control of the 5'-flanking sequences of the human prostate-specific antigen (PSA) gene. Tissue specificity of PARP-DBD expression in human tumor cells was confirmed using the PSA-producing (LNCaP) and PSA-negative (PC-3) prostate cancer cells, as well as cells of nonprostate origin, Ewing's sarcoma (A4573 cells). LNCaP cells stably transfected with the PSA-regulated cDNA for PARP-DBD exhibit an androgen-dependent induction of PARP-DBD expression as determined by Western blotting, reverse transcription-PCR, and in situ immunofluorescence. Furthermore, we found that PARP-DBD sensitized LNCaP cells to DNA-damaging agents, such as ionizing radiation and etoposide. Androgen (R1881) -dependent stimulation of PARP-DBD expression resulted in a 2-fold growth inhibition in LNCaP cells as compared with controls, and an augmented apoptotic cell death in response to ionizing radiation or etoposide. Taken together, the plasmid vector developed in this study permits the expression of the human PARP-DBD in an androgen-inducible and PSA-dependent fashion, and sensitizes prostatic adenocarcinoma cells to DNA-damaging treatments. These results provide proof-of-principle for a novel therapeutic strategy for the treatment of prostate cancer.

Troyer, D. A., J. Mubiru, et al. (2004). "Promise and challenge: Markers of prostate cancer detection, diagnosis and prognosis." *Dis Markers* **20**(2): 117-28.

Approximately 1 man in 6 will be diagnosed with prostate cancer during his life lifetime, and over 200,000 men in the U.S. are diagnosed with prostate cancer annually. Since the widespread adoption of PSA testing, about 60-70% of men at risk in the U.S. have had a blood test for prostate cancer. With this, prostate cancer death rates have decreased, yet only slightly. Thirty thousand men still die each year from this disease. PSA testing fails to identify a small but significant proportion of aggressive cancers, and only about 30% of men with a "positive" PSA have a

positive biopsy. Additionally, of men who are treated for prostate cancer, about 25% require additional treatment, presumably due to disease recurrence. Also of concern is the growing evidence that there are some prostate cancers for which treatment may not be necessary. Very long-term studies from the U.S. and Europe, following men with prostate cancer have found that some tumors do not progress over time. In these individuals, prostate cancer treatment is unnecessary and harmful as these men do not benefit from treatment but will be at risk of treatment-related side effects and complications. They suggest a fundamental problem with prostate cancer: it is not possible, at this time, to predict the natural history of the disease. It is for these reasons that the most important challenge in prostate cancer today is the inability to predict the behavior of an individual tumor in an individual patient. Here we review issues related to performance and validation of biomarkers with a focus on "doing no harm", and bearing in mind that it is the ultimate goal of early detection to save lives. Improved diagnostic and prognostic biomarkers are needed for prostate cancer, and the use of these markers should ultimately translate into increased life span and quality of life. The ultimate goal would be to not only have accurate biomarkers suitable for early diagnosis, but also biomarkers that identify men at greatest risk of developing aggressive disease. Technology has been brought to bear on this problem, and the major approaches are genomics, expression analysis, and proteomics. Proteomics and DNA methylation assays may soon be used in sensitive and specific diagnostic testing of serum and tissues for cancer. Expression arrays may be used to establish both a more specific diagnosis and prognosis for a particular tumor. The proteome is only beginning to be understood, and alternative splicing and post-translational modifications of proteins such as glycosylation and phosphorylation are challenging areas of study. Finally, risk assessment and prognosis are being pursued through analysis of genomic polymorphisms (single nucleotide polymorphisms, SNPs). This huge task is only beginning, and requires the combined expertise of molecular epidemiologists, oncologists, surgeons, pathologists, and basic scientists.

Truman, J. P., N. Gueven, et al. (2005). "Down-regulation of ATM protein sensitizes human prostate cancer cells to radiation-induced apoptosis." *J Biol Chem* **280**(24): 23262-72.

Treatment with the protein kinase C activator 12-O-tetradecanoylphorbol 12-acetate (TPA) enables radiation-resistant LNCaP human prostate cancer cells to undergo radiation-induced apoptosis, mediated via activation of the enzyme ceramide synthase (CS) and

de novo synthesis of the sphingolipid ceramide (Garzotto, M., Haimovitz-Friedman, A., Liao, W. C., White-Jones, M., Huryk, R., Heston, D. W. W., Cardon-Cardo, C., Kolesnick, R., and Fuks, Z. (1999) *Cancer Res.* 59, 5194-5201). Here, we show that TPA functions to decrease the cellular level of the ATM (ataxia telangiectasia mutated) protein, known to repress CS activation (Liao, W.-C., Haimovitz-Friedman, A., Persaud, R., McLoughlin, M., Ehleiter, D., Zhang, N., Gatei, M., Lavin, M., Kolesnick, R., and Fuks, Z. (1999) *J. Biol. Chem.* 274, 17908-17917). Gel shift analysis in LNCaP and CWR22-Rv1 cells demonstrated a significant reduction in DNA binding of the Sp1 transcription factor to the ATM promoter, and quantitative reverse transcription-PCR showed a 50% reduction of ATM mRNA between 8 and 16 h of TPA treatment, indicating that TPA inhibits ATM transcription. Furthermore, treatment of LNCaP, CWR22-Rv1, PC-3, and DU-145 human prostate cells with antisense-ATM oligonucleotides, which markedly reduced cellular ATM levels, significantly enhanced radiation-induced CS activation and apoptosis, leading to apoptosis at doses as low as 1 gray. These data suggest that the CS pathway initiates a generic mode of radiation-induced apoptosis in human prostate cancer cells, regulated by a suppressive function of ATM, and that ATM might represent a potential target for pharmacologic inactivation with potential clinical applications in human prostate cancer.

van Gils, C. H., R. M. Bostick, et al. (2002). "Differences in base excision repair capacity may modulate the effect of dietary antioxidant intake on prostate cancer risk: an example of polymorphisms in the XRCC1 gene." *Cancer Epidemiol Biomarkers Prev* **11**(11): 1279-84.

We propose a hypothesis that differences in base excision repair capacity modulate the effect of dietary antioxidant intake on prostate cancer risk. As a preliminary test of this hypothesis, we conducted a pilot case-control study to evaluate prostate cancer risk in men with polymorphisms in the XRCC1 gene, a key player in base excision repair, across different strata of antioxidant intake. Seventy-seven prostate cancer patients and 183 community controls, for whom we have detailed dietary information, were frequency matched on age and race. We found a somewhat lower prostate cancer risk for men with one or two copies of the variant alleles at the XRCC1 codons 194 and 399 than for those who were homozygous for the common allele [codon 194: odds ratio (OR) = 0.8; 95% confidence interval (CI), 0.4-1.8 and codon 399: OR = 0.8; 95% CI, 0.5-1.3]. The variant at codon 280 was associated with a slightly increased prostate cancer risk (OR = 1.5; 95% CI, 0.7-

3.6). Only the codon 399 polymorphism occurred frequently enough to investigate its joint effect with antioxidant intake. Prostate cancer risk was highest among men who were homozygous for the common allele at codon 399 and had low dietary intake of vitamin E (OR = 2.4; 95% CI, 1.0-5.6) or lycopene (OR = 2.0; 95% CI, 0.8-4.9), whereas low intake of these antioxidants in men without this genotype hardly increased prostate cancer risk. The polymorphism did not modulate risk associated with low intake of vitamin C, A, or beta-carotene. The data give some support for our hypothesis but should be regarded as preliminary, because it is limited by small sample size. We discuss what kind of data and what kind of studies are needed for future evaluation of this hypothesis.

van Leenders, G. J., D. Dukers, et al. (2007). "Polycomb-group oncogenes EZH2, BMI1, and RING1 are overexpressed in prostate cancer with adverse pathologic and clinical features." *Eur Urol* **52**(2): 455-63.

**OBJECTIVES:** Polycomb group (PcG) proteins are involved in maintenance of cell identity and proliferation. The protein EZH2 is overexpressed in disseminated prostate cancer, implicating a role of PcG complexes in tumor progression. In this study, we evaluated the expression of eight members of both PcG complexes in clinicopathologically defined prostate cancer. **METHODS:** Components of both PcG protein complexes PRC2 (EZH2, EED, YY1) and PRC1 (BMI1, RING1, HPH1, HPC1, HPC2) were immunohistochemically identified in tissue microarrays of 114 prostate cancer patients. Protein expression was semi-quantitatively scored and correlated with pathologic parameters and recurrence of prostate-specific antigen (PSA). **RESULTS:** Whereas BMI1, RING1, HPC1 and HPH1 were all abundantly present in normal and malignant prostate epithelium, expression of EZH2 occurred in only <10% of cells. Expression of EZH2, BMI1 and RING1 were all significantly enhanced in tumours with Gleason score (GS) > or = 8, extraprostatic extension, positive surgical margins, and PSA recurrence. When only the subgroup of GS < or = 6 was considered, representing the tumour grade in the majority of needle biopsies, EZH2 and BMI1 were also predictive for PSA recurrence. In a multivariable analysis, BMI1 was the only PcG protein with an independent prognostic value. **CONCLUSIONS:** PcG proteins EZH2, BMI1, and RING1 are associated with adverse pathologic features and clinical PSA recurrence of prostate cancer. Whereas BMI1 and RING1 are abundantly present in prostate cancer, EZH2 is expressed at relatively low levels, making it a less obvious target for therapy.

Vander Griend, D. J., L. Antony, et al. (2009). "Amino acid containing thapsigargin analogues deplete androgen receptor protein via synthesis inhibition and induce the death of prostate cancer cells." *Mol Cancer Ther* **8**(5): 1340-9.

There are quantitative and/or qualitative mechanisms allowing androgen receptor (AR) growth signaling in androgen ablation refractory prostate cancer cells. Regardless of the mechanism, agents that deplete AR protein expression prevent such AR growth signaling. Thapsigargin (TG) is a highly cell-penetrant sesquiterpene-lactone that once inside cells inhibits (IC<sub>50</sub>), approximately 10 nmol/L) critically important housekeeping SERCA 2b calcium pumps in the endoplasmic reticulum. Using a series of five genetically diverse androgen ablation refractory human prostate cancer lines (LNCaP, LAPC-4, VCaP, MDA-PCa-2b, and CWR22Rv1), TG inhibition of SERCA pumps consistently results in depletion of the endoplasmic reticulum Ca<sup>(+2)</sup> coupled with μmol/L elevation in the intracellular free Ca<sup>(+2)</sup> initiating a molecular cascade that: (a) inhibits Cap-dependent AR protein synthesis resulting in 90% depletion of AR protein by 24 hours of TG exposure, (b) arrests the cells in G(0), and (c) induces their apoptotic death. Unfortunately, due to its highly lipophilic nature, TG is not deliverable as a systemic agent without host toxicity. Therefore, TG analogues containing amino acids were developed, which retain ability to deplete AR protein and induce cell death and which can be covalently linked to peptide carriers producing water soluble prodrugs for systemic delivery. Specific amino acid sequences are used to restrict the liberation of cytotoxic amino acid containing TG analogues from the peptide prodrug by prostate-specific proteases, such as prostate-specific antigen and prostate-specific membrane antigen, or cancer-specific proteases, such as fibroblast activation protein, so that toxicity of these prodrugs is selectively targeted to metastatic sites of prostate cancer. Based on these results, these prodrugs are undergoing clinical development.

Vieweg, J. and J. Dannull (2005). "Technology Insight: vaccine therapy for prostate cancer." *Nat Clin Pract Urol* **2**(1): 44-51.

The lack of effective therapies for advanced prostate cancer mandates continued development of alternative treatment strategies. Insights into the regulation of immune responses and the malignant process have facilitated the emergence of new immune-based strategies, currently under investigation in clinical trials. Like other forms of targeted therapy, cancer vaccines hold the promise of achieving cancer control without inducing overt toxicity. Many prostate cancer vaccines at different phases of development have been tested in clinical

trials. Vaccination strategies under consideration include: immunization with defined antigenic preparations such as synthetic peptides, proteins or plasmid DNA; antigen-loaded dendritic cells; manipulated tumor cells; or with viral vectors engineered to express immunogenic genes. Although the underlying mechanisms of immunization may vary, all strategies share the common goal of eliciting immune responses against prostate tumor-associated antigens or of enhancing an otherwise weak antitumor response in the cancer patient. Unlocking the therapeutic potential of cancer vaccines will require a thorough understanding of cellular and molecular mechanisms that modulate the immune response. In this review, we provide an overview of vaccine-based strategies for prostate cancer therapy, discuss their mechanisms of action, and provide relevant clinical trial data.

Walton, T. J., G. Li, et al. (2009). "Quantitative RT-PCR analysis of estrogen receptor gene expression in laser microdissected prostate cancer tissue." *Prostate* **69**(8): 810-9.

**BACKGROUND:** Real-time quantitative RT-PCR analysis of laser microdissected tissue is considered the most accurate technique for determining tissue gene expression. The discovery of estrogen receptor beta (ERbeta) has focussed renewed interest on the role of estrogen receptors in prostate cancer, yet few studies have utilized the technique to analyze estrogen receptor gene expression in prostate cancer. **METHODS:** Fresh tissue was obtained from 11 radical prostatectomy specimens and from 6 patients with benign prostate hyperplasia. Pure populations of benign and malignant prostate epithelium were laser microdissected, followed by RNA isolation and electrophoresis. Quantitative RT-PCR was performed using primers for androgen receptor (AR), estrogen receptor beta (ERbeta), estrogen receptor alpha (ERalpha), progesterone receptor (PGR) and prostate specific antigen (PSA), with normalization to two housekeeping genes. Differences in gene expression were analyzed using the Mann-Whitney U-test. Correlation coefficients were analyzed using Spearman's test. **RESULTS:** Significant positive correlations were seen when AR and AR-dependent PSA, and ERalpha and ERalpha-dependent PGR were compared, indicating a representative population of RNA transcripts. ERbeta gene expression was significantly over-expressed in the cancer group compared with benign controls ( $P < 0.01$ ). In contrast, PGR expression was significantly down-regulated in the cancer group ( $P < 0.05$ ). There were no significant differences in AR, ERalpha or PSA expression between the groups. This study represents the first to show an upregulation of ERbeta

gene expression in laser microdissected prostate cancer specimens. **CONCLUSIONS:** In concert with recent studies the findings suggest differential production of ERbeta splice variants, which may play important roles in the genesis of prostate cancer.

Wang, L., G. Wu, et al. (2006). "Inhibition of CD147 expression reduces tumor cell invasion in human prostate cancer cell line via RNA interference." *Cancer Biol Ther* **5**(6): 608-14.

CD147, also named extracellular matrix metalloproteinase inducer (EMMPRIN), has been proved to be involved in the invasion and metastasis processes of tumor cells in many types of cancers. To determine the role of CD147 in the invasiveness properties of prostate cancer, we successfully downregulated CD147 by RNA interference (RNAi) technology, in PC-3 cell line at high level of CD147 expression. PC-3 cells were transfected with a pSilencer 4.1-CMV neo Vector coding for an RNA composed of two identical 19-nucleotide sequence motifs in an inverted orientation, separated by a 9-bp spacer to form a hairpin dsRNA capable of mediating target CD147 inhibition. Gelatin zymography was employed to determine the effect on reducing secretions of MMP-2 and MMP-9 of the transfected cells. Matrigel invasion assay was performed to evaluate the invasion ability of PC-3 cells in vitro. Our results showed that CD147 expression was significantly inhibited by small interfering RNAs (siRNA) transfectants in PC-3 cells at mRNA and protein levels, which resulted in dramatic reduction of invasion ability in tumor cells. Moreover, downregulation of CD147 resulted in reducing secretions of MMP-2, MMP-9. Taken together, CD147 downregulation by RNAi technology decreases the invasive capability of prostate cancer cells, demonstrating that stable expression of siRNA CD147 could potentially be an experimental approach for prostate cancer gene therapy.

Wang, L. G., A. Beklemisheva, et al. (2007). "Dual action on promoter demethylation and chromatin by an isothiocyanate restored GSTP1 silenced in prostate cancer." *Mol Carcinog* **46**(1): 24-31.

Prostate carcinoma is characterized by the silencing of pi-class glutathione S-transferase gene (GSTP1), which encodes a detoxifying enzyme. The silencing of GSTP1, due to aberrant methylation at the CpG island in the promoter/5'-UTR, occurs in the vast majority of prostate tumors and precancerous lesions. It is a pathologic marker and probably an underlying cause of oxidative damage and inflammation at tumor initiation. Inhibition of the aberrant promoter methylation could therefore be an effective mean to prevent carcinogenesis. Several isothiocyanates,

including phenethyl isothiocyanate (PEITC), found naturally in cruciferous vegetables, induced growth arrest and apoptosis in prostate cancer cells in culture and xenografts. The effects of PEITC to reactivate GSTP1 were investigated. Exposure of prostate cancer LNCaP cells to PEITC inhibited the activity and level of histone deacetylases (HDACs), and induced selective histone acetylation and methylation for chromatin unfolding. Concurrently PEITC demethylated the promoter and restored the unmethylated GSTP1 in both androgen-dependent and -independent LNCaP cancer cells to the level found in normal prostatic cells, as quantified by methylation-specific PCR and pyrosequencing. The dual action of PEITC on both the DNA and chromatin was more effective than 5'-Aza-2'-deoxycytidine, sodium butyrate, or trichostatin A (TSA), and may de-repress the methyl-binding domain (MBD) on gene transcription. The PEITC-mediated cross-talk between the DNA and chromatin in demethylating and reactivating GSTP1 genes, which is critically inactivated in prostate carcinogenesis, underlines a primary mechanism of cancer chemoprevention. Consequently, new approaches could be developed, with isothiocyanates to prevent and inhibit malignancies.

Wang, L. G., E. M. Johnson, et al. (2008). "Androgen receptor overexpression in prostate cancer linked to Pur alpha loss from a novel repressor complex." *Cancer Res* **68**(8): 2678-88.

Increased androgen receptor (AR) expression and activity are pivotal for androgen-independent (AI) prostate cancer (PC) progression and resistance to androgen-deprivation therapy. We show that a novel transcriptional repressor complex that binds a specific sequence (repressor element) in the AR gene 5'-untranslated region contains Pur alpha and hnRNP-K. Pur alpha expression, its nuclear localization, and its AR promoter association, as determined by chromatin immunoprecipitation analysis, were found to be significantly diminished in AI-LNCaP cells and in hormone-refractory human PCs. Transfection of AI cells with a plasmid that restored Pur alpha expression reduced AR at the transcription and protein levels. Pur alpha knockdown in androgen-dependent cells yielded higher AR and reduced p21, a gene previously shown to be under negative control of AR. These changes were linked to increased proliferation in androgen-depleted conditions. Treatment of AI cells with histone deacetylase and DNA methylation inhibitors restored Pur alpha protein and binding to the AR repressor element. This correlated with decreased AR mRNA and protein levels and inhibition of cell growth. Pur alpha is therefore a key repressor of AR transcription and its loss from the transcriptional

repressor complex is a determinant of AR overexpression and AI progression of PC. The success in restoring Pur alpha and the repressor complex function by pharmacologic intervention opens a promising new therapeutic approach for advanced PC.

Wang, L. Z., K. Sato, et al. (2003). "Polymorphisms in prostate-specific antigen (PSA) gene, risk of prostate cancer, and serum PSA levels in Japanese population." *Cancer Lett* **202**(1): 53-9.

Recent studies have indicated that the prostate-specific antigen (PSA) gene polymorphisms may be associated with the risk of prostate cancer in Caucasian populations. To verify the association, we examined the PSA polymorphisms at positions -158 and -252 in 300 prostate cancer cases, 216 benign prostatic hyperplasia (BPH) cases, and 266 controls by the PCR-restriction fragment length polymorphism analysis. Regarding the PSA polymorphism at position -158, the A allele was less common in the Japanese population (A 0.22, G 0.78) than that in other ethnic populations (A 0.37-0.52, G 0.48-0.63). No significant associations were found between the polymorphism and the risks of prostate cancer (P=0.530) and BPH (P=0.740) and between the polymorphism and the serum PSA level (P=0.626). As for the polymorphism at position -252, no significant results were also found. In conclusion, the PSA polymorphisms may not be associated with the risk of prostate cancer development and its disease progression and the risk of BPH in Japanese men, and may also be not related to the serum PSA level in Japanese men with prostate cancer.

Wang, Q., D. I. Holmes, et al. (2002). "Analysis of stromal-epithelial interactions in prostate cancer identifies PTPCAAX2 as a potential oncogene." *Cancer Lett* **175**(1): 63-9.

A PCR-based subtractive hybridisation technique was used to identify genes involved in stromal-epithelial interactions in prostate cancer. Eight genes were identified as being differentially expressed in benign prostatic fibroblast cells after stimulation with tumourigenic LNCaP conditioned media. One of these genes, protein tyrosine phosphatase CAAX2 (PTPCAAX2; also described as PTP4A and OV-1), has recently been shown to be oncogenic in hamster pancreatic epithelial cells. We show that PTPCAAX2 expression is up-regulated 4-fold in benign prostatic fibroblast cells 24 h after stimulation with LNCaP conditioned media and up-regulated 9-fold in prostatic tumour fibroblast cells. PTPCAAX2 overexpression was also detected in both androgen-dependent and androgen-independent prostate cancer cell lines and prostate tumour tissue, as determined by RT-PCR analysis and in situ hybridisation. These observations

of PTPCAAX2 overexpression in prostate tumour cells and tissue suggest that PTPCAAX2 may potentially function as an oncogene in prostate cancer.

Wang, X., V. Tryndyak, et al. (2008). "Sensitivity of human prostate cancer cells to chemotherapeutic drugs depends on EndoG expression regulated by promoter methylation." *Cancer Lett* **270**(1): 132-43.

Analysis of promoter sequences of all known human cytotoxic endonucleases showed that endonuclease G (EndoG) is the only endonuclease that contains a CpG island, a segment of DNA with high G+C content and a site for methylation, in the promoter region. A comparison of three human prostate cancer cell lines showed that EndoG is highly expressed in 22Rv1 and LNCaP cells. In PC3 cells, EndoG was not expressed and the EndoG CpG island was hypermethylated. The expression of EndoG correlated positively with sensitivity to cisplatin and etoposide, and the silencing of EndoG by siRNA decreased the sensitivity of the cells to the chemotherapeutic agents in the two EndoG-expressing cell lines. 5-aza-2'-deoxycytidine caused hypomethylation of the EndoG promoter in PC3 cells, induced EndoG mRNA and protein expression, and made the cells sensitive to both cisplatin and etoposide. The acetylation of histones by trichostatin A, the histone deacetylase inhibitor, induced EndoG expression in 22Rv1 cells, while it had no such effect in PC3 cells. These data are the first indication that EndoG may be regulated by methylation of its gene promoter, and partially by histone acetylation, and that EndoG is essential for prostate cancer cell death in the used models.

Williams, S. A. and D. J. McConkey (2003). "The proteasome inhibitor bortezomib stabilizes a novel active form of p53 in human LNCaP-Pro5 prostate cancer cells." *Cancer Res* **63**(21): 7338-44.

Advanced prostate cancer is resistant to current therapeutic strategies. Bortezomib (Velcade; previously called PS-341) is a potent and specific inhibitor of the 26S proteasome that is currently in clinical trials for treatment of various malignancies, including prostate cancer. We investigated the effects of bortezomib on p53 in the LNCaP-Pro5 prostate cancer cell line. Bortezomib induced strong stabilization of p53, but it did not promote phosphorylation on serines 15 and 20, and p53 remained bound to its inhibitor, mdm2. Nonetheless, bortezomib stimulated p53 translocation to the nucleus (not mitochondria) and enhanced p53 DNA binding, accumulation of p53-dependent transcripts, and activation of a p53-responsive reporter gene. Furthermore, stable LNCaP-Pro5 transfectants of LNCaP-Pro5 expressing the p53 inhibitor human

papillomavirus-E6 displayed reduced bortezomib-induced p53 activation and cell death. Together, our data demonstrate that bortezomib stimulates p53 activation via a novel mechanism.

Woelfelschneider, A., O. Popanda, et al. (2008). "A distinct ERCC1 haplotype is associated with mRNA expression levels in prostate cancer patients." *Carcinogenesis* **29**(9): 1758-64.

Both genetic variants and messenger RNA (mRNA) expression of DNA repair and tumor suppressor genes have been investigated as molecular markers for therapy outcome. However, the phenotypic impact of genetic variants often remained unclear, thus the rationale of their use in risk prediction may be limited. We therefore analyzed genetic variants together with anthropometric and lifestyle factors to see how these affect mRNA levels of ERCC1, MDM2 and TP53 in primary blood lymphocytes. mRNA expression was measured in 376 prostate cancer patients by quantitative real-time polymerase chain reaction after reverse transcription, and ERCC1 rs11615 T>C, ERCC1 rs3212986 C>A, MDM2 rs2279744 T>G and TP53 rs17878362 (p53PIN3) polymorphisms were determined. Considerable interindividual differences in mRNA expression were found (coefficients of variation: ERCC1, 45%; MDM2, 43% and TP53, 35%). ERCC1 expression was positively correlated with plasma levels of beta-carotene (P = 0.03) and negatively correlated with canthaxanthin (P = 0.02) and lutein (P = 0.02). Overall, the polymorphisms affected mRNA expression only weakly. Carriers of a distinct ERCC1 haplotype (CC) showed, however, significantly lower expression values than non-carriers (P = 0.001). Applying logistic regression, we found that CC haplotype carriers had a 1.69-fold increased odds ratio (95% confidence interval: 1.06-2.71) for reduced ERCC1 mRNA levels. This low ERCC1 expression might be associated with reduced DNA repair and better therapy response. In summary, the association we have found between ERCC1 genotype and mRNA expression supports recent clinical observations that genetic variation in ERCC1 can affect treatment outcome and prognosis. Our study further revealed a modulating effect by nutritional factors.

Zellweger, T., K. Chi, et al. (2002). "Enhanced radiation sensitivity in prostate cancer by inhibition of the cell survival protein clusterin." *Clin Cancer Res* **8**(10): 3276-84.

PURPOSE: The purpose of this study is to evaluate the role of the cell survival gene clusterin in radiation-induced cell death in human LNCaP and PC-3 prostate cancer models. Experimental Design: Radiation sensitivities were compared in parental and

clusterin-overexpressing LNCaP cells and in PC-3 cells and tumors treated with antisense or mismatch clusterin oligonucleotides. **RESULTS:** Clusterin-overexpressing LNCaP cells were less sensitive to irradiation with significantly lower cell death rates (23% after 8 Gy) compared with parental LNCaP cells (50% after 8 Gy) 3 days after irradiation. Clusterin expression in PC-3 cells after radiation was found to be up-regulated in a dose-dependent manner in vitro by 70% up to 12 Gy and in vivo by 84% up to 30 Gy. Inhibition of clusterin expression in PC-3 cells using antisense oligonucleotides (ASOs) occurred in a sequence- and dose-dependent manner and significantly enhanced radiation-induced apoptosis and decreased PC-3 cell growth rate and plating efficiency. Compared with mismatch control oligonucleotide treatment, clusterin ASO treatment enhanced radiation therapy and significantly reduced PC-3 tumor volume in vivo by 50% at 9 weeks. In addition, TUNEL staining revealed increased number of apoptotic cells in clusterin ASO-treated and irradiated PC-3 tumors, compared with treatment with mismatch control oligonucleotides plus radiation. **CONCLUSIONS:** These findings support the hypothesis that clusterin acts as a cell survival protein that mediates radioresistance through the inhibition of apoptosis. In vivo results further suggest that inactivation of clusterin using ASO technology might offer a novel strategy to improve results of radiation therapy for prostate cancer patients.

Zerbini, L. F., Y. Wang, et al. (2003). "Constitutive activation of nuclear factor kappaB p50/p65 and Fra-1 and JunD is essential for deregulated interleukin 6 expression in prostate cancer." *Cancer Res* **63**(9): 2206-15.

To date, no effective treatment for patients with advanced androgen-independent prostate cancer is available, whereas androgen ablation therapy, surgery, and radiation therapy are effective in treating local, androgen-dependent tumors. The mechanisms underlying the differences between androgen-dependent and -independent prostate cancer remain elusive. Interleukin (IL)-6 is a pleiotropic cytokine whose expression under normal physiological conditions is tightly controlled. However, aberrant constitutive IL-6 gene expression has been implicated in prostate cancer progression and resistance to chemotherapy and has been directly linked to prostate cancer morbidity and mortality. Particularly striking is the large increase in the expression of IL-6 in hormone-refractory prostate cancer. IL-6, in addition to its role as an immunomodulatory cytokine, functions as a growth and differentiation factor for prostate cancer cells. To determine the molecular mechanisms that lead to deregulated IL-6 expression

in advanced prostate cancer, we examined the regulatory elements involved in IL-6 gene expression in androgen-independent prostate cancer cells. We demonstrate that, in contrast to the androgen-sensitive LNCaP cells, androgen-insensitive PC-3 and DU145 cells express high levels of IL-6 protein and mRNA due to enhanced promoter activity. Deregulated activation of the IL-6 promoter is for the most part mediated by a combined constitutive activation of the nuclear factor (NF)-kappaB p50 and p65 and the activator protein 1 (AP-1) JunD and Fra-1 family members as demonstrated by electrophoretic mobility shift assays, site-directed mutagenesis, and transfection experiments. Mutation of the NF-kappaB and AP-1 sites drastically reduces IL-6 promoter activity in both androgen-independent prostate cancer cell lines. Additionally, inhibition of these transcription factors using adenovirus vectors encoding either the IkappaBalpha repressor gene or a dominant negative JunD mutant leads to a strong down-regulation of IL-6 gene expression at the mRNA and protein level as measured by real-time PCR and ELISA, respectively. Furthermore, the blockade of IL-6 gene expression results in drastic inhibition of the constitutively activated signal transducers and activators of transcription 3 signaling pathway in DU145 cells. Our data demonstrate for the first time that a combined aberrant activation of NF-kappaB p50 and p65 and AP-1 JunD and Fra-1 in androgen-independent prostate cancer cells results in deregulated IL-6 expression, suggesting a novel potential entry point for therapeutic intervention in prostate cancer.

Zu, K., T. Bihani, et al. (2006). "Enhanced selenium effect on growth arrest by BiP/GRP78 knockdown in p53-null human prostate cancer cells." *Oncogene* **25**(4): 546-54.

Redox modification of thiol/disulfide interchange in proteins by selenium could lead to protein unfolding. When this occurs in the endoplasmic reticulum (ER), a process known as unfolded protein response (UPR) is orchestrated for survival through activation of PERK-eIF2alpha (PERK: double-stranded RNA-activated protein kinase-like ER kinase; eIF2alpha: eucaryotic initiation factor 2alpha), ATFalpha (ATFalpha: activating transcription factor 6) and inositol requiring 1 (IRE1)-x-box-binding protein 1 (XBP1) signalings. All three UPR transducer pathways were upregulated very rapidly when PC-3 cells were exposed to selenium. These changes were accompanied by increased expression of UPR target genes, including immunoglobulin heavy chain-binding protein/glucose-regulated protein, 78 kDa and CCAAT/enhancer binding protein-homologous protein/growth arrest-

and DNA damage-inducible gene (CHOP/GADD153). Induction of BiP/GRP78, an ER-resident chaperone, is part of the damage control mechanism, while CHOP/GADD153 is a transcription factor associated with growth arrest and apoptosis in the event of prolonged ER stress. Knocking down BiP/GRP78 induction by small interference RNA produced a differential response of the three transducers to selenium, suggesting that the signaling intensity of each transducer could be fine-tuned depending on BiP/GRP78 availability. In the presence of selenium, CHOP/GADD153 expression was raised even higher by BiP/GRP78 knockdown. Under this condition, the selenium effect on wild-type p53-activated fragment p21 (p21(WAF)), cyclin-dependent kinase (CDK)1 and CDK2 was also magnified in a manner consistent with enhanced cell growth arrest. Additional experiments with CHOP/GADD153 siRNA knockdown strongly suggested that CHOP/GADD153 may play a positive role in upregulating the expression of p21(WAF) in a p53-independent manner (PC-3 cells are p53 null). Collectively, the above findings support the idea that UPR could be an important mechanism in mediating the anticancer activity of selenium.

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12/10/2012