

**PROSTATE CANCER  
(METASTASIZED/LATE STAGE)  
(PAGE 2)**

[Return to the Table of Contents](#)

**Cancer risk factors for selecting cohorts for large-scale chemoprevention trials**

Journal of Cellular Biochemistry (USA), 1996, 63/SUPPL. 25 (29-36)

Many anticipate that application of findings in molecular genetics will help to achieve greater precision in defining high-risk populations that may benefit from chemopreventive interventions. We must recognize, however, that genetic susceptibility, environmental factors, and complex gene-environment interactions are all likely to be risk determinants for most cancers. Cohort studies of twins and cancer indicate that having 'identical' genes is generally not a very accurate predictor of cancer incidence. Data from twin studies support the suggestion that environmental factors such as tobacco use significantly influence cancer risk. The complexities of the genetic contribution to disease risk are exemplified by the development of Duchenne muscular dystrophy in only one of monozygotic twin girls, hypothesized to be the result of X chromosome inactivation, with the distribution patterns of the X chromosome being skewed to the female X in the manifesting twin and to the male X in the normal twin. Evidence from transgenic and genetic-environmental studies in animals support the possibility of genetic-environmental interactions. Calorie restriction modifies tumor expression in p53 knockout mice; a high-fat, low-calcium, low-vitamin D diet increases prepolypyperplasia formation in Apc-mutated mice; and calorie restriction early in life influences development of obesity in the genetically obese Zucker rat (fata). Such environmental modulation of gene expression suggests that chemoprevention has the potential to reduce risk for both environmentally and genetically determined cancers. In view of the growing research efforts in chemoprevention, the NCI has developed a Prevention Trials Decision Network (PTDN) to formalize the evaluation and approval process for large scale chemoprevention trials. The PTDN addresses large trial prioritization and the associated issues of minority recruitment and retention; identification and validation of biomarkers as intermediate endpoints for cancer; and chemopreventive agent selection and development. A comprehensive database is being established to support the PTDN's decision making process and will help to determine which agents investigated in preclinical and early phase clinical trials should move to large-scale testing. Cohorts for large-scale chemoprevention trials include individuals who are determined to be at high risk as a result of genetic predisposition, carcinogenic exposure, or the presence of biomarkers indicative of increased risk. Current large scale trials in well-defined, high-risk populations include the Breast Cancer Prevention Trial (tamoxifen), the Prostate Cancer Prevention Trial (finasteride), and the N-(4-hydroxyphenyl) retinamide (4-HPR) breast cancer prevention study being conducted in Milan. Biomarker studies will provide valuable information for refining the design and facilitating the implementation of future large-scale trials. For example, potential biomarkers are being assessed at biopsy in women with ductal carcinoma in situ (DCIS). The women are then randomized to either placebo, tamoxifen, 4-HPR, or tamoxifen plus 4-HPR for 2-4 weeks, at which time surgery is performed and the biomarkers reassessed to determine biomarker modulation by the interventions. For prostate cancer, modulation of prostatic intraepithelial neoplasia (PIN) by 4-HPR and difluoromethylornithine is being investigated; similar studies are being planned for oltipraz, dehydroepiandrosterone, and vitamin E plus selenomethionine. The validation of biomarkers as surrogate endpoints for cancer incidence in high-risk cohorts will allow more agents to be evaluated in shorter studies that use fewer subjects to achieve the desired statistical power.

**Inhibition of liposomal lipid peroxidation by isoflavonoid type phyto-oestrogens from soybeans of different countries of origin**

Biochemical Society Transactions (United Kingdom), 1996, 24/3 (392S)

**Phytoestrogens: Epidemiology and a possible role in cancer protection**

Because many diseases of the Western Hemisphere are hormone-dependent cancers, we have postulated that the Western diet, compared to a vegetarian or semivegetarian diet, may alter hormone production, metabolism or action at the cellular level by some biochemical mechanisms. Recently, our interest has been mainly focused on the cancer-protective role of some hormonelike diphenolic phytoestrogens of dietary origin, the lignans and the isoflavonoids. The precursors of the biologically active compounds originate in soybean products (mainly isoflavonoids), whole grain cereal food, seeds, and probably berries and nuts (mainly lignans). The plant lignan and isoflavonoid glycosides are converted by intestinal bacteria to hormonelike compounds with weak estrogenic but also antioxidative activity; they have now been shown to influence not only sex hormone metabolism and biological activity but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation, and angiogenesis in a way that makes them strong candidates for a role as natural cancer-protective compounds. Epidemiologic investigations strongly support this hypothesis because the highest levels of these compounds in the diet are found in countries or regions with low cancer incidence. This report is a review on recent results suggesting that the diphenolic, isoflavonoids and lignans are natural cancer-protective compounds.

### **Differential sensitivity of human prostatic cancer cell lines to the effects of protein kinase and phosphatase inhibitors**

Cancer Letters (Ireland), 1995, 98/1 (103-110):

We investigated the effect of protein kinase and phosphatase inhibitors on the growth of six human prostatic cancer cell lines: DU145, PC3, ND1, LNCaP, ALVA31 and JCA1. We studied okadaic acid and sodium orthovanadate as serine/threonine and tyrosine protein phosphatase inhibitors, respectively, and staurosporin and genistein as a serine/threonine and tyrosine protein kinase inhibitors, respectively. All inhibitors examined exhibited a dose-dependent growth inhibitory effect on prostatic cancer cell lines. Our data indicate that prostatic cancer cell lines express unique biochemical properties since the degree of growth inhibition varied greatly and was dependent on the specific cell line and inhibitor studied. In addition, we found that surface expression of endoglin (CD105) changed by treatment with all inhibitors in most of the cell lines. These data also indicate that endoglin appears to be involved both in protein phosphatase and kinase mediated phosphoprotein turnover.

### **Genetic damage and the inhibition of 7,12-dimethylbenz(a)anthracene-induced genetic damage by the phytoestrogens, genistein and daidzein, in female ICR mice**

Cancer Letters (Ireland), 1995, 95/1-2 (125-133)

Populations consuming soybeans have reduced rates of breast, colon and prostate cancer possibly due, in part, to the presence in soybeans of two estrogenic isoflavones, genistein and daidzein. This study investigated the genotoxicity of these soya isoflavones and their interactions with 7,12-dimethylbenz(a)anthracene (DMBA)-induced sister chromatid exchanges (SCE) in bone marrow cells and DNA adduct formations in liver and mammary glands of mice. Groups of female ICR mice were pretreated i.p. with daidzein and/or genistein (10-20 mg/kg per day for 6 days or 50 mg/kg per 12 h for 3 days) or with the solvent, dimethylsulfoxide (DMSO). The mice were implanted with bromodeoxyuridine (BrdU) tablets s.c., and treated with DMBA (50 mg/kg) i.p. and colchicine (4 mg/kg) i.p. 24, 23, and 2 h before sacrifice, respectively. In bone marrow cells, DMBA alone induced 11.73 plus or minus 1.42 SCE/cell compared to 4.35 plus or minus 0.83 SCE/cell in the DMSO treated controls ( $P = 0.001$ ). DMBA induced 20% fewer SCE ( $P < 0.05$ ) in mice pretreated with daidzein, genistein or a combination of genistein and daidzein (6 x 20 mg/kg per day for 6 days) when compared to mice that received no pretreatments. Genistein at 50 mg/kg per 12 h for 3 days also inhibited DMBA-induced SCE by 20%. However, treatment for 3 days with 50 mg/kg per 12 h of genistein or daidzein alone, or a combination of daidzein plus genistein (without DMBA treatment) also induced more SCE than treatment with only the solvent (DMSO,  $P < 0.05$ ). Pretreatment with both the low and the high doses of daidzein plus genistein or the high dose of genistein reduced the replication index of bone marrow cells when compared to pretreatment with DMSO ( $P < 0.05$ ). Pretreatment with genistein reduced DMBA-induced DNA adduct formation by 34%, but this was only marginally significant ( $P = 0.08$ ) due to the large inter-individual variability in adduct levels. These results show that genistein and daidzein suppress SCE and possibly DNA adduct formation induced by the known carcinogen, DMBA. This response to a low dose isoflavone exposure may be partly responsible for the protective effect against endocrine cancers of soya consumption.

## **Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer**

Journal of Cellular Biochemistry (USA), 1995, 58/SUPPL. 22 (181-187)

Pharmacologists have realized that tyrosine kinase inhibitors (TKI) have potential as anti-cancer agents, both in prevention and therapy protocols. Nonetheless, concern about the risk of toxicity caused by synthetic TKIs restricted their development as chemoprevention agents. However, a naturally occurring TKI (the isoflavone genistein) in soy was discovered in 1987. The concentration of genistein in most soy food materials ranges from 1-2 mg/g. Oriental populations, who have low rates of breast and prostate cancer, consume 20-80 mg of genistein/day, almost entirely derived from soy, whereas the dietary intake of genistein in the US is only 1-3 mg/day. Chronic use of genistein as a chemopreventive agent has an advantage over synthetic TKIs because it is naturally found in soy foods. It could be delivered either in a purified state as a pill (to high-risk, motivated patient groups), or in the form of soy foods or soy-containing foods. Delivery of genistein in soy foods is more economically viable (\$1.50 for a daily dose of 50 mg) than purified material (\$5/day) and would require no prior approval by the FDA. Accordingly, investigators at several different sites have begun or are planning chemoprevention trials using a soy beverage product based on SUPRO(TM), an isolated soy protein manufactured by Protein Technologies International of St. Louis, MO. These investigators are examining the effect of the soy beverage on surrogate intermediate endpoint biomarkers (SIEBs) in patients at risk for breast and colon cancer, defining potential SIEBs in patients at risk for prostate cancer, and determining whether the soy beverage reduces the incidence of cancer recurrence. These studies will provide the basis for formal Phase I, Phase II and Phase III clinical trials of genistein and soy food products such as SUPRO(TM) for cancer chemoprevention.

## **A simplified method to quantify isoflavones in commercial soybean diets and human urine after legume consumption**

Cancer Epidemiology Biomarkers and Prevention (USA), 1995, 4/5 (497-503)

Reliable and economical quantification of micronutrients in diets and humans is a critical component of successful epidemiological studies to establish relationships between dietary constituents and chronic disease. Legumes are one of the major dietary components consumed by populations worldwide. Consumption of legumes is thought to play a major role in lowering breast and prostate cancer risk. In this study, a simplified method that uses solid-phase extraction and gas chromatography was developed to measure isoflavones at levels down to 10 microg/5 ml. With the use of this method, 12.5 g miso (a soybean paste), 12 ounces Isomil, and 12 ounces soymilk had daidzin/daidzein levels of 2, 5, and 12.4 mg, respectively, and genistin/genistein levels of 3, 6.5, and 13.7 mg, respectively. In these products, most of the isoflavones were present as glucosides. With the same method, urinary levels of isoflavones in six 15-17-year-old subjects were determined after soymilk ingestion. Each subject was placed on unrestricted nonsoya diets, and three 12-ounce portions of soymilk were given at 12-h intervals. Males excreted 15.02 plus or minus 2.74 (SD) mg of daidzein glucuronides/sulfates (mean recovery, 40.4 plus or minus 7.4% (SD)) by 24 h after the third soymilk ingestion, whereas females excreted 25.56 plus or minus 5.10 mg (68.7 plus or minus 13.7%) of daidzein conjugates, which was more than males ( $P = 0.02$ ). Males and females excreted 7.73 plus or minus 1.95 mg and 9.11 plus or minus 0.84 mg of genistein glucuronides/sulfates (20% recovery of genistin intake), respectively, in the urine. Most of the isoflavones were excreted within 24 h after ingestion. The relative urinary levels of daidzein to genistein excreted were significantly ( $P < 0.05$ ) higher in females than males after the third ingestion. The observed sex difference requires more study since two of the females are siblings. Thus, the method described can be used to measure isoflavones in soya products and urinary excretion after soya ingestion.

## **Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine**

PROC. SOC. EXP. BIOL. MED. (USA), 1995, 208/1 (18-26)

Due to growing evidence suggesting that phytoestrogens might protect against various cancers, particularly against breast and prostate cancer, it is important to measure the exposure of populations to these compounds by determining levels in food and in human tissue or body fluids to assess the possible cancer protective properties of these agents. Therefore, we developed a simple and fast procedure to extract and simultaneously hydrolyze

phytoestrogens and their conjugates from food items, and present a fast and selective high-performance liquid chromatography (HPLC) method for precise determinations of the most common dietary phytoestrogens genistein, biochanin-A, daidzein, formononetin, and coumestrol using flavone as internal standard. For the first time HPLC was applied to measure these phytoestrogens and their most abundant metabolites equol and O-desmethyl-angotensin from human urine. The proposed methodology has been evaluated for losses due to thermal degradation during extraction and hydrolysis and due to sample handling during the entire work-up including solid phase extraction, and values are given for inter- and intra-assay variability. We present isoflavonoid levels of most common peas and beans used in 'western' and 'eastern' diets and compare isoflavonoid and coumestrol levels of raw, canned, and cooked foods which can be used in future epidemiological studies. We also determined human urinary levels with our methodology comparing values before and after soybean intake.

### **Soy intake and cancer risk: A review of the in vitro and in vivo data**

NUTR. CANCER (USA), 1994, 21/2 (113-131)

International variations in cancer rates have been attributed, at least in part, to differences in dietary intake. Recently, it has been suggested that consumption of soyfoods may contribute to the relatively low rates of breast, colon, and prostate cancers in countries such as China and Japan. Soybeans contain a number of anticarcinogens, and a recent National Cancer Institute workshop recommended that the role of soyfoods in cancer prevention be investigated. In this review, the hypothesis that soy intake reduces cancer risk is considered by examining relevant in vitro, animal, and epidemiological data. Soybeans are a unique dietary source of the isoflavone genistein, which possesses weak estrogenic activity and has been shown to act in animal models as an antiestrogen. Genistein is also a specific inhibitor of protein tyrosine kinases; it also inhibits DNA topoisomerases and other critical enzymes involved in signal transduction. In vitro, genistein suppresses the growth of a wide range of cancer cells, with IC50 values ranging from 5 to 40 microM (1-10 microg/ml). Of the 26 animal studies of experimental carcinogenesis in which diets containing soy or soybean isoflavones were employed, 17 (65%) reported protective effects. No studies reported soy intake increased tumor development. The epidemiological data are also inconsistent, although consumption of nonfermented soy products, such as soymilk and tofu, tended to be either protective or not associated with cancer risk; however, no consistent pattern was evident with the fermented soy products, such as miso. Protective effects were observed for both hormone- and nonhormone-related cancers. While a definitive statement that soy reduces cancer risk cannot be made at this time, there is sufficient evidence of a protective effect to warrant continued investigation.

### **Plasma concentrations of phyto-oestrogens in Japanese men**

LANCET (United Kingdom), 1993, 342/8881 (1209-1210)

A low mortality from prostatic cancer is found in Japanese men consuming a low-fat diet with high content of soy products, a rich source of isoflavonoids. We therefore assayed four isoflavonoids in plasma of 14 Japanese and 14 Finnish men. The geometric mean plasma total individual isoflavonoid levels were 7 to 110 times higher in the Japanese than in the Finnish men. Genistein, a tyrosine kinase inhibitor, occurred in the highest concentration (geometric mean 276 nmol/L). We hypothesise that these high phyto-oestrogen levels may inhibit the growth of prostatic cancer in Japanese men, which may explain the low mortality from prostatic cancer in that country.

### **Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture**

STEROIDS (USA), 1993, 58/7 (301-304)

Studies have indicated a correlation between a high level of urinary lignans and isoflavonoid phytoestrogens, particularly genistein, and a low incidence of hormone-dependent cancers, such as breast and prostate cancer. Previously it has been observed that a vegetarian diet is associated with high plasma levels of sex hormone-binding globulin (SHBG), reducing clearance of sex hormones and probably risk of breast and prostate cancer. In the present study we investigated the in vitro effect of genistein on the production of SHBG by human hepatocarcinoma (Hep-G2) cells in culture and its effect on cell proliferation. We found that genistein not only highly significantly increases the SHBG production by Hep-G2 cells, but also suppresses the proliferation of these cancer cells already

at a stage when SHBG continues to be high. We conclude that, in addition to the lignan enterolactone, the most abundant urinary isoflavonoid genistein stimulates SHBG production and inhibits Hep-G2 cancer cell proliferation.

### **Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation**

PROSTATE (USA), 1993, 22/4 (335-345)

The effect of the isoflavones, genistein, daidzein, and biochanin A on the growth of the LNCaP and DU-145 human prostate cancer cell lines has been examined. Genistein and biochanin A, but not daidzein, inhibit both serum and EGF-stimulated growth of LNCaP and DU-145 cells (IC50 values from 8.0 to 27 microg/ml for serum and 4.3 to 15 microg/ml for EGF), but have no significant effect on the EGF receptor tyrosine autophosphorylation. In contrast, tyrphostin 25, a specific EGF receptor tyrosine kinase inhibitor, inhibits EGF-stimulated growth and EGF receptor tyrosine autophosphorylation in these whole cells, but does not inhibit serum-stimulated growth. These data suggest that the mechanism of action of genistein and biochanin A does not depend on inhibition of EGF receptor tyrosine autophosphorylation, but on a more distal event in the EGF receptor-mediated signal transduction cascade.

### **Surrogate endpoint biomarkers for phase II cancer chemoprevention trials**

J. CELL. BIOCHEM. (USA), 1994, 56/SUPPL. 19 (1-9)

Three critical aspects govern successful Phase II cancer chemoprevention trials - well-characterized agents, suitable cohorts, and reliable intermediate biomarkers for measuring efficacy. Requirements for the agent are experimental or epidemiological data showing chemopreventive efficacy, safety on chronic administration, and a mechanistic rationale for the chemopreventive activity observed. The cohort should be suitable for measuring the chemopreventive activity of the agent and the intermediate biomarkers chosen. Also, many cohorts proposed for Phase II trials are patients with previous cancers or premalignant lesions. For such patients, the trials should be conducted within the context of standard treatment to avoid unusual risks. The criteria for biomarkers are that they fit expected biological mechanisms (i.e., differential expression in normal and high-risk tissue, on or closely linked to the causal pathway for the cancer, modulated by chemopreventive agents, and short latency compared with cancer), may be assayed reliably and quantitatively, measured easily, and correlate to decreased cancer incidence. They must occur in sufficient incidence to allow their biological and statistical evaluation relevant to cancer. Since carcinogenesis is a multipath process, single biomarkers are difficult to validate as surrogate endpoints, as they may appear on only one or a few of the many possible causal pathways. Panels of biomarkers, particularly those representing the range of carcinogenesis pathways, may prove more useful as surrogate endpoints. It is important to avoid relying solely on biomarkers representing isolated events that may or may not be on the causal pathway or otherwise associated with carcinogenesis. These include markers of normal cellular processes that may be increased or expressed during carcinogenesis, but are nonspecific. Chemoprevention trials should be designed to fully evaluate the two or three biomarkers that appear to be the best models of the cancer. Additional biomarkers should be considered only if they can be analyzed efficiently and the sample size allows the more important biomarkers to be evaluated completely. Two types of biomarkers that stand out in regard to their high correlation to cancer and their ability to be quantified are measures of intraepithelial neoplasia and indicators of cellular proliferation. Measurements made by computer-assisted image analysis that are potentially useful as surrogate endpoint biomarkers include nuclear pleomorphism comprising nuclear size, shape (roundness), and texture (DNA distribution patterns); nucleolar size and number of nucleoli/nucleus; DNA ploidy; and proliferation biomarkers such as S-phase fraction, bromodeoxyuridine uptake, Ki-67, and proliferating cell nuclear antigen. Phase II chemoprevention trials are currently in progress or planned that will evaluate these biomarkers. The cohorts include patients scheduled for surgery for ductal carcinoma in situ in breast or early breast cancer, patients with previously resected colon tumors or adenomas, patients with prostatic intraepithelial neoplasia, and patients scheduled for prostate cancer surgery.

### **The 16-ene vitamin D analogs**

Current Pharmaceutical Design (Netherlands), 1997, 3/1 (99-123)

Numerous 16-ene vitamin D analogs were investigated as potential anticancer agents. Several structural

modifications have been uncovered that contribute to the improvement in the stimulation of HL-60 cells differentiation, the inhibition of HL-60 cells proliferation and the reduction of calcemic properties in vivo. They include the introduction of 16-, 22E-, 23E- and 23Z-double bonds, 23-triple bond or 22R-allene, and substitution of C26 and C27-hydrogens with fluorine or methyl groups. The biggest gains have been achieved by combination of the 16-double bond with 23-double or triple bond and 26-trifluoro or 26,27-hexafluoro substitution patterns. Separately, the combination of the 16-double bond with 22R-allene has produced a highly active analog. In respect to modifications in the ring A, the high activities in cell differentiation and inhibition of cell proliferation with significant reduction of calcemic properties were observed in the 1 $\alpha$ -fluoro, 3-desoxy, and 19-nor series. It was also shown that the lack of the 1 $\alpha$ -hydroxy group can be overcome by an optimized modification in the ring D and the side chain; 25(OH)-16,23E-diene-26,27-F6D3 is fully active in HL-60 cell differentiation assay with only minimal effects on the cellular calcium homeostasis.

### **Signal transduction inhibitors as modifiers of radiation therapy in human prostate carcinoma xenografts**

Radiation Oncology Investigations (USA), 1996, 4/5 (221-230)

Radiation therapy is very useful in the treatment of prostate cancer; however, local treatment failure still occurs in the majority of patients with locally advanced disease. The growth and progression of tumors involve signaling through protein growth factors and small molecules such as arachidonic acid cascade products. In order to develop novel agents to enhance the efficacy of radiation therapy for patients with prostate cancer, the ability of signal transduction inhibitors including

- (1) the antiandrogen, flutamide;
- (2) the anti-inflammatory agent, ibuprofen;
- (3) the growth factor receptor antagonist, suramin;
- (4) the retinoid, all-trans-retinoic acid; and

(5) the calcium pump inhibitor, thapsigargin to enhance the response of the human prostate carcinoma xenografts DU-145 and LN-CaP, was assessed. Flutamide acted as a radiation protector of the androgen independent DU-145 tumor but produced an additive antitumor effect in combination with fractionated radiation therapy in the androgen dependent LNCaP tumor. Administration of suramin or thapsigargin along with radiation therapy provided little or no tumor growth delay compared with radiation therapy alone. Treatment with all-trans-retinoic acid did not alter the response of the DU-145 to radiation therapy but increased the response of LNCaP tumor to radiation therapy. Administration of ibuprofen along with radiation therapy was most effective. The radiation dose modifying factor for ibuprofen in the DU-145 tumor was 1.8 and 1.7 for a 1-week and a 2-week fractionated regimen, respectively. Administration of ibuprofen along with radiation therapy to animals bearing the LNCaP tumor resulted in a 2-fold increase in tumor growth delay compared with radiation therapy alone. Further investigation of inhibitors of the arachidonic acid cascade as radiation modifiers is warranted.

### **Calcium regulation of androgen receptor expression in the human prostate cancer cell line LNCaP**

Endocrinology (USA), 1995, 136/5 (2172-2178)

Elevation of intracellular calcium levels in the presence of normal androgen levels has been implicated in apoptotic prostate cell death. Since the androgen receptor (AR) plays a critical role in the regulation of growth and differentiation of the prostate, it was of interest to determine whether Ca<sup>2+</sup> would affect the expression of androgen receptor messenger RNA (mRNA) and protein, thus affecting the ability of androgens to control prostate function. AR-positive human prostate cancer cells, LNCaP, were incubated with either the calcium ionophore A23187 or the intracellular endoplasmic reticulum Ca<sup>2+</sup>-ATPase inhibitor thapsigargin. Subsequently, AR mRNA and protein levels were assessed by Northern and Western blot analysis. Both A23187 and thapsigargin were found to down-regulate steady state AR mRNA levels in a time- and dose-dependent manner. AR mRNA began to decrease after 6-8 h of incubation with 10<sup>-6</sup> M A23187 or 10<sup>-7</sup> M thapsigargin, reaching a nadir at 16 and 10 h of incubation, respectively. In contrast, control mRNA (glyceraldehyde 3-phosphate dehydrogenase) did not change significantly during the treatments with either A23187 or thapsigargin. AR protein levels were found to be decreased after 12 h of incubation with either 10<sup>-6</sup> M A23187 or 10<sup>-7</sup> M thapsigargin. The decrease in AR mRNA and protein seemed to precede

apoptosis, since neither A23187 (24 h) nor thapsigargin (30 h) was found to alter cell morphology within the treatment time. Cycloheximide and actinomycin D were unable to change the calcium-mediated decrease in AR mRNA, ruling out the necessity for de novo protein synthesis or a change in mRNA stability. Moreover, the decrease in AR mRNA induced by calcium does not seem to involve protein kinase C- or calmodulin-dependent pathways, since inhibitors of these cellular components had no effect. Nuclear run-on assays demonstrated little or no effects of either A23187 or thapsigargin treatment on AR gene transcription (8 h and 10 h). In conclusion, these studies show that intracellular calcium seems to be a potent regulator of AR gene expression in LNCaP cells.

### **The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin**

CANCER RES. (USA), 1994, 54/23 (6167-6175)

Calcium ( $\text{Ca}^{2+}$ ) accumulates within the endoplasmic reticulum of cells through function of the sarcoplasmic reticulum and endoplasmic reticulum  $\text{Ca}^{2+}$ -dependent ATPase family of intracellular  $\text{Ca}^{2+}$ -pumping ATPases. The resulting pools have important signaling functions. Thapsigargin (TG) is a sesquiterpene gamma-lactone which selectively inhibits the sarcoplasmic reticulum and endoplasmic reticulum  $\text{Ca}^{2+}$ -dependent ATPase pumps with a 50% inhibitory concentration of approximately 30  $\mu\text{M}$ . Treatment of androgen-independent prostate cancer cells of both rat and human origin with TG inhibits their endoplasmic reticulum  $\text{Ca}^{2+}$ -dependent ATPase activity, resulting in a 3-4-fold elevation in the level of intracellular free  $\text{Ca}^{2+}$  ( $\text{Ca}(\text{i})$ ) within minutes of exposure. Due to a secondary influx of extracellular  $\text{Ca}^{2+}$ , this increase in  $\text{Ca}(\text{i})$  is sustained, resulting in morphological (cell rounding) and biochemical changes within 6-12 h (enhanced calmodulin, glucose regulated protein, and tissue transglutaminase expression, and decreased expression of the G(i) cyclins). Within 24 h of exposure, androgen-independent prostatic cancer cells stop progression through the cell cycle, arrest out of cycle in G0, and irreversibly lose their ability to proliferate with a median effective concentration value of 31 nM TG. During the next 24-48 h, the genomic DNA of the G0-arrested cells undergoes double-strand fragmentation. This is followed by the loss of plasma membrane integrity and fragmentation of the cell into apoptotic bodies. During this process, there is no acidification in the intracellular pH. Using cells transfected with the avian M(r) 28,000 calbindin D  $\text{Ca}^{2+}$ -buffering protein, it was demonstrated that the programmed death initiated by TG is critically dependent upon an adequate (i.e., 3-4-fold) sustained ( $>1$  h) elevation in  $\text{Ca}(\text{i})$  and not depletion of the endoplasmic reticulum pools of  $\text{Ca}^{2+}$ . These results demonstrate that TG induces programmed cell in androgen-independent prostatic cancer cells in a dose-dependent manner and that this death does not require proliferation or intracellular acidification but is critically dependent upon an adequate, sustained (i.e.,  $>1$  h) elevation in  $\text{Ca}(\text{i})$ .

### **Programmed cell death as a new target for prostatic cancer therapy**

CANCER SURV. (USA), 1991, 11/- (265-277):

To increase survival of men with metastatic prostatic cancer, a modality that can effectively eliminate androgen independent cancer cells is desperately needed. By combining such an effective modality with androgen ablation, all of the heterogeneous populations of tumour cells within a prostatic cancer patient can be affected, thus optimizing the chances of cure. Unfortunately, such effective therapy for the androgen independent prostatic cancer cell is not yet available. This therapy will probably require two types of agents, one having antiproliferative activity affecting the small number of dividing androgen independent cells, and the other able to increase the low rate of cell death among the majority of non-proliferating (ie interphase) androgen independent prostatic cancer cells present. Androgen dependent prostatic epithelial cells can be made to undergo programmed death by means of androgen ablation, even if the cells are not in the proliferative cell cycle. Androgen independent prostatic cancer cells retain the major portion of this programmed cell death pathway, only there is a defect in the pathway such that it is no longer activated by androgen ablation. If the intracellular free  $\text{Ca}^{2+}$  is sustained at an elevated level for a sufficient time, androgen independent cells can be induced to undergo programmed death. The long term goal is therefore to develop some type of non-androgen ablative method that can be used in vivo to induce a sustained elevation in  $\text{Ca}^{2+}$  in androgen independent prostatic cancer cells. To accomplish this task, a more complete understanding of the biochemical pathways involved in programmed cell death is urgently needed. At present, studies are focusing on the mechanism involved in the  $\text{Ca}^{2+}$  elevation in the normal and malignant androgen dependent cell induced following androgen ablation and the role of the TRPM-2 protein in this process.

### **Hyperparathyroidism in metastases of prostatic carcinoma: A biochemical, hormonal and**

## histomorphometric study

EUR. UROL. (Switzerland), 1990, 17/1 (35-39)

Secondary hyperparathyroidism can develop as a result of bone metastases from prostatic cancer, but this has not been studied from the multiple aspects of biochemistry, hormonal status and histomorphometry. In 20 patients with stage-D prostatic cancer, a transiliac bone biopsy was performed for histomorphometric study. In all of them, molecular parathormone (PTH-M) and osteocalcin were determined by radioimmunoassay together with other parameters considered to be biological markers of bone remodelling. Of these 20 patients, only 2 (10%) had elevated PTH-M (240 plus or minus 20.6 pmol/l), differing significantly from the other 18 (58.6 plus or minus 11.7 pmol/l) and from controls (60.4 plus or minus 7.2 pmol/l). In the high PTH-M patients, corrected calcium was low (7.8 plus or minus 0.4 mg/dl) as compared to normal PTH-M patients (9.2 plus or minus 0.5 mg/dl,  $p < 0.001$ ), and this was also the case for serum phosphorus (2.2 plus or minus 0.6 vs. 3.2 plus or minus 0.3 and 3.4 plus or minus 0.4 mg/dl, respectively  $p < 0.001$ ). Alkaline phosphatase was raised in the patient groups as compared to controls ( $p < 0.001$ ) and was higher in the high PTH-M group (362 plus or minus 58 vs. 224 plus or minus 62 U/l,  $p < 0.001$ ). The same pattern of higher values in the hyperparathyroid patients was repeated for: hydroxyproline/Cr in fasting urine (3.6 plus or minus 0.2 vs. 2.1 plus or minus 0.4 mg/mg,  $p < 0.001$ ); Ca/Cr in fasting urine (0.08 plus or minus 0.02 vs. 0.007 plus or minus 0.01 mg/mg,  $p < 0.001$ , decreased in both patient groups but more so in the high PTH-M group), and for the 24-hour urinary calcium (128 plus or minus 22 vs. 86 plus or minus 11 mg,  $p < 0.001$ ) which was only reduced ( $p < 0.001$ ) in normals. Serum osteocalcin, although raised in both groups, did not differ significantly between patient groups (15.1 plus or minus 2.3 ng/ml for hyperparathyroid patients and 14.4 plus or minus 5.2 ng/ml for normals), but was significantly different between patients and controls (6.8 plus or minus 3.1 ng/ml,  $p < 0.001$ ). Histomorphometrically, trabecular bone volume was elevated in both groups as compared to controls ( $p < 0.001$ ), and the resorption surface was increased in hyperparathyroid patients (9.7 plus or minus 1.1 vs. 4.7 plus or minus 2.8%,  $p < 0.001$ ), as was the osteoid seam thickness index (31.8 plus or minus 6.2 vs. 18.6 plus or minus 5.6,  $p < 0.001$ ). According to the Pearson test, only effected in the normoparathyroid group, the only significant and positive correlations were between osteocalcin and 24-hour urine calcium and between osteocalcin and Ca/Cr (both  $p < 0.001$ ). These results demonstrate the existence of a secondary hyperparathyroidism in 10% of patients with blastic bone metastases due to stage-D prostatic cancer and show that osteocalcin is not an adequate biological bone marker in these patients.

## In vitro studies of human prostatic epithelial cells: Attempts to identify distinguishing features of malignant cells

GROWTH FACTORS (United Kingdom), 1989, 1/3 (237-250)

Recent advances in culture techniques have enabled routine establishment and propagation of epithelial cells derived from normal and malignant tissues of the human prostate. Comparative studies of the responses of normal and cancer-derived cell populations to various growth and differentiation factors in vitro were undertaken to examine the possibility that cancer cells might respond differentially. Clonal growth assays in serum-free medium demonstrated that optimal proliferation of normal as well as cancer cell strains was generally dependent on the presence of cholera toxin, epidermal growth factor, pituitary extract, hydrocortisone, insulin and high levels of calcium in the culture medium, and on the use of collagen-coated dishes. Only one cancer strain responded aberrantly to epidermal growth factor and hydrocortisone. Putative differentiation factors (transforming growth factor-beta and vitamin A) inhibited the growth of all normal and cancer strains. The origin of a cancer-derived cell strain that responded similarly to normal strains was verified by positive labeling with a prostate cancer-specific antibody, validating the conclusion from these studies that normal and cancer prostatic epithelial cells are not distinguishable on the basis of responses to the tested factors.

## Hypocalcemia associated with estrogen therapy for metastatic adenocarcinoma of the prostate

J. UROL. (USA), 1988, 140/5 PART I (1025-1027)

We report 2 cases of true hypocalcemia (not caused by decreased binding protein) associated with metastatic prostate cancer and review previously reported cases. Hypocalcemia is a common but frequently unrecognized complication of prostatic cancer. Estrogen therapy often is associated with the hypocalcemia, which may be asymptomatic. The hypocalcemia is always associated with osteoblastic metastases and usually it is associated with increased serum alkaline phosphatase activity, acid phosphatase activity and serum parathyroid hormone

concentration. Serum concentrations of magnesium, phosphorus and vitamin D frequently are decreased. Patients are in a positive calcium balance. The osteoblastic metastases seem to act as a calcium sink, creating a 'hungry tumor phenomenon'. The role of estrogens may be to stop the resorption of normal bone resulting in lower serum calcium concentrations.

### **Hypercalcemia in carcinoma of the prostate: Case report and review of the literature**

J. UROL. (BALTIMORE) (USA), 1987, 137/2 (309-311)

Hypercalcemia developed in a man with recurrent adenocarcinoma of the prostate. Serum calcium became normal soon after bilateral orchiectomy and the patient was free of disease 18 months later. The absence of radiographically detectable bone metastases in this patient suggested a humoral mechanism for the hypercalcemia. Orchiectomy may be an effective treatment for hypercalcemia complicating prostatic carcinoma.

### **Calcium excretion in metastatic prostatic carcinoma**

BR. J. UROL. (ENGLAND), 1984, 56/6 (687-689)

In 64 men with prostatic carcinoma, calcium excretion per litre of glomerular filtrate (Ca(e)) was persistently lower in those with bone secondaries than in those with soft tissue involvement only, despite a normal range of serum calcium in both groups. In three patients who showed an improvement in their bony metastases on bone scan 6 months after starting treatment, the Ca(e) values had increased slightly but still remained in the low range. In a further five who showed no improvement on bone scan, Ca(e) values were lower than before. In patients with prostatic carcinoma, Ca(e) is an indicator of early changes in calcium homeostasis. It may also provide an objective indication of progression of bone secondaries.

### **Osteomalacia associated with prostatic cancer and osteoblastic metastases**

UROLOGY (USA), 1983, 21/1 (65-67)

A patient with carcinoma of the prostate, extensive bony metastases, and osteomalacia is reported. The diagnosis of osteomalacia was suspected because of generalized weakness and bone pains, hypocalcemia, hypophosphatemia, and raised alkaline phosphatase. It was documented by low 1,25-hydroxyvitamin D level. Furthermore, it was confirmed by improvement in patient's symptomatology and normalization of serum calcium and phosphorus after treatment with 1,25-hydroxyvitamin D<sub>3</sub> (Rocaltrol).

### **Carcinoma of the prostate: The treatment of bone metastases by radiophosphorus**

CLIN. RADIOL. (SCOTLAND), 1981, 32/6 (695-697)

Osseous deposits secondary to advanced carcinoma of the prostate are a common feature of the disease. These deposits are most often seen in the lumbar spine and pelvis and cause severe and intractable pain, often requiring large quantities of strong analgesia for alleviation of pain. Relief of pain can be achieved by external irradiation of these deposits, but this relief may not be permanent and the disease may be so widespread that it is impracticable to treat all the deposits by irradiation. Deposits from carcinoma of the prostate are usually multiple and all may cause pain at the same time. A method of delivering the radiation to all the deposits at the same time has been sought. Previous studies have shown that radioactive phosphorus (P32) can be used to obtain this localisation of radioactivity at sites of osseous activity. In this study 24 patients with bone metastases from carcinoma of the prostate were treated with radiophosphorus and methyl testosterone, or radiophosphorus with parathormone and calcium. An overall response rate of 58% shows this to be an effective palliative treatment. The results suggest there is a greater response when P32 is used in conjunction with parathormone and calcium, than with methyl testosterone.

## **Management of cancer of the prostate**

BRIT.J.HOSP.MED. (ENGLAND), 1974, 11/3 (357-372)

In this article the management of prostatic cancer is discussed according to the clinical stage of the tumor. Ordinarily, treatment of prostatic cancer should not be started until a positive histological diagnosis has been made and the patient has been properly staged. Minimal staging studies include a pretreatment prostatic serum acid phosphatase test and a skeletal survey.

## **Intracavitary irradiation of prostate carcinomas**

REV. MED. SUISSE ROMANDE (SWITZERLAND), 1980, 100/9

A method for the intracavitary irradiation of prostate carcinomas, used at the Central University Hospital in Lausanne in 1979 and 1980 on 10 patients is described. The technique, which is the afterloading type, consists of the positioning of a Cs 137 source in the proximal ureter. This is achieved with the aid of a Foley 26 balloon catheter introduced into the bladder after drainage cystostomy. The source remains in place for about 26 hours and delivers a dose of approximately 3800 rads to the prostate to a depth of 4 cm (NSD=2000 ret) and a maximum of 1700 rads to the rectum (NSD=700 ret).

## **Epidemiology of prostatic cancer: A case-control study**

PROSTATE (USA), 1990, 17/3 (189-206)

A population-based case-control study of prostatic cancer in Alberta was undertaken to determine the risk factors associated with the disease. Cases were 382 newly diagnosed prostatic cancer patients and 625 controls, group-matched to the anticipated age distribution of the cases, chosen at random from the health insurance roster. Subjects were interviewed in their homes by using a pre-tested questionnaire including questions related to ethnic group, education, puberty, marital history, family history, residence, water supply, smoking, and diet. Factors significantly related to the risk of developing prostatic cancer included ethnic group (British high, Ukrainian low), education (elementary high, university low), age at first marriage (early high, late low), family history (high risk for those with relatives with prostatic cancer), and increased masculinity among the children of cases. The results with respect to smoking, occupation, medical history, birthplace, residence, water supply, and diet were generally negative.

## **Demonstration of specifically sensitized lymphocytes in patients treated with an aqueous mistletoe extract (*Viscum album L.*)**

KLIN. WOCHENSCHR. (Germany), 1991, 69/9 (397-403)

Lymphocytes of 25 patients treated with an aqueous mistletoe extract (*Viscum album L.*) for up to 6 months (group 1), up to 2 years (group 2), and more than 2 years (group 3) were examined in 3- and 7-day cultures for specifically sensitized lymphocytes. The whole extract (HM), the lectin-polysaccharide fraction (HM-LP), and the 'viscotoxin' fraction (HM-V) were added at concentrations ranging from 0.5 microg to 12.5 mg extract/ml. Lymphocytes from four of the nine group 2 patients and five of the ten group 3 patients reacted specifically with HM and HM-LP at an optimal dose of 5.0 mg/ml, but did not react with HM-V. Stimulation indices varied between 1.6 and 16. In the patients of group 3 this effect was observed only when their lymphocytes were costimulated in the 3-day cultures with phytohemagglutinin (PHA), in contrast to the four patients of group 2 who reacted only in the 7-day cultures with HM-LP without PHA co-stimulation. Patients' lymphocytes had to be protected from mistletoe lectin-induced cytotoxicity by the addition of their own sera containing anti-mistletoe lectin antibodies. Lymphocytes from tumor patients (n = 18) never treated with mistletoe extracts and healthy individuals (n = 18) showed no specific proliferative response when tested in 3- and 7-day cultures. The production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon-gamma (IFN-gamma) was measured in the supernatants of lymphocytes cultures from all 25 patients and 36 controls exposed to HM, HM-LP, and HM-V in 3- and 7-day cultures. An

### **An urodynamic study of patients with benign prostatic hypertrophy treated conservatively with phytotherapy or testosterone**

WIEN. KLIN. WOCHENSCHR. (AUSTRIA), 1979, 91/18 (622-627)

Conservative therapy of benign prostatic hypertrophy comprises the administration of oestrogens, gestagens, androgens and anti-androgens. Phytodrugs, which contain an extract of *Sabal serrulatum* or *Pygeum Africana* as active substance are without side effects and are, therefore, being used increasingly. 74 patients with irritable or obstructive bladder symptoms due to benign prostatic hypertrophy were treated with a phytodrug (*Sabal serrulatum*) or with testosterone throughout a period of three months. In group one (20 patients given phytodrugs and 10 patients given testosterone) clinical symptoms and measurements of residual urine, residual urine quotient, bladder capacity, micturition pressure and maximum urethral closure pressure were recorded at the beginning and at the end of therapy. In group two 28 patients were treated with the phytodrug in the first and third months with an intervening placebo trial lasting four weeks and 16 patients were given testosterone. Clinical symptoms and uroflow and residual urine only were charted in this group. None of the patients in either group showed an improvement in the urodynamic parameters of obstruction, but all patients felt a subjective alleviation of their symptoms.

### **Phytoestrogens are partial estrogen agonists in the adult male mouse**

Environmental Health Perspectives (USA), 1995, 103/SUPPL. 7

The intake, as well as serum and urinary concentrations, of phytoestrogens is high in countries where incidence of prostate cancer is low, suggesting a chemopreventive role for phytoestrogens. Their significance could be explained by the ability to antagonize the action of more potent endogenous estrogens in initiation or promotion of tumor formation. We have studied estrogenicity and antiestrogenicity of dietary soy and two phyloestrogens, coumestrol and daidzein, in our neoDES mouse model for the study of prostatic neoplasia. Soy was chosen because it is rich in phytoestrogens, is widely used in Oriental diets, and has antiestrogenic and anticarcinogenic properties in the neoDES mouse when given from fertilization onward. In short-term tests with adult animals, no evidence for estrogenicity or antiestrogenicity (capability to antagonize the action of 17beta-estradiol) of soy was found when development of epithelial metaplasia and expression of c-fos protooncogene in prostate were used as end points of estrogen action. Estrogenic activity of coumestrol and daidzein on c-fos expression was subtle. Coumestrol, either given alone or in combination with 17beta-estradiol, had no effect on development of epithelial metaplasia. These marginal or missing effects in adult males could be interpreted by assuming that the neonatal period is more critical for estrogenic or antiestrogenic action of soy and phytoestrogens. Once initiated, estrogen-related lesions would develop spontaneously. Alternatively, the chemopreventive action of soy is not due to antiestrogenicity of soy-derived phytoestrogens.

### **Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet**

AM. J. CLIN. NUTR. (USA), 1991, 54/6

Epidemiologic studies revealed low mortality in hormone-dependent cancer in Japanese women and men consuming a traditional diet. We previously found that certain diphenolic food components, lignans and isoflavonoids, which are converted to biologically active hormone-like substances by intestinal microflora, may be cancer-protective agents. Therefore, we studied urinary excretion of these compounds (enterolactone, enterodiol, daidzein, equol, and O-desmethylangolensin) in 10 women and 9 men in a rural village south of Kyoto, Japan. The subjects consumed a typical low-fat diet with much rice and soy products, fish, and vegetables. An isotope-dilution gas chromatographic-mass spectrometric method was used for the assays. The urinary excretion of lignans was low but that of the isoflavonoids was very high. The excretion of isoflavonoids correlated with soybean-product intake. The low mortality in breast and prostate cancer of Japanese women and men, respectively, may be due to the high intake of soybean products.

## **Control of LNCaP proliferation and differentiation: Actions and interactions of androgens, 1 $\alpha$ ,25-dihydroxycholecalciferol, all-trans retinoid acid, 9-cis retinoic acid, and phenylacetate**

Prostate (USA), 1996, 28/3 (182-194)

There is increasing evidence that growth and differentiation of prostatic carcinoma cells may be modulated not only by androgens and growth factors but also by vitamin D, retinoids, and phenylacetate (PA). The latter agonists may have a role in the prevention and therapy of prostate cancer but their exact therapeutic potential remains unclear. Since both retinoids and vitamin D act via nuclear receptors, the same way androgens do, we studied the interactions of these compounds with androgen-induced proliferation and differentiation using LNCaP cells as a model of androgen-responsive tumor cells. PA was included because of its suspected different mode of action. (3H)-thymidine incorporation was used as a measure of proliferative activity, secretion of prostate-specific antigen (PSA) as a measure of differentiated function. The present data show that 1 $\alpha$ ,25-dihydroxycholecalciferol (VD3), all-trans retinoic acid (atRA), 9-cis retinoic acid (9cRA), and PA stimulated LNCaP cell-differentiated function in the presence or absence of androgens. The effects on cell growth were more complicated. In the absence of androgens growth stimulatory effects were observed for the retinoids and under some conditions for VD3. These effects were limited, however, and tended to be more pronounced at low cell densities. In the presence of androgens nearly exclusively growth inhibitory effects were observed. On a molar basis VD3 was the most effective antiproliferative agonist (ED50 = 10<sup>-9</sup> M). It completely neutralized the stimulatory effects of androgens. Growth inhibition was not due to a decrease in the concentration of androgen receptor: whereas atRA, 9cRA, and PA did not alter androgen receptor levels, VD3 provoked a twofold increase. Neither in the presence nor in the absence of androgens did we observe any cooperativity in the growth stimulatory or inhibitory effects of VD3, atRA, or 9cRA. To test whether treatment with any of the studied agonists resulted in a phenotypic reversion and sustained growth arrest, LNCaP cells were pretreated with VD3, atRA, 9cRA, or PA for 6-12 days and reseeded at equal densities as untreated cells. In all cases tested (3H)-thymidine incorporation was restored within 6 days suggesting that none of these compounds caused irreversible growth inhibition.

## **1,25-Dihydroxy-16-ene-23-yne-vitamin D3 and prostate cancer cell proliferation in vivo**

Urology (USA), 1995, 46/3 (365-369)

**Objectives.** 1,25-Dihydroxyvitamin D can inhibit the proliferation of prostate cancer cells, but its clinical use is limited by hypercalcemia. We examined the effects of a 'noncalcemic' vitamin D analogue, 1,25-Dihydroxy-16-ene-23-yne-cholecalciferol (16-23-D3), on the proliferation of human prostate cancer cells in a mouse model. **Methods.** Twenty-four athymic nude mice were inoculated with human prostate carcinoma cells from the PC-3 cell line. Twelve mice (experimental group) received injections of 1.6 microg of 16-23-D3 on alternate days over a 22-day period. Twelve mice (control group) received sham injections. Tumor volumes, pathologic findings, and terminal serum calcium levels were compared between groups. **Results.** The relative increase in tumor volume was significantly lower in the experimental than in the control group in the first interval following treatment ( $P < 0.01$ ). Mean tumor volumes in the experimental group were approximately 15% smaller than in the control group. Serum calcium levels did not differ between groups. **Conclusions.** 16-23-D3 showed modest antiproliferative effects on prostate cancer cells in this model without evidence of drug-induced hypercalcemia. These findings support the concept that vitamin D analogues can inhibit the proliferation of human prostate cancer cells in vivo.

## **Recent advances in hormonal therapy for cancer**

Current Opinion in Oncology (USA), 1995, 7/6

Hormonal manipulation of cancer is no longer confined to the use of effective antiestrogen therapy for breast cancer or surgical or hormonal castration for prostate cancer. A broader acknowledgment of the potential of different hormonal ligands to evoke cell cycle arrest to prevent the progress of neoplastic transformation, and even to elicit active cell death, has expanded the concept of hormonal therapy. The use of retinoids and deltanoids in conjunction with antiestrogens and antiandrogens is progressing into clinical trials. The use of glucocorticoids in conjunction with cyclic AMP may enhance apoptosis induction. The use of antiandrogens in conjunction with cytotoxic therapy may diminish the risk of bcl-2 mediated resistance in prostate cancer. Innovative use of sequential and synergistic hormonal manipulations based on an expanding understanding of transcriptional regulation promises to advance this science.

## **Endocrine control of prostate cancer**

Cancer Surveys (USA), 1995, 23/- (43-62)

Steroid hormones play an important part in prostate biology. Androgens are crucial for the normal development of the prostate gland and in maintaining its functional state in the adult. It seems that the prolonged presence of androgens might also be an important factor in the development of prostate cancer. In addition, androgens and oestrogens appear to play a part in the development of benign prostatic hypertrophy, although the exact nature of their role has not been clearly defined. Stimulation of prostate cancer growth by androgens is well established with androgen withdrawal therapy being the most effective therapy in men with prostate cancer. Additive steroid therapy of metastatic prostate cancer with oestrogens or progestogens has also proved effective. The effects of androgens on prostate cancer cell growth might be mediated through modulation of growth factor expression and alteration of growth factor receptor levels. Androgen response can be modulated by the expression of mutated oncogenes such as ras. Androgen independence can occur through a loss of AR expression or mutation of the AR; however, the patterns of AR expression in normal prostatic tissue from development to adulthood and in cancer are now just beginning to be described. Other steroids, such as the retinoids, show promise as preventive agents, possibly through the modulation of growth factors. Vitamin D compounds modulate prostate cancer cell growth, but their role in prevention and therapy is unclear.

## **Vitamin D and prostate cancer**

Advances in Experimental Medicine and Biology (USA), 1995, 375/-

Our findings demonstrate the presence of VDR in various human prostate cancer cell lines and in primary cultures derived from normal, BPH and prostate cancer. In addition, 1,25-D induced several bioresponses in these cells including growth inhibition and PSA stimulation. Based on examples in many different malignant cells as well as our data in prostate cells, that vitamin D is anti proliferative and promotes cellular maturation, it seem clear that vitamin D must be viewed as an important cellular modulator of growth and differentiation in addition to its classical role as regulator of calcium homeostasis. In this respect, vitamin D has the potential to have beneficial actions on various malignancies including prostate cancer. Its ultimate role in prostate cancer remains to be determined, but 1,25-D may prove useful in chemoprevention and/or differentiation therapy. We believe the data currently available provide the basis for an optimistic view on the possible use of vitamin D to treat prostate cancer in patients and that further investigation is clearly warranted to belief define its potential therapeutic utility.

## **Actions of vitamin D3 analogs on human prostate cancer cell lines: Comparison with 1,25-dihydroxyvitamin D3**

ENDOCRINOLOGY (USA), 1995, 136/1 (20-26)

Data from epidemiological studies has suggested that vitamin D deficiency may promote prostate cancer, although the mechanism is not understood. We have previously demonstrated the presence of vitamin D receptors (VDR) in three human prostate carcinoma cell lines (LNCaP, PC-3, and DU-145) as well as in primary cultures of stromal and epithelial cells derived from normal and malignant prostate tissues. We have also shown that 1,25-dihydroxyvitamin D3 (1,25-(OH)<sub>2</sub>D<sub>3</sub>) can elicit an antiproliferative action in these cells. In the present study we compared the biological actions of 1,25-(OH)<sub>2</sub>D<sub>3</sub> to those of a series of natural vitamin D<sub>3</sub> metabolites and several synthetic analogs of vitamin D<sub>3</sub> known to exhibit less hypercalcemic activity in vivo. In ligand binding competition experiments, we demonstrated the following order of potency in displacing (3H)1,25(OH)<sub>2</sub>D<sub>3</sub> from VDR: EB-1089 > 1,25-(OH)<sub>2</sub>D<sub>3</sub> > MC-903 > 1,24,25(OH)<sub>2</sub>D<sub>3</sub> > 22-oxacalcitriol (OCT) > 1α,25-dihydroxy-16-ene-cholecalciferol (Ro24-2637) > 25-hydroxyvitamin D<sub>3</sub>, with EB-1089 being similar 2-fold more potent than the native hormone. No competitive activity was found for 25-hydroxy-16,23-diene-cholecalciferol. When compared for ability to inhibit proliferation of LNCaP cells, MC-903, EB-1089, OCT, and Ro24-2637 exhibited 4-, 3-, 3-, and 2-fold greater inhibitory activity than 1,25-(OH)<sub>2</sub>D<sub>3</sub>. Interestingly, although OCT and Ro24-2637 exhibit, respectively, 10 and 14 times lower affinity for VDR than 1,25-(OH)<sub>2</sub>D<sub>3</sub>, both compounds inhibited the proliferation of LNCaP cells with a potency greater than that of the native hormone. The relative potency of vitamin D<sub>2</sub> metabolites and analogs to inhibit cell proliferation correlated well with the ability of these compounds to stimulate prostate-specific antigen secretion by LNCaP cells as well as with their potency to induce the 25-hydroxyvitamin D<sub>3</sub>-24-hydroxylase messenger RNA transcript in PC-3 cells. In

conclusion, these results demonstrate that synthetic analogs of vitamin D<sub>3</sub>, known to exhibit reduced calcemic activity, can elicit antiproliferative effects and other biological actions in LNCaP and PC-3 cell lines. It is noteworthy that although binding to VDR is critical for 1,25-(OH)<sub>2</sub>D<sub>3</sub> action, the analog data indicate that additional factors significantly contribute to the magnitude of the biological response. Finally, the strong antiproliferative effects of several synthetic analogs known to exhibit less calcemic activity than 1,25(OH)<sub>2</sub>D<sub>3</sub> suggest that these compounds potentially may be useful as an additional therapeutic option for the treatment of prostate cancer.

### **Vitamin D and cancer**

REV. FR. ENDOCRINOL. CLIN. NUTR. METAB. (France), 1994, 35/4-5

Receptors for 1 $\alpha$ ,25-(OH)<sub>2</sub>-D<sub>3</sub> have been detected not only in the classical target organs, the intestine, kidney and bone but also in other sites such as the skin, pancreas and certain cells of the immune system. A wide variety of human cancer cell lines (including breast, prostatic cancer and leukemia) also have these receptors. In vitro studies have shown that the biologically active metabolite of vitamin D, 1 $\alpha$ ,25-(OH)<sub>2</sub>-D<sub>3</sub> inhibits cell proliferation and stimulates the differentiation of many cell types. Such studies prompted the suggestion of the use of conventional vitamin D compounds in the treatment of certain malignancies. It is shown in vivo that 1 $\alpha$ ,25-(OH)<sub>2</sub>-D<sub>3</sub> may inhibit the growth of mammary carcinomas but at the risk of hypercalcemia and hypercalciuria. For this reason synthetic analogues have been developed which retain the ability to inhibit cell proliferation and promote cell differentiation but have reduced their calcemic activity. Modifications of the side chain of 1 $\alpha$ ,25-(OH)<sub>2</sub>-D<sub>3</sub> can create superanalogues with enhanced non-calcemic activity (10 to 100-fold) and decreased calcemic potency. These analogues have been successfully used in animal models of leukemia and breast cancer.

### **Human prostate cancer cells: Inhibition of proliferation by vitamin D analogs**

ANTICANCER RES. (Greece), 1994, 14/3 A (1077-1081)

1,25-Dihydroxyvitamin D (1,25(OH)<sub>2</sub>D<sub>3</sub>, calcitriol) can inhibit the proliferation of some human prostate cancer cells but its clinical use is limited by hypercalcemia. We therefore explored the bioactivity of less calcemic vitamin D analogs. We studied the effects of calcitriol and 3 synthetic analogs at concentrations of 10<sup>-6</sup> to 10<sup>-12</sup> M on the in vitro proliferation of 3 human prostate carcinoma cell lines: DU 145, PC-3, and LNCaP. Calcitriol and analogs showed significant antiproliferative activity on PC-3 and LNCaP cells. DU 145 cells were inhibited by the analogs only. We conclude that vitamin D analogs warrant further investigation as therapeutic agents in prostate cancer.

### **Vitamin D and prostate cancer: 1,25 Dihydroxyvitamin D<sub>3</sub> receptors and actions in human prostate cancer cell lines**

ENDOCRINOLOGY (USA), 1993, 132/5 (1952-1960)

It has been suggested that vitamin D deficiency may promote prostate cancer, although the mechanism is not understood. In this study three human prostate carcinoma cell lines, LNCaP, DU-145, and PC-3, were examined both for the presence of specific 1,25 dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) receptors (VDRs) and also employed to study the effects of hormone on cell proliferation and differentiation. Ligand binding experiments demonstrated classical VDR in all three cell lines examined with an apparent dissociation constant of 7.5, 5.4, and 6.3 x 10<sup>-11</sup> M for LNCaP, DU-145, and PC-3 cells, respectively. Corresponding binding capacity for the three prostate carcinoma cell lines were 27, 31, and 78 fmol/mg protein, respectively. The presence of VDR in the three cell lines was also confirmed by immunocytochemistry. In addition, one major 4.6-kilobase messenger RNA transcript hybridizing with a specific human VDR complementary DNA probe was identified in all three cell lines. Interestingly, both DU-145 and PC-3 but not LNCaP cell lines exhibited 1,25(OH)<sub>2</sub>D<sub>3</sub>-stimulated induction of 24-hydroxylase messenger RNA employed as a marker of 1,25(OH)<sub>2</sub>D<sub>3</sub> action. Physiological levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> dramatically inhibited proliferation of the LNCaP and PC-3 cell lines. However, in spite of the presence of high affinity VDR, proliferation of DU-145 cells was not inhibited by 1,25(OH)<sub>2</sub>D<sub>3</sub> at the doses tested. Treatment with 1,25(OH)<sub>2</sub>D<sub>3</sub> caused a dose-dependent stimulation of prostate-specific antigen secretion by LNCaP cells. In conclusion, these results demonstrate that these three human prostate carcinoma cell lines all possess specific VDR and that 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment can elicit both an antiproliferative and a differentiating action on these cancer cells. The findings lend support to the hypothesis that vitamin D might exert beneficial actions on prostate cancer risk.

## **Is vitamin D deficiency a risk factor for prostate cancer? (hypothesis)**

ANTICANCER RES. (Greece), 1990, 10/5 A (1307-1312)

Prostate cancer is a major cause of cancer death among males, yet little is known about its etiology. We hypothesize that Vitamin (Hormone) D deficiency may underlie the major risks for prostate cancer, including age, Black race, and northern latitudes. These factors all are associated with decreased synthesis of Vitamin D. Mortality rates from prostate cancer in the U.S. are inversely correlated with ultraviolet radiation, the principal source of Vitamin D. This hypothesis is consistent with known antitumor properties of Vitamin D, and may suggest new avenues for research in prostate cancer.

## **The in vitro response of four antisteroid receptor agents on the hormone-responsive prostate cancer cell line LNCaP**

Oncology Reports (Greece), 1995, 2/2 (295-298)

Previous reports indicate that flutamide withdrawal is associated with PSA declines and tumor shrinkage in selected patients with 'hormone-refractory' prostate cancer. Though the mechanisms underlying this effect are not clear, investigators have hypothesized that these effects are mediated by mutant androgen receptors recognizing hydroxy-flutamide as an androgenic agonist. Such receptors have been well described in the human prostate cancer cell line LNCaP. Despite the finding that the androgen receptor of LNCaP aberrantly recognizes a variety of steroids, including estrogen and progesterone, as androgenic agonists, there are no studies which examine the effect of estrogen antagonists and progesterone antagonist on baseline and androgen-stimulated LNCaP growth. In this report, LNCaP cells were cultured in phenol red-free media using charcoal-stripped sera. As previously reported, flutamide enhanced LNCaP growth and bicalutamide inhibited androgen-stimulated LNCaP proliferation. Neither tamoxifen nor RU486 influenced LNCaP growth (either in the presence or absence of exogenous androgens). From these data we conclude that antagonists of estrogen and progesterone action have no anti-proliferative effect on LNCaP cells and that the mutant androgen receptor expressed in these cells is quite restrictive in the recognition of compounds with antagonistic activity. The clinical implications of these findings are discussed.

## **Combination treatment in M1 prostate cancer**

CANCER (USA), 1993, 72/12 SUPPL. (3880-3885)

The treatment of advanced prostate cancer is based on hormone manipulation to eliminate the trophic effect of testosterone on sensitive androgen tissue of the tumor. In this study, we evaluated the efficacy of the partial androgen blockage versus the complete androgen blockage. One hundred, twenty- two patients were entered in this study and randomly were treated with buserelin alone or with buserelin and flutamide. The group that received buserelin was given cyproterone acetate (200 mg/day) during first 3 weeks of treatment to avoid 'flare-up'. During the follow-up (range 0-244 plus or minus 1 weeks), we evaluated 59 patients (61.4%) that had positive response and 37 patients (38.6%) that showed progressive disease: There were no statistically significant differences between the two treatment groups, not even in the evaluation of median time to response and of median time to treatment failure. In conclusion, the results emphasize that total androgenic blockage is as effective as a luteinizing hormone-releasing hormone analog used alone.

## **Antiandrogenic drugs**

CANCER (USA), 1993, 71/3 SUPPL. (1046-1049)

Background. Prostate cancer is the most frequent cancer diagnosed in American men today. Currently, about half of all patients with newly diagnosed prostate cancer present with metastatic diseases. Methods. Antiandrogenic drugs, or more appropriately androgen-receptor antagonists, represent a group of compounds that currently have played a limited role in the treatment of metastatic prostate cancer. Their method of action is primarily one of blocking

androgens at their receptor sites in target tissues. They generally are classified as steroidal or nonsteroidal compounds. Cyproterone acetate and megestrol acetate are synthetic steroidal antiandrogenic drugs that, not only compete with testosterone and dihydrotestosterone for androgen receptors, but also have progestational activity and reduce pituitary luteinizing hormone and subsequently plasma testosterone. Nonsteroidal antiandrogenic agents (flutamide, Casodex (ICI Pharmaceuticals, England), and nilutamide) block cellular binding of androgens only, and there is no reduction of testosterone levels. Results. Antiandrogens have been used in numerous trials both in Europe and the United States. This group of compounds have been used as monotherapy and in combination therapy, ie, with orchiectomy or with LHRH agonists. Conclusions. Currently, antiandrogens are used primarily in conjunction with conventional medical or surgical castration to achieve maximal androgen deprivation; however, ongoing clinical studies are comparing these compounds alone against standard hormonal therapy. It seems probable that antiandrogens will play an expanding role in the treatment of metastatic prostate cancer as well as having a role in the treatment of prostate cancer.

### **The effects of flutamide on total DHT and nuclear DHT levels in the human prostate**

PROSTATE (USA), 1981, 2/3 (309-314)

The effects of flutamide, an antiandrogen, on prostate tissue concentrations of total DHT, DHT present in both crude and purified nuclear fractions, prostatic acid phosphatase (PAP) and plasma testosterone were studied and compared to similar parameters in untreated benign prostatic hypertrophy (BPH). Flutamide was given to patients with BPH in a dosage of 750 mg per day by mouth for 10-14 days prior to transurethral resection of the prostate. Total prostate DHT was significantly decreased to 3.95 ng/g in 12 flutamide-treated patients compared to values of 6.61 ng/g in 12 patients with untreated BPH. However, no significant difference was noted in the concentration of DHT present in the crude nuclear fraction of flutamide-treated patients (646 pg/mg DNA, N = 5) and untreated BPH (882 pg/mg DNA, N = 10); nor was DHT in the purified nuclear fraction significantly different in drug versus untreated patients (251 pg/mg DNA for flutamide versus 353 pg/mg DNA for untreated controls). PAP concentration in BPH prostates was 7.11 S.U./mg wet weight and was significantly higher than 2.98 S.U. per mg wet weight noted in flutamide-treated patients. Plasma testosterone tended to rise in the flutamide-treated patients compared to the untreated BPH but this was not statistically significant. The decrease in total prostate DHT without changes in nuclear DHT was unexpected and difficult to explain in terms of current tenets regarding the mechanism of androgen action. The following hypotheses are offered: Flutamide may decrease transport of testosterone into cells, thereby decreasing total prostate DHT. Inhibitory effects of flutamide on receptor-bound DHT translocation to nuclei may be difficult to detect since 95% or more of nuclear DHT may not be bound to a salt extractable receptor. The binding of DHT directly to putative nuclear matrix receptor sites may dilute the effects of flutamide on blocking translocation of receptor bound DHT, resulting in very small differences in DHT present in purified nuclei difficult to detect with current methodology.

### **Endocrine profiles during administration of the new non-steroidal anti-androgen Casodex in prostate cancer**

CLIN. ENDOCRINOL. (United Kingdom), 1994, 41/4 (525-530)

Objective - Casodex (Zeneca) is a new potent, long-acting non-steroidal anti-androgen, which produces androgen deprivation by blocking the androgen receptor. We evaluated the endocrine effects of Casodex 150 mg daily given in monotherapy as primary treatment for patients with prostate cancer. Design - As part of a large, multicentre study comparing the therapeutic effects of surgical castration with 150 mg/day Casodex in monotherapy for patients with prostate cancer, a subgroup of 23 patients on Casodex were studied in detail for changes in endocrine parameters. Serum levels of LH, FSH, testosterone, DHT, oestradiol, prolactin, sex hormone binding globulin and free testosterone were measured at the start of therapy and after 1, 4, 8, 12 and 24 weeks. Effects on libido, sexual activity and the appearance of hot flushes, breast pain and gynaecomastia were recorded. Results - Administration of Casodex resulted in a rise in LH levels in all patients with a mean increase after 24 weeks of 102% ( $P < 0.001$ ). Mean FSH levels showed a limited increase (7%) after 24 weeks, which was significant only after 1 week ( $P < 0.001$ ). As a result of the high LH levels, total testosterone levels increased after 24 weeks by 66% ( $P < 0.001$ ), free testosterone by 57% ( $P < 0.001$ ) and dihydrotestosterone by 24% ( $P = 0.0112$ ). Parallel to testosterone, oestradiol levels rose by a mean of 66% ( $P < 0.001$ ). Mean sex hormone binding globulin and prolactin levels rose by respectively 8% ( $P = \text{NS}$ ) and 65% ( $P < 0.01$ ). Despite an increase in testosterone levels, excellent androgen blockade was obtained, as shown by a decrease in prostate specific antigen levels in 22/23 patients. Libido was maintained in 8/11 patients, and sexual activity in 5/6. No patient complained of hot flushes. However, mild gynaecomastia and/or breast tenderness were seen in 48 and 30% of cases respectively. Conclusion - Casodex 150

mg/day monotherapy resembles surgical castration in achieving androgen deprivation, despite an increase in LH and testosterone levels. In contrast to castration, libido and sexual activity are usually maintained and hot flushes are rare. However, mild gynaecomastia and/or breast tenderness were noted in 48 and 30% of patients.

[Return to the Table of Contents](#)

All Contents Copyright © 1995-2009 Life Extension Foundation All rights reserved.

**LifeExtension®**

These statements have not been evaluated by the FDA. These products are not intended to diagnose, treat, cure or prevent any disease. The information provided on this site is for informational purposes only and is not intended as a substitute for advice from your physician or other health care professional or any information contained on or in any product label or packaging. You should not use the information on this site for diagnosis or treatment of any health problem or for prescription of any medication or other treatment. You should consult with a healthcare professional before starting any diet, exercise or supplementation program, before taking any medication, or if you have or suspect you might have a health problem. You should not stop taking any medication without first consulting your physician.