Molecular Targets for Dietary Prevention of Prostate Cancer

Executive Summary

On June 16, 2000, the National Cancer Institute (NCI) convened a group of scientists in the fields of nutrition, pathology, epidemiology, molecular biology, immunology, and genetics to discuss research relevant to the interdisciplinary study of nutrition and prostate cancer. Drs. Carolyn Clifford, Young Kim, and John Milner moderated the various meeting sessions.

Introduction - Dr. Peter Greenwald

Dr. Greenwald explained that the purpose of the meeting was to develop an agenda for building the transdisciplinary study of prostate cancer and nutrition. In 1998, the Prostate Cancer Progress Review Group created a list of recommendations for the biomedical research community. These recommendations included studies of the role of nutrition in the etiology and primary prevention of prostate cancer. The Prostate Cancer Progress Review Group cited epidemiological studies that suggest a relationship between prostate cancer and fat, lycopene, insulin-like growth factor (IGF), and vitamins A, D, and E. The Nutritional Implementation Group recommended that the NCI sponsor interdisciplinary workshops to link basic biological and nutritional science research with the study of cancer etiology and pathogenesis.

Studies of Diet and Prostate Cancer in Humans: Moderator, Dr. Carolyn Clifford

Epidemiological Evidence for Diet and Prostate Cancer Relationship - Dr. Meir Stampfer

Dr. Stampfer outlined several clues to the relationship between diet and cancer, including:

- Large variations in rates of clinically important prostate cancer between countries
- Migratory studies demonstrating that international variation in prostate cancer rates are not primarily due to genetic differences.
- Environmental changes that are accompanied by changes in cancer rates within countries
- Ecologic studies suggesting relationships between specific nutrients and cancer
- Epidemiological studies (e.g., intervention trials) that found cancer/nutrient correlations.

Dietary Fat Intake

Evidence of a relationship between dietary intake and prostate cancer includes:

- increase in fat consumption and rise in cancer incidence in Japan
- increase in prostate cancer among Japanese immigrants
- correlation between fat intake and prostate cancer mortality rates in different countries
- link between prostate cancer and other cancers that are influenced by fat intake

Correlations between prostate cancer incidence and mortality and fat intake may be due to other nutritional components of foods high in fat. A meta-analysis of prostate cancer and diet studies revealed a stronger link between prostate cancer and fat in dairy foods than fat in meat products.

Dr. Stampfer presented data from a prospective cohort study of 50,000 men that was conducted as part of the Health Professionals Follow-Up Study. This study found strong associations between dietary fat intake and stage C and D prostate cancer after adjusting for age and energy intake. Among the different types of fat examined in this study, alpha-linolenic acid had the strongest link to high stage prostate cancer.

Lycopene Intake

Research suggests a protective effect of lycopene that warrants further study. Lycopene is found primarily in tomatoes and tomato sauce. Dr. Stampfer and colleagues found that total intake of tomato products significantly reduced incidence of total prostate cancer, with an increased risk reduction for advanced cancer. Other studies found a significant inverse relationship between lycopene blood levels and prostate cancer incidence. The Physicians Health Study also found an inverse relationship between circulating blood levels of lycopene and aggressive prostate cancer incidence. Experimental biologic evidence also supports the role of lycopene in cancer prevention. High circulating blood levels of IGF-1, which causes cell proliferation, are strong predictors of prostate cancer. Cell proliferation resulting from high circulating IGF-1decreases as lycopene blood levels increase.

Vitamin D and Calcium Intake

The most active form of vitamin D [1,25 -$(OH)_2$-D_3] is related to reduced prostate cancer risk.

Blood levels of 1,25-$(OH)_2$-D_3 (1,25-D) can be significantly decreased with high dietary calcium intake. The inverse relationship between calcium intake and blood levels of the 1,25-D suggest that high calcium intake may increase prostate cancer risk. Ecologic data support this hypothesis. Prostate cancer mortality is higher in countries with high intake of dairy products, and some research suggests that individuals who consume more dairy products have a higher incidence of prostate cancer. For example, Giovannucci found a significant increase in prostate cancer risk (RR=1.75) with calcium intake of 2 or more grams per day. High calcium intake also was associated with a three-fold increase in relative risk for stages C and D prostate cancer. Analysis of Physicians Health Study data also reveals a modest increase in prostate cancer risk with increased dairy consumption.

Dr. Stampfer used data from the Health Professionals Follow-Up Study to compare the effects of dietary and supplemental calcium intake on prostate cancer risk. He found an association between supplemental calcium intake and increased risk of prostate cancer, even with low dietary calcium intake. This finding suggests that prostate cancer risk is specifically related to calcium intake rather than dairy product consumption.

Selenium

Research indicates that selenium may be important in reducing prostate cancer risk. A randomized skin cancer trial found a marked reduction in prostate cancer with increased selenium intake (Clark). Dr. Stampfer and colleagues also found an association between high levels of selenium in toe nail clippings and decreased incidence of prostate cancer.

Vitamin E

The Alpha-Tocopherol/Beta-Carotene Cancer Prevention (ATBC) Study of Finnish smokers produced data suggesting a relationship between increased vitamin E intake and decreased prostate cancer incidence. Dr. Stampfer and colleagues examined this

relationship in men taking 100 or more IU of vitamin E per day and found no decrease in risk. They found nonsignificant decreased risk in a subsample of smokers in this study.

Conclusions

- Dietary factors differentially affect prostate cancer risk depending on disease stage; therefore, studies of total prostate cancer incidence are likely to miss important associations with dietary factors.
- Prostate specific antigen (PSA) screening is reducing the number of late stage prostate cancers diagnosed, along with the opportunity to observe the effects of dietary factors that only reduce the risk of late stage disease. These dietary factors may provide clues to determinants of the progression of prostate cancer.
- Molecular markers must be identified for metastatic prostate cancer.
- Research should focus on the calcium/prostate cancer connection.

Discussion

Participants discussed the need to balance the suspected role of calcium in increased prostate cancer risk with calcium's role in preventing osteoporosis and perhaps decreasing colon cancer risk. The level of calcium intake at which prostate cancer risk increases should be investigated.

Participants emphasized the need for dietary studies in the African-American population, which experiences higher incidence and mortality from prostate cancer. African-Americans in the United States tend to consume more dietary fat and less tomato products, which may put them at increased risk for prostate cancer.

Selenium and Vitamin E Cancer Prevention Trial (SELECT) - Dr. Howard Parnes

The SELECT trial will be launched in the fall of 2000 to assess the separate and combined effects of selenium and vitamin E on clinical incidence of prostate cancer. About 32,400 patients ages 55 or older (50+ for African Americans) will participate in this trial. Subjects will receive 200 micrograms of selenium in the form of selenomethionine and/or 400 micrograms of vitamin E each day. Secondary endpoints will be cancer-free survival, lung and colon cancer incidence, total incidence of all cancers, all-cancer survival, overall survival, and serious cardiovascular events. Tertiary objectives will focus on quality of life; molecular epidemiology; gene-environment interactions; tissue storage, including lymphocytes, white cells, and DNA; pathology of biomarker studies; assessments of prestudy intake of vitamin E and selenium, including supplement usage and baseline levels. Subjects will be stratified by age, use of finasterides, and family history. Accrual of subjects will take place over 5 years, and the trial is expected to last 12 years. Baseline rates for prostate cancer incidence will be taken from PCPT during the first 3 years of the trial and from the Surveillance Epidemiology and End Results Program (SEER) in years 4 to 12. The trial will have:

- Ninety-six percent power to observe a 25 percent risk reduction for a single agent versus placebo
- Ninety-nine percent power to observe a slightly higher risk reduction with the combination of agents versus placebo
- Eighty-nine percent power to observe a 25 percent risk reduction with the combination of agents versus a single agent.

Selenium

Observational studies suggested a correlation between low selenium levels and increased prostate cancer risk. Case control studies corroborated these findings, but were difficult to interpret because some tumors concentrate selenium. Prospective studies were inconsistent in finding a significant, inverse relationship between selenium intake and prostate cancer risk. The strongest evidence supporting this relationship comes from the Nutritional Prevention of Cancer Study. This prospective, placebo-controlled study was designed to examine the effects of high-selenium yeast consumption on incidence of nonmelanoma skin cancer. Secondary endpoints, established before the trial was completed, were all cause mortality, total cancer incidence, and prostate, lung, and colorectal cancer incidence. Selenium intake was associated with a significant decrease in prostate cancer incidence and a 50 percent reduction in total cancer mortality. Analyses conducted before and after the establishment of secondary endpoints revealed similar reductions in relative risk with increased selenium intake.

Selenium is an essential component of the antioxidant, glutathiol-peroxidase (GSH-peroxidase). Putative mechanisms for protection against prostate cancer include anti-proliferative effects due to inhibition of protein synthesis, increased apoptosis, and possible effects on cellular immunity.

Vitamin E

Vitamin E is the most important fat-soluble antioxidant component of the cell membrane. Vitamin E also may affect immune function and be involved in protein-binding activity that reduces cellular proliferation. In the ATBC Trial, male smokers were administered low doses of either vitamin E, beta-carotene, or both. Prostate cancer incidence, a secondary trial endpoint, decreased by 32 percent in men randomized to the vitamin E

supplement group. Prostate cancer mortality decreased by 41 percent for men in this group.

Discussion

Participants asked about the following aspects of the SELECT trial:

- Primary endpoints, which are clinical detection of prostate cancer either by biopsy or high PSA with a positive bone scan.
- Patients diagnosed with slow-growing (clinically nonsignificant) prostate cancers. Biopsies will not be mandated, thus reducing the risk of diagnosis of subclinical cancer. Most PSA-detected prostate cancer is clinically significant.
- Use of selenomethionine. Selenomethionine represents one-half of the selenium in selenized yeast and is relatively nontoxic compared to other selenium compounds.
- Collection of data on smoking behavior. Smoking has been shown to increase prostate cancer mortality but not incidence, so it is not a focus of the study.

Experimental Evidence Supporting Specific Dietary Factors: Moderator, Dr. Young Kim

Dietary Lipids - Dr. David Heber

Dr. Heber began by presenting his hypothesis that "prostate cancer results from a genetic/ environmental interaction in which diet and lifestyle have a major role in the development and progression of this cancer through the effects of calories, physical activity, fat, fiber, phytochemicals, and micronutrients on cellular and molecular processes." He emphasized that dietary lipids must be examined in the context of the total diet.

Foods that contain large amounts of alpha-linolenic acid and linoleic acid (Omega-6 fatty acid) include nuts, cheese, milk, salad dressing, mayonnaise, butter, and fatty meats and fishes. Linoleic and linolenic acid are specifically involved in pathways for prostaglandin synthesis. Linoleic acid also is a signal protein with a PPAR gamma receptor in the nucleus with the ligand of prostaglandin J3 (PGJ3). PGJ3 is efficiently converted from arachidonic acid in tumor cells.

Arachidonic acid and eicosapentaenoic acid compete for lipoxygenase and cyclooxygenase (COX) enzymes. Biopsies from prostate cancer patients taking fish oil supplements reveal eicosapentaenoic acid in the prostate glands with modulation of COX expression.

A Dietary Intervention for Prostate Cancer Patients

Dr. Heber and colleagues are conducting a dietary intervention trial in which prostate cancer patients are treated with a diet of low fat, high fiber, and soy protein. Patients in the treatment group have done well in complying with the diet long term. Fat intake in the treatment group has been decreased from 28 percent to 15 percent of calories. Dietary fiber intake has been increased from 14 to 28 percent in this group. The treatment group experienced a ten-fold increase in urinary isoflavones without significant change in body fat or weight early on in the very short-term trials. Patients on the diet for 3 to 6 months lost weight.

Animal Studies

A study conducted by Connolly, Coleman, and Rose found a 30 percent increase in prostate cancer in mice that were fed a high fat, linoleic acid rich diet. In this experiment, nude mice were implanted with a human prostate cancer cell. Another animal study by Fleshner, Fair, Huryk, and Heston examined interaction of vitamin E and dietary fat intake in the growth of human prostate tumors implanted in nude mice. They found that vitamin E supplementation with a high fat diet led to significant reduction in tumor growth. These researchers concluded that the mechanism of dietary fat-induced prostate cancer is mediated by oxidative stress. Linoleic acid is a highly oxidizable lipid. Animal research also has shown that conjugated linoleic acid (CLA) can significantly inhibit tumor growth. CLA affects fat metabolism in animals, so that it counteracts obesity. CLA may have an indirect caloric restriction effect on animals that has not been demonstrated in humans.

Dr. Heber and colleagues looked at the interaction effects of dietary fat, soy protein, and isoflavones on the growth of human androgen-sensitive prostate cancer in severe-combined immunodeficient (SCID) mice. Four groups of mice were fed diets of either (1) high-fat;

(2) high-fat, soy protein, and isoflavone extract; (3) low-fat; or (4) low-fat, soy protein, and isoflavone extract. A 15 percent reduction in final xenograft tumor weights was observed in the group that received low-fat diets with soy protein and isoflavone extract. Studies using subcutaneous xenografts tend to produce small reductions in tumor mass. Orthotopic tumor transplantation studies may produce stronger results. At least one orthotopic transplantation study of soy and green tea intake has found 50 percent reductions in tumor growth and metastasis rates (Ronzell).

Conclusions

- A good animal model of prostate cancer does not exist. Domesticated dogs get prostate cancer and may provide good animal models.
- Animal studies should examine dietary fat and fatty acid modulation by reducing dietary fat and supplementing omega-3 fatty acids to reduce prostate cancer progression.
- *In vivo* studies are needed to identify intermediate biomarkers responsive to dietary fat and fatty acid treatment.
- More research is required to investigate the mechanisms by which dietary fat or fatty acid modulation could prevent prostate cancer progression. Research has suggested that fat interacts with other nutrients, including antioxidants, soy phytochemicals, lycopene, green tea, flavanoids, and calcium. For example, high fat binds calcium and lower fat leads to increased bioavailability of calcium.

Discussion

Participants suggested that animal models be used to examine differential gene expression of PSA. Participants also suggested that separate effects of substrates and enzymes be studied. The COX enzyme alone can bioactivate several carcinogens.

Arachidonic Acid and Cell Proliferation - Dr. Charles E. Myers

Dr. Myers noted that all fats have a glycerol backbone with fatty acids attached. Therefore, an important research gap is how each component fatty acid affects the molecular biology of prostate cancer. Dr. Myers and colleagues examined the effects of different fatty acids on various human prostate cancer cell lines. They observed that stearic and linoleic acid bind to all human prostate cancer cell lines. Alpha-linolenic acid and arachidonic acid were found to be powerful mitogens for the prostate cancer cell lines. These acids were more powerful mitogens than testosterone for the hormone-responsive LNCaP cell line. Arachidonic acid also was a powerful survival factor for prostate cancer cells. Further investigation revealed that arachidonic acid converts to various eicosanoids.

Prostate carcinomas exhibit about a ten-fold enhancement of prostaglandin E(2) [PGE(2)] production compared to the surrounding tissue. PGE(2) is lethally toxic to natural killer cells and cytotoxic T-cells. The toxicity of PGE(2) may explain the lack of lymphocytes in high-grade prostate cancer tumors (Gleason 7 or higher). An inverse correlation also exists between PSA levels and circulating levels of CD4 cells and natural killer cells. A patient with widespread metastatic disease has a CD4/CD8 ratio similar to that of an AIDS patient. COX-2 expression also is enhanced in prostate cancer tissue and blood vessels as compared to normal prostate tissue. COX-2 has been shown to be a powerful intermediary of tumor angiogenesis. Only one study has examined the therapeutic benefits of COX-2 inhibitors. This study found that delivery of reasonable levels a COX-2 inhibitor to prostate cancer tissue cultures initiated apoptosis. This finding implicates PGE(2) in the regulation of cell survival.

Animal studies found that presentation of CLA to prostate tumors causes many tumors to turn black and fall off. This effect would be expected from an anti-angiogenesis rather than a cytotoxic agent. Other research indicated that CLA can prevent the conversion of arachidonic acid to eicosanoids. The anti-angiogenesis potential of CLA warrants further investigation.

Dr. Honn and colleagues discovered that prostate cancer cells presented with arachidonic acid express 12-lipoxygenase (12-LOX). 12-LOX metabolizes arachidonic acid to

12(S)-hydroxyeicosateraenoic acid [12(S)-HETE], which may be essential to the invasiveness of prostate cancer cell lines. Dr. Honn engineered a prostate cancer cell line to overexpress 1

2(S)-HETE, which enhanced tumor growth *in vivo* but not *in vitro*. The *in vivo* tumor growth correlated with a dramatic amplification of angiogenesis. 12-LOX expression also was increased in high grade prostate cancer and metastatic disease.

Dr. Myers' research has shown 5-lipoxygenase (5-LOX) to be a powerful mitogen (300 percent increase in cell proliferation). He found that proliferation was not affected by inhibiting production of prostaglandin synthetase, PGE(2), 12(S)-HETE, or 15(S)-HETE *in vitro*. However, when 5-LOX production was inhibited, proliferation stopped. Every available prostate cancer cell line undergoes apoptosis within one hour after inhibiting 5-LOX production. Proliferation halted through the 5-LOX inhibitor could not be rescued by any leukotriene. Proliferation was restored with exogenous 5-hydroxyeicosatetraenoic acid (5-HETE), a metabolite of 5-LOX. These findings indicate that the most important aspect of arachidonic acid biology for fueling proliferation is the synthesis of 5-HETE. Prostate cancer cells die when 5-HETE is removed. Dr. Myers found large amounts of

preformed fas and fas ligand in prostate cancer cells. When 5-HETE is inhibited, fas ligand comes to the cell surface within 15 minutes, and fas comes to the cell surface and death signaling begins within 30 minutes. 5-HETE may force sequestration of the fas death receptor and its ligand at intercellular sites, thus preventing its activation. All prostate cancer cells have enough fas and fas ligand for apoptosis to occur.

5-LOX inhibitors show promise in treating prostate cancer. 5-LOX knockout mice do not go into shock when injected with a platelet activating factor, do not get arthritis or inflammation, but lack osteoclasts. 5-LOX inhibitors appear to increase bone density in humans.

Conclusions

Evidence exists indicating that arachidonic acid can stimulate:

- angiogenesis
- avoidance of immune surveillance
- cell proliferation
- cell survival via 5-HETE and PGE(2).

Further research is needed to:

- determine the arachidonic acid content of various foods
- compare COX-1 and COX-2 for carcinogen activation
- discover how human lipoxygenases affect activation of aromatic carcinogens.

Discussion

Participants asked whether linoleic acid may directly stimulate oxidative changes or activate carcinogens in the prostate without converting to arachidonic acid. Prostate cancer cells express large amounts of low-density lipoprotein (LDL) receptors. Research is needed to explain why prostate cancer is a sink for cholesterol.

Participants also asked about the role of COX-1 and COX-2 inhibitors in reducing prostate cancer risk. Dr. Myers has found that chronic use of combined COX-1 and COX-2 anti-inflammatory drugs causes about a 50 percent reduction in risk of advanced prostate cancer.

Participants noted that the study of genomics and, more importantly, proteomics, may reveal how various dietary components modulate different expression systems. Informatics offers the capability to link biochemical studies with gene information.

Soy Products and Genistein - Dr. Stephen Barnes

Dr. Barnes began by pointing out that cell culture studies generally find no effect of genistein on prostate cancer cell proliferation because levels of isoflavones needed to produce this result are very high (concentrations greater than 50 micromolar). Animal models provide some evidence of the effects of genistein on prostate cancer, including reduction in the amount of invasive tumor and the rate of tumor appearance, and lowered incidence of poorly differentiated tumors.

Dr. Barnes conducted a trial of the use of soy protein in men over age 55 with elevated PSA levels but no histologically confirmed prostate cancer. Soy protein did not reduce PSA levels, but plasma cholesterol levels were significantly reduced. Subjects displayed a wide variability in the serum concentrations of isoflavones, although subjects all were administered the same amount of soy protein.

Many scientists believe that the effect of genistein on prostate cancer is estrogenic. Animal studies do not support this hypothesis. Estrogenic effects are observed in rats and mice only when genistein was administered subcutaneously. High levels of genistein intake also produce minimal estrogenic effects in humans. However, breast cells proliferation was observed in women taking genistein. Genistein binds to estrogen receptors ERα and ERß (found in prostate cells). The beta ring of genistein has been shown to bind to ERß at the same site as the alpha ring of estradiol. Chemists may use this model to improve genistein binding to ERß.

Other potential targets for chlorinated isoflavones include:

- Adenosine receptors
- Hic kinase (3-chloro-isoflavone binds to the site of this tyrosine kinase inhibitor)
- Gamma receptors, which bind chloro-nitro flavanoid.

Genistein is believed to inhibit tyrosine kinase production. Dr. Barnes' research demonstrated that genistein has no effect on the tyrosine kinase or epidermal growth factor receptors in prostate cancer cells. Genistein's effect on prostate cancer may occur at the gene level.

Genistein intake appears to influence specific compounds that are present in prostate tissue. Rats taking genistein exhibit a principal metabolite that may be derived from the genistein molecule (2HPPA) in prostate tissue. Dr. Barnes and colleagues conducted an experiment in which neutrophils were used to produce an oxidated burst that releases carcinogenic compounds from cells. Cells with the genistein isoflavone that received the neutrophil stimulus rapidly produced large amounts of chlorinated and nitrated compounds as the genistein was depleted. Investigators need to examine the properties of these new compounds.

Conclusions

- Genomics and proteomics may yield more focused hypotheses regarding the mechanisms through which genistein affects prostate cancer.
- The effects of metabolism on the process through which soy, genistein (and other compounds) act on prostate cancer must be studied.
- Pure compounds should not be studied in cell cultures because outcomes are misleading.
- Human studies should examine interactions of genistein with other dietary components.

- Tissue studies should be used to identify new compounds because many compounds in the blood are filtered out before reaching the tissue cells.

Discussion

Participants noted that the study that found breast cell proliferation with genistein intake estrogenic effects of this isoflavone. However, cell proliferation alone is not evidence of an estrogenic effect. Studies of humans taking genistein have been poorly controlled nutritionally, so cell proliferation may be explained by factors other than genistein. Participants cited xenograft studies in which genistein was found to produce inhibition of cell proliferation. Scientists are doing transductions with ERα and beta receptors in breast and prostate cancer cells, which may lead to the creation of a model for examining the estrogenic effects of compounds.

Participants recommended studies of the enzymes involved in the breakdown of flavanoid. In both humans and animals, about 80 percent of genistein is converted to alternative metabolites.

Vitamin D - Dr. Robert H. Getzenberg

Dr. Getzenberg noted that the protective effects of vitamin D were first suggested by epidemiological data showing that prostate cancer mortality was higher in northern regions where ultraviolet (UV) light exposure was lower. Other studies indicated that diets high in vitamin D reduced prostate cancer risk. Laboratory studies also produced data showing growth inhibition in most prostate cancer cell lines treated with vitamin D. High levels of melanin in the skin of African Americans inhibit absorption of vitamin D, which leads to lower levels of circulating 1,25-D, and may explain the increased prostate cancer incidence and mortality in this population. Hydroxylation of 1,25-D by the kidney also decreases with age, leading to a reduction in 1,25-D, which may partially explain increased prostate cancer incidence with aging.

Dr. Getzenberg and colleagues conducted an experiment in which castrated mice were treated either with testosterone, vitamin D, or a combination of testosterone and vitamin D. After 2 weeks, mice treated with vitamin D had prostates about 2 times larger than the other mice. Mice treated with vitamin D exhibited primarily stromal cell proliferation in the prostate, as opposed to the normal epithelial cell proliferation produced by androgens. Mice treated with testosterone and vitamin D exhibited increased differentiation in the prostate. Changes in nuclear matrix proteins (NMPs) also were observed in this group. Another study conducted by Dr. Getzenberg and colleagues revealed unusual prostate development in male mice whose mothers were given vitamin D during pregnancy. These males exhibited small body size and increased prostate size. Seventy percent of these males died of cardiac arrest at puberty. Castration prior to puberty reduced these deaths rates.

Dr. Getzenberg conducted a trial in which mouse prostate tumors were treated with calcitriol administered for 3 consecutive days each week, followed by administration of dexamethasone to reduce hypercalcemia. PSAs were reduced by 50 percent in one-third of these mice, 80 percent exhibited a decreased rise in PSA level, and 35 percent showed stabilized disease and PSA levels reduced by more than 50 percent. It is unclear whether reduction in PSA level was due to calcitriol or dexamethasone. Patients in clinical trials were treated with vitamin D doses of about 12 micrograms and suffered minimal hypercalcemia when dexamethasone was administered. The best outcomes were achieved when vitamin D was given on Monday, Tuesday, and Wednesday with dexamethasone given on subsequent days.

Studies using microdialysis probes have revealed that patterns in vitamin D level in prostate tissue differ from those in the blood. Vitamin D levels in the blood tend to peak about 2 hours after administration and then drop. Vitamin D levels in prostate tissue peak within 6 hours after administration, then exhibit a higher peak about 12 hours later.

Discussion

Participants mentioned research by Xue L, Newmark H, Yang K, and Lipkin M that supports

Dr. Getzenberg's findings regarding the increase in stromal/decrease in epithelial cells in prostatic tissue in mice treated with vitamin D.

Molecular Markers for Prostate Cancer - Angelo M. De Marzo

Dr. De Marzo noted that oxidants may cause cancer by forming adducts onto DNA. If that DNA replicates and cannot be repaired it can lead to mutation. Mutation may lead to cell proliferation above the normal baseline. Carcinogens may be inactivated by glutathione-S-transferase (GST). The promoter region of the GSTB gene is hypermethylated in about 90 percent of prostate cancer cells. This hypermethylation irreversibly inactivates GST production. A model has been developed for how GSTB is selected in carcinogenesis. Low levels of chronic oxidative stress over many years were modeled *in vitro* by giving low dose radiation over time. A large increase of 8-hydroxy-guanosine (8-OHGua), an adduct of DNA, was observed in these radiated cells. Transfection with the GSTB clones led to a dramatic reduction in oxidative injury to these cells. Transfection also increased survival of radiated cells over time. The GST therefore appears to reduce radiation-induced cell damage and stimulate apoptosis of damaged cells.

Levels of GST can be significantly increased by diet. Dr. De Marzo and colleagues found that GSTα and GSTμ are present in normal prostates and may be induced by diet. GSTB is methylated so it cannot be induced with normal dietary factors. Research is underway to develop agents that demethylate genes.

Diet also may influence levels of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), a protein present after high temperature cooking of many types of meat. PhIP, when activated by hydroxylation in the liver and converted to a DNA-binding form, can lead to the development of DNA adducts.

N-hydroxy- PhIP added to normal prostate epithelium tissue produces DNA adducts. PhIP added to LnCaP cells increases adduct formation, which can be inhibited by transfecting cells with GST.

In the normal prostate, GSTB is mainly present in the basal cells. Stem cell features apparently switch from basal to secretory in a carcinoma. A population of transiently proliferating cells have been identified in the prostate. These cells are sensitive to androgens. Dr. De Marzo and colleagues hypothesized that these cells may be the targets for transformation of stem cells in carcinomas. They observed that proliferation in prostatic intraepithelial neoplasia (PIN) shifted from basal cell to secretory cell compartments. Secretory cells that proliferate may be more susceptible to genome damage because they normally do not produce GSTπ levels of p27. Normal secretory cells have high levels of p27, which is decreased in high grade PIN cells. Levels of p27 in the primary prostate tumor are prognostic (higher levels indicate a better prognosis). Dr. De Marzo hypothesized that cells that switch to a secretory compartment but continue to proliferate lead to PIN and cancer.

Chronic inflammation has been linked to cancer in many organs. Dr. De Marzo and colleagues hypothesized that long-standing tissue damage results from inflammatory cells' release of reactive oxygen and nitrogen species. Inflammation also may promote cell proliferation in an attempt to repair damaged tissue. Proliferative Inflammatory Atrophy (PIA) has been linked to carcinoma in the prostate. PIA also may be a precursor of high grade PIN because PIA lesions have been found to merge with high grade PIN in a large proportion of cases. PIA is associated with chronic inflammation. Atrophied cells are highly proliferative, although they lack cytoplasm. Nitric oxide synthetase produced by macrophages may damage epithelial cells through the formation of peroxy-nitrate and other radicals that can damage DNA. GSTπ expression

is increased in PIA lesions, which may be a response to oxidative stress. GSTα also is expressed in PIA lesions, but not in normal prostate tissue.

Future Research Directions

- Look at prevalence of PIA with inflammation in prostates with and without PIN and cancer, beginning with South East Asian populations.
- Examine PIA lesions molecularly for evidence of oxidative DNA damage.
- Use animal models to accelerate carcinogenesis of prostate by inducing inflammation. Inflammation may be induced by removing soy isoflavones from the diet of animals prone to autoimmune disease. Imprinting the prostate with estrogen also leads to prostatitis in mice.
- Study the relationship of sexually transmitted diseases (STDs) to prostate cancer. Epidemiological studies found significant increases in relative risk of prostate cancer in men who reported having had gonorrhea or syphilis.
- Conduct trials of anti-inflammatory agents (i.e., genistein, dexamethasone, vitamin D).
- Conduct studies of prostatitis to examine leukotrienes and PGE as well as DNA methyl transferase expression in basal cells.

Transgenic Animal Models - Jeffrey E. Green

Dr. Green noted that transgenic models are useful for examining natural history, biologic characteristics, alterations during tumor progression, and therapeutic approaches. Some key issues in creating transgenic mice models include:

- Targeting of cells in which the transgene is expressed
- Use of hormone dependent promoters, which confounds studies of hormone effects on cancer
- Determining the relevance of the gene under study to the treatment of human cancer.

Dr. Green studied the rat C3(1) transgene, which is androgen responsive. The C3(1) t-antigen (tag) led to the development of PIN in rats at about 3 to 4 months of age. PIN progressed to invasive carcinoma at about 7 to 8 months of age. Almost 20 percent of early PIN lesions demonstrated a RAS mutation in codons that were significant. This mutation rate was about 30 percent in invasive carcinomas. These mutation rates are similar to those found in prostate cancer patients in Japan.

Tag binds to two expressor genes that are relevant to human prostate cancer, and is thought to functionally inactivate them. Several molecular models now use tag to induce prostate lesions. These include C3(1)-SV40 and probasin (428)-SV40, which express T/t antigen (higher expression with the latter model). Another model is probasin (11K), which only expresses T-antigen. Other prostate cancer models include:

- C3(1)-PyMt, which is expressed in many urogenital male organs, and leads to metastasis
- C3(1)-BCl2, which is implicated in human prostate cancer but leads to primarily stromal proliferation (some epithelial) as in benign prostatic hyperplasia (BPH)
- probasin-ras T24, a ras mutation that produces prostatic hyperplasia without nuclear atypia
- MMTV-kgf, which is expressed in the prostate at low levels and over expresses keratin growth factor (kgf). MMTV-kgf causes hyperplasia without atypia.
- MT-TGF, metallothionine fused to a dominant negative form of the TGF$ type 2 receptor, blocks TGF signaling and causes PIN-like lesions and basal carcinoma without metastasis.

Dr. Green and colleagues are using microarray technology to investigate changes in expression profiles during tumor progression, and differences between molecular signatures at different cancer sites with the same oncogene driving the transformation process. They also used microarrays to compare cancer tissues treated and untreated with an agent that controls angiogenesis. Microarrays are problematic because biologically significant cutoff points are hard to determine.

Dr. Green and colleagues are developing tissue arrays from animal cancer models that will allow scientists to better identify the cells responsible for oncogenic changes. Dr. Green also is attempting to identify molecular targets responsible for the chemopreventive effects of DFMO and DHEA.

Future Research Directions

Research is required to compare mice models of prostate cancer with human prostate cancer and to compare breast cancer and prostate cancer. Studies in the fields of biology, histopathology, genomics (genome analysis), and bioinformatics (expression profiling) are needed to make these comparisons.

Discussion

Participants asked about the use of microarrays in examining different stages of cancer. Dr. Green is looking at ways to create microarrays to study end-stage cancer.

Participants questioned the utility of transgenic models for studying chemoprevention of human cancer. Mouse models are useful for controlling and testing genetic alterations. Other animal models may be more applicable to the study of human prostate cancer (i.e., rats, dogs).

Panel Discussion on Recommendations for Future Directions: Moderator, Dr. John Milner

Panel members: Leland W. Chung, Dr. Samson T. Jacob, Barbara C. Pence, Harold Adelman, Meir Stampfer, David Heber, Charles E. Myers, Stephen Barnes, Robert H. Getzenberg, Angelo H. De Marzo, Jeffrey E. Green

Future Directions for Nutrition-Prostate Cancer Prevention Research

Participants recommended that future research into prostate cancer focus on the nutritional interventions, populations, and molecular markers listed below. Participants further recommended that some interventions and populations receive high priority in future research initiatives. These priority interventions and populations are followed by an asterisk (*).

Participants identified ways that preclinical studies can most effectively support human studies of nutrition and prostate cancer. Participants also suggested several ways for the NCI to support interdisciplinary collaboration to study the connection between nutrition and prostate cancer.

Which nutritional interventions appear to be most promising to decrease prostate cancer risk?

- Vitamin D (calcitriol)*
- Obesity
- High fat/high calorie diet*
    - examine compositional and total fatty acids
    - examine fatty acid relative to caloric intake
- High refined carbohydrates
- Selenium * (trials underway)
- Vitamin E * (trials underway)
- Lycopene (from tomato products) *
- Cooked meat (nitrosamines and heterocyclic amines)
- Allyl sulfur (GSH) from cruciferous vegetables
- Garlic
- Tea
- Omega-3 fatty acids (from fish oil)
- Soy/genistein (isoflavones) *
- COX inhibitors.

Which high-risk groups should be targeted?

A model is needed to define prostate cancer risk and confirm target groups. Participants agreed that the following groups should be targeted for chemoprevention trials:

- Men with a Gleason score (PSA level) from 4 to 10 *
- Men with high grade PIN *
- Men ages 30 and older. About 30 percent of men aged 30 are estimated to have microscopic carcinomas. Preventive research and interventions should focus on this age group.
- Men ages 70 and older, to examine effects of oxidants. Endogenous oxidant generation is increased in older people because mitochondrial functioning is less efficient. This group may be an ideal research population because of reduced immune function, susceptibility to nutritional modulation, inflammation, and decreased tendency to treat prostate cancer. *

- First-degree relatives of prostate cancer patients *
- Men of South/West African descent (i.e., African Americans, Jamaicans, etc.) *
- Identical twins, to examine environmental versus genetic risk factors
- Prostate cancer patients after treatment/surgery (for nutritional chemoprevention).

Which potential dietary and/or prostate-specific molecular markers should be explored as intermediate and end point measures?

Assays of needle biopsies, expressed fluid analysis, and nuclear magnetic resonance (NMR), and tissue banks can be used to study the following biomarkers:

- 8-OHGua
- Oxidative DNA damage/abduct formation
- Methylation
- Androgen gene targets
- Changes in prostate-specific genes
- Prostate-specific transcription factor (a late stage, androgen independent marker)
- Proliferation
- Apoptosis
- Angiogenesis.

How can preclinical studies be used to support human studies?

- Cell culture models to compare cancer and normal prostate cells
- Mode of tumor administration
- Comparisons of androgen independent and dependent tumors
- Orthotopic examinations
- Microarray/tissue array studies to examine different stages of cancer development
- Comparative studies of cells/tissues in different populations (i.e., between age groups)
- Identification of GST methylation gene markers
- Identification of prostate-specific gene markers
- Investigation of the role of metalloproteases (MMPs) in cell invasion
- Identification tumor suppression genes
- Examination of the expression of p10 and p27 *in vitro*
- Cytohybridization studies with identified markers
- Microdissection and quantitative RTPCR to study specific genes
- Nutritional/stromal and epithelial cell interaction studies
- Studies of androgen receptor target genes and ways to promote androgen dependence in proliferating prostate cells
- Microarray studies of nutrient/gene interactions
- Transgenic model studies, to test pharmaceuticals and discover additional molecular markers.

What can the NCI do to enhance interdisciplinary collaborations?

- Create working groups (genetics, molecular biology, nutrition, biochemistry). Participants will collaborate in organizing these groups.
- Conduct more meetings like this one to discuss relevant research from a range of fields.
- Fund collaborative studies
- Create tissue banks and gene and protein arrays for prostate cancer
- Train physicians in the field of nutrition.